CHEMICAL ANALYSIS AND BIOLOGICAL STUDIES OF EXTRACTS OF *Smallanthus sonchifolius* (POEPP.) H. ROB. FROM NEPAL

A DISSERTATION SUBMITTED TO THE

DEPARTMENT OF CHEMISTRY

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By

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DECLARATION

This dissertation entitled **"CHEMICAL ANALYSIS AND BIOLOGICAL STUDIES OF EXTRACTS OF *Smallanthus sonchifolius* (POEPP.) H. ROB. FROM NEPAL"** submitted to the Department of Chemistry, Amrit Campus, Institute of Science and Technology (IOST), Tribhuvan University, Nepal for the partial fulfillment of the Master of Science (M.Sc.), a degree in Chemistry is a research work carried out by me under the supervision of Assoc. Prof. Dr. Ram Lal (Swagat) Shrestha Department of Chemistry, Amrit Campus, Tribhuvan University, Nepal.

I, Ashika Tamang, declare that this work presented herein is genuine and initially done by me and has not been published or submitted in part or complete in this or any other form to any university or institute, here or elsewhere, to award any degree.

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RECOMMENDATION LETTER

This is recommended that the dissertation work entitled "**CHEMICAL ANALYSIS AND BIOLOGICAL STUDIES OF EXTRACTS OF *Smallanthus sonchifolius* (POEPP.) H. ROB. FROM NEPAL**" had been carried out by **Ms. Ashika Tamang** as partial fulfillment for the Master's Degree in Chemistry under my supervision. This material has not been submitted to any institution for a degree.

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LETTER OF APPROVAL

This dissertation entitled "**CHEMICAL ANALYSIS AND BIOLOGICAL STUDIES OF EXTRACTS OF *Smallanthus sonchifolius* (POEPP.) H. ROB. OF NEPAL**" submitted by **Ms. Ashika Tamang**, under the supervision of **Assoc. Prof. Dr. Ram Lal (Swagat) Shrestha**, Department of Chemistry, Amrit Campus, Institute of Science and Technology (IOST), Tribhuvan University, Nepal, is now approved for the partial fulfilment of the Master of Science (M.Sc.) Degree in Chemistry. This dissertation has never been submitted to another university or organization to confer a degree.

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ABSTRACT

Smallanthus sonchifolius (Poepp.) H. Rob., often known as "yacon" is a member of the Asteraceae family endemic to the Andes region. It has been used in folk medicine to treat diabetes, digestive disorders, obesity, and other diseases. In the current work, *S. sonchifolius* leaf and root extracts were prepared using hexane, chloroform, ethyl acetate, methanol, and distilled water as solvents using air-dried, powdered plant material and tested for different chemical and biological activities. The distilled water root extract had a high yield percentage of 6.37% whereas the lowest yield was found of ethyl acetate with 0.05% yield. The qualitative phytochemical screening had shown that methanol extract of plant contains phytochemicals like flavonoids, phenolic compounds, terpenoids, tannins, steroids, and carbohydrates. Hexane leaf extract's GC-MS analysis revealed 17 primary potential components where 7,8,8-trimethyl-4,5-diazatricyclo [4.2.1.$0^{3,7}$] non-4-ene was abundant (27.02%). The GC-MS analysis of methanol leaf extract identified 4 main possible components where 4-bromobutyric acid and 3-methylbut-2-yl ester were abundant (91.70%). Hexane root extract's GC-MS analysis, revealed 30 major potential components where 3,7,7-Trimethyl-bicyclo [2.2.1] hept-2-yl)-methanol (23.33%) and Cyclocopacamphenol (15.10%) were most abundant. The quantitative analysis showed that the chloroform root extract had higher total phenolic content i.e. 69.97 mg GAE/g whereas chloroform root extract had the highest total flavonoid content i.e. 350.87 mg QE/g. From the DPPH free radical scavenging method, the potential antioxidant property was shown by methanol leaf and root extract with the IC_{50} value below 500 µg/mL. From the α-amylase inhibition experiment, methanol leaf extract showed medium antidiabetic activity with the lowest IC_{50} value of 733.83 µg/mL. In the cytotoxicity test by using brine shrimp lethality assay, three extracts (MeOH leaf, chloroform root and MeOH root) showed medium toxicity with the LC_{50} value between 100-500 µg/mL whereas chloroform leaf extract showed high toxicity with LC_{50} value less than 100 µg/mL (92.76 µg/mL). The antimicrobial test showed that ethyl acetate root extract had better antimicrobial action against all the tested micro-organisms. The highest ZOI was observed against B*acillus subtilis* with ZOI of 1.1 cm. TLC showed different numbers of spots for various extracts indicating the presence of several compounds. UV-Vis analysis of the fractions from column chromatography has shown the existence of

multiple bonds, aromaticity, conjugation, and unsaturated organic molecules in the plant extracts. FT-IR analysis of fractions verified the presence of alcohols (-OH), nitro group (-NO_2), peroxides, carbonyls, and amines in the fraction compounds. From all above results, it can be concluded that this plant has potential therapeutic and pharmacological importance in the future drug discovery.

Keywords: *Smallanthus sonchifolius*, phytochemicals, GC-MS, column chromatography, biological activity.

सोधसार

भुइँ स्याउ (*Smallanthus sonchifolius* (Poepp.) H. Rob.,) प्राय 'याकोन' भनेर चिनिने एस्टेरेसी परिवारको सदस्य हो जुन एन्डिज क्षेत्रमा उपायोग गरिएको पाइएको थियो । एस बिरुवालाई घरेलु औषधीको रुपमा मधुमेह, पाचन प्रणालिको समस्याहरु, मोटोपना लगाएत अन्य रोगहरुको उपचारको लागी प्रयोग गरिएको पाईएको छ । यस अनुसन्धानमा बिरुवाको पात र जराका एक्स्ट्र्याक्टहरु छायादार, सुकेको पातको पाउडरलाई ५ वटा विभिन्न सोल्भेन्टहरू, हेक्जेन, क्लोरोफर्म, इथाइल एसीटेट, मिथानोल र डिस्टिल्ड वाटर, प्रयोग गरेर अल्ट्रासोनिक निकासी विधिबाट प्रशोधन गरियो र विभिन्न रसायनिक तथा बायोलोजिकल गतिविधिहरुको परीक्षण गरियो । बिरुवाको पाच एक्स्ट्र्याक्टहरु मध्ये डिस्टिल्ड वाटर एक्स्ट्र्याक्टको उपज प्रतिशत बढी पाइयो (६.३७ %) भने सबसे कम इथाइल एसीटेटको (०.०५ %) पाईयो । गुणात्मक फाइटोकेमिकल स्क्रिनिङले मिथानोल एक्स्ट्र्याक्टमा एल्कालोइड्स, फ्लेभोनोइड्स, फेनोलिक कम्पाउन्डहरू, ट्यानिन्स, स्टेरोइड्स, र कार्बोहाइड्रेटको उपस्थिति जनायो । GC-MS परीक्षणमा हेक्जेन पातको एक्स्ट्र्याक्टमा १७ वोटा प्रबल कम्पाउन्डहरू देखियो भने मिथानोल पातको एक्स्ट्र्याक्टमा ४ वोटा र हेक्जेन जराको एक्स्ट्र्याक्टमा ३० वोटा प्रबल कम्पाउन्डहरू देखियो । फाइटोकेमिकल्सको मात्रात्मक परीक्षणबाट उच्चतम कुल फेनोलिक कन्टेन्ट (TPC) ६९.९७ mg GAE/g मात्रा सहित क्लोरोफर्म जराको एक्स्ट्र्याक्टमा पाईयो भने उच्चतम कुल फ्लेभोनोइड कन्टेन्ट (TFC) ३५०.८७ mg QE/g मात्रा सहित क्लोरोफर्म जराको एक्स्ट्र्याक्टमा पाईयो । एन्टिअक्सिडेन्ट गतिविधिको परीक्षण DPPH फ्री रेडिकल स्क्याभेन्जिङ विधिबाट गरियो जसमा मिथानोल पातको एक्स्ट्र्याक्टले २४२.७४ µg/mL को IC_{50} मानको साथ संभाव्यतात्मक एन्टिअक्सिडेन्ट क्षमता देखायो । एन्टि-डाइबेटिक गतिविधिको परीक्षण

DNSA विधिबाट गरियो जसमा मिथानोल पातको एक्स्ट्र्याक्टले ७३३.८३ µg/mL को IC_{50} मानको साथ मध्यम α-amylase अवरोधक क्षमता देखायो । एक्स्ट्र्याक्टहरुको बिषाक्तताको परख ब्राइन श्रीम्प (brine shrimp) एस्सेबाट गरियो जसमा मिथानोल पात र जरा अनि क्लोरोफर्म जराको एक्स्ट्र्याक्टको LC_{50} को मात्रा १००-५०० µg/mL सहित मध्यम मात्रामा विषाक्त पाइयो भने क्लोरोफर्म पातको एक्स्ट्र्याक्ट IC_{50} मात्रा १०० µg/mL भन्दा कम सहित बडी विषाक्त पाइयो । एन्टिमाइक्रोबियल गतिविधि परीक्षण अगार दिफ्फुजन विधिबाट गरियो जसमा इथाइल एसीटेटले सम्पूर्ण तीन माइक्रोअर्गानिजममा उच्चतम जोन अफ इन्हिबिशन (ZOI) सहित राम्रो एन्टिमाइक्रोबियल गतिविधि देखायो जसमध्धे उच्च ZOI १.१ cm सहित B*acillus subtilis* मा देखियो । TLC परीक्षणले बिभिन्न एक्स्ट्रयाक्रटमा धेरै स्पट संख्याहरू देखायो । मिथानोल पातको एक्स्ट्र्याक्टको कोलुमं क्रोम्याटोग्रफीबाट प्राप्त फ्राक्सनहरुको UV विश्लेषणले फ्राक्सनहरूमा अन्स्याचुरेशन, यारोम्याटिसिटी, कन्जुगेसन तथा मल्टिपल बोन्ड समूहको अणुहरुको उपस्थिति पुष्टि गर्यो भने FT-IR विश्लेषणले अल्कोहल (-OH), नाइट्रो ग्रुप ($-NO_2$), इथर (-O-), पेरोक्साईड, कार्वोनाइल, अमाइन्स, लगायतका फंगसनल ग्रुपहरुको उपस्थिति प्रमाणित गर्यो । माथि प्राप्त परिणामहरुबाट हामी यो भन्न सक्छौ कि एस बिरुवा भविष्यमा हुने औषधी खोजमा थप अनुसन्धानको लागि एक महत्तोपुर्ण सम्भावना भएको बिरुवाको रुपमा देखिन्छ ।

कीवर्डहरु: भुइँ स्याउ, फाइटोकेमिकल्स, GC-MS, कोलुमं क्रोम्याटोग्रफी, बायोलोजिकल गतिविधि

LIST OF ACRONYMS AND ABBREVIATIONS

AOA/AA	:	Antioxidant Activity
AST	:	Antimicrobial Susceptibility Test
ATCC	:	American Type Culture Collection
AC	:	Ante Cibum
ABTS	:	2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid
AUC	:	Area Under the Curve
BHA	:	Butylated hydroxy anisole
CBMN	:	Cytochalasine B-Blocked Micronucleus
CHO-K1	:	Chinese Hamster Ovary Cells
DPPH	:	2, 2- Diphenyl-1-picrylhydrazyl
DCFH	:	2,7-Dichloro-dihydro fluorescein
DMSO	:	Dimethyl Sulfoxide
DNSA	:	3,5-Dinitrosalicylic Acid
FOS	:	Fructooligosaccharides
FCR	:	Folin-Ciocalteu Reagent
FTIR	:	Fourier-Transform Infrared Spectroscopy
GAE	:	Gallic Acid Equivalent
GC-MS	:	Gas Chromatography-Mass Spectroscopy
HPLC-DAD	:	High-Performance Liquid Chromatography Photodiode Array Detection
IC_{50}	:	Inhibitory Concentration 50% Inhibition
LC_{50}	:	Lethal Concentration for 50% Mortality
LDL	:	Low-Density Lipoprotein
MRSA	:	Methicillin-resistant *Staphylococcus aureus*
MAE	:	Microwave Assisted Extraction
MHA	:	Muller Hinton Agar
MIC	:	Minimum Inhibitory Concentration
MTT	:	Methyl Tetrazolium Bromide Test
MNi	:	Micronuclei
NaEDTA	:	Disodium Ethylene Diamine Tetraacetate
NMR	:	Nuclear Magnetic Resonance

NBUDs	:	Nuclear Buds
NPBs	:	Nucleoplasmic Bridges
PLE	:	Pressurized Fluid Extraction
QE	:	Quercetin Equivalent
R_f	:	Retention factor
ROS	:	Reactive Oxygen Species
RS	:	Reducing Sugar
RO	:	Reactive Oxygen
RV	:	Rota-vapour
SFC	:	Supercritical Fluid Extraction
STLs	:	Sesquiterpene Lactones
STZ	:	Streptozotocin
TAC	:	Total Antioxidant Capacity
TFC	:	Total Flavonoid Content
TPC	:	Total Phenolic Content
TLC	:	Thin Layer Chromatography
UV	:	Ultraviolet
VLDL	:	Very Low-Density Lipoprotein
ZOI	:	Zone of Inhibition
M	:	Meter
Cm	:	Centimeter
mL	:	Milliliter
L	:	Litre
H	:	Hour
nm	:	Nanometer
g	:	Gram
mg	:	Milligram
kg	:	Kilogram
kHz	:	Kilohertz
W	:	Watt
ppm	:	Parts Per Million

LIST OF SYMBOLS

α	:	Alpha
β	:	Beta
γ	:	Gamma
μ	:	Micro
%	:	Percent
^{o}C	:	Degree Celsius
π	:	Pi

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CHAPTER I: INTRODUCTION

1.1 Background

Nepal is a landlocked country with a variety of topography. Terai, the middle Hills, and the upper Himalayas are its three separate physiographic zones. Nepal has a wide range of altitudes, ranging from around 60 m to 8,848.86 m, which has resulted in a diverse plant population. With 118 ecosystems, 12 of the 867 worldwide terrestrial eco-regions, 8 climatic zones, 35 forest types, and 75 vegetation, Nepal is the world's 31st richest country in terms of biodiversity (Kalauni & Joshi, 2018). Nepal has a vast number of plant species due to its biodiversity. There are an estimated 9,000 different kinds of flowering plants in Nepal, making it the ninth most florally diverse country in Asia. There is a total of 6,653 flowering plant species known. Catalogs in Nepal list 1,792 to 2,331 medicinal and aromatic plants (Kunwar *et al.*, 2022). The medicinal plant contains various types of chemical compounds which are responsible for the medicinal value of respective plants.

Natural products are an extensive group of heterogeneous chemical substances possessing a broad range of biological properties. These products have been applied in several fields, including agriculture, veterinary medicine, and human health (Katz & Baltz, 2016). Alkaloids, flavonoids, polyphenols, sugar, and other compounds are examples. The synthesis of primary metabolites is an essential component of regular growth and is produced by microorganisms throughout the exponential phase of growth. Amino acids, nucleotides, vitamins, solvents, and organic acids are the most significant primary metabolites in the industrial sector. These are produced by a wide variety of bacteria and fungi and are widely used in the chemical, food, and nutraceuticals sectors (Sanchez & Demain, 2008). Secondary metabolites are a diverse range of substances that are produced by the secondary metabolic pathways in plants. They contain a broad class of structurally diverse molecules that are not necessary for the growth and reproduction of organisms but do have an ecological function (Pang *et al.*, 2021). Natural product chemistry is concerned with the secondary metabolites of plants and animals, including overflow metabolism products (M. R. Berenbaum, 1995). Natural products cover a wide spectrum of chemical substances obtained and extracted from biological sources. Such molecules have found use in a variety of disciplines, including medicine, cosmetics, agriculture, etc.

(Sarker *et al.*, 2006). As a source of remedies, medicinal plants are widely used as alternative therapeutic tools for the prevention and treatment of various diseases. It is gaining attention in medicine due to the presence of natural products with nutritional and pharmacological properties. Plants used as medicine may be dated back at least 60,000 years in humans, according to fossil records. Natural products have an important role in traditional medicine. For hundreds or thousands of years, people have used these treatments all around the world. The first pharmacologically active molecule, morphine, was discovered from the opium plant at the beginning of the 19th century, starting the era of the "modern" drug. As a result, a large number of active chemicals have been isolated from natural products and used in the field of medicine. Hence great attention has been devoted to the use of natural compounds obtained from plants (Yuan *et al.*, 2016).

The act of identifying, isolating, and quantifying a sample's constituent parts in order to determine its nature and composition is known as chemical analysis (*Chemical Analysis: Definition & Examples | StudySmarter*). The analysis includes phytochemical analysis, total phenolic content, total flavonoid content, GC-MS etc. The techniques used to calculate a substance's impact on living things are known as biological analyses which have a great significant in the pharmaceutical sector (Rosso, 2010). The analysis includes the analysis of antioxidant activity, antidiabetic activity, cytotoxicity etc.

Phytochemicals are chemical components found in natural plant products, and photochemistry is the study of these phytochemicals. They can be classified as flavonoids, alkaloids, tannins, terpenoids, and so on by their properties. Phytochemical screening utilizing various chemicals can be used to characterize these compounds. Various extraction methods, such as soxhlet extraction, percolation, steam distillation, and others, can be used to isolate or recover the purest form of such a set of compounds. Thin layer chromatography (TLC), column chromatography, Gas chromatography-Mass spectroscopy (GC-MS), and other chromatographic techniques can be used to isolate the resulting compound extract which can be used to study their various biological activities. GC-MS is the method that combines two methods i.e. gas chromatography and mass spectrometry which can be used to carry out the chemical and biological analysis of the plant extract.

1.2 Introduction of the Plant *Smallanthus sonchifolius* (Poepp.) H. Rob

Smallanthus sonchifolius (Poepp.) H. Rob. *(S. sonchifolius)* commonly known as yacon, is a perennial herbaceous plant native to the Andes Mountains that has spread to Venezuela, Argentina's northeast, and the west. It is related to plants found in Europe, New Zealand, and other parts of the world (Lachman *et al.*, 2003). It's a member of the Asteraceae family. Jicama, aricoma, strawberry, Chicama, etc. are other common names for it. Yacon is derived from the Quechua word Yacu which means "watery". It was at first classified under the genus *Polymnia* but Robinson later put it under the genus *Smallanthus*, which is now favored over the old one and regarded as a synonym. Robinson included at least 21 species under *Smallanthus* (Ferraz *et al.*, 2020). It's a tough plant with huge dark green leaves that thrives in a warm, temperate climate between 880 and 3500 m, with a plant height of 1.5-3 m. It grows well in rich, medium-deep to deep soils that are well structured and drained. *S. sonchifolius* flowers range in color from yellow to orange (Duarte *et al*., 2008). It produces two kinds of roots: edible tuberous roots that look like sweet potatoes and are used for food storage, and fibrous roots that are used for vegetative multiplication. Each plant yields 4–20 edible tubers weighing up to 20 kg (*Russo et al.*, 2015). Tubers have a sweet flavor and a crisp, juicy texture that has been compared to a cross between a fresh apple and a watermelon. As a result, it is sometimes referred to as the "apple of the earth" (Ruan *et al.*, 2010). A defense mechanism in the leaves and glands of *S. sonchifolius* cultivation hinders insect attacks and plant access. It also helps maintain a pesticide-free culture with minimal chemical contamination, among other advantages (Ferraz *et al.*, 2020). It is reported that *S. sonchifolius* has antioxidant, anti-inflammatory, antimicrobial, and antidiabetic activities as it contains bioactive substances like fructooligosaccharides, inulin, and phenolic compounds with various phytochemicals (Cruz *et al.*, 2019). Because of such activities, it can be used for the treatment of diabetes, for growth and activity of intestinal flora, and obesity, for tea production, and for various health-promoting foods (Choi *et al.*, 2010).

1.2.1 Classification of the Plant

Kingdom	:	Plantae
Division	:	Angiosperm
Class	:	Eudicots
Order	:	Asterales
Family	:	Asteraceae
Genus	:	*Smallanthus*
Species	:	*sonchifolius*
Binomial name	:	*Smallanthus sonchifolius* (Poepp.) H. Rob.
Common names	:	Yacon, Ground apple, Bhui syau,
Synonym	:	*Polymnia sonchifolia*

Figure 1: *S. sonchifolius plant*

a) **b)**

Figure 2: a) Leaves **b)** Root of *S. sonchifolius*

1.2.2 Traditional and Medicinal Use

During older Inca feasts, the plant *S. sonchifolius* was traditionally consumed as food for ritualistic purposes. It's also been utilized in Andean and Bolivian traditional medicine for kidney and liver cancer treatment, as well as diabetes and digestive issues (Moreira Szokalo *et al*., 2020). Due to the nutritional and functional relevance of the *S. sonchifolius* plant, it has been employed in the food industry and many other disciplines to produce health-promoting foodstuffs and dietary food. It also has been used as medicated green tea worldwide for diabetes patients (Myint e*t al*., 2019). Isolation of bioactive compounds from this plant in recent years has been used for the development of novel pharmaceuticals due to its various biological and pharmacological activities.

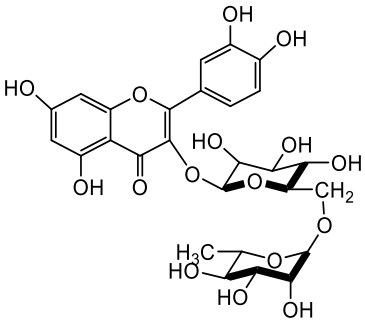
1.2.3 Chemical Constituents of *S. sonchifolius*

Chemical compounds have been discovered and characterized in several parts of the *S. sonchifolius* plant. A plant's chemical composition changes depending on elements including location, farming, growing season, harvest period, and post-harvest time. In general, macronuclei such as carbohydrates, lipids, proteins, fibers, and high water content can be found in every region of the plant (Ferraz *et al*., 2020).

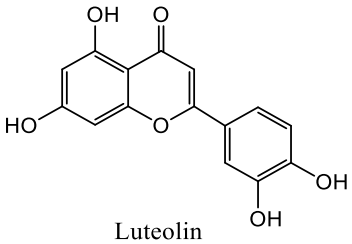
The tuberous root is high in fructooligosaccharides and inulin, both of which are fructans that include kestose and nystose (Contreras-Puentes N, 2020). Other carbohydrates found in the tuberous root include sucrose, fructose, glucose, and oligosaccharides with a low polymerization degree that fluctuates during the growing cycle and harvest. It also contains minerals such as calcium, and phosphorous as well as essential amino acids in juices (Ferraz *et al*., 2020).

Terpenes, flavonoids, phenolic compounds, and other bioactive substances can be found in the leaves. The plant's leaves contain compounds that give it its antioxidant and antidiabetic properties. These include gallic acid, caffeic acid, rosmarinic acid, chlorogenic acid, protocatechuic acid, p-coumaric acid, quercetin, and L-tryptophan (Dou *et al*., 2010). The ethanol extracts and decoction extract of the plants included higher concentrations of flavonoids, including luteolin 3', 7-O-diglucoside, luteolin 7, 6-glucoside, apigenin, and luteolin. Terpenes are commonly classified as

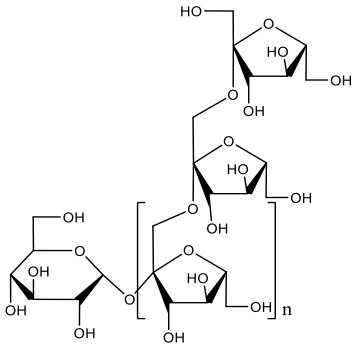
monoterpenes, sesquiterpenes, and diterpenes. Terpenes physically contribute to the plant's antibacterial and pest-resistant properties (Lim, 2015). The studies have also shown the presence of isolated compounds like oxalic acid, tannins, carotenoids, sesquiterpene-lactones, 4 kaurenoids, and 3 isomeric decaffeoylquinic compounds in the plant (Contreras-Puentes N, 2020). Together with essential oils like β-pinene, β-caryophyllene, β-cubenene, β-bourbonene, and γ-cadinene, it has also been shown to contain polyphenols in the form of polyphenolic ions of saturated and unsaturated fatty acids (Ferraz *et al*., 2020; Lim, 2015).



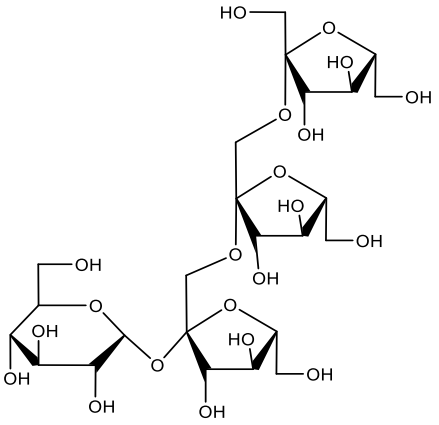
Quercetin3-O rutinoside



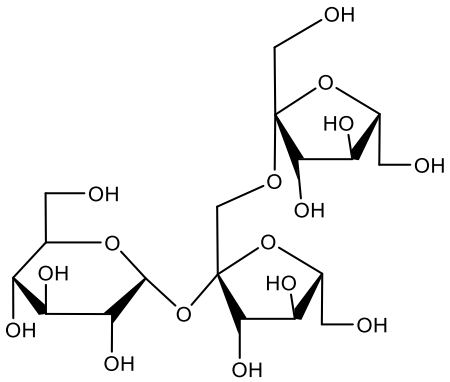
Luteolin



Inulin



Nystose



1-Kestose

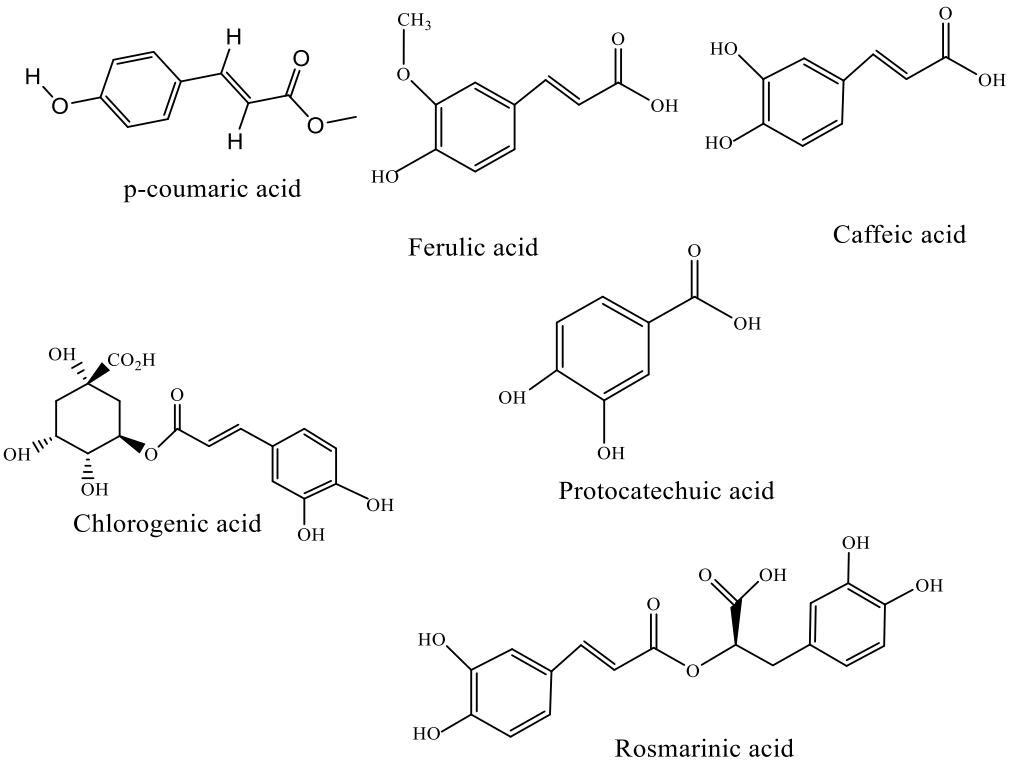


Figure 3: Molecular structure of some chemical constituents found in *S. sonchifolius*

1.3 Extraction Process

Extraction is the process of removing an active ingredient or waste material from a solid or liquid mixture using a liquid solvent. One or more components are removed from a liquid or solid mixture using a liquid immiscible solvent. It is a technique for separating organic components from mixtures. This method allows for the selective dissolution of one or more compounds in a suitable solvent. Solvent extraction is the method by which chemical components present in two immiscible liquids are separated based on their relative solubilities. This method also helps to remove different harmful chemicals present in two immiscible liquids. In Pharmacology, the term extraction refers to the process of selectively extracting the medicinally active components of plant or animal tissues from inert or inactive components using the traditional extraction method. Recent or ecologically friendly extraction methods for removing desired components from natural materials include supercritical fluid extraction (SFC), pressurized liquid extraction (PLE), and microwave-assisted extraction (MAE). It is important to extract natural products to use them as herbal treatments, assess the biological activities of secondary metabolites, or isolate a

known mixture of components (Patel *et al*., 2019). Some common extraction methods are as follows:

- Maceration: The isocratic, cold extraction process known as maceration involves submerging a plant sample in a specific solvent in order to recover the constituent ingredients. This method is generally used for the extraction of thermally moisturizing compounds (Patel *et al.*, 2019).
- Percolation: This is the extraction process in which the extraction of components is carried out by gravity force in a glass vase called a percolator. This method involves the soaking of dried and pulverized plant material for a certain time duration where the gravity force pushes the solvent through the plant material downward. With the help of decantation and filtration techniques, the extract is separated. (Abubakar & Haque, 2020; Patel *et al*., 2019; Poojar *et al*., 2017).
- Decoction: This is the short and continuous hot extraction method that uses a certain volume of water as a solvent. It involves the addition and blending of dried, and powdered plant material with water in the ratio of 4:1 or 16:1. The extraction is subsequently accelerated by the use of heat throughout. It is applicable for the extraction of plant material that is water-soluble and heat-stable (Poojar *et al*., 2017).
- Ultrasonic extraction (Sonication): It is an interesting technique for obtaining high-value compounds within an initial few minutes. This method involves the use of ultrasound whose frequency ranges from 20 kHz to 200 kHz. The main advantages will be lower energy consumption due to improved extraction efficiency, and heat-sensitive chemicals will benefit from the usage of moderate temperatures. Many aspects need to be considered in ultrasonic-assisted extraction, but the most important ones are the frequency, extraction temperature, solvent-sample interaction, reactor properties, and applied ultrasonic power. Even though it is an efficient method, its use is limited due to its high cost (Esclapez *et al.*, 2011).

1.4 Phytochemicals

The bioactive chemical elements present in naturally occurring plant products are called phytochemicals and their study is called phytochemistry. A vast range

of secondary metabolites found in plants include tannins, terpenoids, alkaloids, and flavonoids. Plants frequently use these substances as defenses against pathogens, pests, and herbivores. Based on their metabolic origin, they can be categorized as flavonoids, alkaloids, tannins, terpenoids, and so forth. These substances can be identified by the test using different chemicals, called phytochemical screening. A detailed analysis of medicinal plants with a folkloric reputation is required to promote the appropriate use of herbal medicine and reveal their potential as a source of novel medications (Gul *et al*., 2017).

1.5 Separation of Compounds

Mixtures can be separated using a variety of processes, such as filtering, funnel separation, sublimation, basic distillation, and chromatography methods. These methods are all physical.

1.5.1 Thin Layer Chromatography (TLC)

Thin-layer chromatography is a type of adsorption chromatography that uses a solid-liquid interface. The stationary phase in this procedure is a solid adsorbent material spread out over glass plates. Glass, plastic, or aluminum foil sheets that have been gently coated with an adsorbent material—most commonly cellulose, silica gel, or aluminum oxide (alumina) are used for the procedure. The idea behind it is that different compounds will partition into two phases with different adsorption properties and solubilities. It is a solid-liquid method in which the two phases are a liquid (moving phase) and a solid (stationary phase). Using capillary action, the stationary phase facilitates the upward migration of the mobile phase. If the molecules in the sample are colorless, their locations on the chromatogram can be determined by using fluorescence, radioactivity, or a particular chemical substance to produce a visible colored reactive result, like ninhydrin or black-light viewing (Akash & Rehman, 2020).

One of the most straightforward, rapid, and affordable chromatographic techniques is TLC. It works great for both natural ingredient identification (such as essential oils and alkaloids) and biochemical analysis (such as isolating or separating biological metabolites).

1.5.2 Column Chromatography

A common chromatographic method for separating components of interest from a mixture is column chromatography, which is analogous to adsorption chromatography. Both small- and large-scale applications of this technique are possible for the separation and purification of constituents (Akash & Rehman, 2020). Column chromatography is invaluable in the production or isolation of novel compounds since it requires minimal knowledge of the drug's physical characteristics before the purification process. When developing new medications, the pharmaceutical industry frequently uses this method to purify substances (Silverman & Holladay, 2015).

1.5.3 Gas Chromatography-Mass Spectroscopy

Gas chromatography (GC) and mass spectrometry (MS) are two distinct analytical methods that are used to examine complex organic and biological mixes. This process is known as GC-MS. Mass spectrometry is a method that measures the ratio of mass to charge (m/z) of the charged particles (Singha & Deka, 2024), whereas gas chromatography (GC) is a commonly used analytical technique in numerous academic and industrial research facilities for quality assurance as well as the identification and quantification of components in a mixture (Al-bukhaiti *et al.*, 2017). The examination of esters, fatty acids, alcohols, aldehydes, terpenes, etc. is limited to GC-MS. The use of GC-MS for tracking and monitoring organic contaminants in the environment has grown in popularity. In order to check urine samples from athletes for illegal performance-enhancing substances like anabolic steroids, sports anti-doping laboratories employ it as a primary tool. A rare chance to conduct novel chemical analysis for characterization and identification of synthesized or derivatized substance is presented by the GC-MS, a distinctive and potent technique (Al-Rubaye *et al*., 2017).

1.6 Biological Study of the Compound

The natural activity of a variety of plant species is attributed to the presence of secondary metabolites. As established medical concepts are emphasized, natural

ingredients are currently garnering a lot of attention in the pharmaceutical creation process.

1.6.1 Antimicrobial Activity

An antimicrobial agent is a substance that either kills or stops bacteria from growing. Antimicrobial drugs are categorized according to the microorganisms they are most effective against. If fungi are to be treated, antifungals are employed, and antibiotics are used to treat bacteria. Additionally, they might be categorized into groups according to their roles. The vast majority of medications used in medicine today are synthetic. Drug-resistant microbes have led to new clinical situations involving the treatment of microbial diseases. Since multiple drug resistance has increased and hampered the development of new synthetic medicine, it has become vital to hunt for novel antimicrobials generated from natural plant sources. In light of this, current research screens for natural compounds present in medicinally significant plants to develop innovative and effective medications for the treatment of microbial infections and illnesses (Hassan & Zainab Kazmi, 2015).

1.6.2 Antioxidant Activity

Antioxidants are described as "those compounds that greatly delay or prevent oxidation of an oxidizable substrate when present at low concentrations compared to that substrate." Since its introduction into chemistry, the fields of epidemiology, biology, medicine, and nutrition have all embraced the idea of antioxidant capacity. Antioxidants have several health benefits, making them important nutraceuticals. They are also widely employed as lipid peroxidation inhibitors in the food industry. Potential sources of antioxidants have been studied for vegetables, fruits, seeds, woods, barks, roots, leafy spices, and herbs (Cutrim *et al*., 2019). In degenerative diseases such as aging, cancer, cardiovascular disease, cataracts, degenerative neuron disorders, liver disease, and inflammation, reactive oxygen species (ROSs) play a pivotal role. Free radicals are created when the body's levels of ROS and antioxidants are out of equilibrium. Thus, in a balanced diet, antioxidants are required to lower the risk of pathological illnesses brought on by free radicals (Balamurugan *et al*., 2012). It is crucial to select an antioxidant assay method for assessing antioxidants that considers the particular function being evaluated. More order and consistency are

needed for this vital topic in the form of stricter and more dependable standards and test methodologies, given the wide range of results for natural antioxidants in food systems. To get the same result, an analyst must employ many methodologies for elemental analysis parameters, which differ from TAC and AOA (Pisoschi & Negulescu, 2012).

1.6.2.1 Principle of DPPH Assay

The DPPH free-radical method is an electron-transfer-based antioxidant assay that produces a violet solution in alcohol. When an antioxidant molecule is present, the violet solution turns colorless. Like the majority of other free radicals, it is a stable free radical that does not dimerize. The deep violet color resulting from the delocalization is indicated by an absorption band in the methanol solution with a center at approximately 517 nm. A substance with hydrogen atom donation potential reacts with a DPPH solution, changing its structure and turning it from violet to pale in the end due to the pecryl residue (Molyneux, 2004).

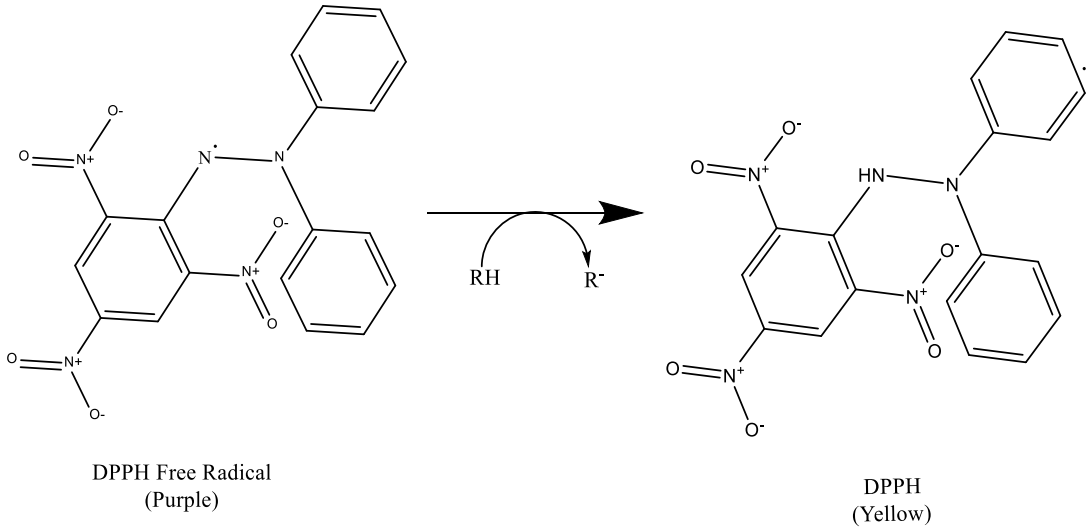


Figure 4: Mechanism showing DPPH radical scavenging

Ascorbic acid, also known as vitamin C, is an antioxidant that occurs naturally with a molecular mass of 176.12 g/mole and the chemical formula $C_6H_8O_6$. The DPPH radical scavenging method uses it as a standard.

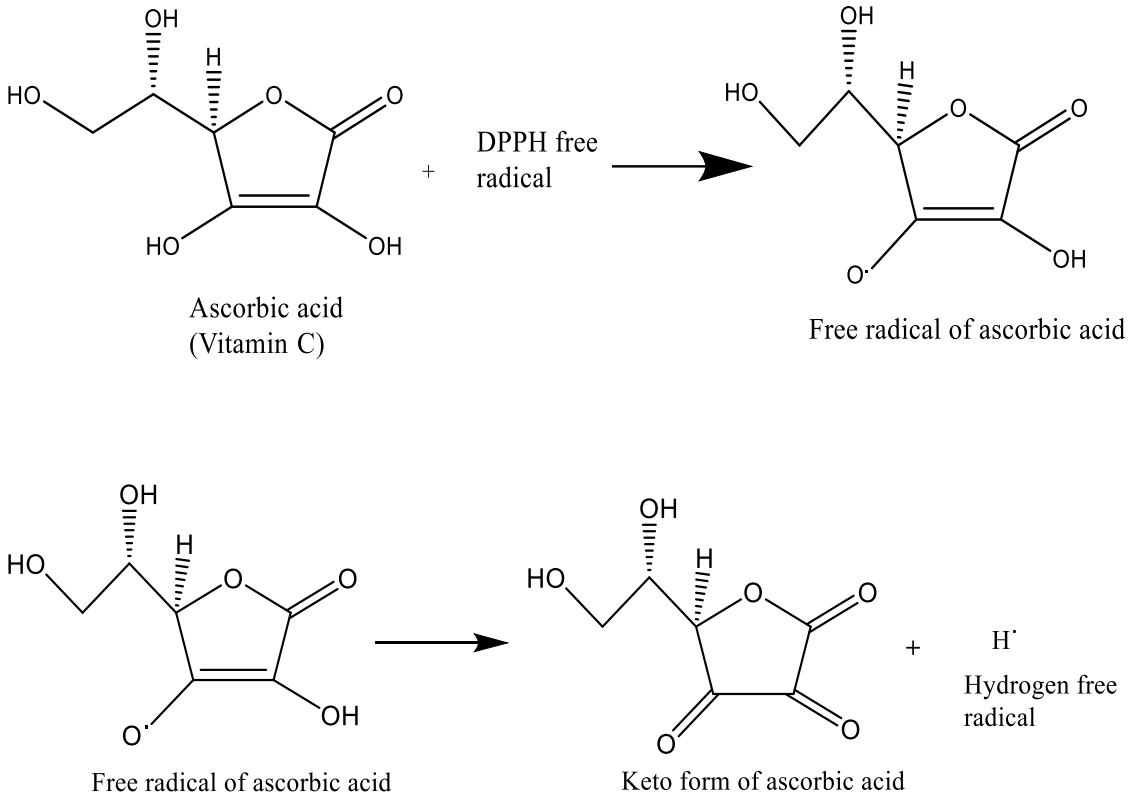


Figure 5: Mechanism showing DPPH radical scavenging by ascorbic acid

1.6.3 Antidiabetic Activity

Diabetes mellitus is a chronic condition characterized by elevated blood glucose levels and inadequate insulin synthesis and/or activity. The pancreatic islets of Langerhans produce the polypeptide hormone insulin, which influences the metabolism of protein, glycogen, and triacylglycerol. It has anabolic effects on a range of tissues (Ferraz *et al.,* 2020). It is a widespread, chronic condition that harms different human tissues, organs, and systems. It could result in consequences that considerably affect the patient's life, both financially and physically (Kasali *et al*., 2022). Studies into the phytochemistry of the plant have revealed several unusual compounds. These substances include fatty acids, glycosides, alkaloids, polysaccharides, saponins, steroids, and various amino acids. Furthermore, none of these drugs are thought to be especially efficient anti-diabetic agents (Wickramaratne *et al.,* 2016).

1.6.4 Polyphenols and Flavonoids

Identification of plant phenolics and flavonoids is essential due to their potent biological effects. Plant materials with an aromatic ring and one or more hydroxyl groups are known as phenolic compounds. Of the 8,000 or so naturally occurring phenolics, flavonoids comprise around half of them. Phenolics have a variety of biochemical properties, including antioxidant, antimutagenic, and anticarcinogenic actions, in addition to their ability to change gene expression. The phytochemical class that comprises the majority of antioxidant activity found in plants and plant-derived products is called phenolics.

Similarly, flavonoids are the most common class of naturally occurring phenolic chemicals. They exist in both free-state and glycoside forms in numerous plant areas. Their polyphenolic content enables them to scavenge harmful free radicals such as superoxide and hydroxyl radicals. Polyphenolic substances have more antioxidant capacity than monophenols. Phenolic chemicals improve food's quality and nutritional worth by preserving its nutritional value while increasing its color, flavor, smell, and taste (Sulaiman & Balachandran, 2012).

1.6.5 Brine Shrimp Lethality Assay

Assays for the mortality of brine shrimp are frequently employed to ascertain the cytotoxicity of various chemicals. This is the preliminary screening for toxicity against dental materials, pesticides, plant extracts, cyanobacteria toxins, fungal toxins, heavy metals, and nanostructures. This is a cost-effective, user-friendly test that works well. Known by several names, nauplii or nauplius, the larvae measure about 22 mm in length, which makes them big enough to see without a magnifying glass yet small enough to hatch in huge quantities without taking up much space in a lab. Since 1982, the lethality assay has guided the bioassay for anti-tumor and cytotoxic medications. This is a fast and comprehensive test to find out if the bioactive component is synthetic or natural. Since aseptic procedures are not needed, the test is very easy to perform and reasonably priced. It only requires a small sample size (2–20 mg or less), is easy to utilize a wide variety of organisms for statistical validation, and doesn't require specific equipment (Phulpoto *et al*., 2017).

1.7 Objectives

1.7.1 General Objective

- To determine the medicinal values of *S. sonchifolius*.

1.7.2 Specific Objectives

- The ultrasonic extraction with different solvents like hexane, chloroform, ethyl acetate, methanol, and distilled water.
- Phytochemical screening, GC-MS, TPC, and TFC of the extracts.
- Study of the bioactivity of the extracts like antibacterial, antioxidant, antidiabetic, and cytotoxicity.
- Column chromatography of the extract.
- UV and FT-IR analysis of the fractions from the column chromatography.

CHAPTER II: LITERATURE REVIEW

2.1 History

Eduard Friedrich Poepping initially identified the *S. sonchifolius* plant as *Polymnia sonchifolius* Poepp. in 1845. Harold Ernest Robinson created the genre Smallanthus in 1987 by dividing Polymnia into two distinct species, *Smallanthus* and *Polymnia,* which is known by the name *Polymnia edulis* Wedd. because it was first described as a botanical synonym in 1857. There were also records of the use of *S. sonchifolius* centuries before the Incas (NRC, 1989). The oldest yacon representation has been found in the archeological reserve Nazca (500-1200AC) (Ferraz *et al.,* 2020; Lim, 2015).

Yacon is native to Andes inhabitants as a root crop. It has been used not only as food but also used in folk medicine. In colonial times, yacon also was used for religious purposes. Later it was used for the treatment of liver and kidney diseases by Andes people. It was transferred from New Zealand to Japan from the Andes in the 20th century. It was then cultivated in Italy, Germany, France, and the USA for cultivation. Later in 1993, it was introduced into the Czech Republic in the form of caudices originating from New Zealand (Lim, 2015).

S. sonchifolius has received recognition during the past two decades due to its nutritional, functional constitutes, and health-beneficial effects (Khajehei, Hartung, *et al.,* 2018). It was cultivated as a new perspective plant in Europe as a natural sugarcane substitute, used for diabetes and the prevention of obesity (Moreira Szokalo *et al.,* 2020). *S. sonchifolius* tubers have gained great attention due to their potential not only as a part of the diet for those who are suffering from digestive disorders but also as a health-promoting food for dieters whereas leaves were used in the food industry and folk medicine to produce teas mostly. In recent years there has been a growing interest in the pharmacological properties of yacon, mainly as a hypoglycaemic agent. For this reason, the cultivation of this species has been expanded worldwide (Khajehei, Merkt, *et al.,* 2018).

2.2 Extraction and Isolation Process Used in *S. sonchifolius*

In 2003, leaves and tubers of *S. sonchifolius* were extracted in various ways. Soxhlet extraction was done using ethyl acetate as a solvent whereas percolation, superficial fluid extraction, decoction, and tea infusion extraction were done with boiling distilled water. The phytochemical screening was done for the extracts, analyzed by HP-TLC, and identified by using HPLC/MS or GC-MS technique (Fengqiu *et al*., 2003). The analysis showed the presence of various phenolic acids, flavonoids, and antimicrobial melampolide compounds (Simonovska *et al.,* 2003). The phenolic compounds' antioxidant activity was studied using DPPH, xanthin/XOD, and superoxide radical scavenging assays (Valentova *et al.,* 2003).

In 2010, leaves of *S. sonchifolius* were extracted by using solvents having different polarities such as methanol, n-hexane, n-butanol, ethyl acetate, and water. Phytochemical screening was done for the extracts and the compounds were isolated by using HPLC or column chromatography technique (Choi *et al.,* 2010). The structures of the isolated compounds were identified based on chemical and phytochemical evidence (Dou *et al.,* 2010). The isolated compounds consist of two new diterpenes, Enhydrin, polymatin B, allo-schkuhriolide, new phenylpropanoid smallanactone A, smallanthaditerpenic acids, etc (Joung *et al*., 2010; Ruan *et al*., 2010; Schwartz, 2010).

In 2012, tuber flesh of 35 different *S. sonchifolius* accessions and leaves were extracted with methanol and isolated by column chromatography. The compounds were found to be new lignin from leaf extract, FOS, and phenolic compounds from the flesh extract. Structures of the compounds were identified by mass of extensive NMR and antioxidant activity was determined by DCFH assay. The presence of FOS and phenolic compounds were analyzed by HPLC-IR and HPLC-PAD respectively. For use in nutraceuticals, the identification of S. sonchifolius growers rich in FOS, AC, or both was investigated (Campos *et al.,* 2012). The AC of 35 *S. sonchifolius* accessions was determined by ABTS assay, TPC by Folin-Ciocalteu method, and RS by using diastrosalicilic acid as reagent and fructose as standard. Using a guinea pig model, the prebiotic effects of FOS were investigated in vivo (Lv *et al.,* 2012).

In 2014, ethanol extract of the leaves of *S. sonchifolius* was obtained by maceration and percolation of dry pulverized leaves whereas the flower of yacon was extracted by three methods: infusion, decoction with boiling distilled water, and soxhlet extraction with methanol. The extracts were used for phytochemical testing which showed the presence of phenols, flavonoids, leucoanthocyanidin, tannin, triterpenes, coumarins, lactones, sesquiterpenes, and amino acids. The phenolic content was characterized by HPLC-DAD, and TPC by using the Folin-Ciocalteu method and TFC by UV-visible colorimetric method. The antioxidant activity was determined by DPPH and ABTS radical scavenging assays. The α-amylase inhibitory activity was determined by the colorimetric method using acarbose as a reference inhibitor.

In 2017 and 2019, leaves of *S. sonchifolius* were extracted using ethanol, and methanol as a solvent and characterized by using the HPLC method. The isolated sesquiterpene lactones from ethanol extract were assayed with the H22 tumor mice model in vivo at different doses for the study of anticancer action and pharmacokinetics of these compounds. Using artemisinin as an internal standard, the pharmacokinetics of the extract's enhydrin and uvedalin were investigated following oral administration at doses of 200 and 100 mg/kg, respectively. Methanol extract's anticancer potential was investigated by evaluating its cytotoxicity activity against malignant cell lines by the use of the MTT assay, which demonstrated a noteworthy impact on these cells (Bai *et al.,* 2017). Numerous assays, such as an in vitro cell migration assay, a colony formation assay, cell cycle analysis, and a ROS assay, were used to further explore it (Myint *et al.,* 2019).

In 2018, the extraction of roots and herbs of *S. sonchifolius* was done by using methanol solvent and characterized the presence of carboxylic acids for the first time by using the GC-MS technique. The studies have shown that 9 out of 12 components of roots and 18 out of 41 components of herbs were carboxylic acids (Demeshko *et al.,* 2018). This shows the presence of high carboxylic acids in the yacon plants. In the same year, the effects of 4 different treatments including storage duration after harvest, pre-treatment before drying, drying method, and convective hot air drying, cultivar on the quality of *S. sonchifolius* chips in terms of their TPC and AA. The TPC was found in high amounts in chips proceeded using pre-treatment with diluted lime

juice and freeze-drying. The AA was determined by using ABTS, DPPH, and FRAP assays (Khajehei, Hartung, *et al.*, 2018).

The method used to make tea bags in popular medicine was also used in 2020 to create the aqueous extract of S. sonchifolius. Thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC-MS/MS) were used to identify the main compounds. The MTT test was utilized to establish the dose range, and the Cytochalasine B-blocked micronucleus (Cytome assay) was performed to quantify genotoxicity. Sesquiterpene lactones (STLs) enhydrin and dimer enhydrofolin were identified as the primary constituents of the extract, along with phenolic chemicals, based on chemical analysis. This is the first time that enhydrofolin has been detected in the aqueous extract of yacon (Moreira Szokalo *et al.*, 2020).

2.3 Biological activities

2.3.1 Antioxidant Activity

The yacon plant has promising antioxidant properties because of its phenolic compound and flavonoid content. The antioxidant activity of the phenolic compounds was investigated by means of tests for superoxide radical scavenging, xanthin/XOD, and DPPH (Valentova *et al.*, 2003). Using ethyl acetate leaf extract, the novel phenylpropanoid smallanactone A demonstrated strong anti-oxidation activity, with an IC_{50} value of 0.46 μg/mL (Ruan *et al.*, 2010). In the DPPH assay, the decoction of yacon leaves showed the highest level of antioxidant activity, with an EC_{50} value of 220.50 g dw. Compared to the other extracts tested, the infusion of leaf extract shown greater antiradical activity in the ABTS assays (422.13 M equiv. Trolox/g dw). In summary, these findings suggest that yacon leaf and flower infusions and decoctions are excellent providers of phenolic acids and flavonoids with potent antioxidant qualities (De Andrade E.F. *et al.*, 2014).

2.3.2 Antidiabetic Activity

The plant's antidiabetic efficacy was demonstrated by the inhibitory effect of Smallantha diterpenic acids, which were extracted from the extract (Schwartz, 2010). When the two diterpenes from the leaf extract were given orally to alloxan-induced hypoglycemic mice, the amount of STZ-induced hyperglycemia decreased, suggesting

that they may have antidiabetic properties (Dou *et al.*, 2010). When yacon tea extracts in percentages ranging from 2 to 10% were administered to rats with STZ-induced hyperglycemia, it significantly improved lipid profiles and caused significant changes in albumin and creatinine levels in the exposed individuals. Consequently, when compared to the control groups used, *S. sonchifolius* extracts and a diet rich in the plant significantly lower levels of triacyl glycerides, total cholesterol, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and glycemia, according to comparable reports of hypoglycemic and lipid-lowering activity (Contreras-Puentes N, 2020). This was because of the fructooligosaccharides (FOS) and inulin present in the yacon.

2.3.3 Antimicrobial Activity

Using the paper disc assay, 8β-methacryoxymelampolid-14-oic acid ester has demonstrated antibacterial activity against *Pyricularia oryzae* and *Bacillus subtilis* among the two antimicrobial melampolide compounds (Choi *et al.,* 2010).The antibacterial efficacy of Smallanthus sonchifolius leaf extracts against methicillin-resistant staphylococcus aureus (MRSA) was demonstrated by both the disc diffusion method and the micro-dilution broth method in presence of light (Fengqiu *et al.,* 2003). It was because the S. sonchifolius leaf extract contained enhydrin. The extract has demonstrated a synergistic impact at all fractional inhibitory concentrations when combined with ampicillin or oxacillin, with the value being less than 0.5 (Joung *et al.,* 2010).

2.3.4 Prebiotic Effect

Short fatty acid levels in caecal material increased as a result of the introduction of root methanol extract from 35 *S. sonchifolius* accessions rich in FOS, which stimulated the growth of lactobacilli and bifidobacteria. Additionally, it improved the caecum tissue's cell density and crypt formation, both of which are signs of improved colon health (Campos *et al.,* 2012).

2.3.5 Anticancer Activity

Using an MTT assay, the IC_{50} value of *Smallanthus sonchifolius* leaf extract was found to be 58.21.9 g/mL, indicating a significant impact on a malignant cell line. It demonstrated dose-dependent inhibition of HepG2 cell migration and proliferation,

cell cycle arrest, and necrosis. Furthermore, using artemisinin as an internal reference, it decreased RO synthesis in HepG2 cells following oral dosing at dosages of 200 and 100 mg/kg, respectively. In the investigation of the enhydrin and uvedalin pharmacokinetics, the pharmacokinetics parameters were computed as Enhydrin's and uvedalin's t_{max} and C_{max}. t_{max} was found to be 1.5 hours for both dosages (200 and 100 mg/kg), and their C_{max} ranged from 13.416 and 8313.31 mg/mL for high doses to 6.887 and 4231.45 mg/mL for low doses, respectively (Bai *et al.*, 2017). AUC_{0-t} values for uvedalin and enhydrin ranged from 43.426 mg/L/h at low dose to 17345.375 mg/L/h at high dose, respectively (Myint *et al.*, 2019). This shows yacon has anticancer properties.

2.3.6 Cytotoxicity

Higher dosages of the aqueous extract of *S. sonchifolius* were cytotoxic to CHO-K1 and HepG2 cells in the MTT experiment. 144 μg/mL was the cytotoxic concentration in 50% of the cells (CC_{50}) for CHO-K1 cells. The extract concentrations selected for CBMN allowed around 80% viability in the MTT experiment. However, MNi frequency rose by a significant amount in HepG2 cells with three of the four tested dosages, but only with the highest dose of NPBs and NBUs in the CBMN assay. In CHO–K1 cells, the frequency of MNi, NBUDs, and NPBs increased statistically and significantly (Moreira Szokalo *et al.*, 2020).

CHAPTER III: MATERIALS AND METHODOLOGY

3.1 Materials

3.1.1 Chemicals Required

- Analytical-grade chemicals such as hexane, chloroform, ethyl acetate, and methanol, were utilized.
- Ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), KOH, conc. H_2SO_4, conc. HCl, $AlCl_3$, and phenol were employed as chemicals and reagents of laboratory grade.
- Reagents like Mayer's, Dragendroff's, Fehling's, etc., were made in the lab using chemicals that are provided in the laboratory of laboratory reagent grade.

3.1.2 Instrument and Equipment

- Rotavapour (IKA, RV 10 D S96)
- Sonicator, Refrigerator
- UV lamp (UV 2510TS)
- Heating Bath, Digital weighing balance, Hot air Oven, Electric grinders
- Measuring cylinders, beakers, conical flasks, test tubes, burettes, micropipettes, pipettes, thermometers, water baths, and vial tubes.
- Double beam UV-Vis spectrophotometer (Labtronics LT-2802)
- FTIR (PerkinElmer Spectrum IR; Version 10.6.2)
- GC-MS

3.2 Methods

3.2.1 Sample Collection and Identification of Plant Materials

The plant was collected from the Kashikhanda municipality, Kavrepalanchwok district, Nepal to represent the whole species of the plant. The herbarium of the plant was prepared, determined by Prof. Dr. Bipana Devi Acharya, Botany Department, Amrit Campus and verified by the National Herbarium and Plant Laboratory, Lalitpur, Nepal.

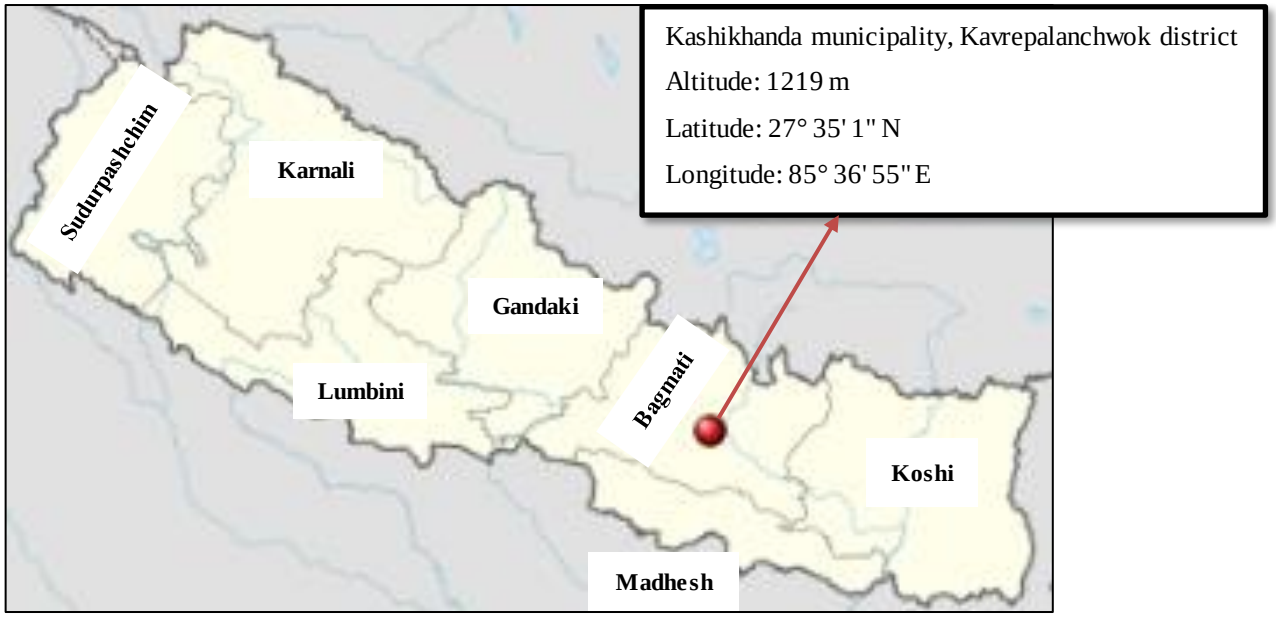


Figure 6: Map of Nepal showing collection site of *S. sonchifolius*

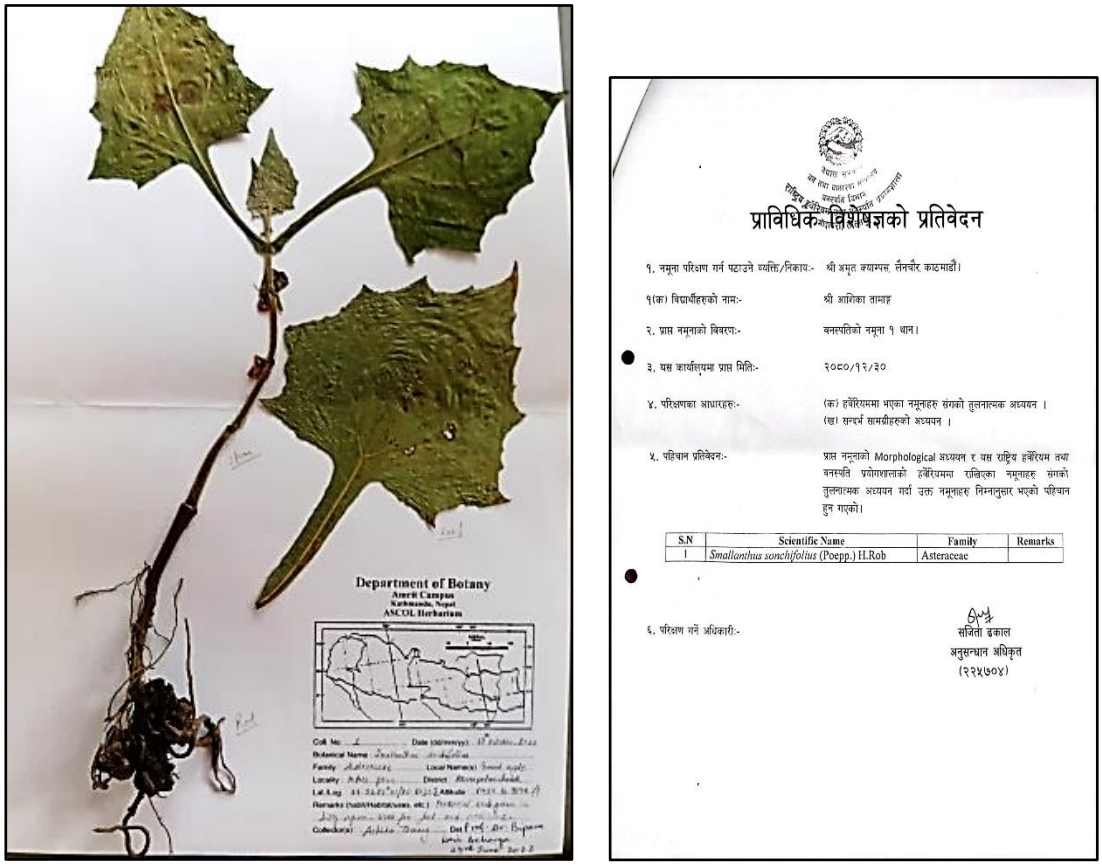


Figure 7: Plant collection and verification

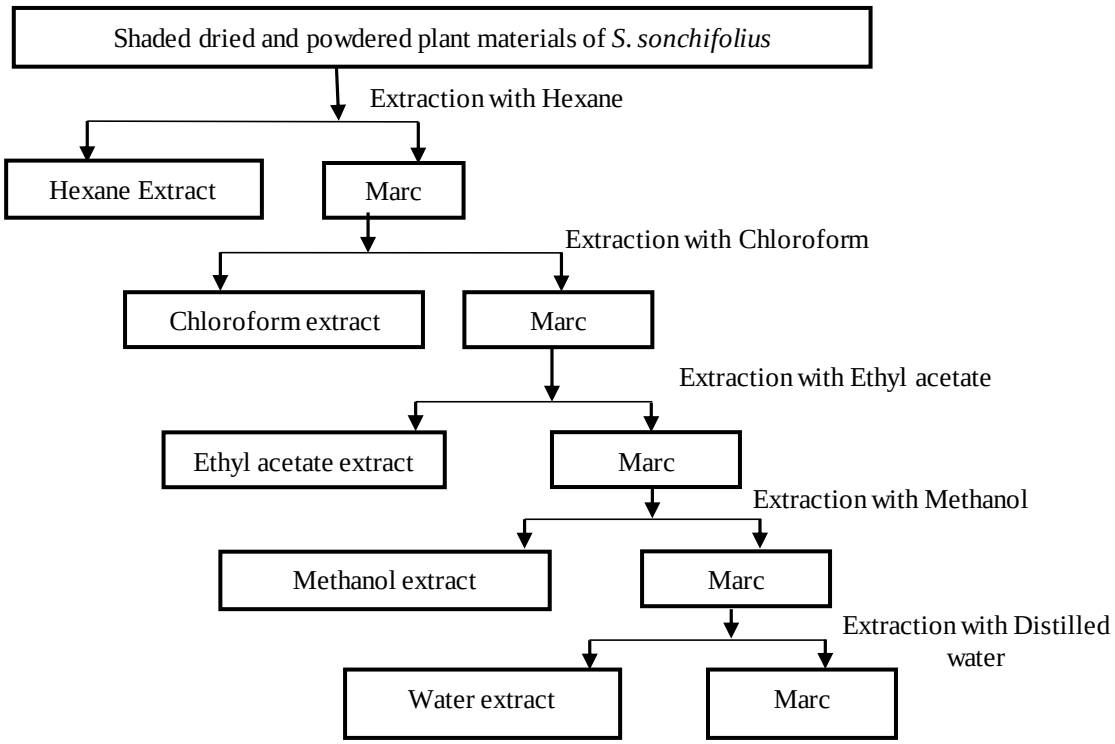
3.2.2 Sample Preparation

Plants were collected locally, their leaves (almost 6 kg) and roots (6 kg) cleaned, and then they were set to air dry in the shade for a few days. The roots and leaves of the

plant were dehydrated in the shade, ground into a powder using an electric grinder, weighed (almost 500 g for the roots and 600 g for the leaves), and stored in a clean, low-temperature plastic container until required.

3.2.3 Extraction Process

The ultrasonic extraction method was used among other extraction techniques. 400 g of powdered plant leaf and root were maintained separate during this procedure in a sterile, dry beaker. After thoroughly mixing the hexane in the beaker, it was stored in a 30 x 15 x 20 cm ultrasonic cleaner tub. The tub was filled to about one-third full with distilled water. Three modules of 50 W each made up the total power, and the operating frequency was 40 kHz. The beaker was filtered and given time to decant following extraction. The filtrate was concentrated using a rotary evaporator. After being weighed and dried, the concentrated extract was placed in an airtight vial tube for future use. In this way, the filtered residue was mixed with hexane prior to sonication. The crude extract was obtained using the same process for three batches. A different solvent, such as hexane, chloroform, ethyl acetate, methanol, or water extracts, was made using the same procedure in light of the solvents' increasing polarity. The process is shown in the scheme diagram below.



Scheme 1: Extraction process

The extracts were then screened, analyzed, and tested as follows:

Phytochemical Screening: All

GC-MS Analysis: Hexane (leaf and root) and methanol (leaf)

TPC: Chloroform (root), ethyl acetate (leaf), and methanol (leaf and root)

TFC: Chloroform (leaf and root) and methanol (leaf and root)

Antimicrobial Activity: All

Antioxidant Activity: Chloroform (leaf and root) and methanol (leaf and root)

Antidiabetic Activity: Chloroform (leaf and root) and methanol (leaf and root)

3.2.4 Gas Chromatography-Mass Spectroscopy (GC-MS)

The technologies of mass spectrometry and gas chromatography combine two methods. It is helpful in many different circumstances. Nevertheless, its main use and best suited application is the analysis and separation of multicomponent mixtures, like those containing hydrocarbons, solvents, and essential oils. In mass spectrometry, compounds' exact molecular mass can be determined by their mass-to-charge ratio; in gas chromatography, on the other hand, chemicals at low concentrations can be quantitatively measured by using the flame ionization detector in combination with the electron capture detector *(*Al-Rubaye *et al.*, 2017).

Among application disciplines, pollution research comes in second, general traces analysis in third, and forensics in fourth. Gas chromatography is an essential chemical technique due to its sensitivity, ease of use, and capacity to separate components of a mixture. Some advantages of this method include the ability to detect thermochemical variables such as vapor pressures, activity coefficients, solution, and vaporization temperatures, and the ability to analyze mixtures both quantitatively and qualitatively. Additionally, impurities can be removed from compounds (Sermakkani & Thangapandian, 2012).

3.2.4.1 GC-MS Analysis of Hexane and Methanol Extract

A tiny sample of concentrated methanol root extract, methanol leaf extracts, and hexane from the rota-evaporator were dissolved in chloroform and put through GC-MS analysis. The GC-MS analysis was carried out by Thapathali, Nepal's Department of Plant Resources.

Column type: RTX-5MS

Dimension of column: 60 m×0.32 mm×0.25 μm
Detector type: MS
Sample amount: 1 mL
Injection volume: 1.00 μL
Packing of sample: Eppendorf tube
Library used: NIST17

3.2.5 Phytochemical Screening

A number of extracts underwent phytochemical screening. The procedure was followed in order to direct the phytochemical screening process (Banu & Cathrine, 2015). In summary, phytochemical screening facilitates the identification of the bioactive compounds present in plants. Significant groups of natural compounds were detected in the various plant extracts by examining the color reaction using a number of specific reagents. The method is explained in detail in Appendix A.

3.2.6 Total Phenolic Content (TPC)

The total phenolic content of the plant extract was determined using the Folin-Ciocalteu colorimetric technique, which is based on the oxidation-reduction reaction. Concentration of gallic acid is utilized as a benchmark (Balasundram *et al.*, 2006).

3.2.6.1 Preparation of Folin-Ciocalteau Reagent

1 mL of the Folin-Ciocalteau reagent was dissolved in 10 mL distilled water, i.e. (1:10 dissolved in distilled water).

3.2.6.2 Preparation of Standard Gallic Acid Solution

The first step was dissolving 1 milligram of gallic acid in 1 milliliter of methanol to produce a stock solution with a 1000 μg/mL (ppm) concentration. Gallic acid in a variety of quantities was produced by dilution of the stock solution numerous times. The concentrations ranged from 500, 250, 150, 100, 50, and 25 μg/mL.

3.2.6.3 Construction of the Calibration Curve

Initially, 20 µL of a gallic acid solution was added to each test tube. After that, 100 µL (10%) of Folin-Ciocalteu reagent (FCR) in a test tube was kept and let it stand in the dark for five minutes at room temperature. To get a final volume of 200 µL, the mixture was then combined with 80 µL of an aqueous 7% Na_2CO_3 solution. After giving the mixture a good shake, it was left in the dark for two hours. The reactant absorbance at 760 nm was determined using a control (20 µL solvent + 100 µL F-C reagent + 80 µL sodium carbonate incubated for two hours). Gallic acid was chosen as the standard. The total phenolic content of the sample was determined as milligrams of gallic acid equivalent per gram of dry weight of extract (mg GAE/g of dry extract), using the gallic acid calibration curve as a guide.

3.2.6.4 Preparation of the Sample Solution

To make a stock solution of extract (ethyl acetate and methanol) with a concentration of 1000 µg/mL (ppm),1 milligram of extract was first diluted in 1 mL of methanol. Multiple quantities of extracts were generated by serial dilution of the stock solution, and the same method used for gallic acid was applied to evaluate the absorbance.

3.2.6.5 Calculation of the Total Phenolic Content

The total phenolic content in the sample expressed as milligrams of gallic acid equivalent was calculated using the equation below.

$$C = \frac{cV}{m} \quad \ldots\ldots\ldots\ldots (1)$$

where,

C = Total content of the phenolic compounds (mg/g) in gallic acid equivalent

c = Concentration of the gallic acid established from the calibration curve (µg/mL)

V = Volume of the extract (mL)

m = Weight of the plant extract (µg)

For each concentration, the results were recorded as the mean of absorbance. The linear correlation coefficient (R^2) value was calculated from these values. The regression equation appears as follows:

$$y = mx + c \quad \ldots\ldots\ldots\ldots (2)$$

where,

y = Absorbance of the extract

m = Slope from the calibration curve

x = concentration of the extract

c = Intercept

This regression equation was used to calculate the concentration of the extract. Therefore, using the concentration of the extract as an input, equation (2) was developed to determine the substance's total phenolic content.

3.2.7 Total Flavonoid Content (TFC)

The plant extract's total flavonoid content was assessed using a colorimetric test with aluminium chloride (Heim *et al*., 2002). Quercetin was the standard that was used. The procedure yields a sample in which the hydroxyl groups of flavonoids combine with aluminum chloride to form a chemical.

3.2.7.1 Preparation of the Standard Quercetin Stock Solution

One milligram of quercetin (stock concentration: one milligram per milliliter) was dissolved in one milliliter of pure methanol to create the stock solution. Next, serial dilution was performed on the different doses, which were 250 µg/mL, 150 µg/mL, 100 µg/mL, 50 µg/mL, and 25 µg/mL. An aliquot of 100 µL of each methanol concentration was placed into a microplate well. An extra 100 µL of 2% aluminum chloride (in methanol) was added to reach a total volume of 200 µL. The yellow-colored mixture was left in total darkness for ten minutes. Ultimately, a spectrophotometer was used to measure the absorbance at 425 nm against the blank solution (control), which consisted of 100 µL of 2% aluminum chloride and 100 µL of solvent in place of the extract sample. The sample and the control were both incubated at the same time. The calibration curve was plotted using the average absorbance values for the various quercetin concentrations. As a positive control, the quercetin was employed. The total flavonoid concentration was calculated as quercetin equivalent (mg QE/g of dried plant material) using a quercetin standard curve.

3.2.7.2 Preparation of the Sample Solution

First, 1 mg of extract (chloroform and methanol) was dissolved in 1 mL of methanol to create a stock solution of an extract with a concentration of 1000 µg/mL (ppm). By diluting the stock solution several times, different amounts of extracts were produced, and their absorbance values were assessed using the same procedure as quercetin above.

3.2.7.3 Measurement of Total Flavonoid Content

The total flavonoid content in the extract was calculated using the equation below:

$$C = \frac{cV}{m} \quad \ldots\ldots\ldots\ldots\ldots (3)$$

where,

C = Total Flavonoid Content (in mg/g) in Quercetin Equivalent (QE)

c = Concentration of quercetin established from calibration curve in mg/m

V = Volume of the extract (in µg/mL)

m = Weight of the plant extract (in µg)

The data were expressed as the mean absorbance for each concentration. The linear correlation coefficient (R^2) value was calculated using these values.

The following is how the regression equation looks:

$$y = mx + c \quad \ldots\ldots\ldots\ldots (4)$$

where,

y = Absorbance of the extract

m = Slope from the calibration curve

x = concentration of the extract

c = Intercept

A calculation was performed to find the extract's concentration using this regression equation. Consequently, equation (4) was developed to determine the total flavonoid content of the material by entering the concentration of the extract as an input.

3.2.8 Antioxidant Activity

The antioxidant approach was based on a protocol described by Blois M.S. (1958).

The DPPH free-radical method, reliant on electron transfer, serves as an antioxidant assay. In alcohol, it yields a violet solution; however, the presence of an antioxidant molecule causes the solution to transition to a colorless state (Otohinoyi *et al*., 2014).

The many techniques used to gauge a plant's antioxidant capacity might rely on the free radical being used as a reactant to produce different results.

The percentage of the DPPH free radical scavenging activity was determined By using the following equation:

$$\text{Radical scavenging } (\%) = \frac{A_0 - A_S}{A_0} \times 100 \quad \ldots\ldots\ldots\ldots\ldots (7)$$

where,

A_0 = Absorbance of the control (DPPH solution + methanol)

A_S = Absorbance of the test sample

The IC_{50} (50% inhibitory concentration) value is the effective sample concentration required to scavenge 50% of the DPPH free radicals. The IC_{50} values were calculated by plotting the extract concentration against the relevant scavenging effect using an inhibition curve.

3.2.8.1 Preparation of the 0.1 mM DPPH Solution

DPPH has a molecular weight of 394.32 g/mol. Hence, a 0.1 mM DPPH solution was prepared by precisely weighing 0.0019716 g of DPPH, dissolving it in methanol, and adjusting the volume to 50 mL. After then, this solution was kept out of the light until needed.

3.2.8.2 Preparation of Standard Ascorbic Acid

To make the stock solution with a concentration of 1 mg/ml, 10 milligrams of ascorbic acid were measured and diluted in 10 mL of methanol. After then, solutions of ascorbic acid were serially diluted having concentrations of 30 µg/mL, 20 µg/mL, 10 µg/mL, 5 µg/mL, and 2.5 µg/mL.

3.2.8.3 Preparation of Sample Solution

Methanol and chloroform extracts (10 mg) were weighed and dissolved in 10 mL of methanol to make the stock solution (1 mg/mL). Following that, solutions containing extracts were diluted in steps comprising 20 µg/mL, 10 µg/mL, 5 µg/mL, and 2.5 µg/mL.

3.2.8.4 Measurement of DPPH Radical Scavenging Activity

A 96-well plate was filled with 150 µL of a methanol solution of DPPH after 50 µL an ascorbic acid solution from every concentration had been pipetted and dissolved. This yielded 200 µL. The solutions were then left in the dark for a duration of 20 minutes. Next, a spectrophotometer was used to measure their absorbance value at 520 nm employing methanol and DPPH solution as a blank. The method used to calculate the absorbance value of ascorbic acid was also applied to the extracts and DPPH solution. The calibration curve was created using the concentrations of the sample solution and ascorbic acid as the X- and Y-axes, respectively. The Y-axis indicated the percentage (%) of radical scavenging activity, and IC_{50} values were also ascertained.

3.2.9 Antimicrobial Assay

Some dangerous germs can be prevented from growing by using plant extracts. Antimicrobial activity is a useful metric for evaluating a plant's or essential oil's ability to prevent microbiological growth (Kalemba & Kunicka, 2005). During the biological screening step, a variety of organism species were used to assess the effects of a crude plant extract or fraction administered at a certain dose level. A microorganism test was performed during this checkup. The paper disc diffusion method was utilized for leaf extracts and the well diffusion method was employed for root extracts in the screening process and evaluation of the unprocessed plant extracts' antibacterial activity. The ZOI (Zone of Inhibition) value was obtained by measuring the amount of bacterial growth inhibition using the paper disc diffusion method for leaf extract and the well diffusion method for root extracts (Balouiri *et al.*, 2016).

3.2.9.1 Collection of Standard Culture

Three standard strains of microbes were given from Himalayan Research Centre, Koteshwor, in activated cultures. The strain investigation comprised the following three categories of bacteria:

Gram-positive bacteria: *Bacillus subtilis* ATCC 6051

Gram-negative bacteria: *Escherichia coli* ATCC 8739

Fungi: *Candida albicans* ATCC 2091

3.2.9.2 Preparation of Media

The media for the research were produced in accordance with the manufacturer's guidelines. The specific steps are as follows:

A. Nutrient Agar

For this preparation, 2.5 grams of agar had been suspended in 100 milliliters of distilled water. It was then heated to dissolve the agar and sterilized to 45 minutes at 121 degrees Celsius to sterilize it. Once it had cooled to about 40°C, it was spread out throughout the petri dish. The petri dish cemented after being left to dry in a laminar stream for 20 minutes.

B. Nutrient Broth Media (Liquid Media)

100 milliliters of purified water were used to dissolve two grams of powdered nutritional broth. Then, it was thoroughly combined to dissolve completely, and it was autoclaved for 45 minutes at 15 pounds of pressure (121 degrees Celsius) to sanitize it. Thereafter, it was let to cool.

3.2.9.3 Preparation of Working Solution

For leaf extract:

15 mg of each crude leaf extract was dissolved in 100 µL of DMSO solution.

For root extract:

100 mg extract was dissolved in 1 mL DMSO solvent.

The vials were filled with the solution, sealed, and refrigerated (2–8 °C) until needed. The positive control was made with Kanamycin. To produce it, 5 mg of Kanamycin was dissolved in 1000 mL (5 µg/mL) of distilled water.

3.2.9.4 Screening and Evaluation of Antimicrobial Activity

The antimicrobial activity was assessed by the Agar Disc Diffusion method for leaf extract and the Agar Well Diffusion method for root extract using both gram-positive and gram-negative test organism assay. First, the microbial strains namely *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 8739), and *Candida albicans* (ATCC 2091) were cultured in liquid nutrient broth media at 37°C for 24 hours. 100 µL

culture broth of each strain was then plated over nutrient agar plate and kept for 15 min at 37°C. After 15 min of incubation at 37°C, the bioplastic sample as a paper disc (8 mm) in the case of leaf extract (10 µL) and a well of 8mm in the case of root extracts (30 µL) was held on the nutrient agar plate and incubated overnight at 37°C. The next day, the antimicrobial activities of the bioplastic against *B. subtilis, E. coli,* and *C. albicans* microbial strains were investigated. Kanamycin (5 µL) was used as a positive control. DMSO was used as a negative control. The ZOI formed by the antimicrobial activity of plant extracts was then seen on the plates, and the inhibition zones were measured using a scale.

3.2.10 α-Amylase Inhibition Assay

Defects in the synthesis or function of insulin cause abnormalities in the balance of lipid and carbohydrate metabolism in diabetes mellitus. It is a serious metabolic non-communicable disease with a high death rate and significant healthcare expenses (Poovitha & Parani, 2016). The primary source of blood glucose is the hydrolysis of dietary starch, where the key enzymes involved in starch breakdown and intestine absorption are α-amylase and α-glycosidase, respectively. Inhibiting these enzymes is thought to be a useful tactic in the treatment of hyperglycemia associated with type II diabetes (Lordan *et al.*, 2013).

3.2.10.1 A General Protocol for α-Amylase Inhibition Assay

The 3,5-dinitrosalicylic acid (DNSA) technique was used to carry out the α-amylase inhibition experiment. The *S. sonchifolius* plant extract (chloroform and methanol) was diluted in a minimum of 10% DMSO. The mixture of DMSO and the sample was dissolved in buffer and NaCl once more at a pH of 6.9 to produce concentrations of a varying range. Following a 20-minute incubation period at 30°C, 200 µL of the extract and 200 µL α-amylase solutions were combined. After that, each tube received 200 µL of the starch solution, which was left there for three minutes. 200 µL DNSA reagent was added to halt the reaction. A water bath heated to 85–90°C was used to boil the combined sample for ten minutes. Once the combined sample had cooled to room temperature, distilled 5 mL water was added in order to dilute it. The value of the absorbance at 540 nm was determined using a UV spectrophotometer in comparison to the blank solution. To construct a blank having 100% enzyme activity,

200µL of buffer was substituted for the plant extract. An enzyme-free blank reaction is produced by utilizing the sample plant extract at each concentration.

The following equation was used to determine the % inhibition of α-amylase inhibitory activity. IC50 values were determined by graphing the extract concentration versus the percent of α-amylase inhibition.

$$\% \text{ α-amylase inhibition} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

3.2.11 Brine Shrimp Lethality Assay (BSLA)

BSLA was conducted using the same procedures as (Ashfak *et al*., 2016; Abhijit, 2015). The brine shrimp lethality experiment, which measures the ability of plant extracts and other substances (nauplii) to destroy larvae cultured in a lab, is a helpful method to determine possible cytotoxicity. For 24 hours, the nauplii were treated with various doses of plant extract. To assess the extract's effectiveness, the quantity of nauplii that could migrate was counted. It simply needs a minimal amount of test material and is easy and affordable to use (Sarah *et al*., 2017). It calculates the LC_{50} (Lethal concentration) values for the crude extracts in milligrams per milliliter. The toxicity evaluation of plant extracts was based on Clarkson's toxicity criterion (Hamidi *et al.*, 2014) which states that extracts with an LC_{50} more than 1000 µg/ml are non-toxic, 500–1000 µg/ml are low harmful, 100–500 µg/ml are medium toxic, and 0–100 µg/ml are very hazardous (Clarkson *et al.*, 2004).

3.2.11.1 General Procedure for Brine Shrimp Lethality Assay

Prior to usage, all of the experiment's equipment was sterilized.

3.2.11.1.a Preparation of Artificial Sea Water

Approximately 3.2 grams of rock salt was crushed, weighed, and then dissolved in 100 mL of distilled water. The mixture was filtered to obtain a clear solution, removing any undissolved particles or impurities.

3.2.11.1.b Hatching of Brine Shrimp Egg

For hatching, the beaker containing the simulated saltwater was covered with aluminum foil, and then 50 mg of brine shrimp (*Artemia salina*) eggs were scattered

over it. To improve heat and light conductivity, a few tiny pores were created in the material. After that, the beaker was exposed to light from the 60-watt lamp for 48 hours at room temperature.

3.2.11.1.c Preparation of Samples

A stock solution was created with a 1000 ppm (mg/mL) concentration by adding 2 mL of DMSO (dimethyl sulphoxide) to 2 mg of the extract (chloroform and methanol). Solutions with concentrations ranging from 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, along with 0.0625 mg/mL were generated from the original stock solution using the serial dilution technique. For every concentration, three test tubes containing two milliliters of each of the five solutions were used, in addition to three tubes containing two milliliters of DMSO (as a blank). After labeling, the tubes were stored for a full day.

3.2.11.1.d Calculation of Assay

A bioassay defines mortality as the inability to go forward under controlled conditions for 30 seconds. For every concentration and control, the lethality percentage of nauplii was calculated. The number of dead and living nauplii in each tube may be used to calculate the proportion of mortality. Next, the following equation was used to get the fraction of mortality.

$$\% \text{ Mortality} = \frac{No.\ \text{of dead shrimps}}{\text{Total No. of shrimps}} \times 100$$

3.2.12 Chromatographic Separation of Compound

Chromatography is the process of separating molecules in a solution by depositing them onto a surface or into a solid or liquid stationary phase, and then moving the molecules with the aid of a mobile phase. Because of these variations, some combination components exit the chromatographic equipment and reach the mobile phase more quickly. On the other hand, some mixture components move through the system more slowly and stay in the stationary phase longer than others (Coskun, 2016).

Thin-layer chromatography (TLC) was applied to each extract to determine the elements that were present in each one. In this case, TLC was performed on a TLC aluminum sheet that was 0.2 mm thick. The plates were made in different solvent

ratios by altering the liquids' polarity. The concentration of the solvent system is shown in Table 2. The plates were visualized using a UV fluorescent lamp.

Table 1: Concentration of solvent system of TLC

S.N.	Solvent system of TLC	% Ratio
1.	Acetone: Hexane	5%,10%,15%,20%
2.	Ethyl acetate: Hexane	5%,10%,15%,20%,
3.	Methanol: Hexane	5%,10%,15%,20%,
4.	Chloroform: Hexane	5%,10%,15%,20%,
5.	Methanol: Chloroform	5%,10%,15%,20%,

In this case, a thin layer chromatographic analysis was carried out by applying the solute as a spot. Once the TLC plate was in the beaker containing an adequate amount of the previously indicated solvent, the solvent was run. Information on each of the mixture's constituent parts can be found in the chromatogram that has been produced. A UV light was used to view the resulting chromatogram. The retention factor (R_f) value constant was used to determine the properties of the material, which shows how the material moves toward the solvent in a particular chromatographic system.

$$\text{Retention factor } (R_f) = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by the solvent}}$$

3.2.13 Column Chromatography

In the field of chemistry, certain chemical components are extracted from mixtures distributed in a liquid medium using a process known as column chromatography. With the use of the compounds' differential adsorption to the adsorbent as they pass through the column at different rates, separates the materials into fractions (Coskun, 2016). The stationary phase (silica gel) was packed along with the used solvent, hexane, in a glass column that had a cylindrical shape. For the first column chromatography, a 2 feet 2-foot-long glass column of 40 mm diameter, and for the second column chromatography, a 2 2-foot long glass column with 20 mm diameter was used. When the column was ready, a small amount of sand was placed over the top layer of the silica gel to collect the sample. The sample was added from the top and consisted of methanol extract combined with a tiny bit of silica gel using a solvent with a 1% ethyl acetate: hexane ratio. The mixture was concentrated using

the Rota vapor after more than 25 mL of fraction had been collected. A UV lamp was used to check the TLC once it had been completed. As the sample moved across the TLC plate, the concentration of the solvent was increased from 1% to 2% then close to 100%.

3.2.14 UV-Visible Spectroscopy

An analytical technique called UV-Vis spectroscopy counts the number of unique UV or visible light wavelengths that a sample transmits or absorbs with a reference or blank sample (Tom, 2021). It is widely used to determine if organic molecules are unsaturated. It can provide crucial information on the electronic structure of a chemical, including the presence of π-bonds and the conjugation of double bonds (Pratiwi & Dani Nandiyanto, 2022).

The instrument utilized was a Labtronics LT-2802 double-beam ultraviolet-visible (UV-visible) spectrometer from the Department of Chemistry, Amrit Campus, Kathmandu for spectroscopic analysis of the compounds recovered from column chromatography, where the aromaticity and unsaturation were determined. For the recording of spectra, the 10^{th} fraction and 9^{th} fraction from first and second-column chromatography were used respectively.

3.2.15 FT-IR Spectroscopy

In order to determine the type of bonding present in organic molecules, more specifically, the functional group, FTIR spectroscopic spectroscopy is a helpful technique. The FTIR spectra of the 10^{th} and 9^{th} fractions from the first and second respective column chromatography fractions were generated using an FTIR spectrometer (PerkinElmer Spectrum IR; Version 10.6.2) at the Department of Chemistry, Amrit Campus, Kathmandu. The development of FTIR spectra as a tool for the simultaneous determination of organic components, including chemical bonds, and organic content has allowed for the components' functional groups, the presence of aromatic or aliphatic structures, and the π-bond conjugate system (Nandiyanto *et al*., 2019).

CHAPTER IV: RESULTS AND DISCUSSION

4.2 Yield Percentage

Using the ultra-sonication method of extraction, the *S. sonchifolius* plant was extracted. The percentage yield of hexane, chloroform, ethyl acetate, methanol, and aqueous extract was 3.18%, 1.64%, 0.11%, 2.50%, and 6.40% for leaf extract whereas 0.21%, 0.38%, 0.05%, 1.63% and 6.37% for root extract respectively which are shown below:

Table 2: Table showing percentage yield of various extract

Plant parts	Extract yield (in %)				
	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous
Leaf Yield (g)	12.71	6.54	0.43	10.0	25.59
Leaf Yield (%)	3.18	1.64	0.11	2.50	6.40
Root Yield (g)	0.82	1.51	0.21	6.50	25.50
Root Yield (%)	0.21	0.38	0.05	1.63	6.37

4.3 Qualitative Analysis of Phytochemicals

A set of phytochemicals was discovered by the micro-chemical analysis of the crude extract obtained from *S. sonchifolius* plant using different solvent systems, as indicated in Tables 3 and 4. The phytochemical content of each *S. sonchifolius* extract was examined, and color appearance indicated the presence of phytochemicals.

Table 3: Phytochemical analysis of leaf extract of *S. sonchifolius*

S.N.	Class of phytochemicals	Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Methanol Extract	Aqueous Extract
1	Volatile oils	+	+	+	+	-
2	Alkaloids	-	-	-	+	+
3	Carbohydrates	+	+	+	+	+
4	Phenolic compounds	+	+	+	+	+
5	Tannins	-	-	-	+	+
6	Flavonoids	+	+	+	+	-
7	Terpenoids	+	-	-	+	+
8	Quinones	-	+	+	+	+
9	Reducing sugar	-	+	-	+	-
10	Saponins	-	-	-	+	+

Where '+' means presence and '-' means absence.

From Table 3, methanol leaf extract has shown the presence of all phytochemicals. Carbohydrates and phenolic compounds were found in all extracts. Flavonoids and volatile oils were found in almost all extracts except in aqueous extract. Alkaloids were found to be present in methanol and aqueous extract only.

Table 4: Phytochemical analysis of root extract of *S. sonchifolius*

S.N.	Class of phytochemicals	Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Methanol Extract	Aqueous Extract
1	Volatile oils	+	-	+	+	-
2	Alkaloids	+	-	+	+	+
3	Carbohydrates	-	+	+	+	+
4	Phenolic compounds	-	+	+	+	+
5	Tannins	-	-	-	+	+
6	Flavonoids	-	+	-	+	+
7	Terpenoids	+	+	+	+	+
8	Quinones	-	-	+	+	+
9	Reducing sugar	-	-	+	+	-
10	Saponins	-	-	+	+	+

Where '+' means presence and '-' means absence.

From above Table 4, methanol root extract has shown the presence of all phytochemicals. Terpenoids were found to be present in all extracts, and carbohydrates and flavonoids were found to be in all other extracts except hexane extract. Alkaloids were found in all of the extracts except chloroform extract.

4.4 GC-MS Spectra Analysis

The GC-MS analysis was done in hexane and methanol extract of the leaf and hexane extract of the root of *S. sonchifolius.*

4.4.1 GC-MS Spectra Analysis of Hexane Leaf Extract

A NIST library search and GC-MS analysis of the *S. sonchifolius* hexane leaf extract's chemical makeup identified 17 main components. Based on GC-MS analysis, the important chemicals are listed below:

Table 5: Components based on GC-MS analysis of hexane leaf extract

S.N.	Name of compound	Retention time	Molecular formula	Area%
1.	Cyclofenchene	6.300	$C_{10}H_{16}$	2.17
2.	7,8,8-trimethyl-4,5-diazatricyclo [4.2.1.$0^{3,7}$] non-4-ene	7.067	$C_{10}H_{16}N_2$	27.02
3.	9,10-Dimethylenetricyclo [4.2.1.1(2,5)] decane	15.925	$C_{12}H_{16}$	2.37
4.	1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-(1. α.,4. α.,4a. α.,10a. α.)-	17.983	$C_{13}H_{20}N_2$	8.22
5.	3,3,6,6,9,9-hexamethyltetracyclo [6.1.0.$0^{2,4}$.$0^{5,7}$] nonane	18.726	$C_{25}H_{24}$	3.93
6.	Diazoprogesterone	20.583	$C_{21}H_{30}N_4$	7.19
7.	1.8-Cyclotetradecadiyne	20.900	$C_{14}H_{20}$	3.67
8.	7,10,10-Trimethyl-4-oxa-3,5-diazatricyclo [5.2.1.0(2,6)] deca-2,5-dien-3-one	21.800	$C_{10}H_{14}N_2O_2$	
9.	Trans-β-terpeneol	23.125	$C_{10}H_{18}O$	5.60
10.	2-isopropyl-3-methylcyclohexanol	24.292	$C_{10}H_{20}O$	7.91
11.	Pentadecafluorooctanoic acid, dodecyl ester	24.425	$C_{20}H_{25}F_{15}O_2$	
12.	Pentadecafluorooctanoic acid, dodec-2-en-1-yl ester	25.042	$C_{20}H_{23}F_{15}O_2$	2.56
13.	8-Methylnonanoic acid, methyl ester	25.750	$C_{11}H_{22}O_2$	2.39
14.	Decanoic acid, silver (1+) salt	26.292	$C_{10}H_{19}AgO_2$	2.16
15.	Ethyl-4-methyl-octanoate	26.842	$C_{11}H_{22}O_2$	5.08
16.	δ-Hexadecanesultone	27.642	$C_{16}H_{32}O_3S$	
17.	3,7-Dimethyl-6-nonen-1-ol	28.800	$C_{11}H_{22}O$	3.00

4.4.1.1 Mass Spectral Data of Constituents Identified by GC-MS

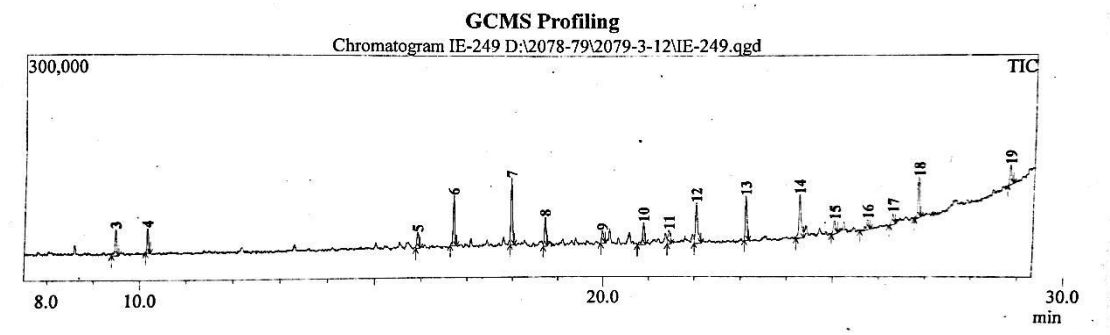


Figure 8: Chromatogram of hexane leaf extract of *S. sonchifolius*

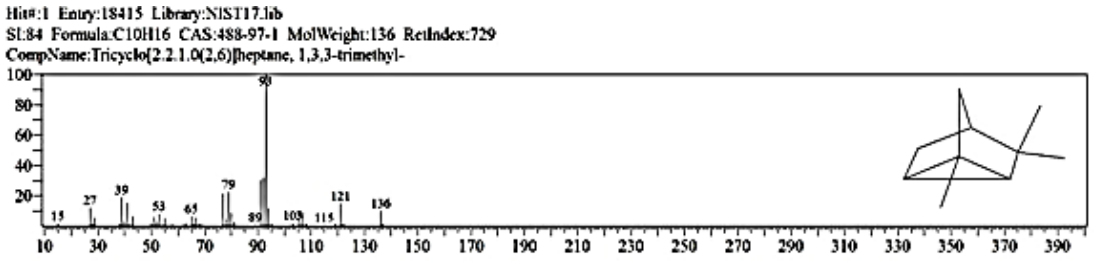


Figure 9: Chromatogram of Cyclofenchene

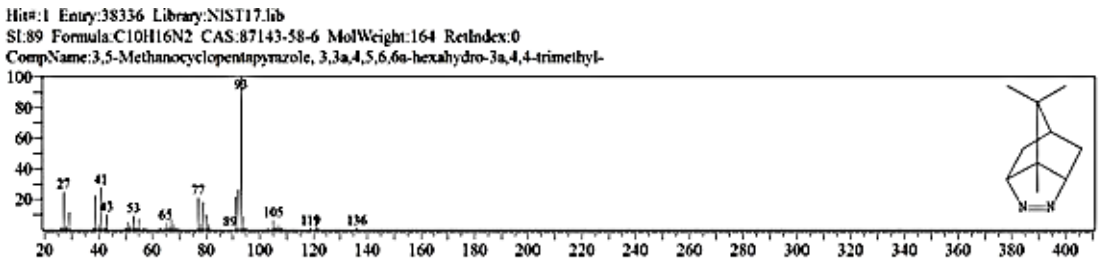


Figure 10: Chromatogram of 7,8,8-trimethyl-4,5-diazatricyclo [4.2.1.0^{3,7}] non-4-ene

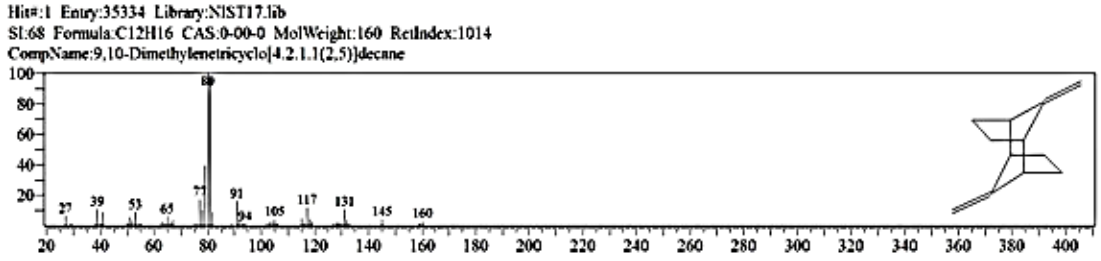


Figure 11: Chromatogram of 9,10-Dimethylenetricyclo [4.2.1.1(2,5)] decane

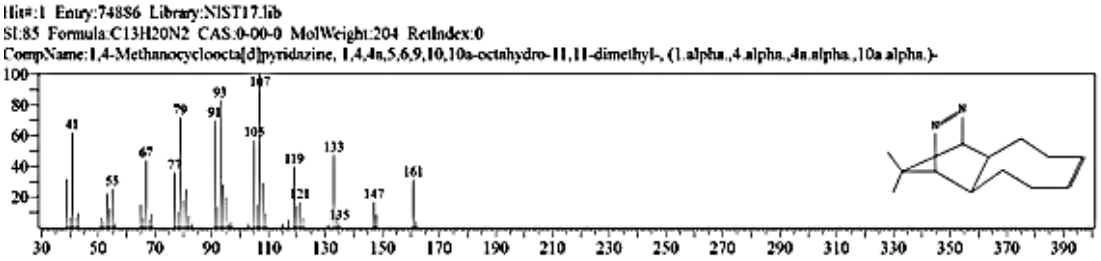


Figure 12: Chromatogram of 1,4-Methanocycloocta[d]pyridazine, 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-(1. α.,4. α.,4a. α.,10a. α.)-

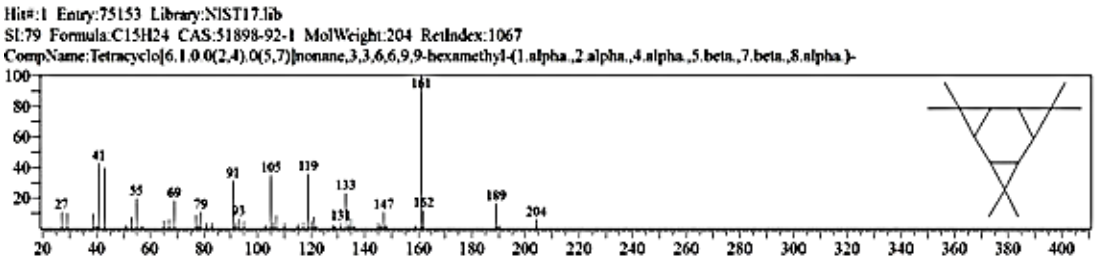


Figure 13: Chromatogram of Tetracyclo [6.1.0.0(2,4).0(5,7)] nanone,3,3,6,6,9,9-hexamethyl-(1. α.,2. α.,4. α.,5. β.,7. β.,8. α.)-

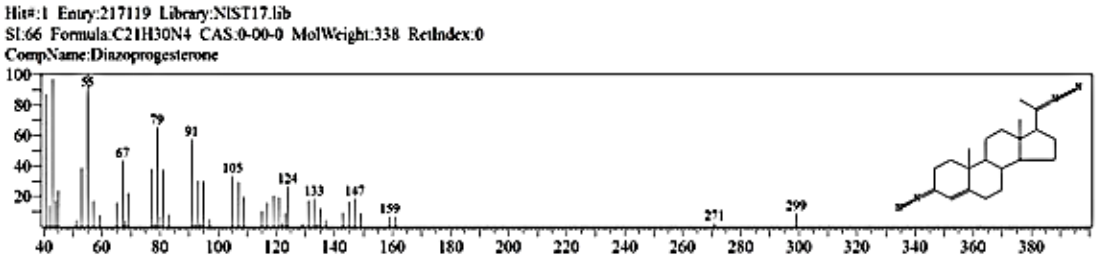


Figure 14: Chromatogram of Diazoprogesterone

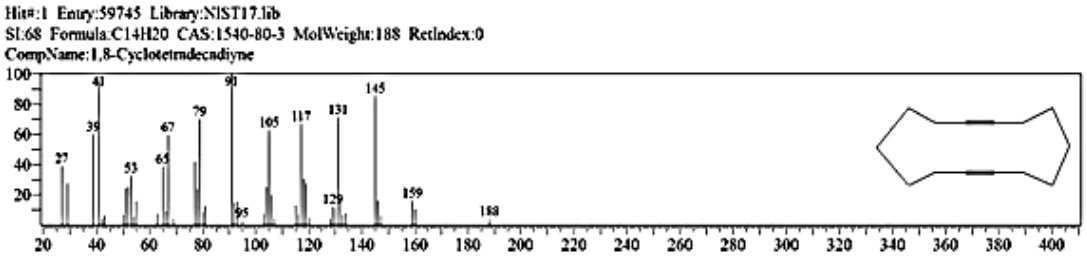


Figure 15: Chromatogram of 1.8-Cyclotetradecadiyne

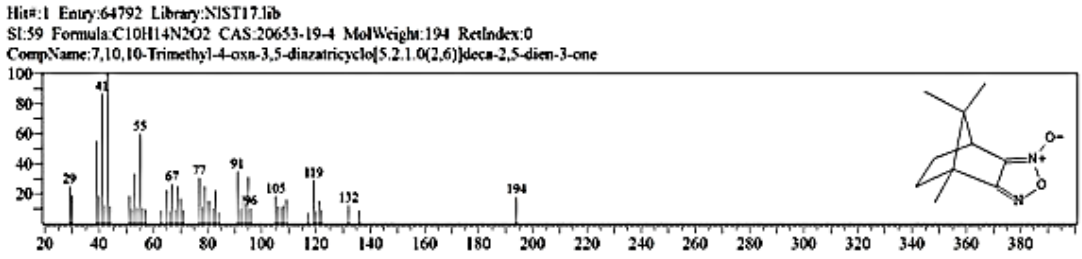


Figure 16: Chromatogram of 7,10,10-Trimethyl-4-oxa-3,5-diazatricyclo [5.2.1.0(2,6)] deca-2,5-dien-3-one

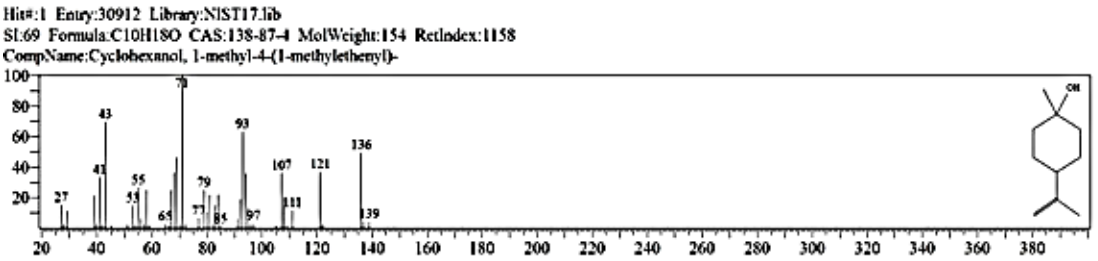


Figure 17: Chromatogram of Trans-β-terpeneol

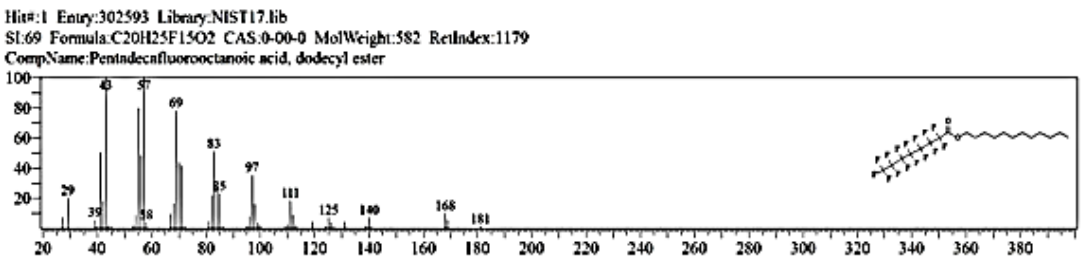


Figure 18: Chromatogram of Pentadecafluorooctanoic acid, dodecyl ester

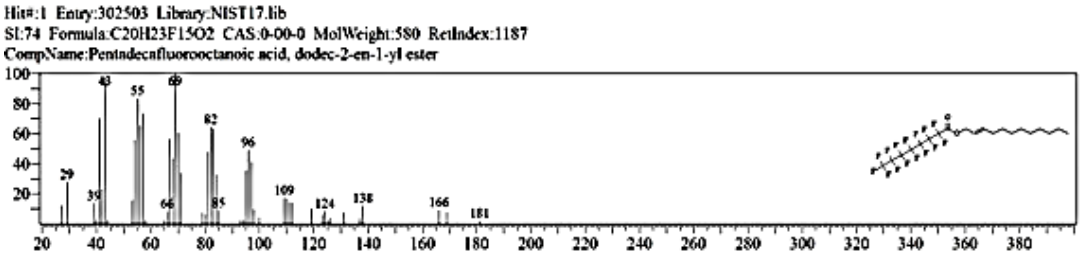


Figure 19: Chromatogram of Pentadecafluorooctanoic acid, dodec-2-en-1-yl ester

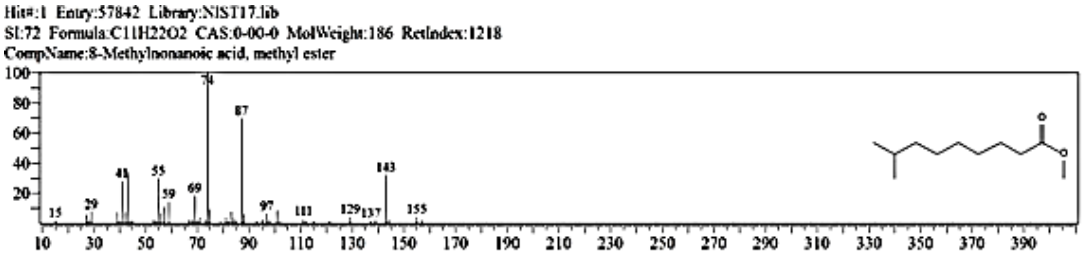


Figure 20: Chromatogram of 8-Methylnonanoic acid, methyl ester

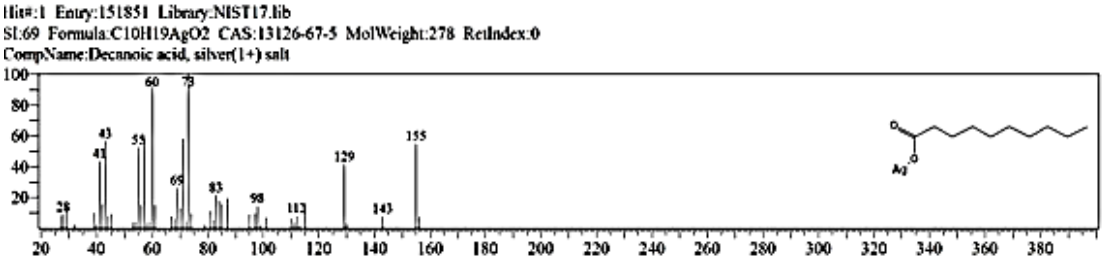


Figure 21: Chromatogram of Decanoic acid, silver (1+) salt

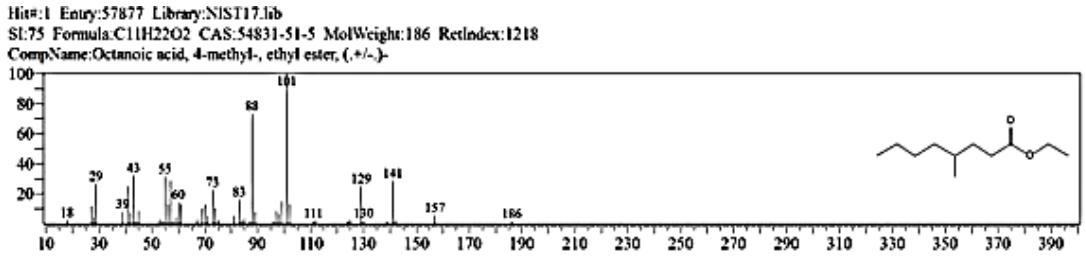


Figure 22: Chromatogram of Octanoic acid, 4-methyl-, ethyl ester, (+/-.)-

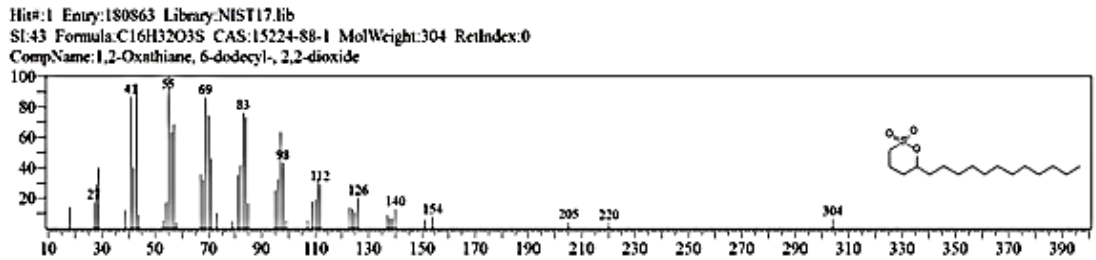


Figure 23: Chromatogram of δ-Hexadecanesultone

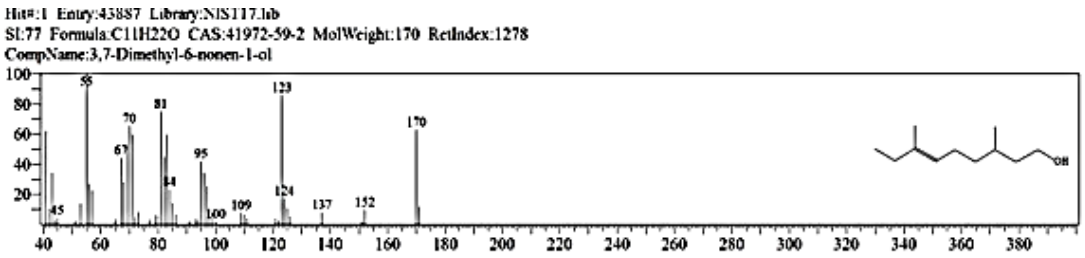


Figure 24: Chromatogram of 3,7-Dimethyl-6-nonen-1-ol

4.4.1.2 Major Compounds Found in GC-MS Analysis of Hexane Leaf Extract:

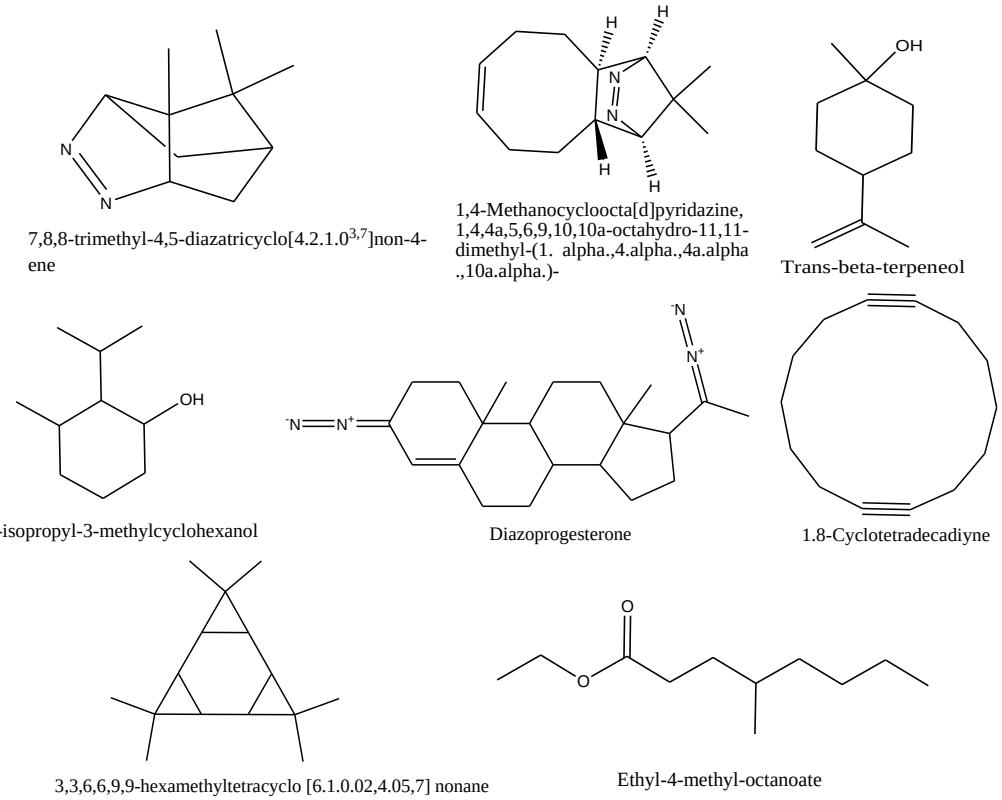


Figure 25: Structures of major compounds found in GC-MS analysis of hexane leaf extract

4.4.2 GC-MS Spectra Analysis of Methanol Leaf Extract

A NIST library search and GC-MS analysis of the *S. sonchifolius* methanol leaf extract's chemical make-up identified 4 main components. Based on GC-MS analysis, the list of important compounds is given below:

Table 6: Components based on GC-MS analysis of methanol leaf extract

S.N.	Name of compound	Retention time	Molecular formula	Area%
1.	Nonanoic acid, methyl ester	24.473	$C_{10}H_{20}O_2$	0.38
2.	Cyclopentyl-methyl-phosphinic acid, 2-isopropyl-5-methyl-cyclohexyl ester	26.167	$C_{16}H_{31}O_2P$	7.21
3.	2-Nonen-1-ol, 2-methyl-	27.514	$C_{10}H_{20}O$	0.71
4.	4-Bromobutyric acid, 3-methylbut-2-yl ester	28.308	$C_9H_{17}BrO_2$	91.70

4.4.2.1 Mass Spectral Data of Constituents Identified by GC-MS

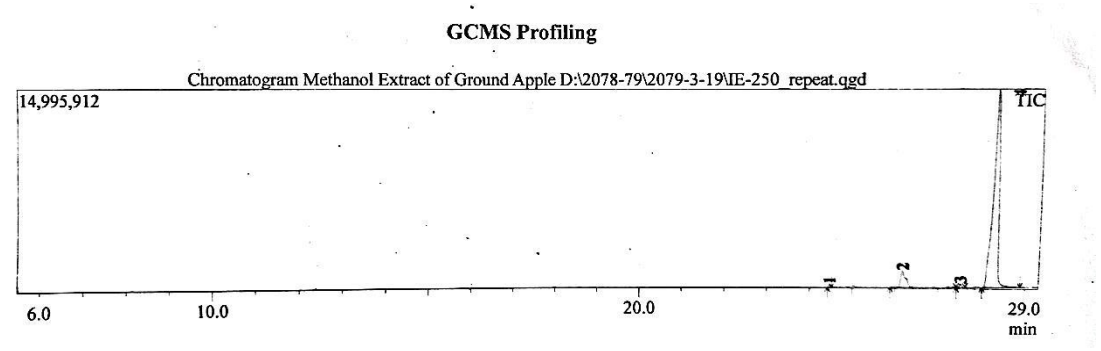


Figure 26: Chromatogram of methanol leaf extract of *S. sonchifolius*

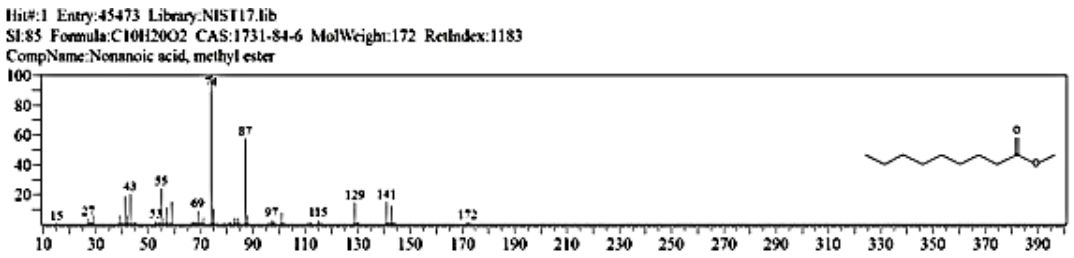


Figure 27: Chromatogram of Nonanoic acid, methyl ester

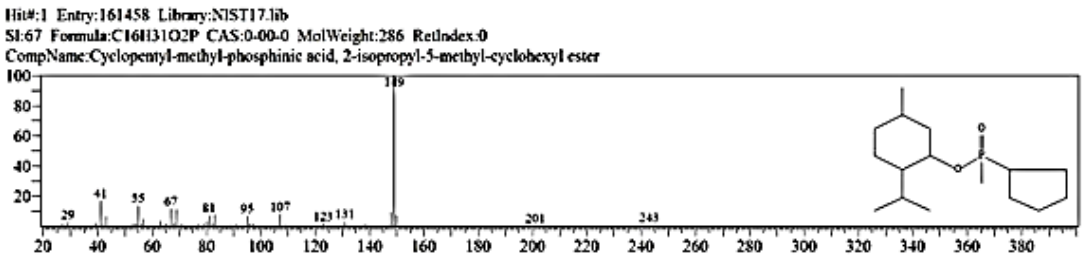


Figure 28: Chromatogram of Cyclopentyl-methyl-phosphinic acid, 2-isopropyl-5-methyl-cyclohexyl ester

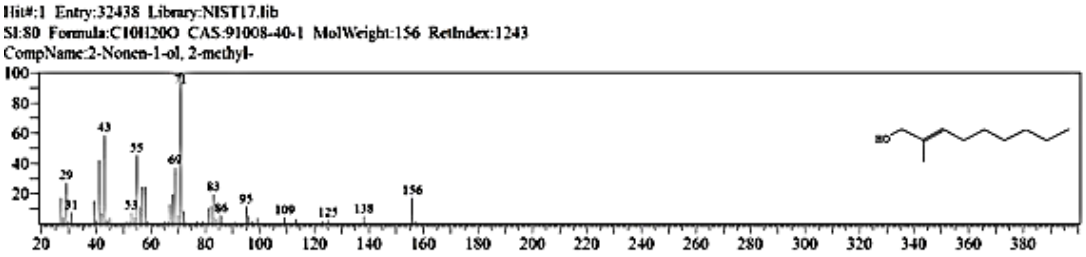


Figure 29: Chromatogram of 2-Nonen-1-ol, 2-methyl-

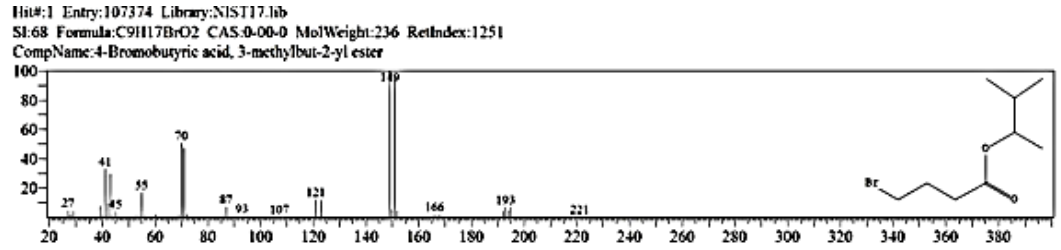
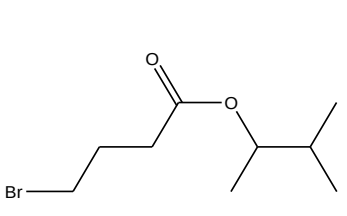
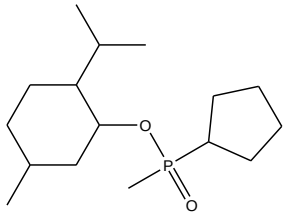


Figure 30: Chromatogram of 4-bromobutyric acid, 3-methylbut-2-yl ester

4.4.2.2 Major Compounds Found in GC-MS Analysis of Methanol Leaf Extract:



4-Bromobutyric acid, 3-methylbut-2-yl ester



Cyclopentyl-methyl-phosphinic acid, 2-isopropyl-5-methyl-cyclohexyl ester

Figure 31: Structures of the major compounds found in GC-MS analysis of methanol leaf extract

4.4.3 GC-MS Spectra Analysis of Hexane Root Extract

A NIST library search and GC-MS analysis of the *S. sonchifolius* hexane root extract's chemical makeup identified 30 main components. The following list of significant substances is based on GC-MS analysis:

Table 7: Components based on GC-MS analysis of hexane root extract

S.N.	Name of compound	Retention time	Molecular formula	Area%
1.	2-Methyl-2,4-dimethoxybutane	8.100	$C_7H_{16}O_2$	0.47
2.	Octane-2,3,3-trimethyl-	14.517	$C_{11}H_{24}$	0.21
3.	1,4-Methanocycloocta[d]pyridazine. 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1. α.,4. α.,4a. α.,10a. α.)-	19.567	$C_{13}H_{20}N_2$	0.28
4.	Plinol C	20.558	$C_{10}H_{18}O$	0.29
5.	δ-Hexadecanesultone	23.733	$C_{16}H_{32}O_3S$	0.48

6.	Silver decanoate	24.042	$C_{10}H_{19}AgO_2$	6.83
7.	Cyclopentyl-methyl-phosphinic acid, 2-isopropyl-5-methyl-cyclohexyl ester	33.675	$C_{16}H_{31}O_2P$	2.54
8.	Nonane, 3-methyl-5-propyl-	25.633	$C_{13}H_{28}$	0.45
9.	(-)-β-Copaene	25.792	$C_{15}H_{24}$	0.51
10.	1,5-Dodecadiene	26.625	$C_{12}H_{22}$	0.96
11.	Decane, -5-propyl-	27.208	$C_{13}H_{28}$	0.21
12.	5-Dodecyne	27.817	$C_{12}H_{22}$	4.57
13.	5-Decen-1-ol, (E)-	27.875	$C_{10}H_{20}O$	2.35
14.	Stearyltrimethylammonium chloride	27.317	$C_{21}H_{46}ClN$	0.24
15.	3,7,7-Trimethyl-bicyclo [2.2.1] hept-2-yl)-methanol	28.192	$C_{11}H_{20}O$	23.33
16.	Isoaromadendrene epoxide	28.500	$C_{15}H_{24}O$	1.82
17.	Dodecane, 4,6-dimethyl-	28.700	$C_{14}H_{30}$	0.69
18.	Tricyclo [4.3.0.0(7,9)] nonane, 2,2,5,5,8,8-hexamethyl-, (1.α.,6. β.,7. α.,9. α.)-	29.075	$C_{15}H_{26}$	0.98
19.	Pregnane-3,11,20,21-tetrol, cyclic 20,21-(methylboronate)-, (3.α.,5. α.,11. β.,20R)-	29.200	$C_{22}H_{37}BO_4$	1.26
20.	5,7-dimethyl-1,3,4-triazaindolizine	29.400	$C_7H_8N_4$	0.27
21.	Cyclohexane, 3,4-bis(1-methylethenyl)-1,1-dimethyl-	29.842	$C_{14}H_{24}$	0.24
22.	Cyclohexane, 1,2-dimethyl-3,5-bis(1-methylethenyl)-, (1. α.,2. β.,3. β.,5. α.)-	30.033	$C_{14}H_{24}$	0.40
23.	Pregnane-3,11,20,21-tetrol, cyclic 20,21-[(1,1-dimethylethyl) boronate],(3. α.,5. α.,11. β.,20S)	32.850	$C_{25}H_{43}BO_4$	6.07
24.	Cyclodecacyclotetradecene, 14,15-didehydro-1,4,5,8,9,10,11,12,13,16,17,18,19,20-tetradecahydro-	30.933	$C_{22}H_{32}$	2.02
25.	7-Nonenamide	31.225	$C_9H_{17}NO$	3.68
26.	9-Isopropyl-1-methyl-2-methylene-5-oxatricyclo [5.4.0.0(3,8)] undecane	31.758	$C_{15}H_{24}O$	2.03
27.	Trans-2-Dodecen-1-ol, heptafluorobutyrate	31.992	$C_{16}H_{23}F_7O_2$	1.19
28.	Cyclocopacamphenol	32.417	$C_{15}H_{24}O$	15.10
29.	Cyclohexane, 1,1,2-trimethyl-3,5-bis(1-methylethenyl) - (2. α.,3. β.,5. βα.)-	32.692	$C_{15}H_{26}$	2.93
30.	Dodecanal	34.667	$C_{12}H_{24}O$	1.72

4.4.3.1 Mass Spectral Data of Constituents Identified by GC-MS

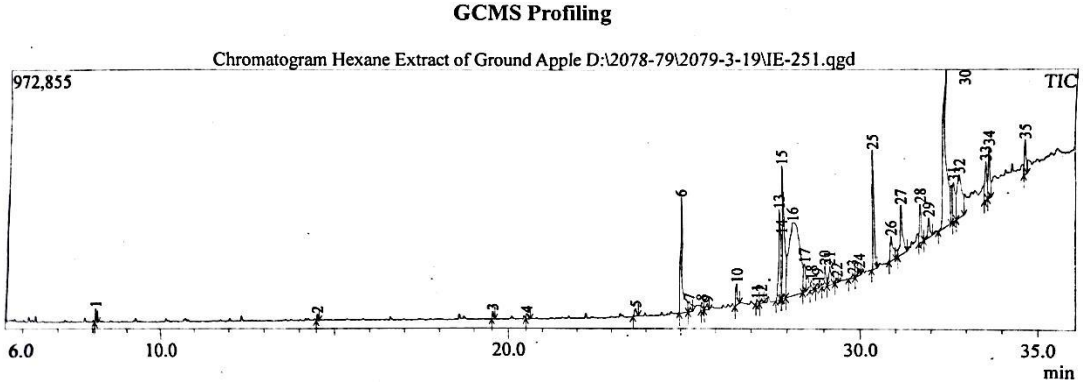


Figure 32: Chromatogram of hexane root extract of *S. sonchifolius*

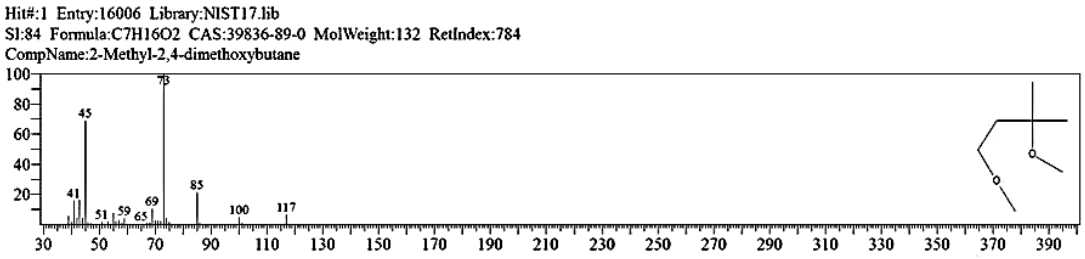


Figure 33: Chromatogram of 2-Methyl-2,4-dimethoxybutane

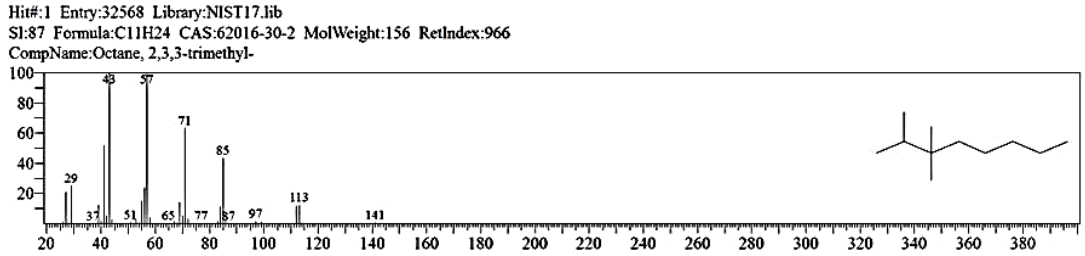


Figure 34: Chromatogram of Octane-2,3,3-trimethyl-

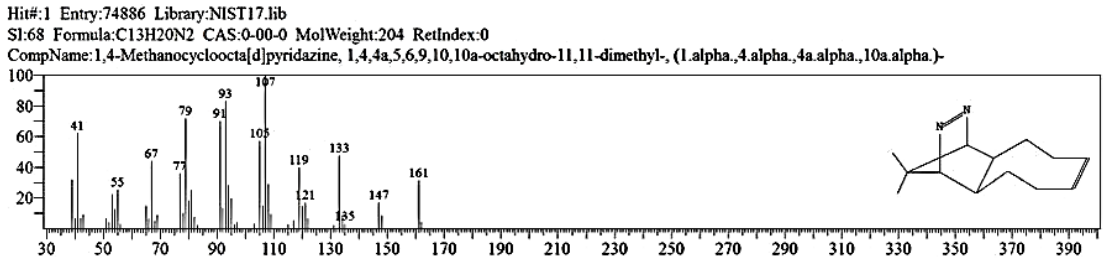


Figure 35: Chromatogram of 1,4-Methanocycloocta[d]pyridazine. 1,4,4a,5,6,9,10,10a-octahydro-11,11-dimethyl-, (1.α.,4. α.,4a.α.,10a.α.)-

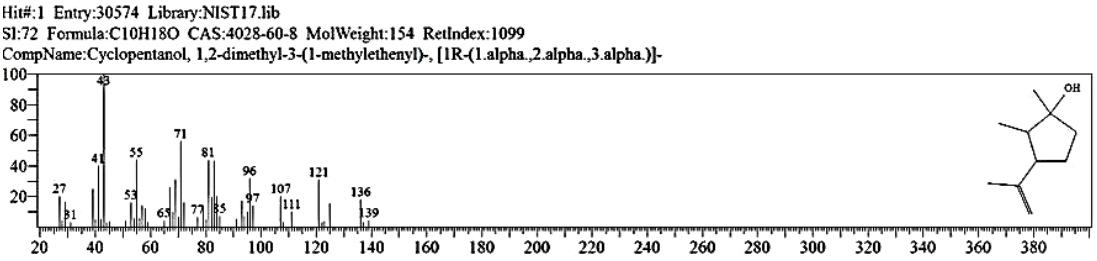


Figure 36: Chromatogram of Pinol-C

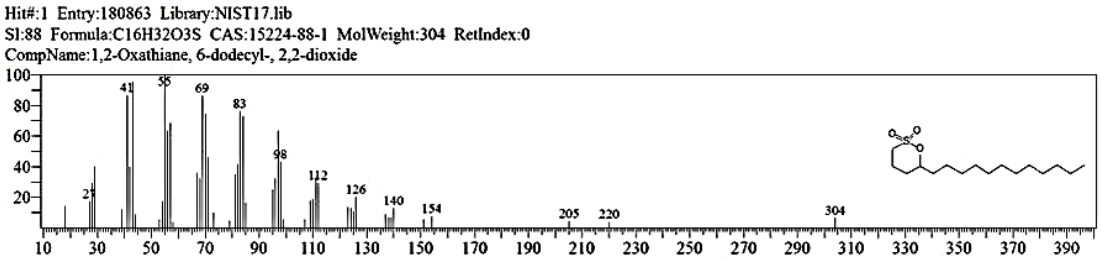


Figure 37: Chromatogram of δ-Hexadecanesultone

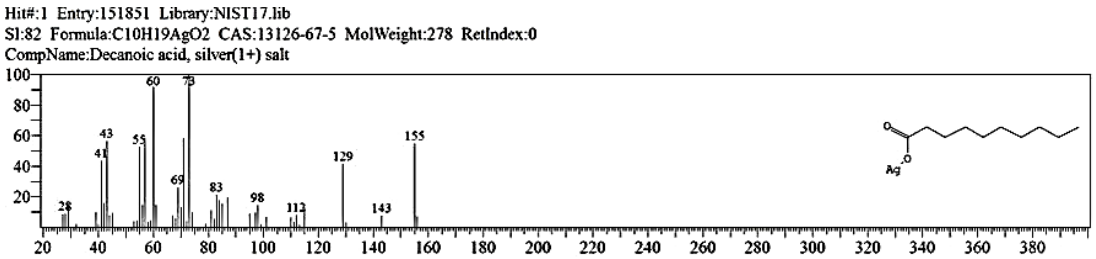


Figure 38: Chromatogram of Silver decanoate

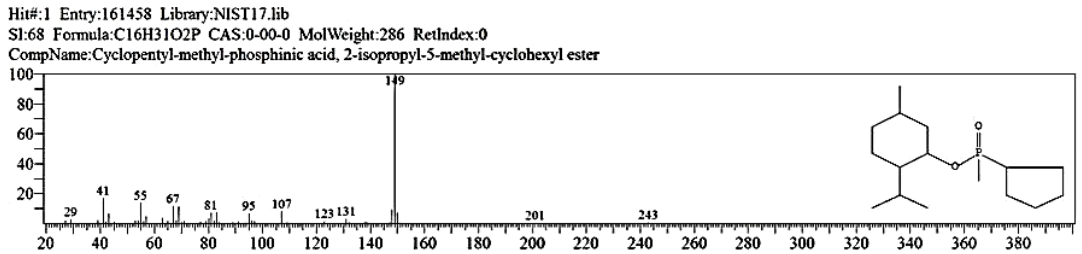


Figure 39: Chromatogram of Cyclopentyl-methyl-phosphinic acid, 2-isopropyl-5-methyl-cyclohexyl ester

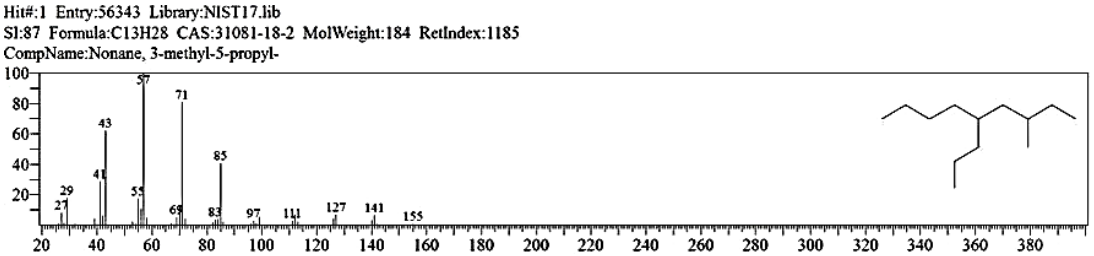


Figure 40: Chromatogram of Nonane, 3-methyl-5-propyl-

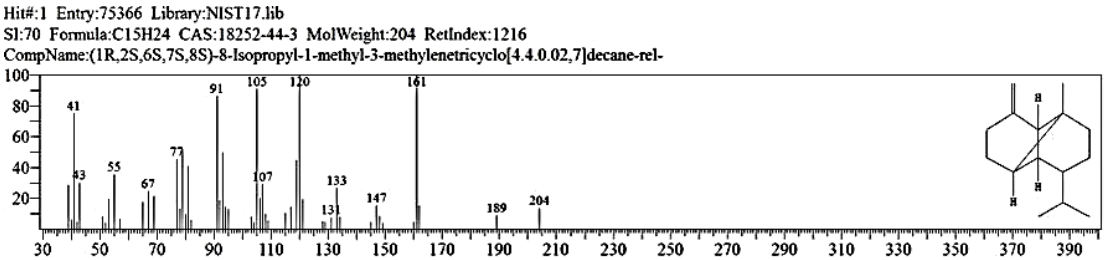


Figure 41: Chromatogram of (-)-β-copaene

Hit#:1 Entry:40328 Library:NIST17.lib
SI:86 Formula:C12H22 CAS:0-00-0 MolWeight:166 RetIndex:1212
CompName:1,5-Dodecadiene

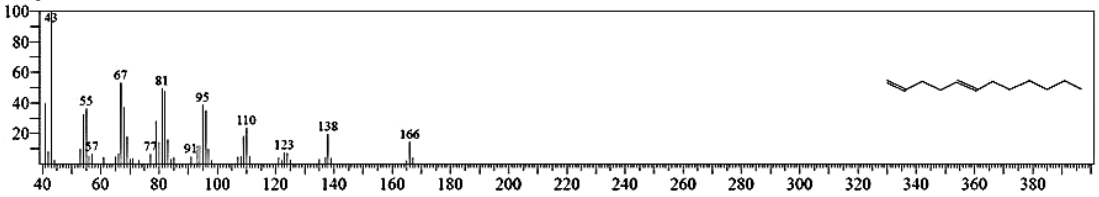


Figure 42: Chromatogram of 1,5-Dodecadiene

Hit#:1 Entry:56341 Library:NIST17.lib
SI:86 Formula:C13H28 CAS:17312-62-8 MolWeight:184 RetIndex:1249
CompName:Decane, 5-propyl-

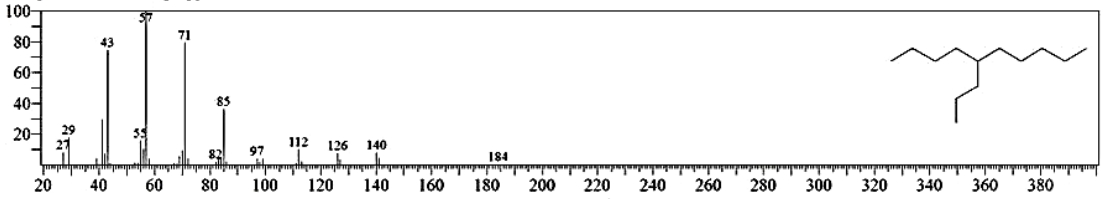


Figure 43: Chromatogram of Decane, -5-propyl-

Hit#:1 Entry:40410 Library:NIST17.lib
SI:82 Formula:C12H22 CAS:19780-12-2 MolWeight:166 RetIndex:1231
CompName:5-Dodecyne

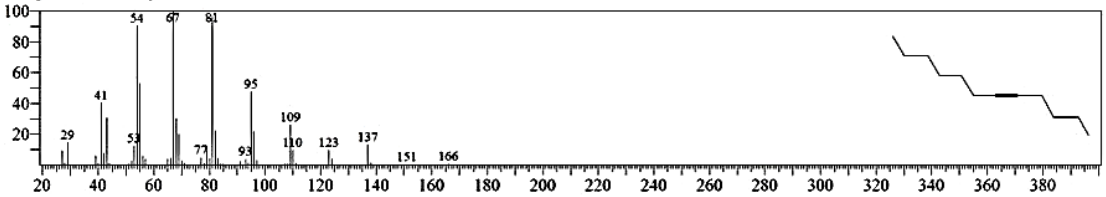


Figure 44: Chromatogram of 5-Dodecyne

Hit#:1 Entry:32424 Library:NIST17.lib
SI:73 Formula:C10H20O CAS:56578-18-8 MolWeight:156 RetIndex:1266
CompName:5-Decen-1-ol, (E)-

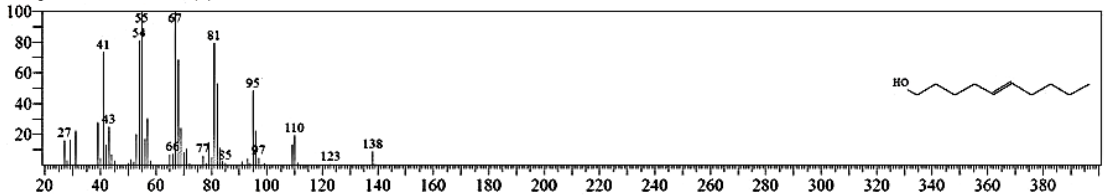


Figure 45: Chromatogram of 5-Decen-1-ol, (E)-

Hit#:1 Entry:226088 Library:NIST17.lib
SI:67 Formula:C21H46ClN CAS:112-03-8 MolWeight:347 RetIndex:0
CompName:Stearyltrimethylammonium chloride

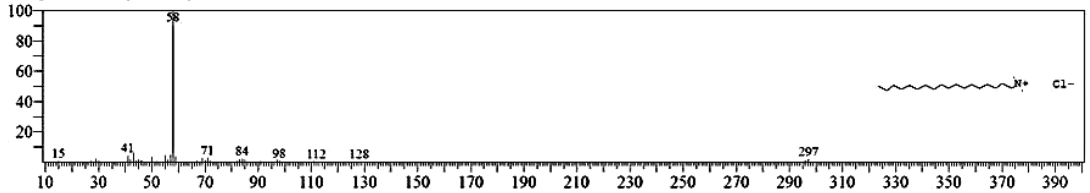


Figure 46: Chromatogram of Stearyltrimethylammonium chloride

Hit#:1 Entry:42015 Library:NIST17.lib
SI:69 Formula:C11H20O CAS:0-00-0 MolWeight:168 RetIndex:1241
CompName:(3,7,7-Trimethyl-bicyclo[2.2.1]hept-2-yl)-methanol

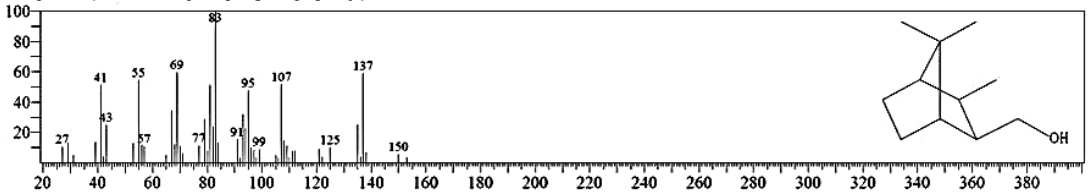


Figure 47: Chromatogram of 3,7,7-Trimethyl-bicyclo[2.2.1]hept-2-yl)-methanol

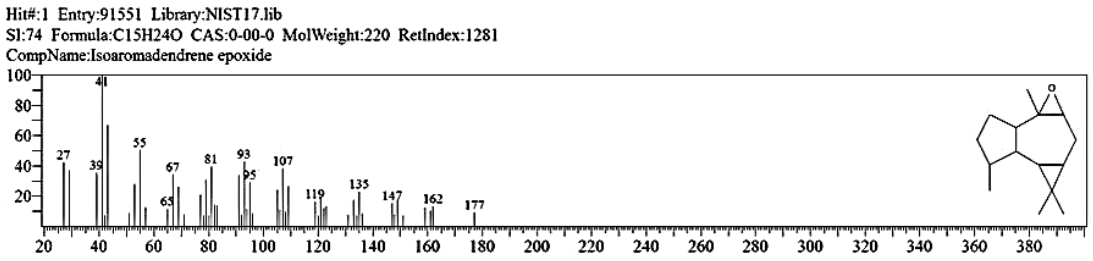


Figure 48: Chromatogram of Isoaromadendrene epoxide

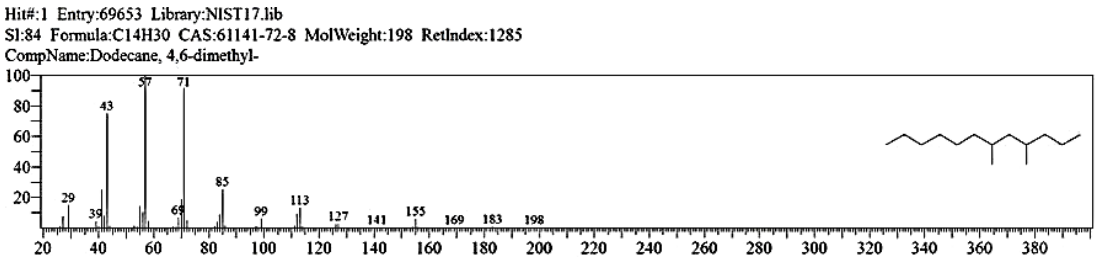


Figure 49: Chromatogram of Dodecane,4,6-dimethyl-

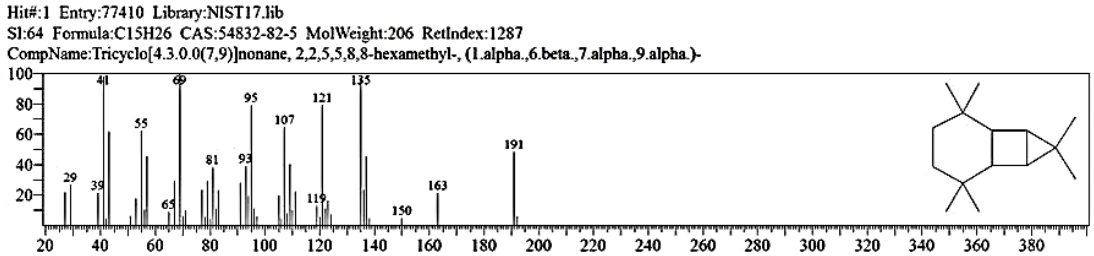


Figure 50: Chromatogram of Tricyclo [4.3.0.0(7,9)] nonane,2,2,5,5,8,8-hexamethyl-, (1.α.,6. β.,7. α.,9. α.)-

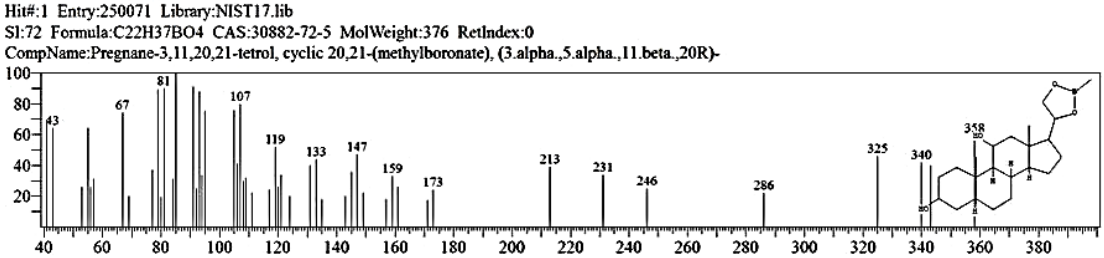


Figure 51: Chromatogram of Pregnane-3,11,20,21-tetrol, cyclic 20,21-(methylboronate)-, (3.α.,5. α.,11. β.,20R)-

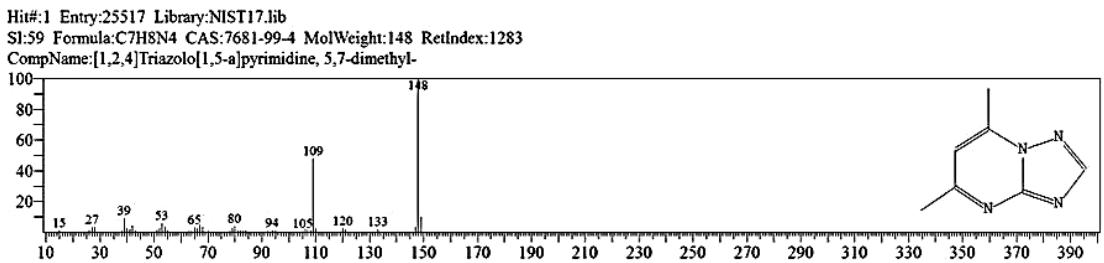


Figure 52: Chromatogram of 5,7-dimethyl-1,3,4-triazaindolizine

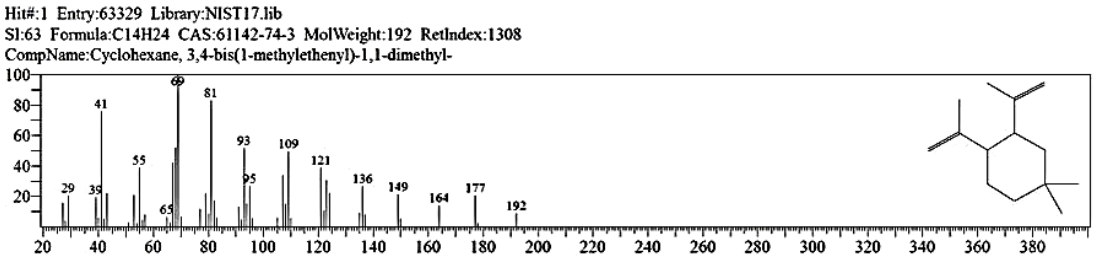


Figure 53: Chromatogram of Cyclohexane,3,4-bis(1-methylethenyl)-1,1-dimethyl-

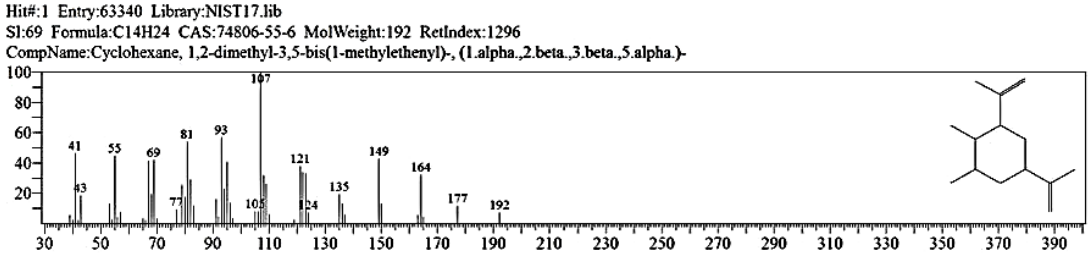


Figure 54: Chromatogram of Cyclohexane, 1,2-dimethyl-3,5-bis(1-methylethenyl)-, (1.α.,2. β.,3.β.,5.α.)-

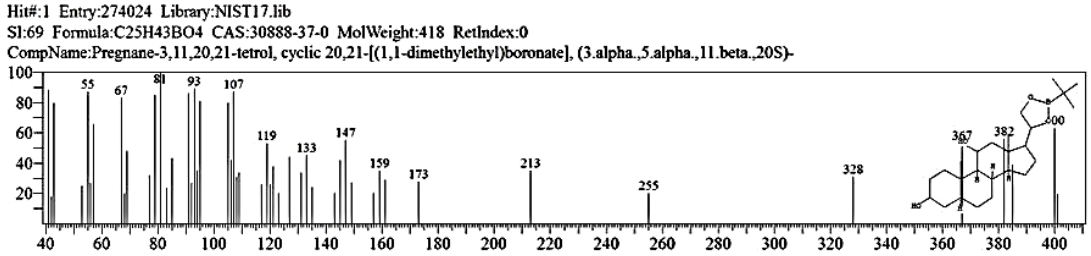


Figure 55: Chromatogram of Pregnane-3,11,20,21-tetrol, cyclic 20,21-[(1,1-dimethylethyl) boronate], (3.α.,5. α.,11. β.,20S)-

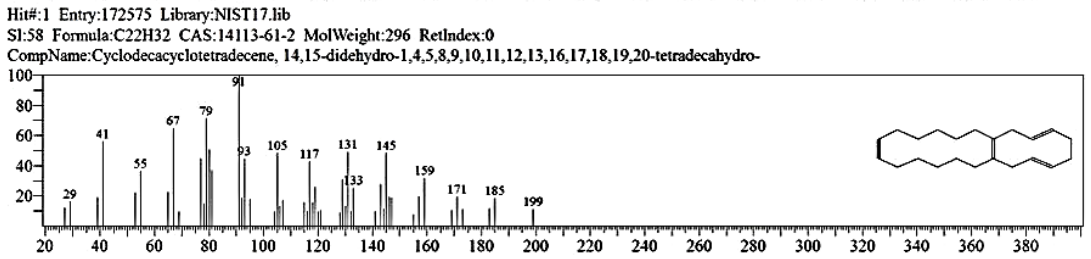


Figure 56: Chromatogram of Cyclodecacyclotetradecene, 14,15-didehydro-1,4,5,8,9,10,11,12,13,16,17,18,19,20-tetradecahydro-

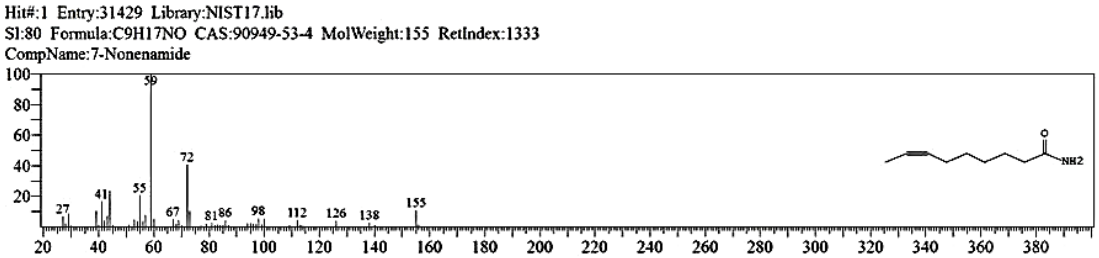


Figure 57: Chromatogram of 7-Nonenamide

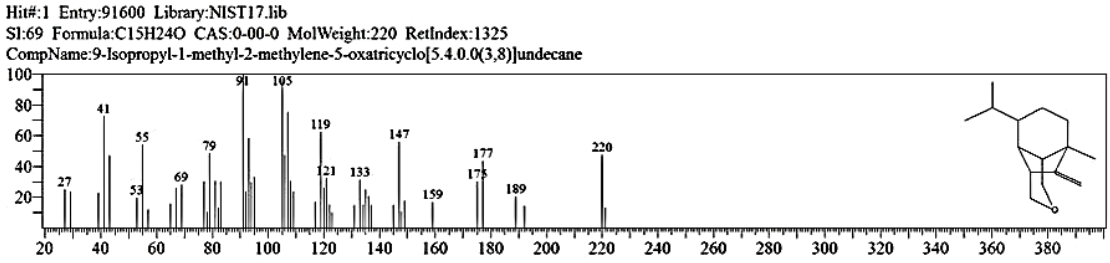


Figure 58: Chromatogram of 9-Isopropyl-1-methyl-2-methylene-5-oxatricyclo [5.4.0.0(3,8)] undecane

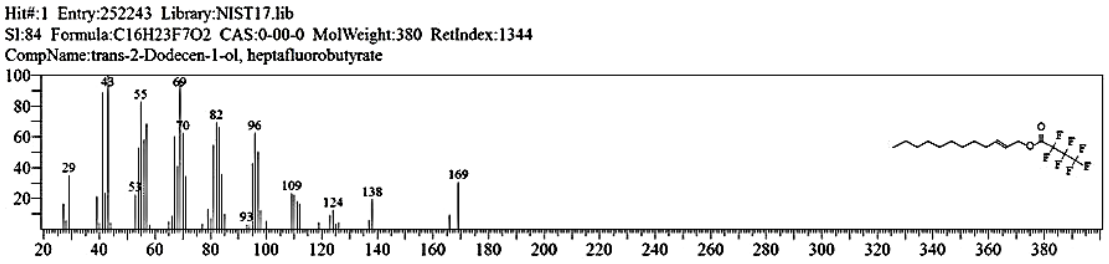


Figure 59: Chromatogram of Trans-2-Dodecen-1-ol, heptafluorobutyrate

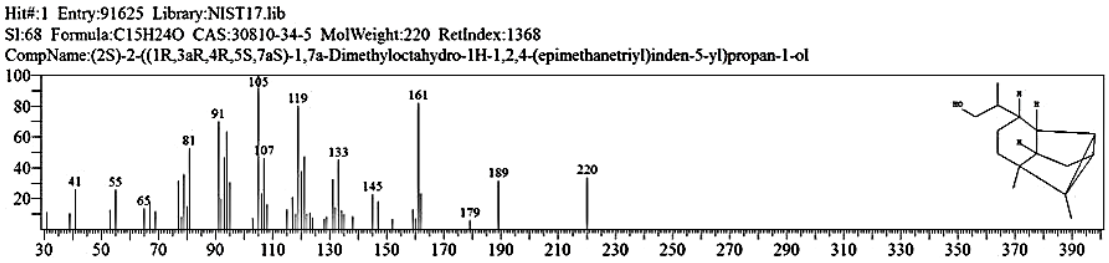


Figure 60: Chromatogram of Cyclocopacamphenol

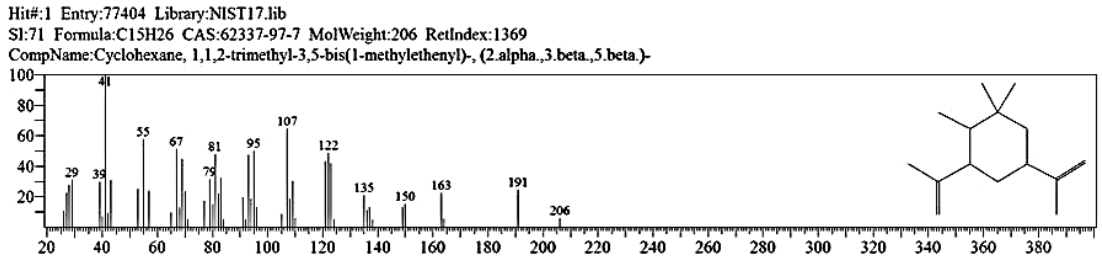


Figure 61: Chromatogram of Cyclohexane,1,1,2-trimethyl-3,5-bis(1-methylethenyl) - (2. α.,3. β.,5. β.)-

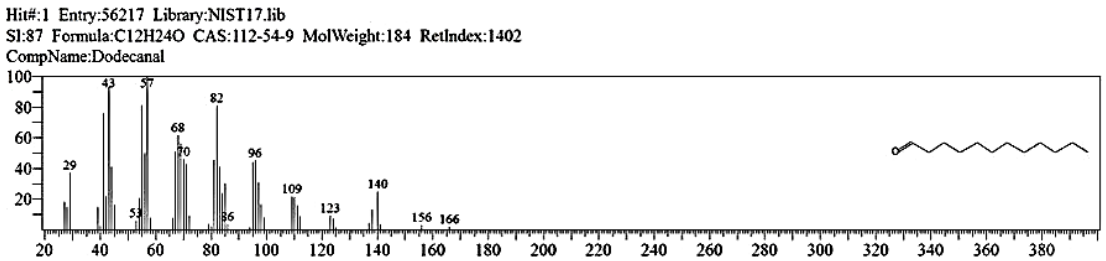


Figure 62: Chromatogram of Dodecanal

4.4.3.2 Major Compounds Found in GC-MS Analysis of Hexane Root Extract:

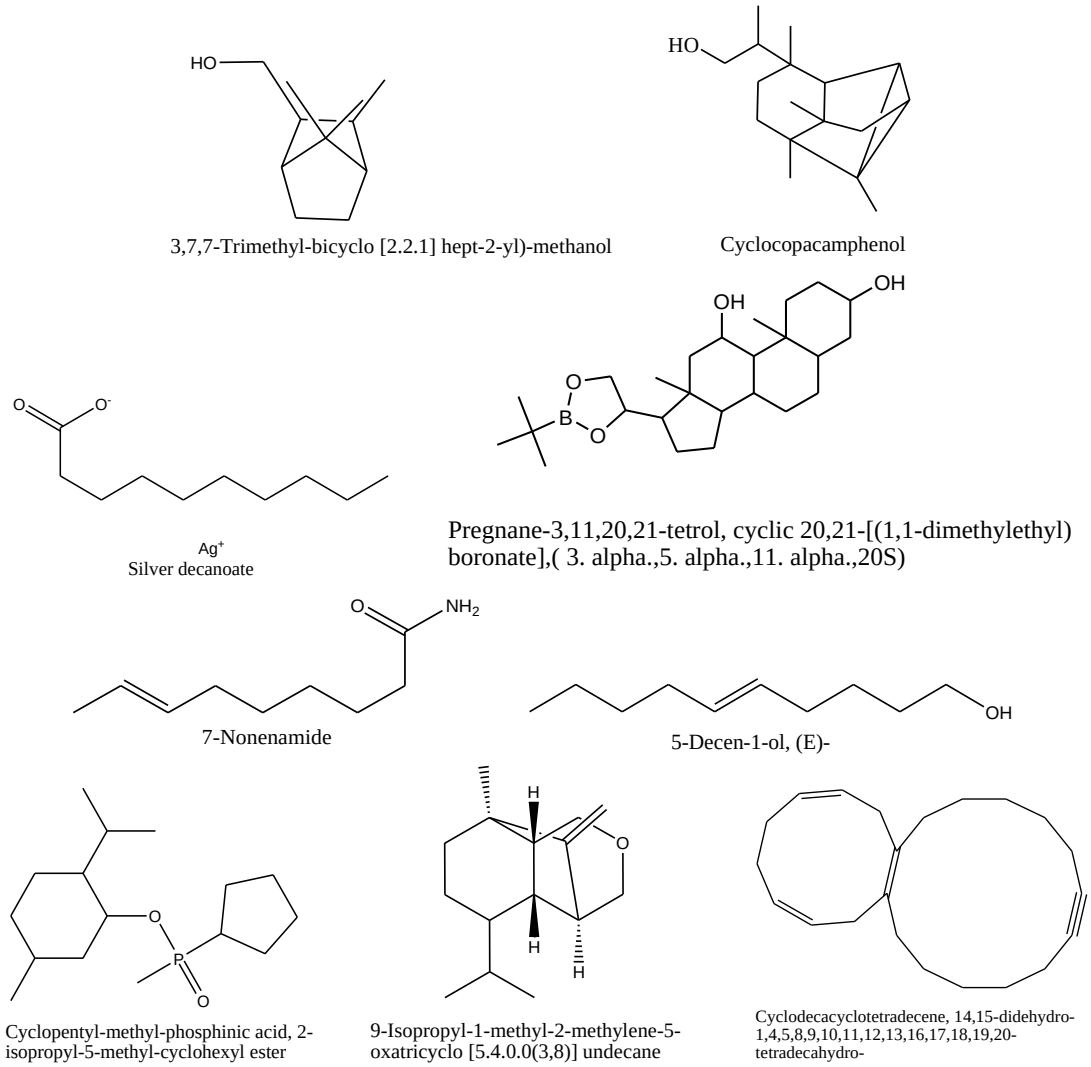


Figure 63: Structures of the major compounds found in GC-MS analysis of hexane root extract

4.5 Quantitative Analysis of Phytochemicals

4.5.1 Estimation of Total Phenolic Content

4.5.1.1 Construction of the Calibration Curve

The total phenolic content of plant extracts was evaluated using the Folin-Ciocalteu colorimetric (FCC) technique, which was based on the oxidation-reduction method. The calibration curve created using this approach was based on gallic acid as the standard. The phytochemicals in plants that serve as antioxidants and lower the risk of oxidative-induced illnesses contain the most excellent phenolic content (Balasundram *et al*., 2006). Using a UV-Vis spectrophotometer, the absorbance of gallic acid at 760

nm wavelengths was measured at different concentrations (500, 250, 150, 100, 50, and 25 µg/mL) to build the calibration curve. UV-visible spectrometry may be used to quantify the number of polyphenols in plant extracts that react with a particular redox reagent (FCR) to form a blue complex having a maximum light absorption of 760 nm. The degree of light absorption at that wavelength is negatively associated with the concentrations of phenols. A graph was used to show the absorbance at different concentrations of standard gallic acid; the Y-axis showed absorbance and the X-axis showed concentration. The absorbance curve of standard gallic acid is shown in the figure.

Table 8: Gallic acid standard

Conc. (µg/mL)	Triplicates Absorbance data			Average Absorbance
500	2.332	2.121	2.208	2.22
250	1.3	1.344	1.369	1.338
150	0.891	0.854	0.821	0.855
100	0.675	0.635	0.664	0.658
50	0.544	0.554	0.53	0.543
25	0.374	0.366	0.353	0.364

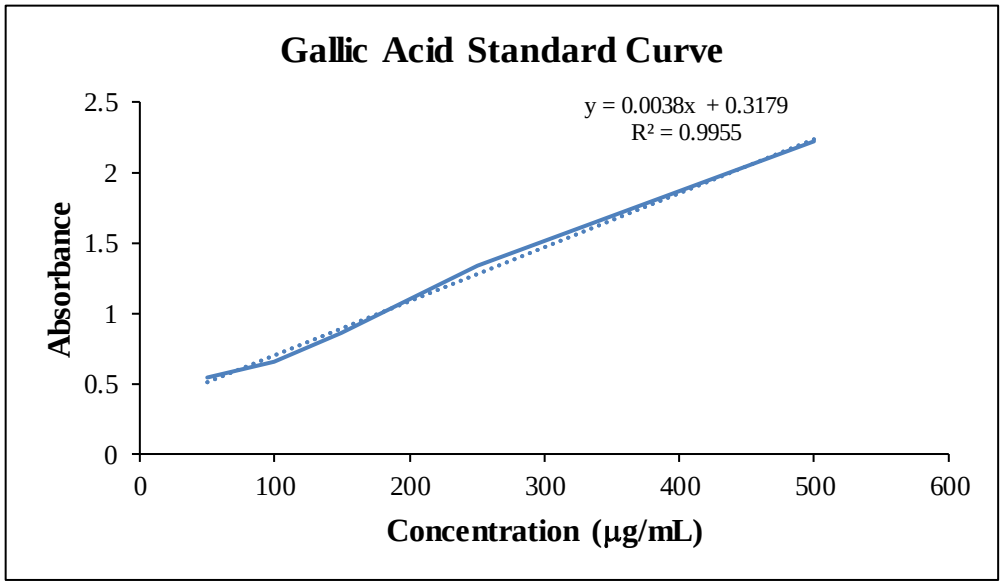


Figure 64: Calibration curve for standard gallic acid

The concentration of ethyl acetate and methanol extract was determined by using an equation that is obtained from the standard gallic acid curve in Figure 59.

4.5.1.2 Calculation of Total Phenolic Content in Different Extracts

Table 9: Total phenolic content in ethyl acetate and methanol leaf extract

Extracts	Concentration (µg/mL)	Observed Data (O.D) Sample	O.D control	O.D value
Ethyl acetate	1000	0.332	0.0597	0.0273
Methanol	1000	0.1247	0.0597	0.065

The total phenolic content of ethyl acetate leaf extract of *S. sonchifolius* from the data was 55.26 mg GAE/g, and that of Methanol leaf extract was 2.10 mg GAE/g.

Table 10: Total phenolic content in chloroform and methanol root extract

Extracts	Concentration (µg/mL)	Observed Data (O.D) Sample	O.D control	O.D value
Chloroform	1000	0.389	0.0597	0.329
Methanol	1000	0.119	0.0597	0.06

The total phenolic content of chloroform root extract of *S. sonchifolius* from the data was 69.97 mg GAE/g, and that of methanol root extract was 0.82 mg GAE/g.

From the above result, it was found that the chloroform root extract of *S, sonchifolius* contains high phenolic compounds 69.97 mg GAE/g. Chloroform root extract had the highest phenolic content among the four extracts. This finding suggests that, in general, phenolic chemicals are more soluble in polar organic solvents than non-polar solvents.

Table 11: Total phenolic content of *S. sonchifolius* extracts

Extract	Ethyl acetate leaf (mg GAE/g)	Methanol leaf (mg GAE /g)	Chloroform root (mg GAE/g)	Methanol root (mg GAE/g)
Total phenolic content (mg GAE /g extract)	55.26	2.10	69.97	0.82

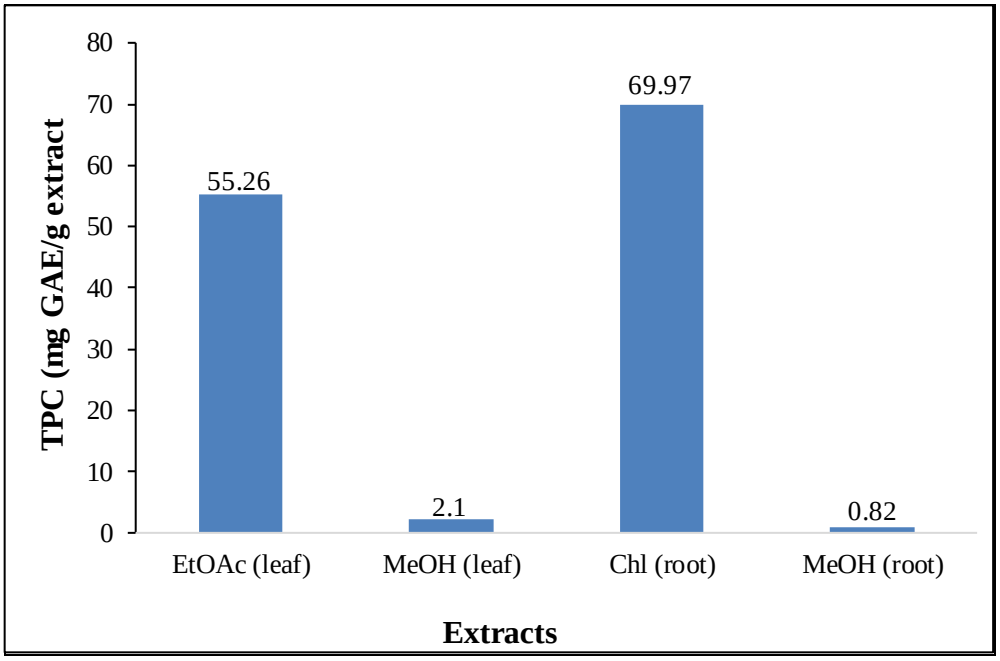


Figure 65: Total phenolic contents of *S. sonchifolius* extracts

The results show that chloroform root extract has shown higher TPC than methanol root extract i.e. 69.97 mg GAE/g whereas ethyl acetate leaf extract has shown higher TPC than methanol leaf extract i.e. 485.17 mg GAE/g.

4.5.2 Estimation of Total Flavonoid Content

4.5.2.1 Construction of Calibration Curve

An assay for measuring the total flavonoid concentration in plant extracts was conducted using a colorimetric aluminum chloride assay. By using this technique, quercetin was chosen to act as the reference substance while creating the calibration curve (Heim *et al*., 2002). The flavonoids in the plant extracts produce an acid liable complex when aluminium chloride is present. By measuring the complex's yellow fluorescence at 425 nm in the UV spectrum, we can see that it is quite strong. The number of flavonoids and the amount of light absorbed at that wavelength are directly correlated. The flavonoid components display a wide range of biological and pharmacological actions. The graph plots in the figure below are in various concentrations (250 µg/mL, 150 µg/mL, 100 µg/mL, 50 µg/mL, and 25 µg/mL).

Table 12: Quercetin standard

Conc (µg/mL)	Triplicates Absorbance data			Average Absorbance
250	1.731	1.76	1.829	1.773
150	1.117	1.089	1.093	1.100
100	0.762	0.771	0.76	0.764
50	0.416	0.416	0.422	0.418
25	0.225	0.228	0.23	0.228

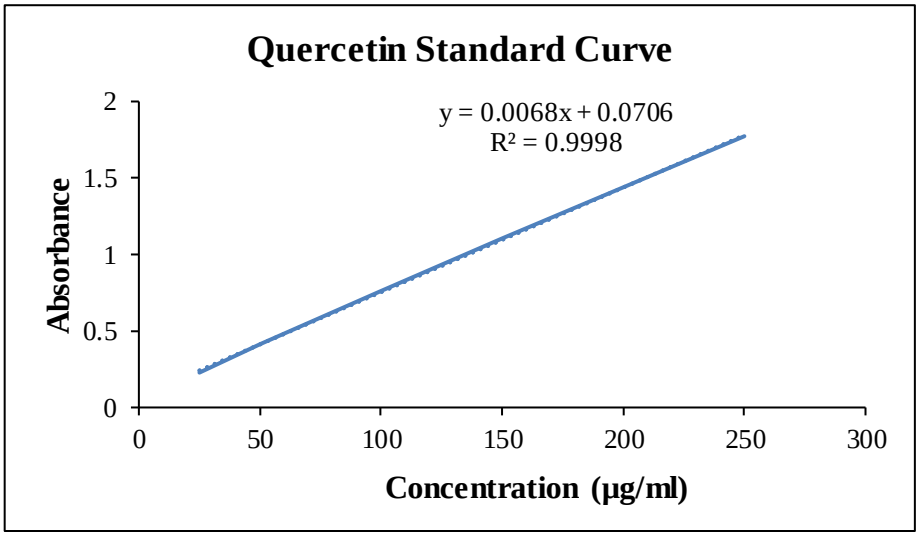


Figure 66: Calibration curve for standard quercetin

The concentration of chloroform and methanol extract was determined by using an equation that is obtained from the standard quercetin curve in Figure 61.

4.5.2.2 Calculation of Total Flavonoid Content in Different Extracts

Table 13: Total flavonoid content in chloroform and methanol leaf extract

Extracts	Concentration (µg/mL)	Observed Data (O.D) Sample	O.D control	O.D value
Chloroform	1000	2.464	0.0697	2.394
Methanol	1000	0.636	0.0697	0.566

The total flavonoid content of chloroform leaf extract was found to be 350.87 mg QE/g, and that of Methanol leaf extract was 82.049 mg QE/g.

Table 14: Total flavonoid content in chloroform and methanol root extract

Extracts	Concentration (µg/mL)	Observed Data(O.D) Sample	O.D control	O.D value
Chloroform	1000	0.121	0.0697	0.0517
Methanol	1000	1.255	0.0697	1.185

The total flavonoid content of chloroform root extract was found to be 6.36 mg QE/g, and that of methanol root extract was 173.03 mg QE/g.

They were calculated from the regression equation, R^2 equation followed by the formula C= cV/m and expressed as mg quercetin equivalents (QE) per g of extract in dry weight (mg/g). The total flavonoid content was calculated in Chloroform and Methanol extracts of *S. sonchifolius* which is tabulated below:

Table 15: Total flavonoid content of *S. sonchifolius* extracts

Extracts	Chloroform leaf (mg QE/g)	Methanol leaf (mg QE/g)	Chloroform root (mg QE/g)	Methanol root (mg QE/g)
Total flavonoid content (mg QE/g extract)	350.87	82.049	6.36	173.03

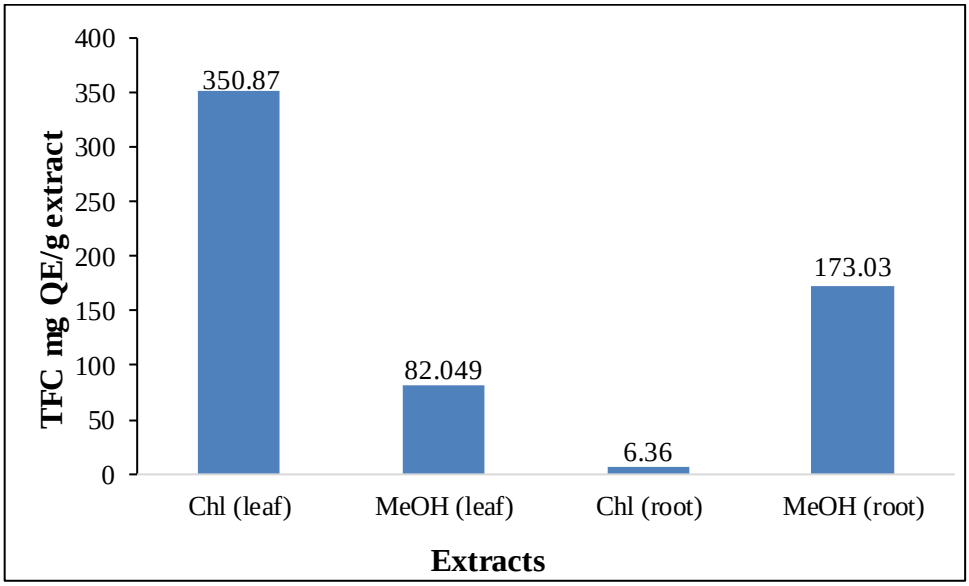


Figure 67: Total flavonoid content of *S. sonchifolius* extracts

From the above data, it was found that chloroform leaf extract has the highest TFC value i.e. 350.87 mg QE/g compared with chloroform leaf extract, methanol leaf extract, and methanol root extract.

4.5.3 Antioxidant Activity

Using the DPPH free radical scavenging test, the amount of antioxidant activity was determined. Antioxidants change DPPH into its reduced form through proton sacrifice. The dark violet absorption band in DPPH solutions is located at 520 nm. The purple-to-yellow color shift is reflected in the decrease in 520 nm absorption. The degree of decolorization indicates the anti-inflammatory and free radical-

scavenging properties of the sample (Molyneux, 2004). Several *S. sonchifolius* plant extracts were subjected to the DPPH assay, with ascorbic acid serving as the standard. At 520 nm, the absorbance of various extract solutions and ascorbic acid solutions was measured after they had been incubated at room temperature: antioxidant concentration and activity lower absorption.

The antioxidant potential, inversely proportional to the IC_{50} value, can be ascertained using the linear regression of % inhibition vs. antioxidant activity. The linear regression method is used to determine this. If the IC_{50} is low, the substance is an antioxidant. DPPH and methanol were employed as controls, but no extracts were used.

Each solution's absorbance was measured and noted as follows:

Table 16: Antioxidant activity of ascorbic acid

Sample	Concentration (µg/mL)	Absorbance(nm)			Average absorbance(nm)	% of scavenging
1	Control	0.48	0.524	0.47	0.491	18.33
2	2.5	0.433	0.433	0.433	0.433	11.872
3	5	0.385	0.362	0.366	0.371	24.491
4	10	0.303	0.304	0.309	0.305	37.856
5	20	0.21	0.195	0.177	0.194	60.516
6	30	0.16	0.166	0.162	0.163	66.892
IC_{50} = 14.38 µg/mL						

The percentage of inhibition and graph were used to derive the IC_{50} values for various extracts. Free radicals were scavenged at different concentrations using ascorbic acid, and the measured absorbance was plotted in a graph, as seen in the figure.

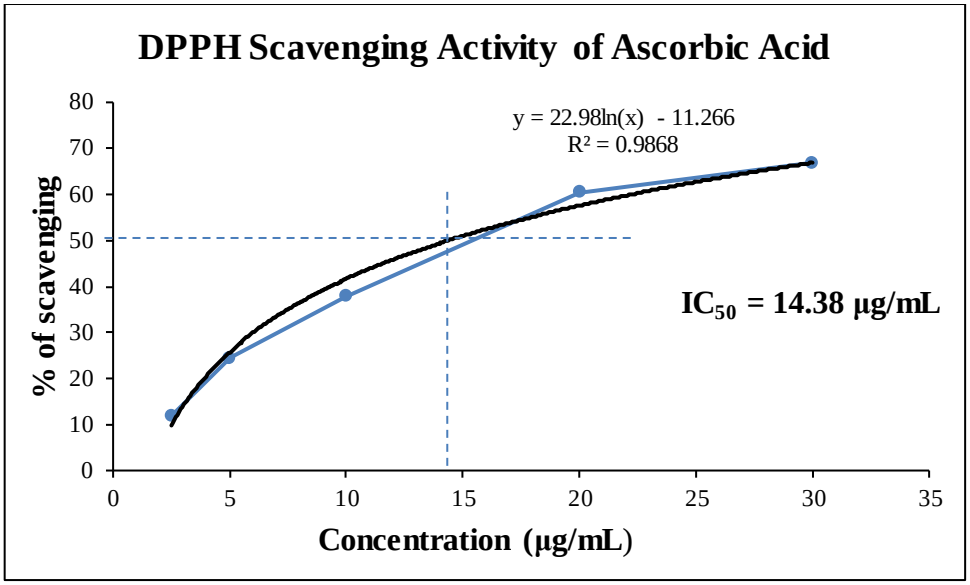


Figure 68: Antioxidant activity of ascorbic acid

From this graph, the IC_{50} value of ascorbic acid was found to be 14.38 µg/mL. At 520 nm, absorbance values of various chloroform extract concentrations were determined. The following table displays the % inhibition achieved by DPPH radicals against the sample, which was determined using these values:

Table 17: Antioxidant activity of chloroform leaf extract

S.N.	Concentration (µg/mL)	% of scavenging
1	2000	52.244
2	1000	46.795
3	250	33.526
4	125	31.282
IC_{50}= 1555.73 µg/mL		

The IC_{50} value of chloroform leaf extract of *S. sonchifolius* was found to be 1555.73 µg/mL. This is shown in the figure below:

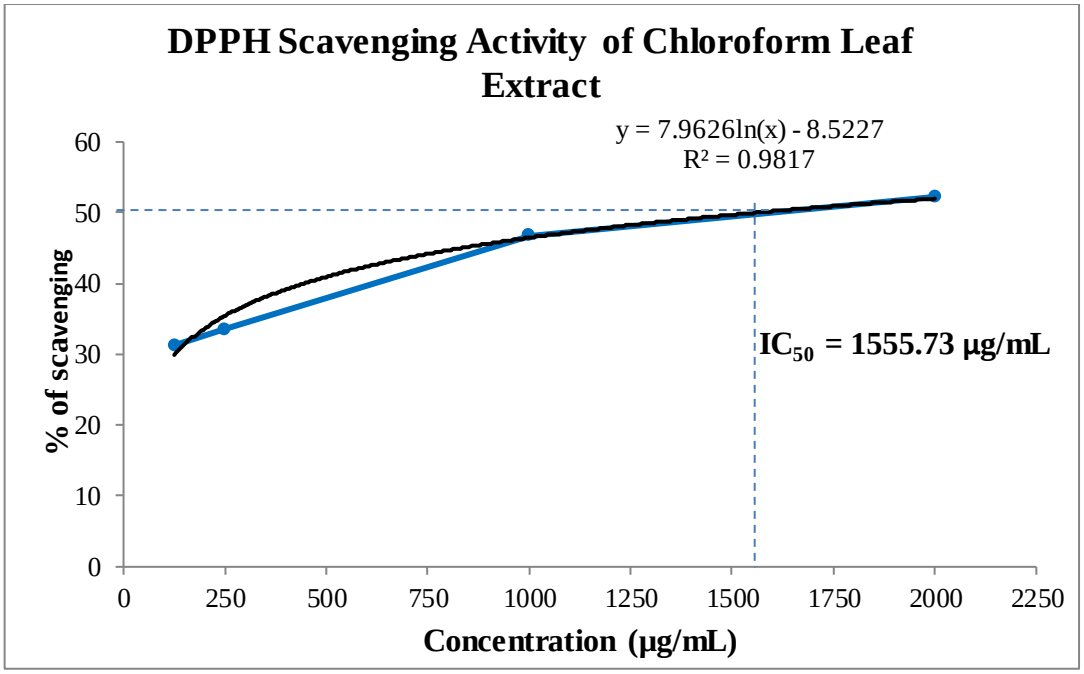


Figure 69: Antioxidant activity of chloroform leaf extract

At 520 nm, absorbance values of various methanol leaf extract concentrations were determined. The % inhibition achieved by DPPH radicals against the sample, which is displayed in the table below, was computed using these values:

Table 18: Antioxidant activity of methanol leaf extract

S.N.	Concentration (µg/ml)	% of scavenging
1	1000	77.692
2	500	65.512
3	250	48.077
4	125	38.205
IC_{50}= 242.74 µg/mL		

The IC_{50} value of methanol leaf extract was found to be 242.74 µg/mL. This is shown in the figure below.

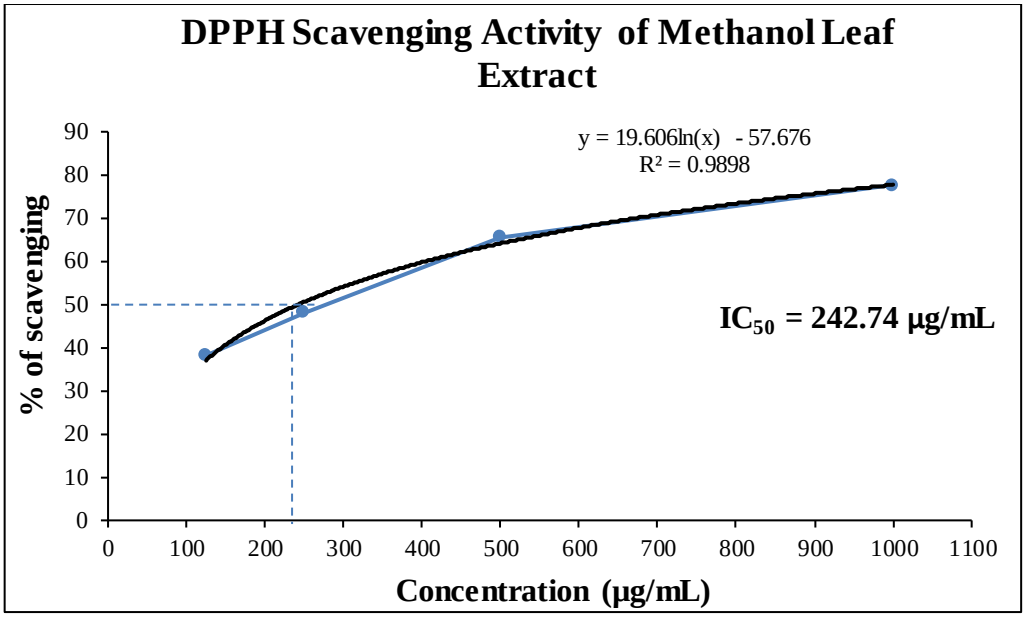


Figure 70: Antioxidant activity of methanol leaf extract

Absorbance values of various chloroform root extract concentrations were determined at 520 nm. The table below displays the % inhibition achieved by DPPH radicals against the sample, which was determined using these values.

Table 19: Antioxidant activity of chloroform root extract

S.N.	Concentration (µg/mL)	% of scavenging
1	2000	65.897
2	1000	50.987
3	500	41.730
4	125	24.167
IC_{50}= 797.75 µg/mL		

The IC_{50} value of methanol extract was 797.75 µg/mL. This is shown in the figure below.

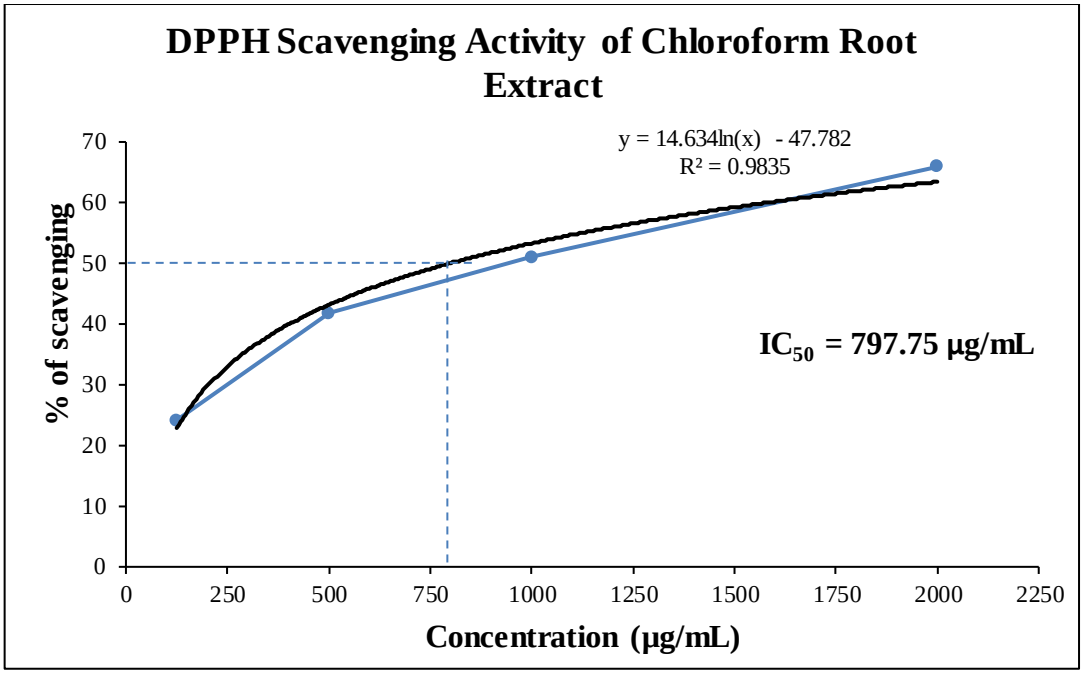


Figure 71: Antioxidant activity of chloroform root extract

At 520 nm, absorbance values of various methanol root extract concentrations were determined. The % inhibition achieved by DPPH radicals against the sample, which is displayed in the table below, was computed using these values:

Table 20: Antioxidant activity of methanol root extract

S.N.	Concentration (µg/ml)	% of scavenging
1	1000	70.961
2	500	55.192
3	250	37.692
4	125	36.8590
IC_{50}= 349.91 µg/mL		

The IC_{50} value of methanol root extract was found to be 349.91 µg/mL. This is shown in the figure below.

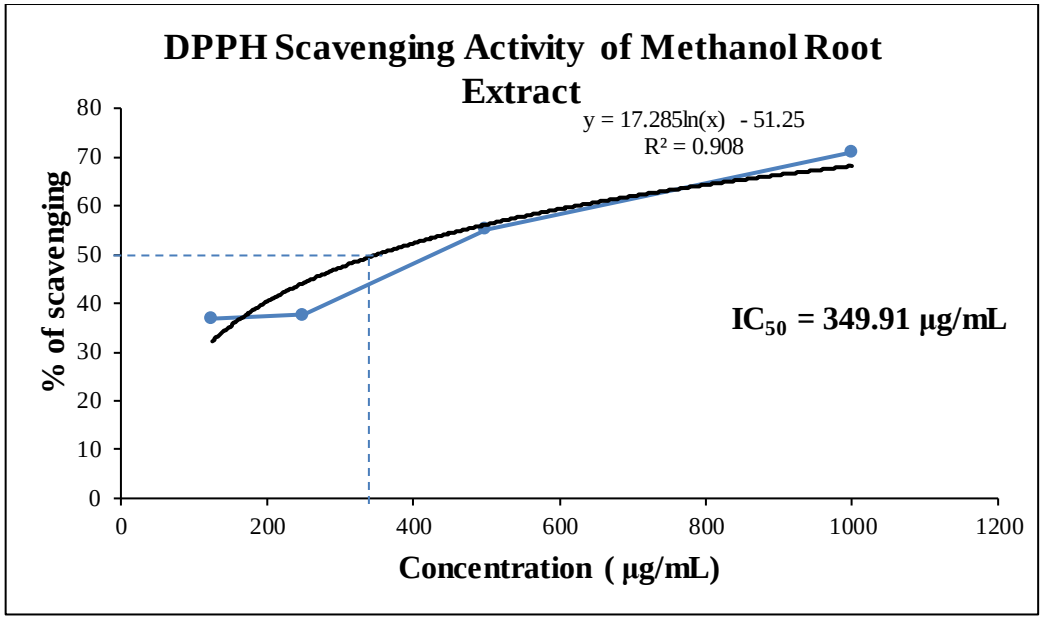


Figure 72: Antioxidant activity of methanol root extract

The IC_{50} values of different extracts with standard ascorbic acid are listed below:

	Ascorbic acid	Chloroform leaf extracts	Methanol leaf extracts	Chloroform root extracts	Methanol root extracts
IC_{50} (µg/mL)	14.38	155.73	242.74	797.75	349.91

The plant extract's and standard ascorbic acid's IC_{50} value are shown in the figure below.

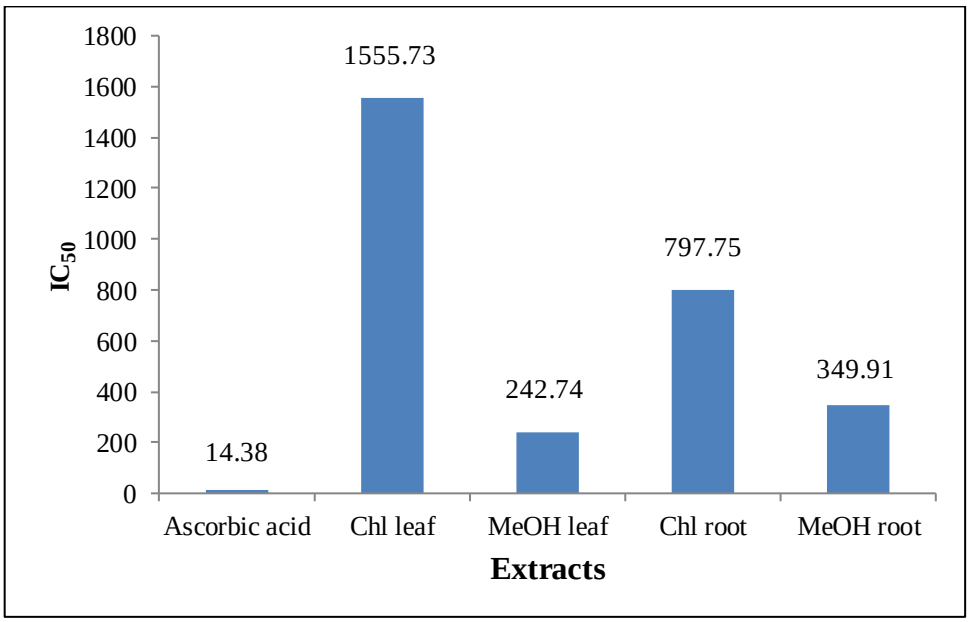


Figure 73: IC_{50} values of different extracts along with ascorbic acid

The IC_{50}, also known as the extract concentrations, required to inhibit DPPH free radicals by 50%, was calculated from the inhibition of DPPH radicals generated by the various concentrations of the extract. Antioxidant activity is negatively correlated with IC_{50} values, meaning that extracts with low IC_{50} values have higher antioxidant potential than those with high IC_{50} values. The above data has shown that methanol leaf (242.74 µg/mL) extract and methanol root extract (349.91 µg/mL) have a lower IC_{50} value than others which indicates potential antioxidant activity than other extracts. Chloroform leaf has shown the least antioxidant property with a high IC_{50} value of 1555.73 µg/mL.

4.5.5 Antimicrobial Activity

The width of a zone of inhibition, or ZOI, that plant extracts produced on certain bacteria was measured to assess the antimicrobial capacity of the extracts. The ability of various *S. sonchifolius* leaf and root extract fractions to prevent microbial growth at a fixed concentration of 10 µL for leaf extract and 30 µL for root extract was evaluated by the process outlined in the portion, using 5 µL kanamycin (5 µg/mL) as a positive control. The outcomes were reported as the diameter of the inhibitory zone, including the well's diameter (8 mm).

The antimicrobial activity of the different extracts of leaf and root of *S. sonchifolius* was performed towards one gram-positive bacteria, i.e., *B. subtilis* (ATCC 6051), one gram-negative bacteria, i.e., *E. coli* (ATCC 8739) and one fungus, i.e., *C. albicans* (ATCC 2091). Centimeters were used to assess the zone of inhibition or ZOI. As a negative control, the solvent DMSO was used.

The positive control was taken as Kanamycin, and its zone of inhibition was found to be 0.95 cm for leaf extracts and 1.1 cm for root extracts. They all exhibited antimicrobial behavior toward the microbes. However, the sort of bacteria can affect the antimicrobial activity differently.

Table 21: Antimicrobial activity shown by the different leaf extracts in diameter (cm) of the inhibition zone (ZOI)

Micro-organisms	Zone of Inhibition (ZOI) in cm						Positive control
	Negative control DMSO	Hexane extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract	Kanamycin ZOI (cm)
B. subtilis (ATCC 6051)	0	0.55	0.5	0.5	0.45	0.45	0.95
E. coli (ATCC 8739)	0	0.55	0.55	0.5	0.35	0.35	0.95
C. albicans (ATCC 2091)	0	0.9	0.9	0.55	0.3	0.55	0.95

The antimicrobial property of leaf extract in 1.5 mg/mL was observed. Hexane extract and chloroform extract have shown high antimicrobial action on *E. coli* and *C. albicans* with a ZOI value of 0.9 cm.

Table 22: Antimicrobial activity shown by the different root extracts in diameter (cm) of the inhibition zone (ZOI)

Micro-organisms	Zone of Inhibition (ZOI) in cm						Positive control
	Negative control DMSO	Hexane extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract	Kanamycin ZOI (cm)
B. subtilis (ATCC 6051)	0	0.2	0.4	1.1	0.5	0.5	1.1
E. coli (ATCC 8739)	0	0.2	0.4	1.0	0.8	0.5	1.1
C. albicans (ATCC 2091)	0	0.2	0.3	1.0	0.6	0.2	1.1

Antimicrobial activity of root extract in 100 mg/mL concentration was observed. The results have shown that ethyl acetate extract has more antimicrobial potential than other extracts against all three microorganisms taken with a ZOI value of 1.1 cm which was equivalent to the standard taken i.e. kanamycin. Hexane extract has shown

the lowest antimicrobial potential against all microorganisms with a ZOI value of 0.2 cm.

4.5.6 α-Amylase Inhibition Assay

The 3,5-dinitrosalicylic acid (DNSA) technique was used to carry out the α-amylase inhibition experiment. Various extract solutions and standards were incubated at room temperature, and their absorbance was measured at 540 nm. The concentration and activity of α-amylase inhibition reduce absorption. An inverse relationship between the IC_{50} value and the antidiabetic potential may be found through a linear regression analysis of the percentage of inhibition vs the α-amylase inhibition activity. This was determined through linear regression. Acarbose is used as the standard. A low IC_{50} shows that the chemical has antidiabetic potential. Controls used include DPPH and methanol. To construct a blank having 100% enzyme activity, 200 µl of buffer was used in the place of plant extract. At 540 nm, absorbance values of various acarbose concentrations were determined. The % inhibition of α-amylase versus the sample displayed below was computed using these data.

Table 23: α-amylase inhibition of acarbose

Concentration (µg/mL)	% of Inhibition
62.5	52.48
31.25	45.7
15.625	35.02
7.8125	29.08
IC_{50} = 52.019 µg/mL	

The IC_{50} value of acarbose standard was found to be 52.019 µg/mL which is shown by the figure below:

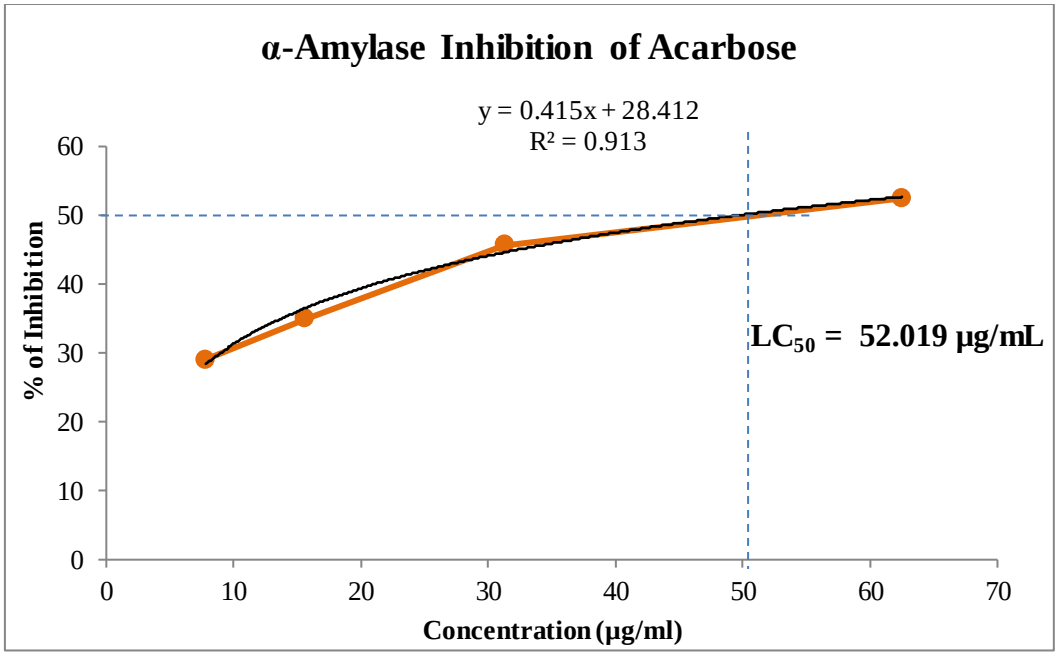


Figure 74: α-amylase inhibition of standard acarbose

At 540 nm, absorbance values of various chloroform leaf extract concentrations were determined. The % inhibition of α-amylase versus the sample was computed using these parameters, and the result is displayed in the table below.

Table 24: α-amylase inhibition of chloroform leaf extract

Concentration (µg/mL)	% of Inhibition
2000	55.918
1000	30.642
500	19.582
250	5.760
IC_{50} = 1819.11 µg/mL	

The IC_{50} value of chloroform leaf extract of *S. sonchifolius* was 1819.11 µg/mL which is shown by the figure below:

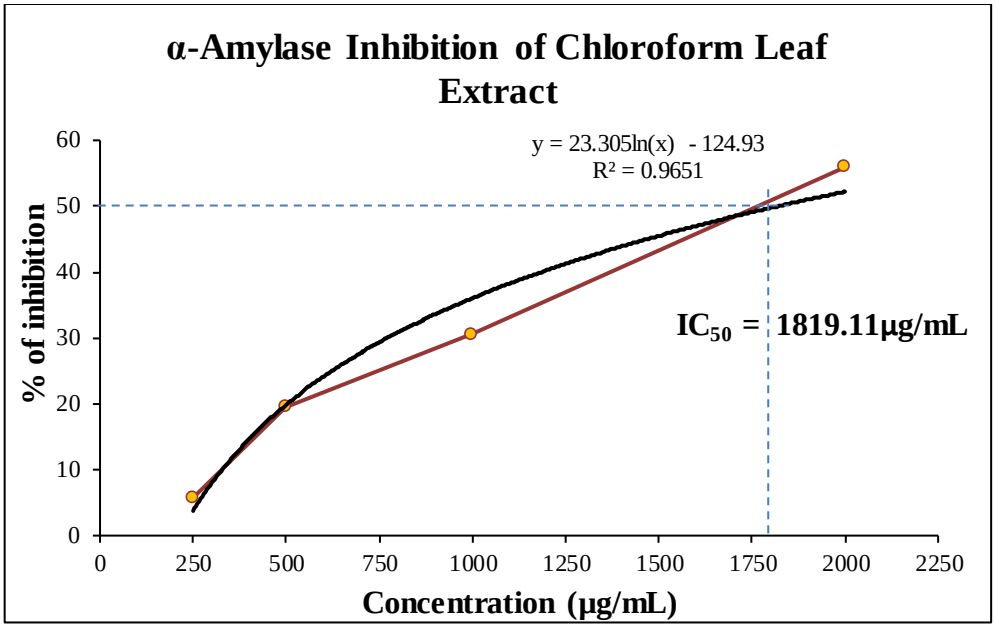


Figure 75: α-amylase inhibition of chloroform leaf extract

At 540 nm, absorbance values of various methanol leaf extract concentrations were determined. The % inhibition of α-amylase versus the sample was computed using these parameters, and the result is displayed in the table below.

Table 25: α-amylase inhibition of methanol leaf extract

Concentration (µg/mL)	% of Inhibition
1000	58.959
500	36.383
250	37.260
125	25.589
IC_{50}= 733.83 µg/mL	

The IC_{50} value of the methanol leaf extract of *S. sonchifolius* was 733.83 µg/mL which is shown by the figure below:

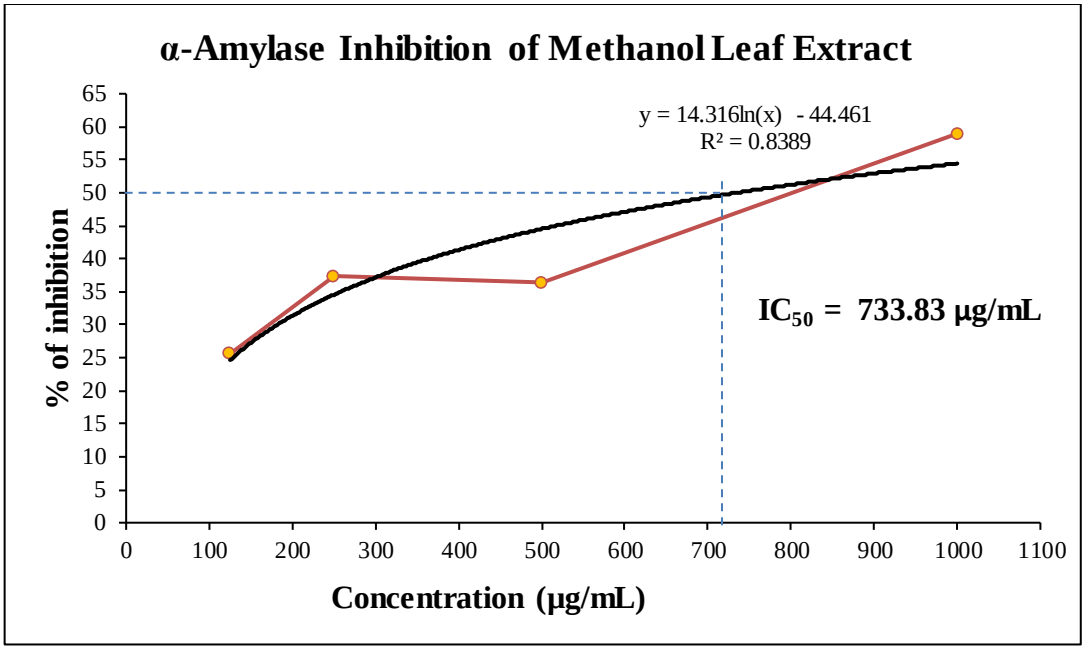


Figure 76: α-amylase inhibition of methanol leaf extract

At 540 nm, absorbance values of various chloroform root extract concentrations were determined. The % inhibition of α-amylase versus the sample displayed in the table below was computed using these data.

Table 26: α-amylase inhibition of chloroform root extract

Concentration (µg/mL)	% of Inhibition
2000	54.454
1000	48.055
500	42.409
250	40.318
IC_{50}= 1253.38 µg/mL	

The chloroform extract of the root of *S. sonchifolius* has an IC_{50} value of 1253.38 µg/mL. The diagram below demonstrates this:

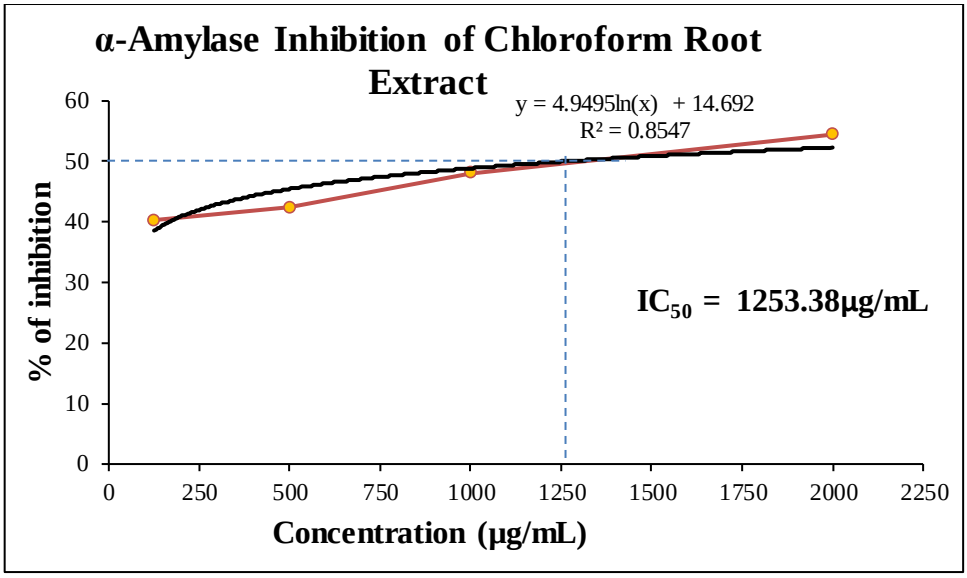


Figure 77: α-amylase inhibition of chloroform root extract

At 540 nm, absorbance values of various methanol root extract concentrations were determined. The % inhibition of α-amylase versus the sample displayed in the table below was computed using these values:

Table 27: α-amylase inhibition of methanol root extract

Concentration (µg/mL)	% of Inhibition
2000	44.907
1000	32.342
500	22.007
250	17.55
IC_{50}= 3364.72 µg/mL	

The methanol extract of the root of *S. sonchifolius* has an IC_{50} value of 3364.72 µg/mL. The following figure illustrates this:

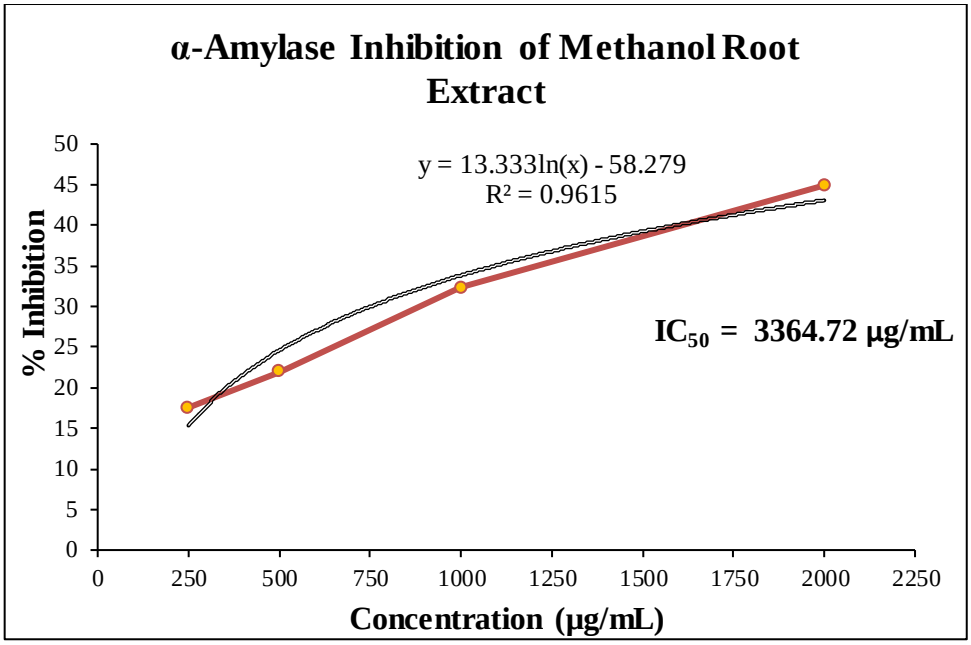


Figure 78: α-amylase inhibition of methanol root extract

The IC_{50} values of different extracts with standard acarbose are listed below:

	Acarbose	Chloroform leaf extracts	Methanol leaf extracts	Chloroform root extracts	Methanol root extracts
IC_{50} (µg/mL)	52.019	1819.11	733.83	1253.38	3364.72

The following figure displays the plant extracts' corresponding IC_{50} value:

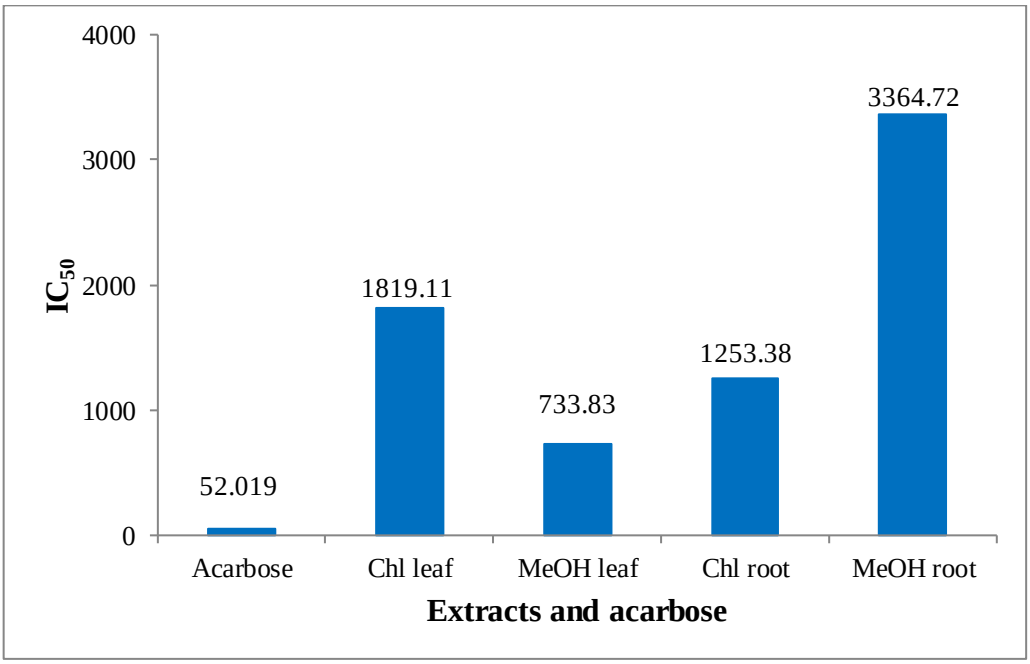


Figure 79: IC_{50} values of different extracts along with acarbose

The IC_{50}, also known as the concentration of the plant extract required to inhibit α-amylase by 50%, was calculated from the α-amylase inhibition generated by the various extract concentrations. Antidiabetic activity is negatively correlated with IC_{50} values, meaning that extracts with low IC_{50} values have higher antidiabetic potential than those with high IC_{50} values.

From the above data, methanol leaf extract was found to have moderate antidiabetic property with low IC_{50} value of 733.85 µg/mL for α-amylase inhibition whereas methanol root extract has shown least α-amylase inhibition potential with IC_{50} value of 3364.72 µg/mL.

4.5.7 Brine Shrimp Bioassay

After the freshly hatched live nauplii were exposed to doses of 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, and 62.5 µg/mL, respectively, the LC_{50} value of the methanol, acetone, and chloroform extracts was determined. It appeared that extracts having LC_{50} values under 1000 µg/mL were pharmacologically active. It was shown that the degree of lethality was closely connected with the level of concentration of that extract. The larvae of brine shrimp died in the greatest numbers at concentrations of 1 mg/mL and least mortalities at 0.0625 mg/mL, respectively. The table below provides a summary of the LC_{50} value computation for the chloroform leaf extract:

Table 28: Calculation of mortality % of chloroform leaf extract

Concentration (µg/mL)	No. of dead nauplii			% Mortality
1000	10	10	10	100
500	9	10	10	96.667
250	8	7	8	76.667
125	5	5	6	53.333
62.5	4	4	4	40
LC_{50} = 92.76 µg/mL				

The chloroform extract of the leaves of *S. sonchifolius* has an LC_{50} value of 92.76 µg/mL. The following figure illustrates this:

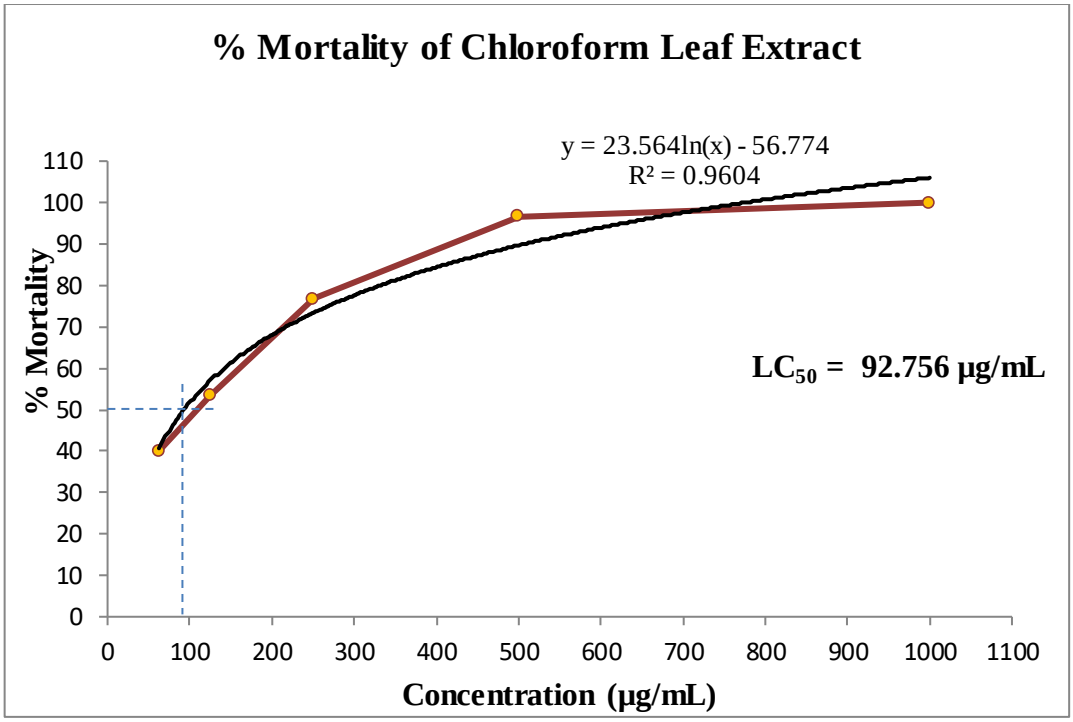


Figure 80: Cytotoxicity activity of chloroform leaf extract

The LC_{50} result of the methanol extract of leaves calculation is shown in the data table below:

Table 29: Calculation of mortality % of methanol leaf extract

Concentration (µg/mL)	No. of dead nauplii			% Mortality
1000	9	10	10	96.667
500	8	9	10	90.0
250	6	7	7	66.667
125	3	2	2	23.333
62.5	0	0	0	0
LC_{50} = 216.81 µg/mL				

The methanol extract of the leaves of *S. sonchifolius* has an LC_{50} value of 216.81 µg/mL. The following figure illustrates this:

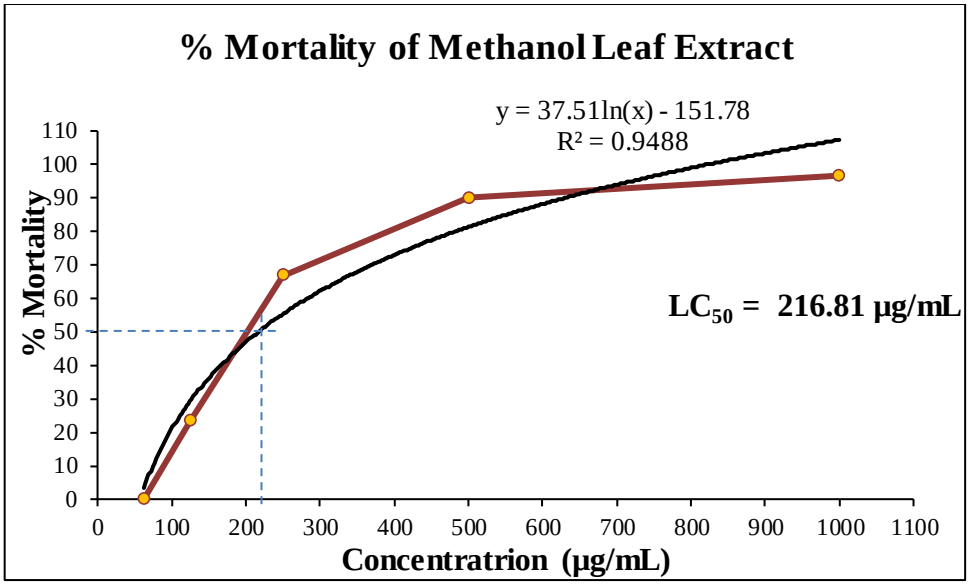


Figure 81: Cytotoxicity activity of methanol leaf extract

The table below provides a summary of the LC_{50} value computation for the chloroform root extract:

Table 30: Calculation of mortality % of chloroform root extract

Concentration (µg/mL)	No. of dead nauplii			% Mortality
1000	10	10	10	100
500	10	10	10	100
250	9	8	7	80
125	4	4	4	40
62.5	2	2	2	20
LC_{50} = 141.81 µg/mL				

The chloroform extract of the root of *S. sonchifolius* has an LC_{50} value of 141.81 µg/mL. The following figure illustrates this:

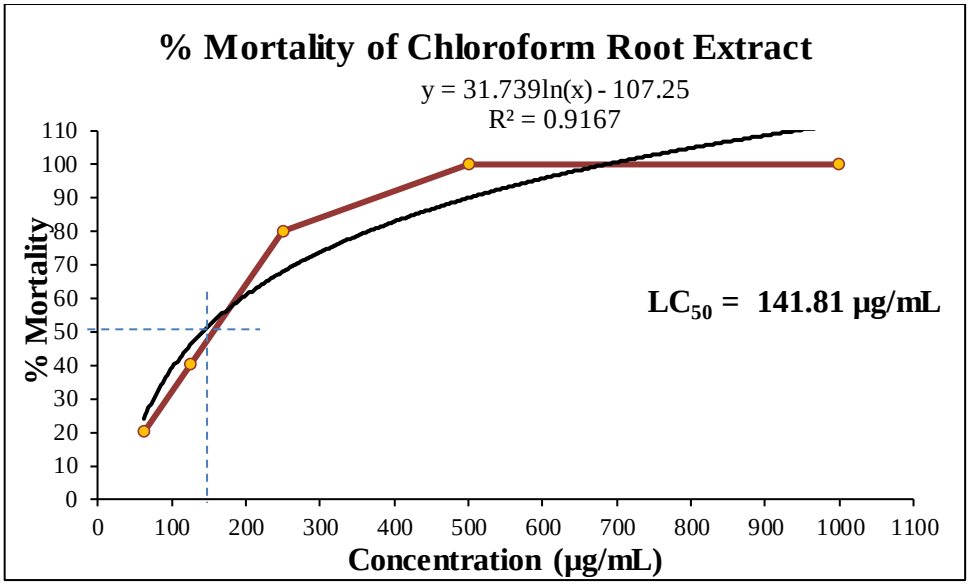


Figure 82: Cytotoxicity activity of chloroform root extract

The table below provides a summary of the LC_{50} value computation for the methanol root extract:

Table 31: Calculation of mortality % of methanol root extract

Concentration (µg/mL)	No. of dead nauplii			% Mortality
1000	9	10	10	96.667
500	6	6	7	63.333
250	1	5	5	36.667
125	3	3	4	33.333
62.5	0	2	1	10
LC_{50} = 267.62 µg/mL				

The methanol extract of the root of *S. sonchifolius* has an LC_{50} value of 267.62 µg/mL. The following figure illustrates this:

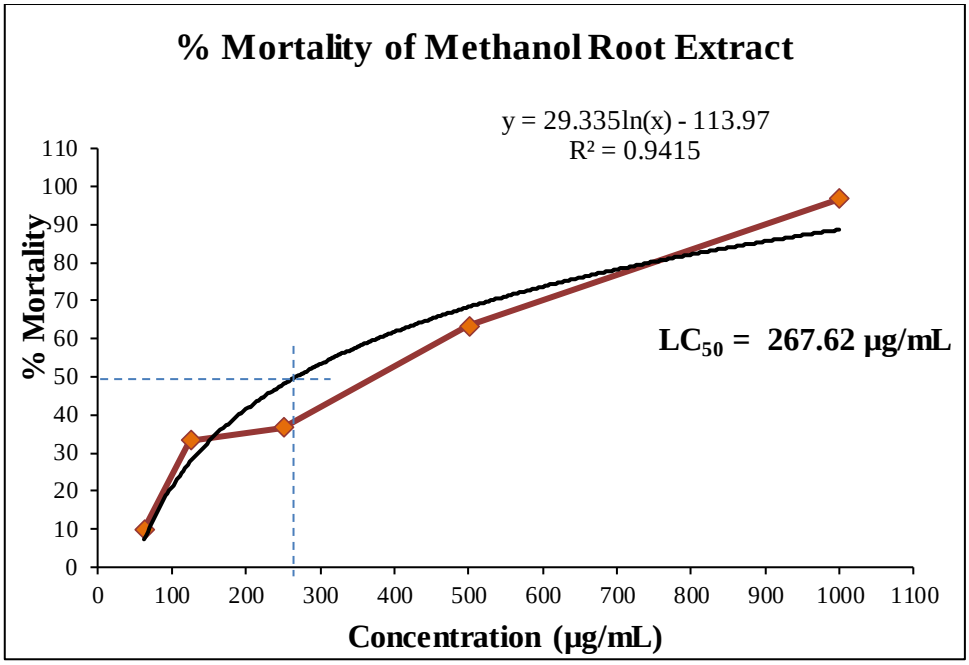


Figure 83: Cytotoxicity activity of methanol root extract

The following figure displays the plant extracts' corresponding LC_{50} value:

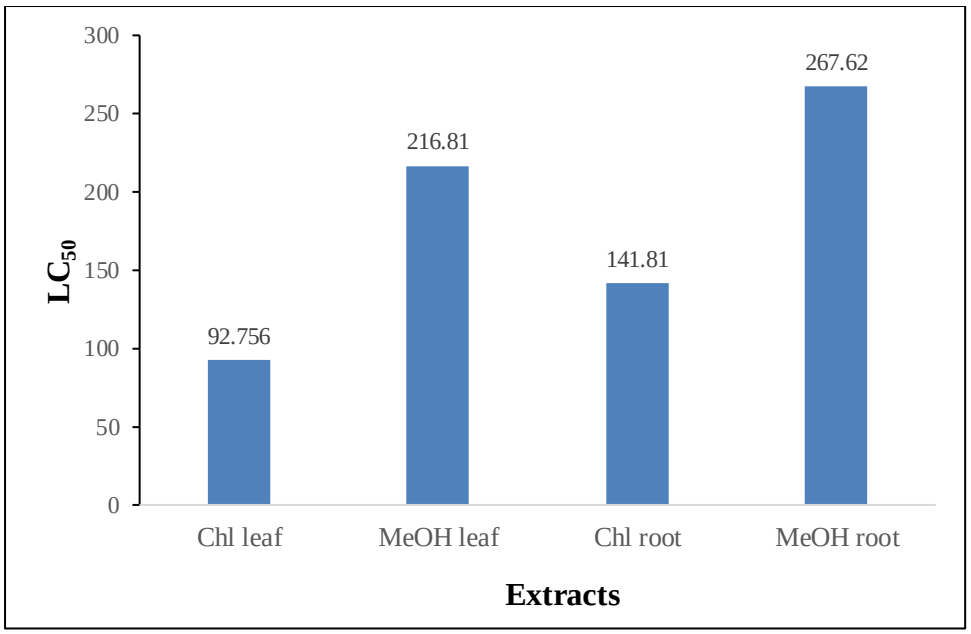


Figure 84: LC_{50} values of different extracts for cytotoxicity

The brine shrimp lethality experiment is an easy way to monitor the biological activity of different plant species. This technique, while not fully addressing the system of toxic action, is especially helpful in determining the potentially hazardous nature of different plant extracts. Once the active chemicals are identified, this approach yields preliminary screening results that may be verified by more focused bioassays.

Consequently, chloroform leaf extract was discovered to be highly toxic to brine shrimp larvae with LC_{50} value below 100 µg/mL whereas other three extracts were found to be moderate toxic with LC_{50} value greater than 100 µg/mL and below 500 µg/mL.

4.5.8 Thin layer Chromatography

The polar ratio of ethyl acetate: hexane was gradually raised throughout the TLC quantitative evaluation of the S. sonchifolius methanol extract. Before the plates were seen under a UV fluorescent light, they were subjected to several solvent treatments. The visualized plates are shown in the picture:

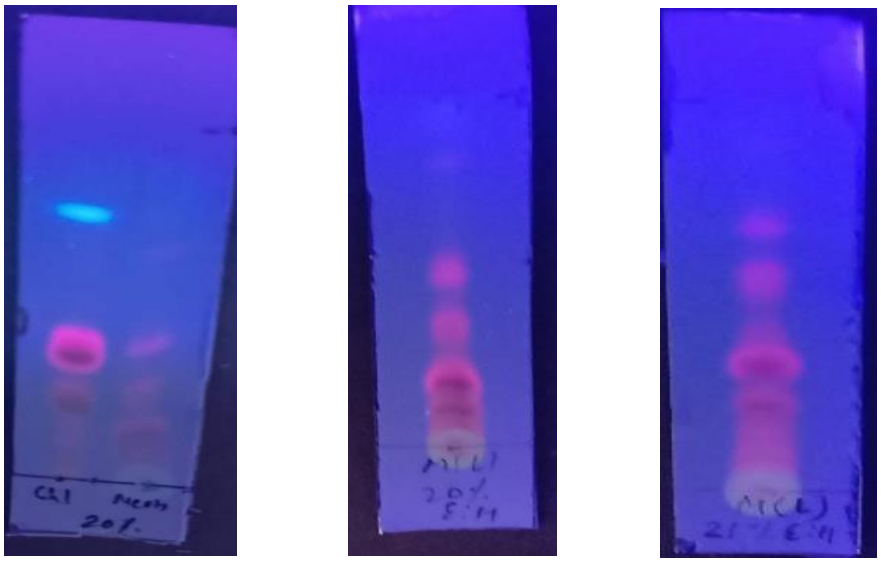


Figure 85: TLC plate at 20% and 21% ethyl acetate: hexane of leaf extract of *S. sonchifolius*

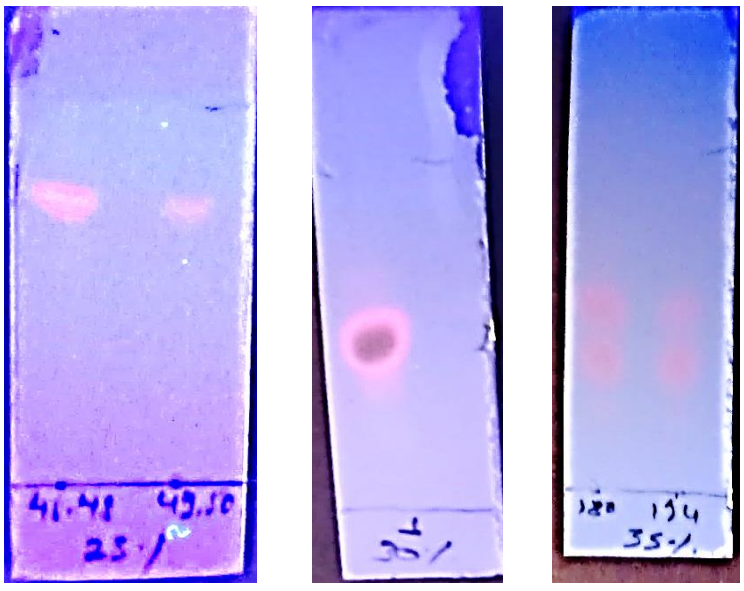
4.5.9 Column Chromatography

Column chromatography was used to analyze the methanol leaf extract of *S. sonchifolius*. For column chromatography, hexane and ethyl acetate generated the perfect solvent combination. Different ratios of the solvent solution were generated and allowed to flow through the column. After the fractions were collected in a conical flask and concentrated using rota-vapors, a thin-layer chromatographic test was conducted. Solvent systems with different ratios are shown in the table below:

Table 32: Column chromatography of methanol leaf extracts of *S. sonchifolius*

S. N	Solvent System	Ratio	Fraction
1	100% Hexane	100	1-4
2	1% Ethyl Acetate: Hexane	1:99	5-16
3	3% Ethyl Acetate: Hexane	3:97	17-20
4	5% Ethyl Acetate: Hexane	5:95	21-37
5	7.5% Ethyl Acetate: Hexane	7.5:92.5	38-54
6	10% Ethyl Acetate: Hexane	10:90	55-65
7	12.5% Ethyl Acetate: Hexane	12.5:87.5	66-96
8	15% Ethyl Acetate: Hexane	15:85	97-143
9	17.5% Ethyl Acetate: Hexane	17.5:82.5	144-179
10	20% Ethyl Acetate: Hexane	20:80	180-198
11	25% Ethyl Acetate: Hexane	20:75	199-202

The TLC of the different fractions were taken and some are given below:



a) 5th fraction b) 9th fraction c) 10th fraction

Figure 86: TLC of different fractions

The 9th fraction i.e. 17.5% EtOAc: hex fraction and 10th fraction i.e. 20% EtOAc: hex fraction were collected. The TLC of 9th fraction showed dark single pink spot in 30%

TLC at 30% TLC solvent (EtOAc: hex). The 10th fraction named as 10th fraction (1) was sent for the UV and FT-IR analysis. The 9th fraction (200 mg) was not pure hence it was run for second column chromatography as done previously.

The solvent system with different ratios is shown below in the table:

Table 33: Column chromatography of 17.5% EtOAc: hex fraction of methanol leaf extract

S. N	Solvent System	Ratio	Fraction
1	100% Hexane	100	1-2
2	1% Ethyl Acetate: Hexane	1:99	3-5
3	2.5% Ethyl Acetate: Hexane	2.5:97.5	6-8
4	5% Ethyl Acetate: Hexane	5:95	9-35
5	7.5% Ethyl Acetate: Hexane	7.5:92.5	36-44
6	10% Ethyl Acetate: Hexane	10:90	45-60
7	12.5% Ethyl Acetate: Hexane	12.5:87.5	62-64
9	17.5% Ethyl Acetate: Hexane	17.5:82.5	65-80

From the second column chromatography, different fractions were performed for TLC. The 9th fraction i.e. 17.5% EtOAc: hex was collected, named as 9th fraction (2) and sent for UV and FT-IR analysis.

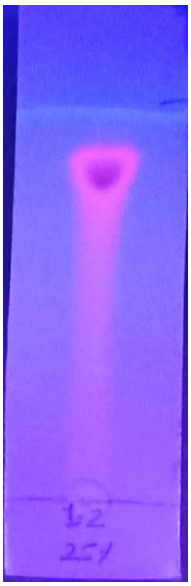


Figure 87: TLC of 9th fraction at 25% EtOAc: hex

4.5.10 UV-Vis Spectroscopic measurements

With the use of the UV measurement, one can ascertain whether an organic compound is unsaturated or not.

The 9th fraction (2) and 10th fraction (1) were subjected to UV-Vis Spectroscopy. The obtained data are given below:

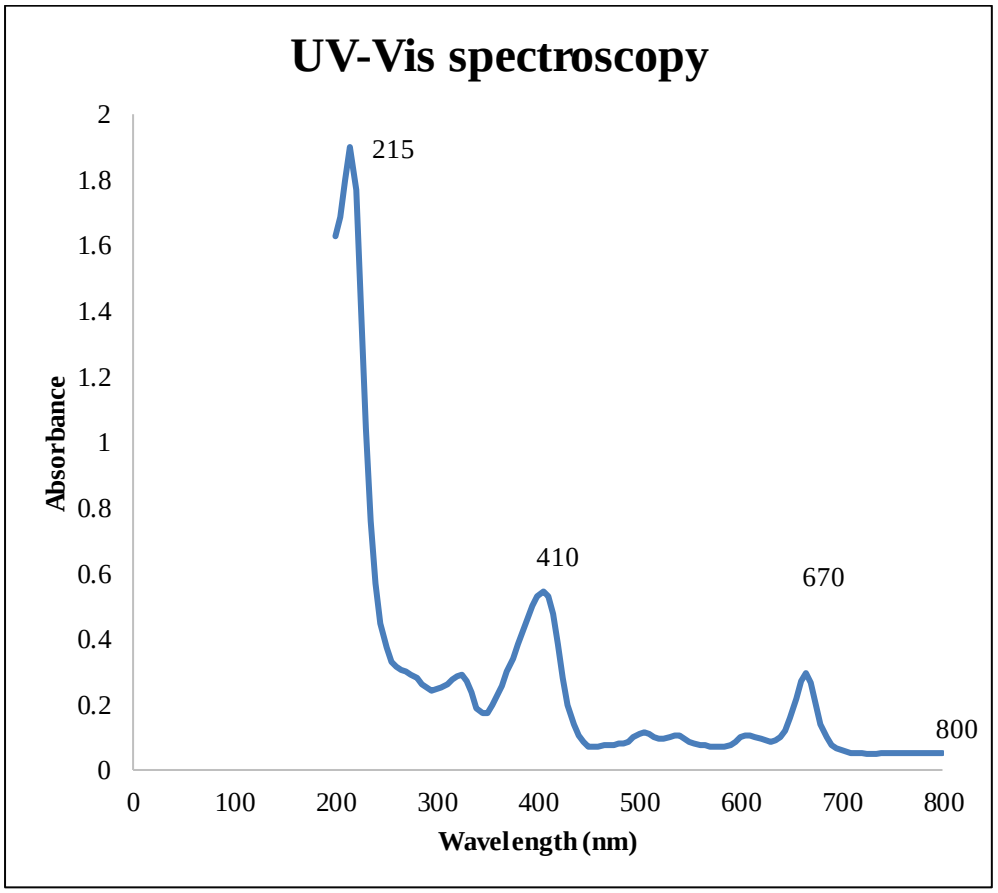


Figure 88: UV spectrum of 9th fraction (2)

The peak at 215 nm, 410 nm, and 670 nm in Figure 88 shows the presence of unsaturation, conjugation, and aromaticity in the 9th fraction (2)

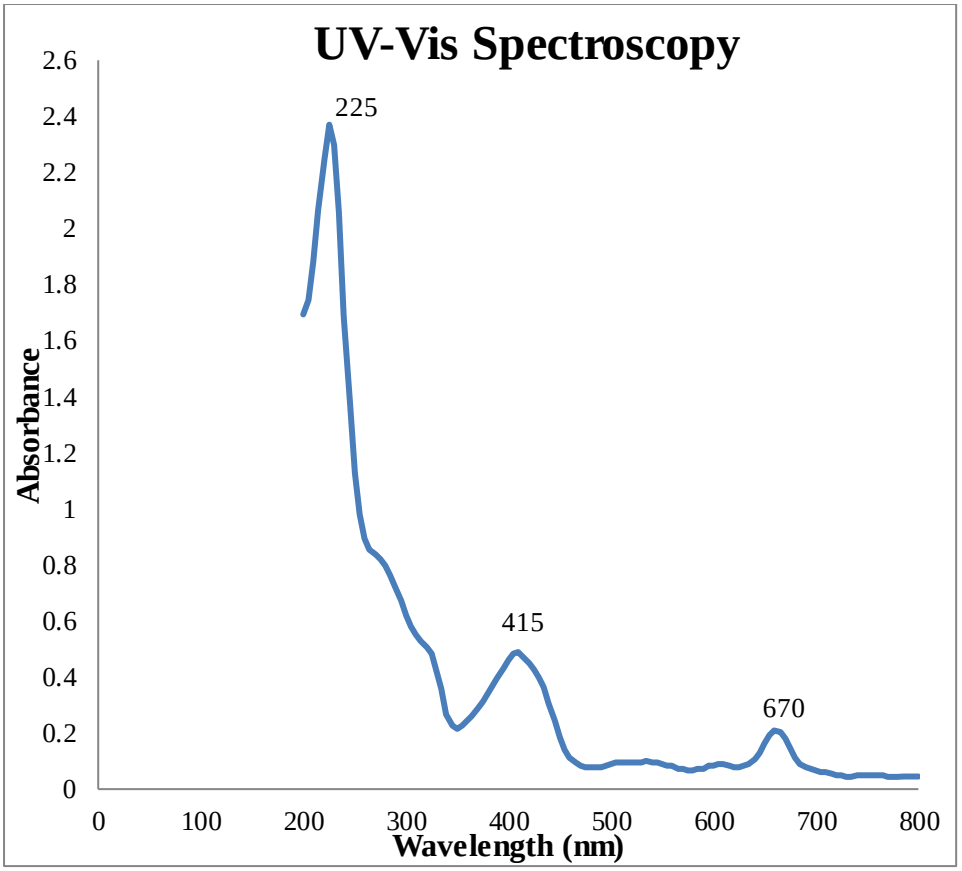


Figure 89: UV spectrum of 10th fraction (1)

The peak at 225 nm, 415 nm, and 670 nm in Figure 89 shows the presence of presence of unsaturation, conjugation, and aromaticity in the 10th fraction (1).

4.5.11 FT-IR Analysis

Organic compounds may be examined using FTIR spectroscopy to determine their kind of bonding, aromatic or aliphatic structures, and most importantly their functional group. In addition to confirming the existence of several organic molecules and functional groups, the FTIR spectra also show the chemical structure of the sample. The stretching frequency in the spectrum of FTIR may be used to identify the binding nature between heteroatoms and carbon (Nandiyanto *et al*., 2019).

The FT-IR spectroscopy of 9th fraction (2) and 10th fraction (1) were taken and the data observed are listed below:

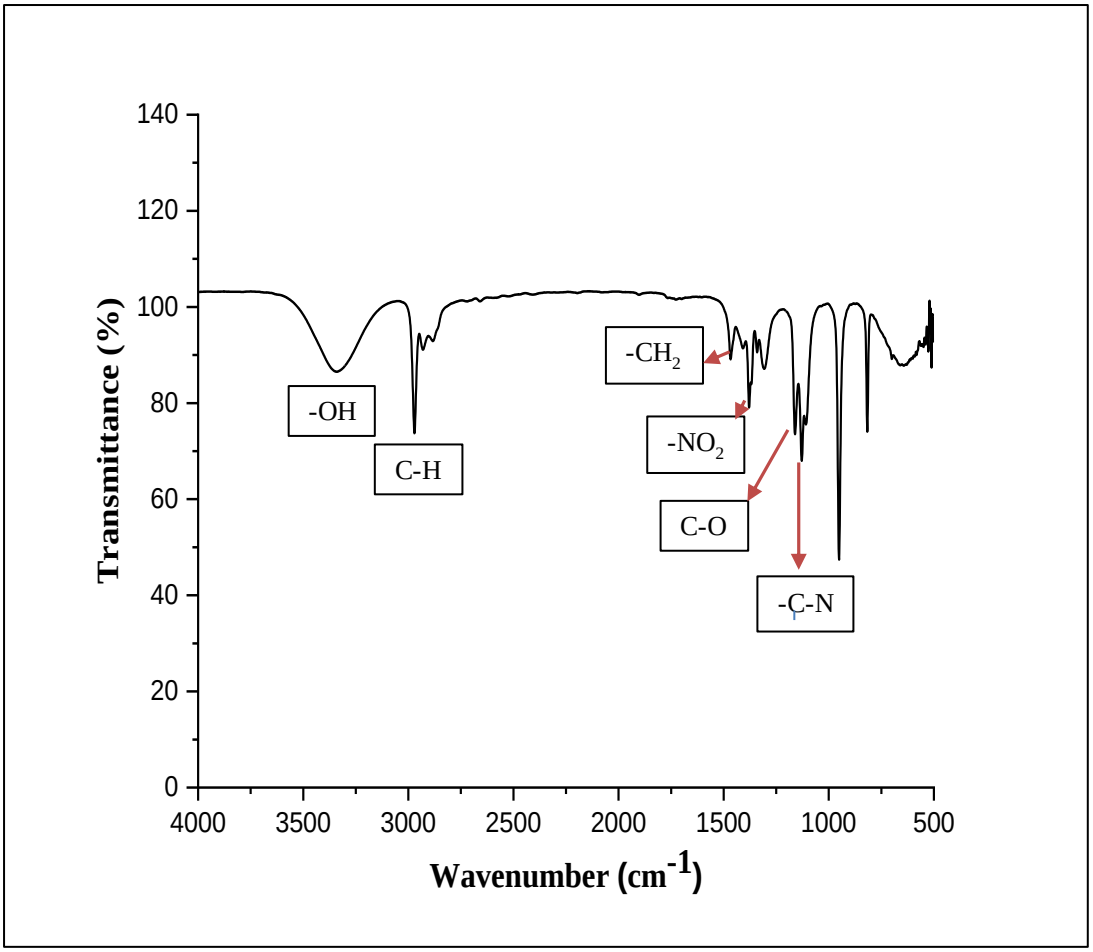


Figure 90: FT-IR spectrum of 9th fraction (2)

The absorption, forms of stretching, and general appearance of 9th fraction (2) are displayed in the table below:

Table 34: FT-IR peak value and functional groups of 9th fraction (2)

Absorption (cm^{-1})	Types of stretching	Appearance
3340.64	O-H stretching	Broad and Medium
2970.69 2930.26 2883.44	C-H stretching	Medium to Strong
1467.03	$-CH_2$ stretching	Medium
1378.96	$-NO_2$ stretching	Strong
1306.93	-C-N stretching (Aromatic primary)	Medium
1160.54	-C-N stretch (Secondary)	Medium
1128.65	C-O stretching (Cyclic ether)	Medium

Utilizing an FT-IR analysis infrared chart, the outcomes were analyzed where peak at 3340.64 cm^{-1} for –OH stretching, 2970.69 cm^{-1}, 2930.26 cm^{-1}, and 2883.44 cm^{-1} for

C-H stretching, 1467.03 cm^{-1} for $–CH_2$ stretching, 1378.96 cm^{-1} for $–NO_2$ stretching, 1306.93 cm^{-1} for –C-N stretching (aromatic primary amine), 1160.54 cm^{-1} for –C-N stretching (secondary amine) and 1128.65 cm^{-1} for C-O stretching. This confirmed that the 9th fraction (2) contains alcoholic group, amines, ether and nitro groups.

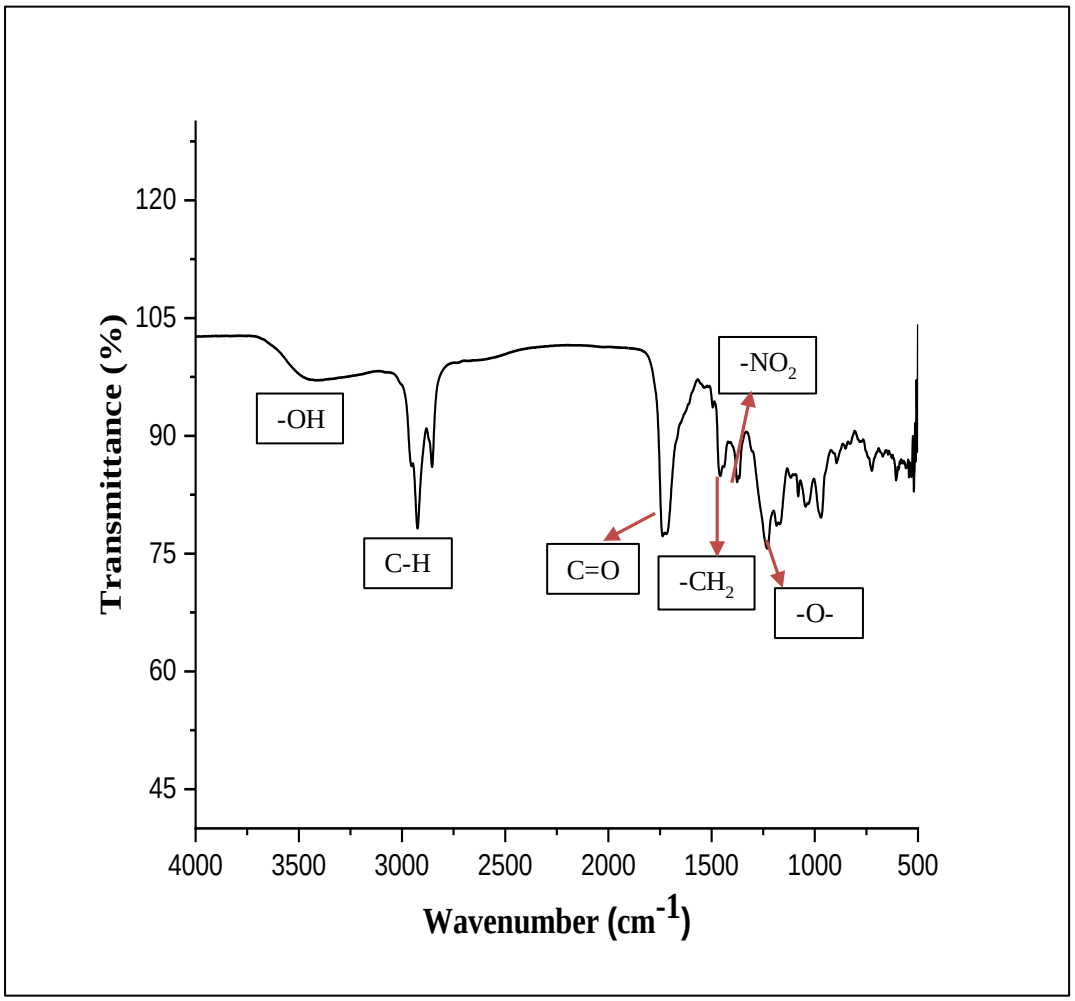


Figure 91: FT-IR spectrum of 10th fraction (1)

The absorption, forms of stretching, and general appearance of 10th fraction (1) are displayed in the table below:

Table 35: FT-IR peak value and functional groups of 10th fraction (1)

Absorption (cm^{-1})	Types of stretching	Appearance
3405.28	O-H	Broad and weak
2924.77 2854.38	-C-H	Medium Weak
1737.31	-CO- (carbonyl)	Medium
1457.16	$-CH_2$	Weak
1376.42	$-NO_2$	Weak
1230.55	-O- stretching (Aryl ether)	Weak
1184.60 1080.40 1044.88	-C-N (Secondary amine)	Weak

Utilizing an FT-IR analysis infrared chart, the outcomes were analyzed where peak at 3405.28 cm^{-1} for –OH stretching, 2924.77 cm^{-1}, and 2854.38 cm^{-1} for C-H stretching, 1737.31 cm^{-1} for –CO- stretching (carbonyl), 1457.16 cm^{-1} for $–CH_2$ stretching, 1376.42 cm^{-1} for $–NO_2$, stretching, 1230.55 cm^{-1} for –O- stretching (aryl ether) and 1184.60 cm^{-1}, 1080.40 cm^{-1} and 1044.88 cm^{-1} for -C-N stretching (secondary amine). This confirmed that the 10th fraction (1) contains alcoholic groups, amines, aromatic ether, carbonyl, and nitro groups.

Summarized table for the biological activities data:

Extracts	**Biological Activities**		
	Antioxidant (IC_{50}) in µg/mL	**A-amylase inhibition (IC_{50}) in µg/mL**	**Cytotoxicity (LC_{50}) in µg/mL**
Chloroform leaf	1555.73	1819.11	92.756
Methanol leaf	242.74	733.83	216.81
Chloroform root	797.75	1253.38	141.81
Methanol leaf	349.91	3364.72	267.62

CHAPTER V: CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Phytochemical analysis of all the leaf and root extracts of *S. sonchifolius* plant in hexane, chloroform, ethyl acetate, methanol, and distilled water showed the presence of volatile oils, polyphenols, flavonoids, terpenoids, quinones, saponins, glycosides, reducing sugars, and tannins. Hexane leaf extract's GC-MS analysis revealed 17 primary potential components where 7,8,8-trimethyl-4,5-diazatricyclo [4.2.1.$0^{3,7}$] non-4-ene was abundant (27.02%). GC-MS analysis of methanol leaf extract identified 4 main possible components where 4-bromobutyric acid and 3-methylbut-2-yl ester were abundant (91.70%). Hexane root extract was subjected to GC-MS analysis, which revealed 30 major potential components where 3,7,7-Trimethyl-bicyclo [2.2.1] hept-2-yl)-methanol (23.33%) and Cyclocopacamphenol (15.10%) were most abundant. In the TPC analysis of four extracts (ethyl acetate leaf, methanol leaf, chloroform root, and methanol root), the chloroform root extract was found to have the highest phenolic content (69.97 mg GAE/g) whereas methanol root extract had the lowest (0.82 mg GAE/g). Additionally, in TFC analysis of four extracts (chloroform leaf, methanol leaf, chloroform root, and methanol root), the chloroform leaf extract was found to have the highest flavonoid content (350.87 mg QE/g) whereas the lowest was shown by chloroform root extract (6.36 mg QE/g). In the DPPH scavenging activity of four extracts (chloroform leaf, methanol leaf, chloroform root, and methanol root), methanol extracts of both leaf and root showed good antioxidant activity with IC_{50} value of 242.74 µg/mL and 349.91 µg/mL respectively. The least antioxidant activity was shown by chloroform leaf with an IC_{50} value of 1555.73 µg/mL. The α-amylase inhibition experiment showed that the methanol leaf extract had a better capacity for inhibition with an IC_{50} value of 733.83 µg/mL compared to the other three extracts (chloroform leaf, chloroform root, and methanol root). All the extracts showed good antimicrobial activity against the three tested microorganisms. A higher zone of inhibition (ZOI), i.e., 1.1 cm was observed in *B. subtilis* by ethyl acetate root extract. The cytotoxicity assay showed the lowest toxicity of methanol leaf extract with the highest lethality concentration (LC_{50}) of 267.62 µg/mL as compared to the other three extracts (chloroform leaf, chloroform root, and methanol

root). Methanol extract of leaves in the EtOAc: hexane solvent system demonstrated the best TLC, which was subsequently applied in a column.

The 9th fraction (Compd. 1.2) was obtained from second-column chromatography at 17.5 % EtOAc: hex and the 10th fraction (Compd. 5) obtained from first-column chromatography at 20% EtOAc: hex was sent for UV and FT-IR analysis. The results of the UV spectrophotometer indicated that the fractions included unsaturated organic molecules, numerous bonds, aromaticity, and conjugation in the UV-visible region. FT-IR results confirmed the existence of O-H, C-H, -CO-, -CH_2, -C-N, -NO_2, and –O- groups. All the above results concluded that this plant can have the important role for the future drug discovery.

5.2 Suggestions for Further Works

Phytochemical studies identified several biologically active chemicals in the plants, which may have both pharmacological and biological functions. Therefore, it is desirable to produce extracts from plants in primary solvent using different extraction techniques than those covered here to extract a wider variety of phytochemicals in higher amounts. For the extraction of unique active chemicals from this plant, which could lead to the development of a new treatment for several chronic illnesses, more investigation is needed. My dissertation subjects include UV-Vis, FT-IR analysis, GC-MS, antibacterial, antioxidant, and antidiabetic properties of phytochemical screening. Future study topics that have been identified include the following:

- For the antimicrobial activity assays, just three microbes were employed. Additionally, more research on different microorganisms can be done, which helps in the creation of novel antimicrobials for the treatment of microbial illnesses.
- There are several important compounds that the plant *S. sonchifolius* might possess. As a result, identification, isolation, and structural elucidation can be done.

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APPENDICES

A. Phytochemical Screening Protocol

1. Test for Volatile Oils

0.5 mL of methanol was added to approximately 500 mg of extract, agitated well, and filtered. A capillary tube was used to place a few drops of the filtrate onto a piece of filter paper. Volatile oils are indicated by a yellow mark that remains after the solvent has evaporated.

2. Test for Alkaloids

3 mL of 2% (v/v) HCl was used to dissolve about 500 mg of extract. After the solution was filtered, the experiments listed below were carried out:

i. Mayer's Test

To the first portion, a few drops of Mayer's reagent were applied. The presence of alkaloids is indicated by the production of a pale-yellow precipitate.

ii. Dragendroff's Test

In the second section, a few drops of Dragendroff's reagent were added. The presence of alkaloids is indicated by the appearance of an orange-red precipitate.

3. Test for Terpenoids

Carefully, 2 mL of the chloroform solution ($CHCl_3$) and 3 mL of pure sulfuric acid (H_2SO_4) were introduced to about 200 mg of extract. The development of a reddish-brown color near the contact is indicative of terpenoids.

4. Test of Flavonoids (Shinoda's Test)

2 mL of methanol was used to dissolve around 200 mg of extract. A little bit of magnesium and a couple of drops of strong hydrochloric acid (HCl) were added to this mixture. The development of an orange color signifies the existence of flavonoids.

5. Test for Phenolic Compounds/ $FeCl_3$ Test

A small amount of a 10% by volume of ferric chloride ($FeCl_3$) reagent was added after 1 mL of extract and 1 mL of distilled water were combined. The existence of phenolic compounds is indicated by the emergence of a greenish-blue coloring.

6. Test for Glycosides

After dissolving around 500 mg of extract in 2 mL of methanol and splitting it into two halves, the following experiments were carried out:

i. Molisch's Test

Molisch's reagent (5 mL) was used to treat the first section, and then conc. H_2SO_4 was introduced drop to drop from the test tube's side without disrupting the solution. The existence of glycosides is indicated by the formation of a violet-colored ring at the intersection of two liquids, which, when shaken, transforms the solution violet in color.

ii. A 2 mL solution of 25% (v/v) NH_4OH was added for the second portion, and it was vigorously shaken. The existence of glycosides is indicated by the emergence of a cherry red color.

7. Test for Reducing Sugars

1 mL of distilled water and 1 mL of Fehling's reagent (1:1 ratio of Fehling's solution A with B) were added to about 1 mL of extract. After that, the mixture was heated for 30 minutes over a water bath. When reducing sugars are present, a red brick precipitation will form.

8. Test for Quinones

A small amount of the ammonium thiocyanate (NH_4SCN) crystals and 1 mL of newly made ferrous sulfate ($FeSO_4$) solutions were added to around 2 mL of extract. The mixture was then treated drop by drop with concentrated sulfuric acid (H_2SO_4). Quinones are present when a rich red coloration appears and stays that way.

9. Test for Saponins

After treating 500 mg of extract with hot water, it was shaken for 30 seconds. The presence of saponins is indicated by the development of thick forth.

10. Test for Tannins

After boiling around 200 mg of extract, 10 mL of distilled water was added. After the mixture had cooled, it was filtered, and the filtrate was mixed with a few droplets of $FeCl_3$ solution. There is indication of tannins when a blue-black precipitate appears.

B. Preparation of reagents

1. Mayer's Reagent

Using a 50 mL volumetric flask, 0.679 g of mercury chloride, or $HgCl_2$, was weighed and then dissolved into distilled water. 2.5 g of potassium iodide (KI) was added to this mixture. Shaking was used to dissolve the crimson-red precipitate, and distilled water was added to bring the volume up to the required level.

2. Dragendroff's Reagent

To create solution A, 4.000 g of bismuth nitrate, $Bi(NO_3)_3$, was dissolved in 10 mL of 5 N nitric acid. To create solution B, potassium iodide, or KI (13.5 g), was then dissolved in 20 mL of distilled water. The 50 mL volumetric flask was used to combine these two solutions.

An aqueous solution of picric acid was prepared by dissolving 0.25 g of picric acid in 50 mL of distilled water. Sodium bicarbonate was used to neutralize the solution (NaHCO3).

3. Molisch's Reagent

Molisch's reagent was made by dissolving 5.0 g of α-Naphthol in 50 mL of methanol.

4. Neutral Ferric Chloride ($FeCl_3$) Solution

In 100 mL of pure water, 1.0 g of ferric chloride crystals were dissolved. Crystals of sodium carbonate were gradually added to this solution while being stirred until slight

turbidity persisted. The mixture was then passed through a filter and its colorless filtrate was added to a solution of neutral ferric chloride.

Photos



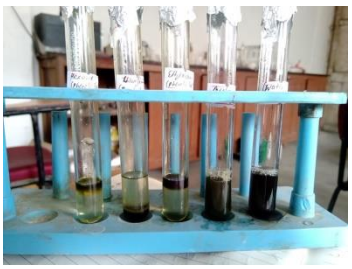

Collection of samples

Ultrasonic extraction


Filtration of extract


Rota evaporator


Collection of extracts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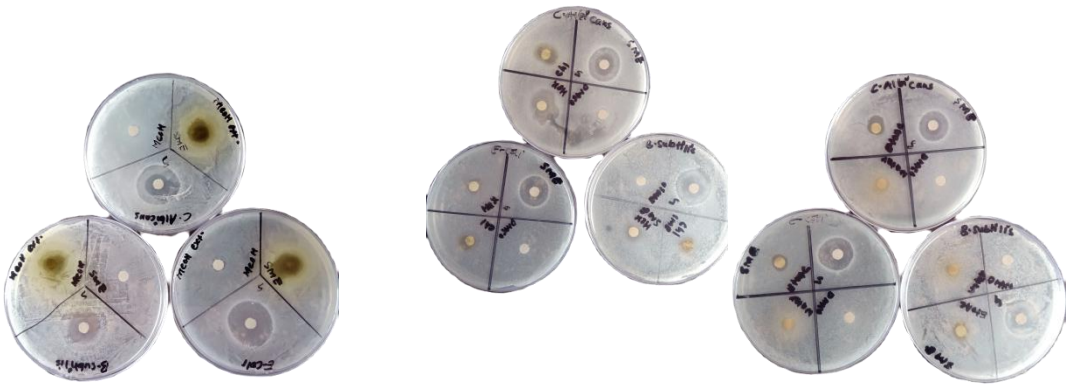
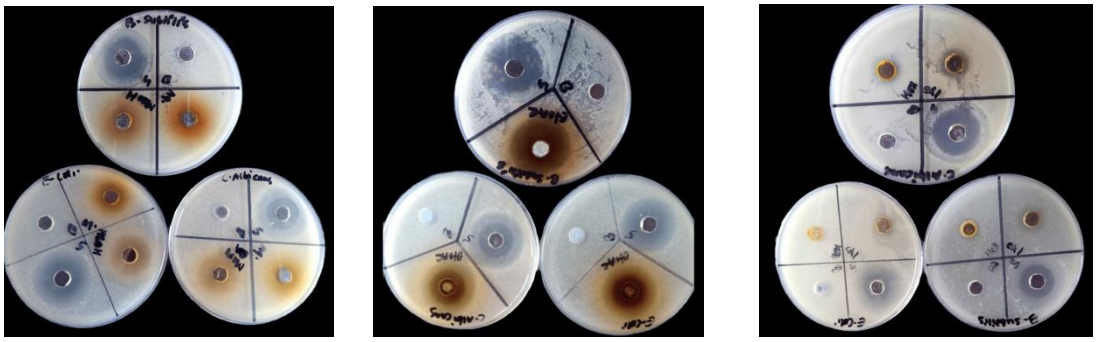


ZOI of leaves extracts of *S. sonchifolius*



ZOI of root extracts of *S. sonchifolius*

Performing TLC

Column chromatography