GC-MS Profiling and Evaluation of Antioxidant and Thrombolytic Activity of Methanolic Leaf Extract of Ricinus Communis L

PROJECT REPORT

[In the partial fulfillment of the requirements for the degree of Bachelor of Pharmacy]

Submitted To

The Department of Pharmacy

Faculty of Health & Life Sciences

Daffodil International University

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April 2024

APPROVAL

This project paper, **"GC-MS Profiling and Evaluation of Antioxidant and Thrombolytic Activity of Methanolic Leaf Extract of *Ricinus Communis L*",** submitted to the Department of Pharmacy, Faculty of Health & Life Sciences Daffodil International University, has been accepted as satisfactory for the partial fulfillment of the requirements for the degree of Bachelor programmed and approved as to its style and contents.

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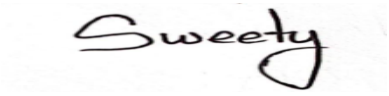
DECLARATION

I hereby declare that this project report **"GC-MS Profiling and Evaluation of Antioxidant and Thrombolytic Activity of Methanolic Leaf Extract of *Ricinus Communis L*",** is done by me under the supervision of Mr. Subrato Kumar Barman Lecturer Department of Pharmacy, Faculty of Health & Life Sciences, Daffodil International University. I am declaring that this project is my original work. I also declare that neither this project nor any part therefore has been submitted elsewhere for the award of Bachelor or any degree.

Submitted By



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ACKNOWLEDGEMENT

I might want to communicate my profound applause to the All-powerful Allah who has given me the capacity to finish my undertaking work and the chance to concentrate in this subject.

I'm a lot of thankful to my honorable thesis supervisor **Mr. Subrato Kumar Barman Lecturer** Department of Pharmacy, Faculty of Health & Life Sciences, Daffodil International University for his brilliant direction and steady oversight just as for giving essential data in regards to the task and furthermore for her help in finishing the project.

I also wish to offer my respect to all of the teachers of Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University and thankful to other members for their excellent cooperation with us.

Finally, I would like to express my gratitude towards my parents and other family members for their kind cooperation and encouragement which helped me in completion of this project.

My Parents,

The persons who always encourage me in every sphere of my life.

My teacher,

The persons who guided me in this process and the committee who kept me on track.

Abstract

This study investigates the chemical composition, antioxidant potential, and thrombolytic activity of the methanolic leaf extract of *Ricinus communis L*. Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed the presence of diverse bioactive compounds. A phytochemical examination of *Ricinus communis L* revealed the presence of flavonoids, phenol, carbohydrates, tannin, glycoside, and alkaloids. In this investigation about 61 compounds were found in this extract. This plant exhibited thrombolytic activity, with an output of 77.03%, compared to the usual streptokinase value of 76%. Additionally displayed is the antioxidant activity (IC_{50}=9.072), which is contrasted with the standard ascorbic acid (IC_{50}=17.937). It represent the analytical Mean ±SD data for flavonoid, tannin & phenol was 366.65, 128.01 & 184.22 respectively. More compound separation is necessary to validate the effects of particular compounds. These findings suggest that the methanolic leaf extract of *Ricinus communis L*. holds promise as a natural source of bioactive compounds with potential therapeutic applications in oxidative stress-related ailments and cardiovascular disorders. Further research is warranted to elucidate underlying mechanisms and optimize its utilization in pharmaceutical and nutraceutical sectors.

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Introduction

1. Introduction

Gas Chromatography-Mass Spectrometry (GC-MS) profiling is a powerful analytical technique used in the field of phytochemistry to identify and quantify the chemical constituents present in complex mixtures such as plant extracts. This method combines the separation capabilities of gas chromatography with the detection and identification capabilities of mass spectrometry, providing detailed information about the composition of natural products [1]. *Ricinus communis L*., commonly known as castor bean, is a plant species renowned for its diverse pharmacological properties. Among its various parts, the leaves of *Ricinus communis* have garnered significant attention due to their potential therapeutic benefits. Methanolic leaf extracts of *Ricinus communis* have been investigated for their antioxidant and thrombolytic activities, which are indicative of their potential use in the management and prevention of oxidative stress-related diseases and thrombotic disorders, respectively. Antioxidants are compounds that can neutralize harmful free radicals and protect cells from oxidative damage, thus playing a crucial role in maintaining overall health and preventing various diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders [2]. Thrombolytic agents, on the other hand, are substances capable of dissolving blood clots and restoring normal blood flow, making them essential in the treatment of thrombotic events such as heart attacks and strokes. The evaluation of antioxidant and thrombolytic activities of methanolic leaf extracts of *Ricinus communis* involves assessing their ability to scavenge free radicals and dissolve blood clots, respectively. Understanding the chemical composition of these extracts through GC-MS profiling can provide valuable insights into the bioactive compounds responsible for their pharmacological activities [3]. In this study, we aim to characterize the chemical constituents present in the methanolic leaf extract of *Ricinus communis* using GC-MS analysis and evaluate its antioxidant and thrombolytic activities. By elucidating the chemical composition and biological properties of this extract, we seek to contribute to the growing body of knowledge on the medicinal potential of *Ricinus communis* and facilitate its further exploration as a source of natural antioxidants and thrombolytic agents [4].

Figure 1: *Ricinus communis*

1.1 Medicinal plant in Bangladesh

Bangladesh, nestled in the lush greenery of South Asia, boasts a rich diversity of flora that includes an extensive array of medicinal plants. For centuries, the country's diverse ecosystems, ranging from the fertile plains of the Ganges Delta to the hills of the Chittagong region, have nurtured a treasure trove of botanical remedies integral to traditional healing practices. Medicinal plants hold a profound cultural and practical significance in Bangladesh, serving as vital components of indigenous healthcare systems and providing remedies for a myriad of ailments [5]. The use of medicinal plants in Bangladesh traces back to ancient times, with indigenous communities harnessing the therapeutic properties of plants to treat various illnesses and maintain well-being. Over the years, this traditional knowledge has been passed down through generations, contributing to the rich tapestry of herbal medicine practiced throughout the country. In contemporary Bangladesh, amidst the rapid advancements in modern medicine, there is a resurgence of interest in the therapeutic potential of medicinal plants. Researchers, healthcare professionals, and traditional healers alike are rediscovering the value of these botanical treasures, exploring their pharmacological properties, and integrating them into modern healthcare practices [6]. The significance of medicinal plants in Bangladesh extends beyond healthcare; they also play a crucial role in biodiversity conservation and sustainable development. Many medicinal plants are deeply intertwined with the country's ecosystems, providing habitat and sustenance for diverse flora and fauna. Furthermore, the cultivation

and sustainable harvesting of medicinal plants offer economic opportunities for rural communities, contributing to livelihoods and fostering environmental stewardship. However, the conservation of medicinal plants in Bangladesh faces challenges such as habitat loss, overexploitation, and climate change [7]. Efforts to address these challenges involve a combination of scientific research, community engagement, and policy interventions aimed at promoting sustainable practices and preserving biodiversity. In this exploration of medicinal plants in Bangladesh, we delve into the rich heritage, therapeutic potential, conservation efforts, and the intricate interplay between tradition and modernity. Through understanding and preserving this botanical wealth, Bangladesh continues to uphold its heritage of holistic healing while embracing the opportunities for sustainable healthcare and environmental stewardship in the 21st century [8].

1.2 Plants in traditional medicine

"Plants in traditional medicine" is a captivating exploration into the historical, cultural, and medicinal significance of botanical remedies across diverse civilizations. For millennia, humans have turned to plants not only as a source of sustenance but also as a potent arsenal against ailments and maladies. This symbiotic relationship between humanity and flora has fostered a rich tapestry of traditional medicinal practices, each deeply rooted in local customs and knowledge. From the rainforests of the Amazon to the steppes of Mongolia, traditional medicine has thrived as a holistic approach to health, viewing the individual as an integral part of their environment [9]. Plants, revered for their innate healing properties, form the cornerstone of these age-old practices. Passed down through generations via oral traditions and empirical observations, the wisdom of plant-based remedies has endured the test of time. The allure of traditional medicine lies not only in its efficacy but also in its reverence for nature and the interconnectedness of all living beings. Herbalists, healers, and shamans harness the potency of botanicals, drawing upon a vast reservoir of knowledge to address ailments ranging from the mundane to the mystical. Whether brewed into teas, ground into powders, or applied topically, plants offer a diverse pharmacopoeia that transcends geographical and cultural boundaries [10]. Moreover, the study of plants in traditional medicine provides valuable insights into biodiversity conservation and sustainable healthcare practices. As modern medicine continues to evolve, there is a growing recognition of the complementary role that traditional remedies can play in

augmenting conventional treatments. However, the integration of traditional knowledge into contemporary healthcare systems also raises questions of intellectual property rights, ethical sourcing, and scientific validation. Balancing respect for indigenous cultures with the need for evidence-based practices presents a complex but necessary endeavor in the pursuit of global health equity. In essence, the study of plants in traditional medicine is not merely an academic pursuit but a profound exploration of humanity's relationship with the natural world and a testament to the enduring wisdom found within the leaves, roots, and flowers that surround us [11].

Scientific classification	
Kingdom:	Plantae
Phylum:	Magnoliophyta
Class:	Magnoliopsida
Order:	Malpighiales
Family:	Euphorbiaceae
Subfamily:	Acalyphoideae
Tribe:	Acalypheae
Subtribe:	**Ricininae**
Genus:	***Ricinus***
Species:	***R. communis***
Binomial name	
Ricinus communis L.	

1.3 Chemical Composition of *Ricinus communis L*

The chemical composition of Ricinus communis L., commonly known as the castor bean plant, can vary depending on factors such as soil composition, climate, and plant variety. However, some key components typically found in castor beans include:

1. **Ricin**: This is one of the most well-known toxins present in castor beans. Ricin is a highly toxic protein that can cause severe health effects if ingested, inhaled, or injected [12].
2. **Ricinine**: This alkaloid compound is found in castor beans and is toxic to humans and animals in large doses.

3. **Fatty acids**: Castor beans contain high levels of fatty acids, particularly ricinoleic acid, which makes up about 85-95% of the total fatty acids present. Ricinoleic acid is unique to castor oil and is responsible for many of its therapeutic properties [13].
4. **Proteins**: Apart from ricin, castor beans contain various proteins, some of which may have industrial or pharmaceutical applications.
5. **Carbohydrates**: Castor beans also contain carbohydrates, including sugars and fibers, which contribute to the overall nutritional profile of the plant.
6. **Phytochemicals**: Castor beans contain a variety of phytochemicals, including flavonoids, phenolic compounds, and other secondary metabolites, which may have antioxidant or other biological activities [14].

It's important to note that while castor oil extracted from the beans has various industrial and medicinal uses, the raw beans themselves are highly toxic and should not be ingested. Proper processing methods are required to extract the oil and remove toxins before it can be safely used [15].

1.4 Therapeutic use and pharmacological activity of *Ricinus communis L*

Ricinus communis L., commonly known as the castor bean plant, has a long history of use in traditional medicine due to its various therapeutic properties. However, it's important to note that while castor oil derived from the plant has several medicinal applications, other parts of the plant, including the seeds, contain ricin, a highly toxic protein, and should be handled with extreme caution [16].

Castor Oil: This is the most well-known medicinal product derived from Ricinus communis. It is extracted from the seeds of the plant and has several therapeutic uses:

Laxative: Castor oil is commonly used as a laxative to relieve constipation. It works by stimulating the intestines and promoting bowel movements.

Anti-inflammatory: When applied topically, castor oil has anti-inflammatory properties and can help reduce inflammation and pain associated with conditions like arthritis and muscle soreness [17].

Moisturizer: Castor oil is often used in skincare products due to its moisturizing properties. It can help hydrate the skin and improve its texture.

Hair Care: Castor oil is believed to promote hair growth and thickness when applied to the scalp. It is often used in hair care products and treatments [18].

Pharmacological Activity:

Ricin: Although highly toxic, ricin derived from castor beans has attracted attention for its potential pharmacological applications, particularly in cancer treatment. Research has focused on utilizing ricin as a targeted toxin to destroy cancer cells. However, due to its extreme toxicity, developing safe and effective delivery methods for ricin-based therapies remains a challenge.

Antimicrobial Activity: Some studies have reported that extracts from Ricinus communis possess antimicrobial properties against various pathogens, including bacteria, fungi, and viruses. These antimicrobial properties could potentially be harnessed for medicinal purposes, such as in the development of new antimicrobial agents [19].

Anti-inflammatory Effects: Beyond its traditional use as a topical anti-inflammatory agent, research suggests that compounds present in Ricinus communis may have broader anti-inflammatory effects when administered orally or systemically. These effects could have implications for the treatment of inflammatory conditions such as rheumatoid arthritis and inflammatory bowel disease.

While Ricinus communis and its derivatives offer promising therapeutic benefits, it's crucial to use them responsibly and under the guidance of healthcare professionals due to the potential toxicity associated with certain parts of the plant [20].

1.4 In vivo antioxidant evaluation

In vivo antioxidant evaluation is a crucial aspect of assessing the potential health benefits of various compounds, foods, or pharmaceuticals. Antioxidants play a vital role in neutralizing harmful molecules called free radicals, which can cause oxidative stress and damage to cells and tissues in the body. Oxidative stress has been linked to numerous chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative

disorders [21]. In vivo studies involve the examination of antioxidant activity within living organisms, such as animals or humans, providing a more comprehensive understanding of how antioxidants interact with biological systems. These studies often utilize animal models to simulate the physiological conditions of humans and investigate the effectiveness of antioxidants in preventing or mitigating oxidative damage [22].

Several methods are employed to evaluate the in vivo antioxidant activity, including:

Biochemical assays: These assays measure various biomarkers of oxidative stress and antioxidant defense mechanisms in biological samples, such as blood, tissues, or urine. Common biomarkers include levels of reactive oxygen species (ROS), antioxidant enzymes (e.g., superoxide dismutase, catalase), and oxidative damage products (e.g., lipid peroxidation products).

Histological analysis: Histological examination of tissues allows researchers to assess structural changes and cellular damage caused by oxidative stress. Techniques such as staining with specific dyes can highlight areas of oxidative damage or inflammation within organs [23].

Animal behavior studies: In some cases, the effects of antioxidants on animal behavior and cognitive function are evaluated to understand their potential neuroprotective properties against oxidative stress-induced damage to the brain and nervous system.

Health outcome assessments: In long-term studies, researchers may measure overall health outcomes, such as lifespan, incidence of diseases, or improvement in symptoms, to evaluate the effectiveness of antioxidants in promoting health and longevity [24].

The findings from in vivo antioxidant evaluation provide valuable insights into the potential therapeutic applications of antioxidants in preventing or treating various diseases associated with oxidative stress. However, it's essential to interpret these results in the context of other factors influencing antioxidant activity, such as bioavailability, metabolism, and interactions with other nutrients or medications. Overall, in vivo antioxidant evaluation serves as a crucial step in the development of antioxidant-based interventions for improving health and reducing the risk of chronic diseases in humans [25].

1.5 In vivo thrombolytic evaluation

In vivo thrombolytic evaluation is a fundamental aspect of assessing the efficacy and safety of potential treatments aimed at dissolving blood clots, a process known as thrombolysis. Blood clots, or thrombi, can obstruct blood flow within blood vessels, leading to serious medical conditions such as myocardial infarction (heart attack), ischemic stroke, and pulmonary embolism. Thrombolytic therapy aims to restore blood flow by breaking down these clots, thereby preventing tissue damage and improving patient outcomes [26]. In vivo studies play a pivotal role in evaluating the thrombolytic potential of various compounds, drugs, or therapeutic strategies within living organisms, typically utilizing animal models that closely mimic human physiology and pathology. These studies provide valuable insights into the effectiveness, mechanisms of action, and potential side effects of thrombolytic agents under physiological conditions.

Several methods are commonly employed to evaluate in vivo thrombolytic efficacy:

Animal models: Various animal models, including rodents (such as mice and rats) and larger animals (such as rabbits or pigs), are used to simulate thrombotic conditions relevant to human diseases. These models may involve inducing blood clots in specific blood vessels, such as the coronary arteries or cerebral arteries, followed by treatment with thrombolytic agents to assess clot dissolution and restoration of blood flow [27].

Blood flow measurements: Techniques such as Doppler ultrasound or laser Doppler flowmetry are utilized to monitor blood flow before and after thrombolytic treatment, allowing researchers to quantify the extent of clot lysis and assess the functional outcomes in terms of vascular patency.

Histological analysis: Histological examination of thrombi and surrounding tissues provides insights into the structural changes induced by thrombolytic treatment, including clot dissolution, tissue reperfusion, and potential adverse effects such as hemorrhage or tissue damage [28].

Coagulation parameters: In addition to assessing clot dissolution, in vivo thrombolytic evaluation often involves monitoring coagulation parameters, such as activated partial

thromboplastin time (aPTT) and prothrombin time (PT), to evaluate the systemic effects of thrombolytic agents on blood coagulation and hemostasis.

The findings from in vivo thrombolytic evaluation contribute to the development and optimization of thrombolytic therapies, guiding the selection of promising candidates for further clinical investigation. Additionally, these studies help identify potential risks and limitations associated with thrombolytic treatment, informing strategies to improve efficacy and safety in clinical practice. Overall, in vivo thrombolytic evaluation plays a critical role in advancing our understanding of thrombus dissolution mechanisms and facilitating the translation of novel thrombolytic therapies from preclinical research to clinical application, with the ultimate goal of reducing the burden of thrombotic diseases and improving patient outcomes [29].

Chapter two
Purpose of the study

2. Objective of the study

The purpose of research on "GC-MS Profiling and Evaluation of Antioxidant and Thrombolytic Activity of Methanolic Leaf Extract of Ricinus Communis L" could encompass several objectives:

- To identify Chemical Composition by GC-MS analysis
- To know the Medicinal Potential of this plant leaf
- Phytochemical Profiling
- To assess Thrombolytic activity
- To assess Anti-oxidant activity

Overall, this research aims to deepen our understanding of the chemical composition, biological activities, and potential medicinal applications of *Ricinus Communis L*, ultimately contributing to both scientific knowledge and potential practical applications in medicine or related fields.

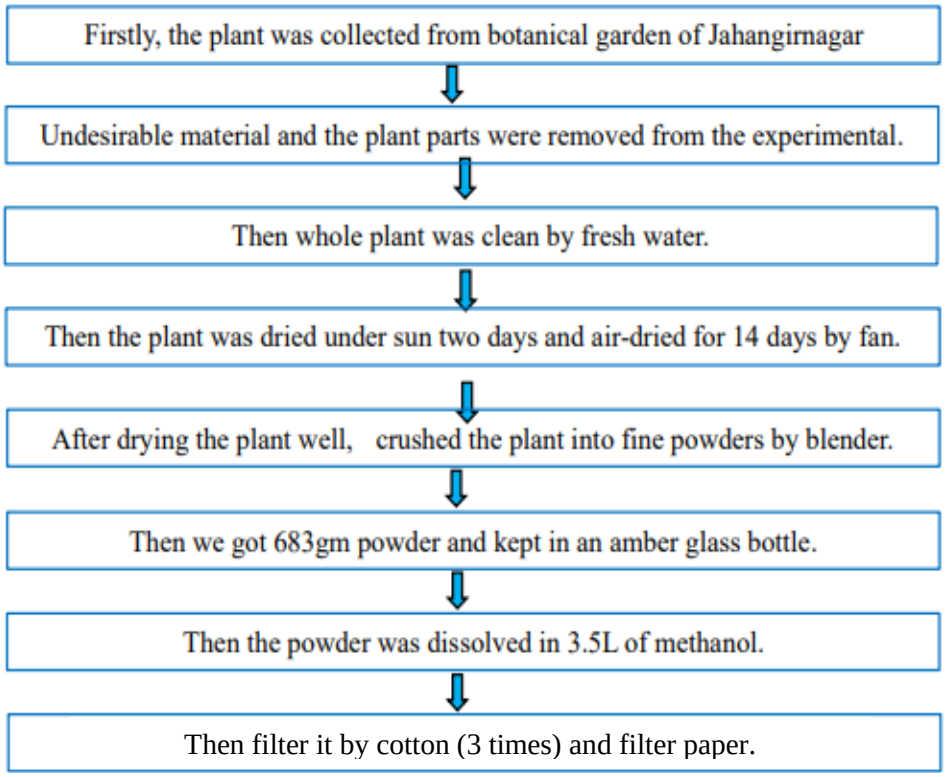
Chapter three
Materials & Methodology

3. Materials & methodology

Analyzing phytochemicals entails studying the bioactive substances found in plants. These chemicals are extracted, isolated, and characterized using a variety of techniques and equipment.

3.1 Description of plant extraction is given below:

Unprocessed medicine is extracted from the plant and animal and used without modifying its chemical composition. I organized raw concentration in several steps. Here's a general description of the process:



3.2 Extraction:

A glass jar with a plastic top was thoroughly cleaned before being given a methanol rinse. Subsequently, 1500 milliliters of methanol were poured to the jar containing 52 grams of the dry powder, before the surface of the sample was sufficiently coated, reaching a height of one inch above the jar. The jar was appropriately airtight, with a plastic cover that was coated with aluminum foil. The procedure described above was done for twelve days. To enhance the extraction procedure, the jar was shook nearly twice a day.

3.2.1 Filtration

After the process of extraction, sterile cotton and filter paper were used to filter the plant's extract. Both items were subsequently immersed in the required solvents and put within a funnel. The filtered material was stored in a glass jar.

Figure 2: Filtration of extract via funnel with filter paper

3.2.3 Evaporation

The liquid extraction was evaporated using an evaporator that rotates. At a temperature of 65 to 670 degrees Celsius and a spinning speed of 34 to 38 rotations per minute, methanol was evaporated. The liquefied material was transferred to a 50 milliliter beaker. After the pure material was gathered, the resultant solution was put just beneath the fan in beakers wrapped with aluminum foil. A cold hair drier was then used to completely eliminate the leftover water after the rest of the component in the resulting extract had begun to evaporate.

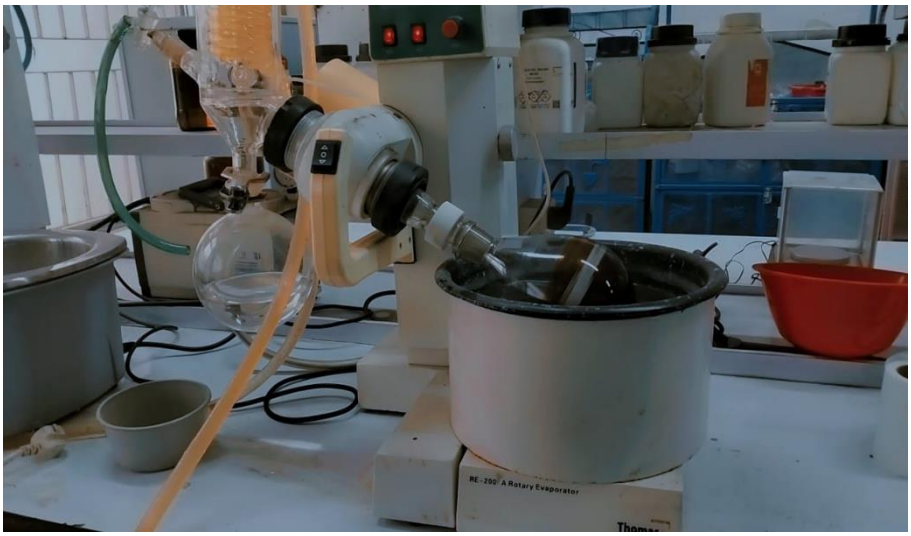


Figure 3: Evaporation of extract via Evaporator

3.3 My investigation

In my investigation I have been gadget

- Phytochemical Screening test
- In vivo thrombolytic activity
- Anti-oxidant activity

3.4 Phytochemical Assessment

As part of the phytochemical evaluation process, plant phytochemicals are examined and assessed. Plants naturally contain bioactive molecules called phytochemicals, which may have positive effects on human health. These compounds help the plant prevent disease,

maintain links with its environment, and strengthen its defense mechanisms. Phytochemicals encompass a wide range of compounds, including as phenolic acids, alkaloids, flavonoids, terpenoids, saponins, and more.

3.4.1 Reagents applied for the diverse chemical group analysis

3.4.1 Assessment for alkaloids

Mayer's evaluation

Two milliliters of extort solution and 0.2 milliliters of diluted hydrochloric acid were put into a test tube. Then, 1 milliliter of Mayer's reagent was applied. The appearance of a precipitate with a yellow tint indicated the existence of alkaloids.

Dragendroff's examination

Two milliliters of extort solution and two milliliters of diluted hydrochloric acid were put into a test tube. Dragendroff's reagent (1 mL) was then added. An orange-brown precipitated was observed, indicating the possible involvement of alkaloids.

[a]

[b]

A=Mayer's analysis

B= Dragendroff's analysis of extract

3.4.2 Assessment for Flavonoids

The presence of flavonoids is shown by the red color, which was obtained by adding 2 mL of droplets of strong hydrochloric acid to a 5 mL amount of an alcoholic solution of the plant component.

Figure 4: Flavonoids analysis of extract

3.4.3 Assessment for Saponins

After diluting 5 milliliter of the aqueous solution with 5 milliliters of distilled water, it was agitated in a glutted container for fifteen minutes. A foam layer just one centimeter thick indicates the presence of saponins.

Figure 5: Saponins analysis of extract

3.4.4 Assessment for Tannins

10 ml of purified water will be used to absorb 5g of extraction (Equivalent 2 mL). The mixture will then be filtrated, and the resultant filtrate will be treated with 2-3 drops of ferric chloride reagents. The formation of a blue-black, green, or blue-green precipitation suggests the presence of tannins.

Figure 6: Tannins analysis of extract

3.4.5 Assessment for Glycosides

0.2 mL of concentrated HCL was added after 2 mL of the sample was initially taken. The evaluation of yellow color indicated the existence of glycosides. Fehling liquid contains a tiny amount of a heated alcohol extract. The amount of red brick that precipitated was measured to ascertain the presence of glycosides. After the substance was combined with alcohol and water, a little portion was heated with a few drops of diluted sulfuric acid. After neutralizing Fehling solution with sodium hydroxide water, bring it to a boil. The red brick precipitated was assessed as a glycoside indication.

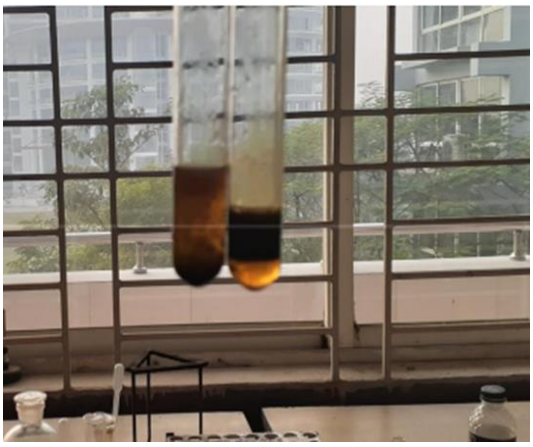


Figure 7: Glycosides analysis of extract

3.4.6 Test for Phenol

A 2-3 drops neutral ferric chloride solution was diluted with 2 milliliters of the extract; the dark blue coloring indicated the presence of phenolic chemicals.

3.4.7 Test for Steroid

One milliliter of concentrated H2SO4 was added to two milliliters of extract in a test tube. Bright yellow or red coloration.

3.4.8 Test for gums

Sulfuric acid was added after the resultant extract and Molish reagent were combined in five milliliters. A reddish-violet ring formed when two liquids were combined, indicating the existence of gums and carbs.

3.4.9 Test for Reducing Sugar (Benedict's test)

After mixing Benedict's reagent with one milliliter of the analyte sample, place the mixture in a boiling water bath and heat it for three to five minutes. The development of a brick-red-colored cuprous oxide precipitate indicates the presence of reducing sugars in the analyte.

3.4.10 Test for Carbohydrates

2 ml of concentrated H2SO4 plus 2-3 drops of Molisch's reagent and 2 ml of extract. The existence of carbohydrates was indicated by a red or purple ring.

3.5 Experimental procedure of GC-MS Analysis

The Ethanolic leaf extract of *Ricinus Communis L* was examined using the GC-MS electron impact ionization (EI) technique at the Bangladesh Reference Institute for Chemical Measurements (BRiCM), employing a GC-2010 gas chromatograph coupled to a GCMS-TQ8040 mass spectrometer from Shimadzu. The gas chromatograph utilized a DB-5MS column composed of 5% phenyl-95% methylpolysiloxane (with a film thickness of 0.25 μm, a length of 30 m, and a diameter of 0.25 mm).

The GC parameters were set as follows: inlet temperature of 250°C, initial oven temperature of 50°C held for 1 minute, increased to 200°C at a rate of 15°C/min for 2 minutes, and finally raised to 300°C at a rate of 5°C/min for 7 minutes. Helium was used as the carrier gas at a constant pressure of 53.5 kPa, with a total flow rate of 11.0 mL/min and a column flow rate of 1.0 mL/min. The split ratio was 5.0, with a sampling time of 1.0 minute and a solvent cut time of 3.50 minutes. Methanol was employed to dissolve the samples, and the total retention time for chromatographic analysis was 40 minutes.

The MS parameters were configured as follows: interface temperature of 250°C, ion source temperature of 230°C, acquisition mode set to Q3 scan mode, scan speed of 2000, and a mass range of 50 - 600 m/z. Compound identification was performed using the "NIST-MS Library" for mass spectra analysis. The relative percentage of separated compounds was determined from the peak areas of the total ionic chromatogram (TIC).

3.6 Thrombolytic Activity Test

3.6.1 Equipment of thrombolytic activity test

Serial No.	Name
1	Test Tube
2	Glass Rod
3	Vortex Mixture
4	Eppendorf Tube
5	Eppendorf Tube Rack
6	Beaker (50ml)
7	Incubator
8	Cotton
9	Syringe
10	Syringe Filter
11	Micropipette

Figure 8: Equipment of thrombolytic activity test

3.6.2 Reagent of thrombolytic activity test

Serial No.	Name
1	Blood (Human)
2	Streptokinase
3	Distilled Water
4	Sample Extraction

Table 1: Reagent of thrombolytic activity test

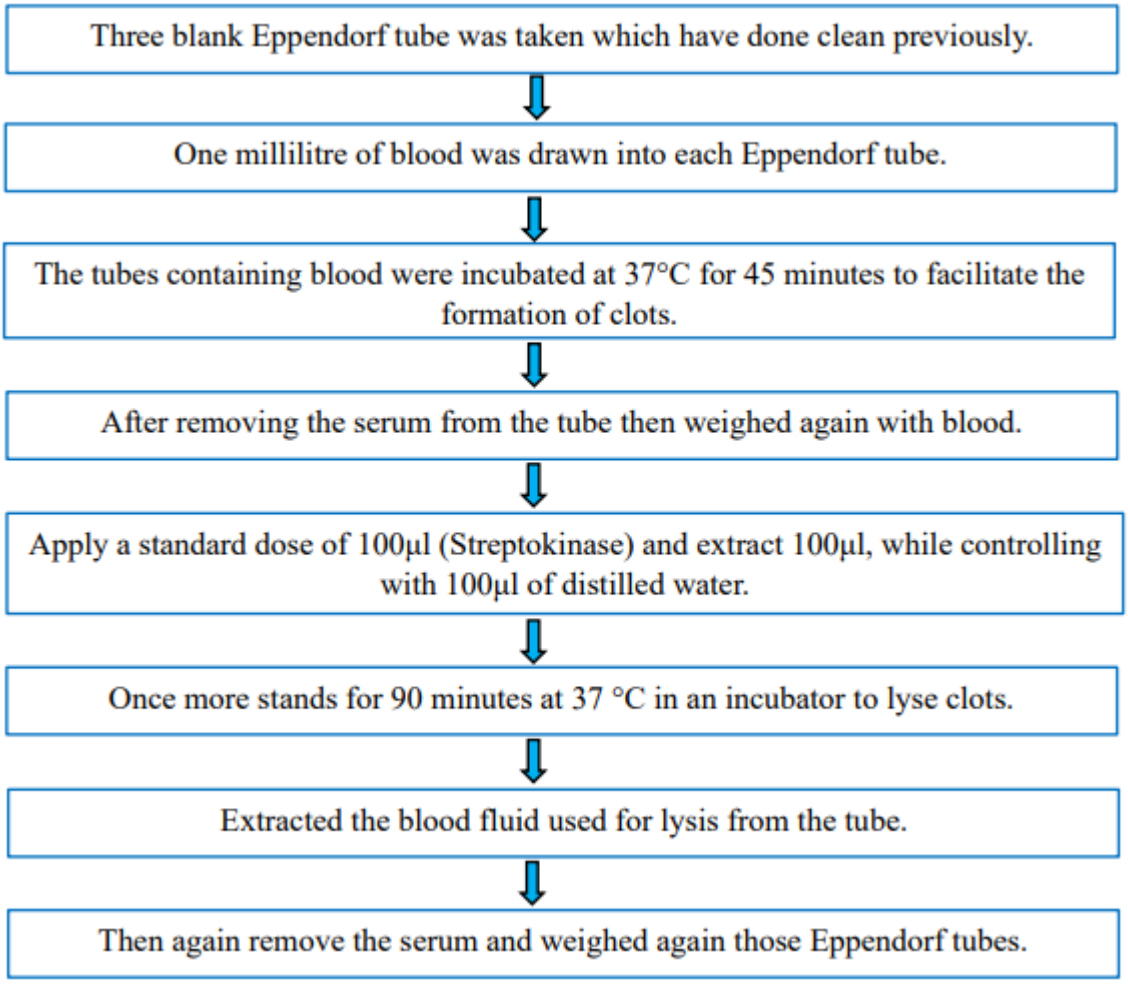
3.6.3 Preparation of test sample

In the beginning, 25 mg of crude extract suspended in 2.5 ml of distilled water were stirred with a vortex mixer. The suspension was allowed to settle overnight before being filtered using syringe filter paper to collect the soluble supernatant. With the solution, ex-vivo cardioprotective action could now be accomplished.

3.6.4 Preparation of Streptokinase Solution as Standard

The readily accessible 15, 00,000 I.U. lyophilized ATPase (Streptokinase) alpine tube (Beacon Pharmaceutical Ltd.) served as the standard solution. After that, five water was added to the streptokinase vial and carefully stirred. This suspension was used in 100µl (30,000 I.U.) for in vitro thrombolysis.

3.6.5 Working Procedure of Thrombolytic Test



3.7 Antioxidant Test

The plant extraction stock solution (10 mg/ml) was prepared in methanol and used in a serial dilution. The first six concentration kinds are prepared using six volumetric flasks: 1, 5, 10, 50.100, and 500 ug/ml. We encircle volumetric flasks and test tubes with foil paper. Six volumetric flasks are prepared with varying extract dilutions and labeled appropriately. Two milliliters of the sample for every concentration level and two

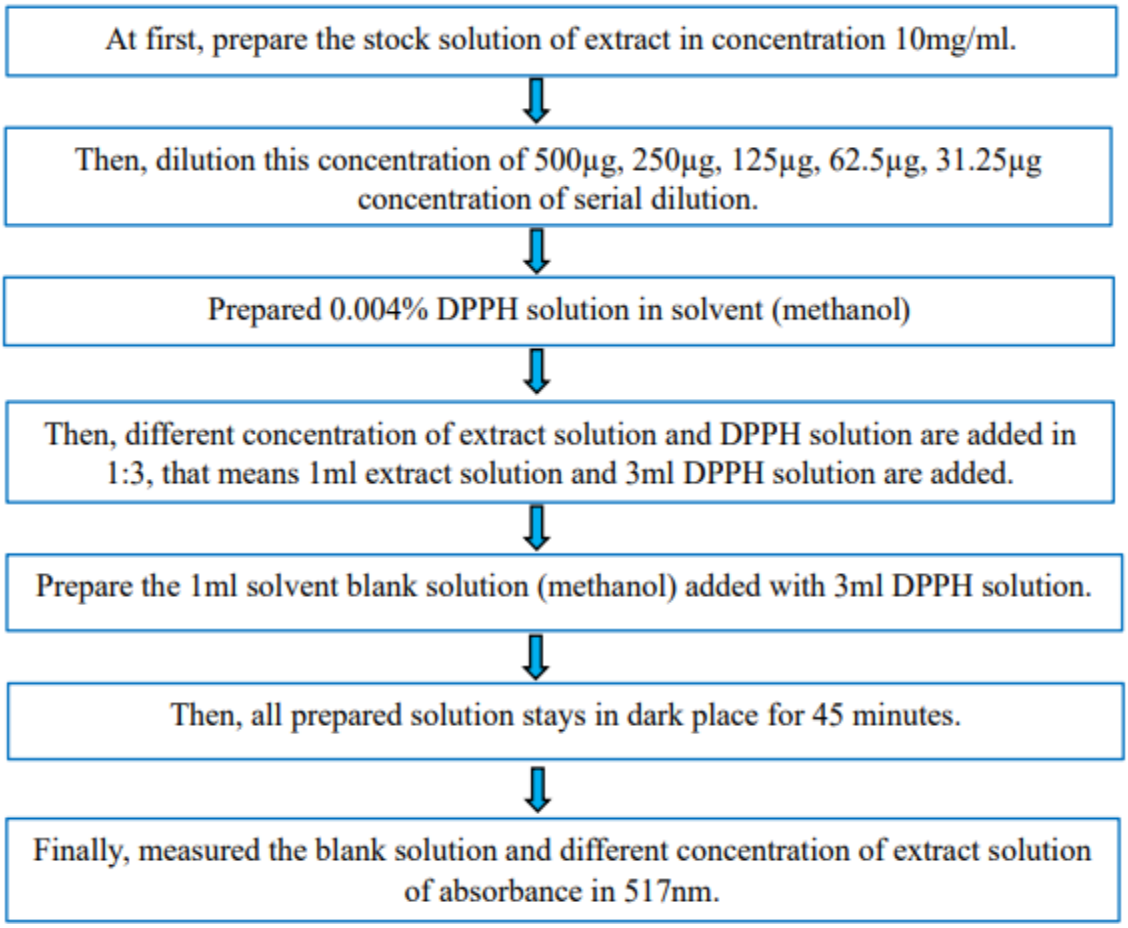
milliliters of the 0.004% DPPH solution are pipetted into each of the six test tubes. Each test tube is wrapped in foil paper, and the solution is then left in a dark room for half an hour. In another tube for testing, combine two milliliters of 0.004% DPPH and two milliliters of methanol to create the blank solution. Next, UV Spectroscopy is used to measure absorption.

3.7.1 Equipment & Reagent of Antioxidant test

Serial No.	Name
1	Test Tube
2	Glass Rod
3	Vortex Mixture
4	Pipette Filter
5	Test Tube Rack
6	Beaker (100ml)
7	Micropipette
8	Ascorbic Acid
9	Methanol
10	Plant Extract

Table 2: Equipment & Reagent of Antioxidant test

3.7.2 Working Procedure of Antioxidant Test



Measurement of % of inhibition:

$$\%\ of\ Inhibition = Blank\ Absorbance - Extract\ Solution\ Absorbance\ Blank\ Absorbance \times 100$$

Chapter four
Results and Discussion

4.1 Result and Discussion of phytochemical evaluation

Serial No.	Test Name	Result
1	Alkaloid (Mayer's & Dragendorff's test)	+
2	Glycoside	+
3	Tannin	+
4	Saponin	-
5	Steroid	+
6	Phenol	+
7	Carbohydrate	+
8	Gum	+
9	Reducing Sugar (Benedict & Fehling Test)	+
10	Flavonoid	-

Table 3: Result of phytochemical evaluation

The following table lists the several ***Ricinus Communis L*** extracts together with their matching dry extract amounts, physical attributes, and qualitative chemical analyses.

4.2 GC-MS analysis

Using a database of more than 62000 patterns from the National Institute of Standard and Technology (NIST), the mass spectrum of the GC-MS instrument was interpreted. The spectra of the unknown molecule were compared to the spectrum of the reported component stored in the NIST collection. The spectrum of the known component from the NIST library was compared to the spectrum of the extracts to identify the components.

Forty (40) components from the ethanolic leaf extract of *Ricinus Communis L* were identified and quantified by the GC-MS analysis.

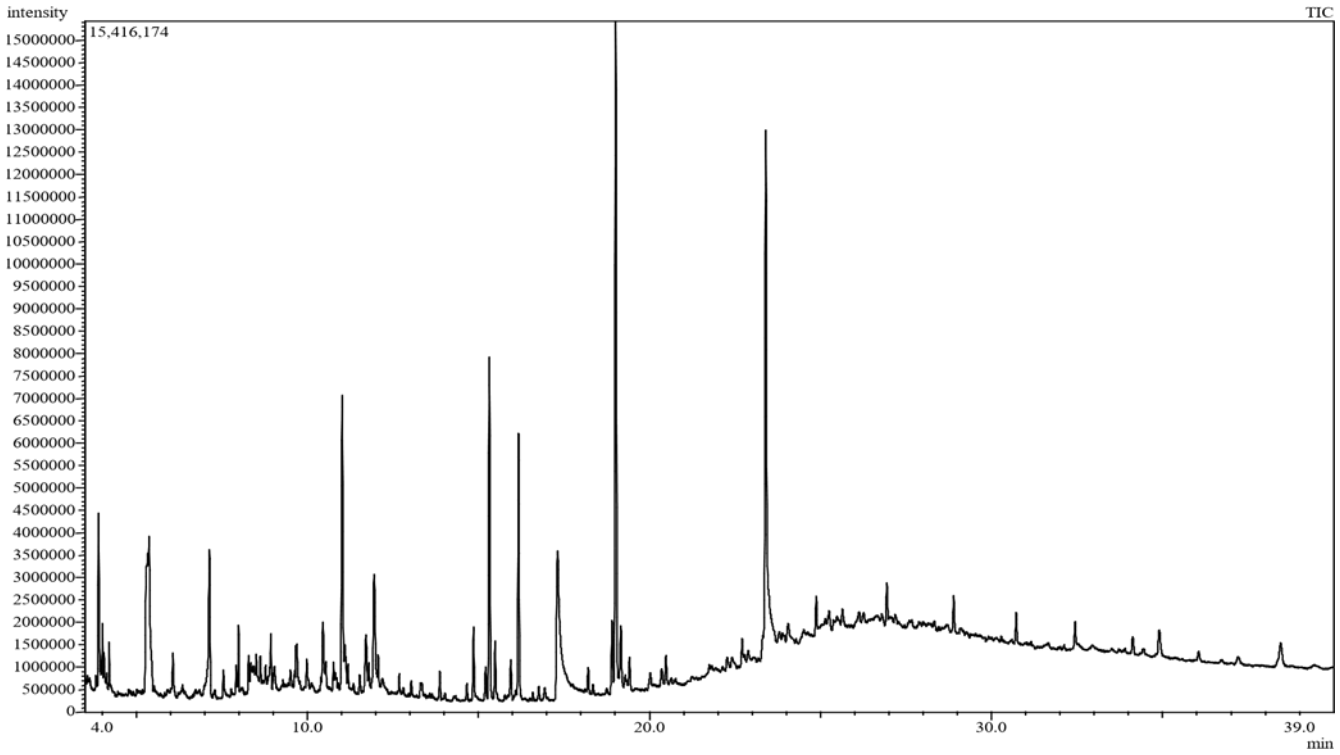


Figure 9: Chromatograph (GC/MS) of ethanolic leaf extract of Ricinus Communis L

Table 4: Constituents of ethanolic leaf extract of Ricinus Communis L identified by GC–MS analysis

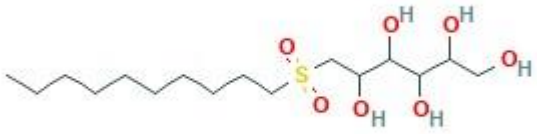
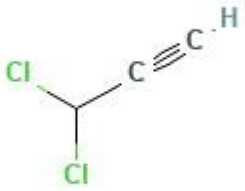
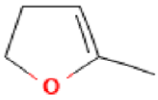
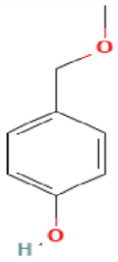
Sl. No.	Retention Time	Peak Area (%)	Name of constituent	Molecular Formula	Molecular Weight	PubChem CID
1	3.525	0.51	d-Mannitol, 1-decylsulfonyl-	C16H34O7S	370.5	568528
2	3.575	0.48	3,3-Dichloropropyne	C3H2Cl2	108.95	33035
3	3.65	0.38	Furan, 2,3-dihydro-5-methyl-	C_5H_8O	84.12	15145
4	3.827	0.26	Phenol, 4-(methoxymethyl)-	C8H10O2	138.16	79310
5	3.901	3.24	Furfural	C5H4O2	96.08	7362
6	4.013	1.11	Isobenzofuran, 1,3-dihydro-1,3dimethoxy-	C10H12O3	180.2	11629690
7	4.057	0.72	Sulfuric acid, dimethyl ester	C2H6O4S	126.13	6497

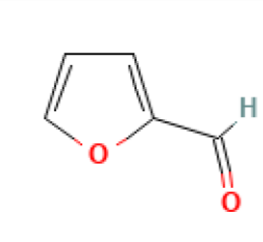
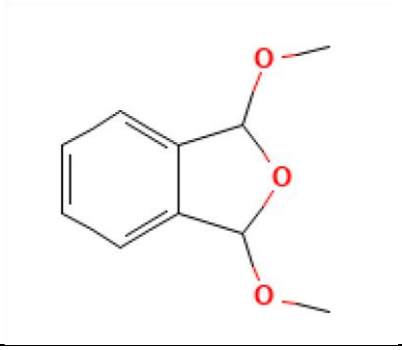
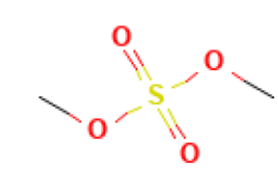
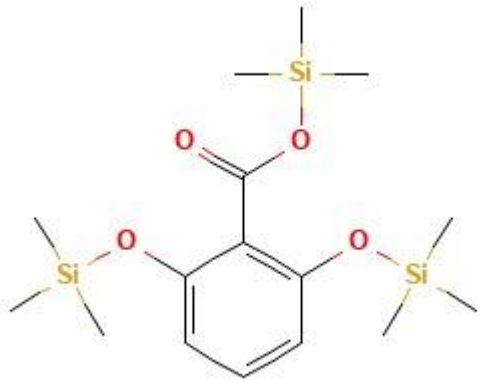
8	7.01	0.43	2,6-Dihydroxybenzoic acid, 3TMS derivative	C16H30O4Si3	370.66	520869
9	7.549	0.47	Hepta-2,4-dienoic acid, methyl ester	C8H12O2	140.18	5370266
10	7.77	0.22	4-Penten-1-ol, TMS derivative	$C_8H_{18}OSi$	158.31	10866616
11	8.285	0.68	1,4:3,6-Dianhydro-.alpha.-dglucopyranose	C6H8O4	144.12	22213879
12	8.347	0.73	Benzaldehyde, 3,5-dimethyl-	C9H10O	134.17	34225
13	8.423	0.73	Cyclohexanol, 2-(1-methylethyl)-	C9H18O	142.24	95331
14	8.507	0.79	Benzothiazole	C_7H_5NS	135.19	7222
15	8.623	0.57	2-Amino-4,6-dimethoxypyrimidine	C6H9N3O2	155.15	118946
16	8.781	0.23	Dodecane, 2,6,11-trimethyl-	C15H32	212.41	35768
17	8.9	0.27	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9decamethyl-	C10H30O4Si5	354.77	6329089
18	9.005	0.21	5-(Hydroxymethyl)-2-(dimethoxymethyl)furan	C 8H12O4	172.18	588127
19	9.039	0.40	.alpha.-L-Galactopyranoside, methyl 6-deoxy-	C7H14O5	178.18	446578
20	9.506	0.30	2-Bromomethyl-2phenyl[1,3]dioxolane	C10H11BrO2	243.1	291574
21	9.666	0.49	6,6,8,8-Tetramethyl-2,5,7,9,12pentaoxa-6,8-disilatridecane	C10H26O5Si2	282.48	91724015
22	9.691	0.96	2H-Pyran-3,4,5-triol, tetrahydro-2methoxy-6-methyl-	C7H14O5	178.18	242517
23	9.79	0.10	3-Hydroxymethyl-4-(1-hydroxy-2methylprop-2-enyl) toluene, TBDMS derivative	C18H30O2Si	306.5	554606
24	9.984	0.61	1,6-Anhydro-.beta.-d-talopyranose	C6H10O5	162.14	1777528

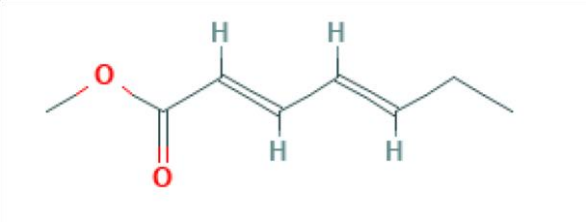
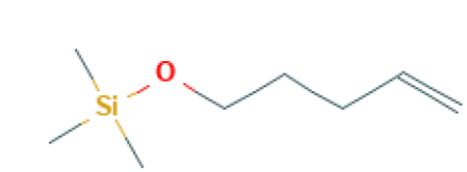
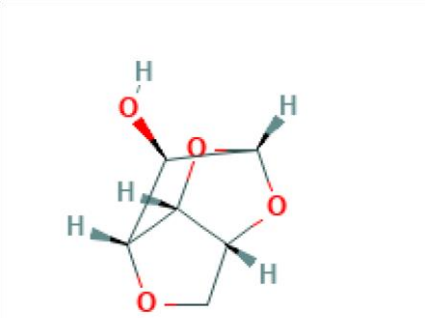
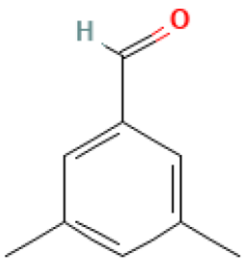
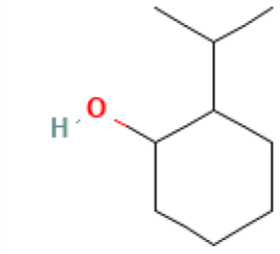
25	10.398	0.20	Propiolic acid, 3-phenyl-, methyl ester	C10H8O2	160.17	138378
26	10.455	1.86	2-(Isobutoxymethyl)oxirane	C7H14O2	130.18	98155
27	10.764	0.39	1-Dodecanol	C12H26O	186.33	8193
28	10.814	0.36	Scyllo-Inositol, 1-C-methyl-	C7H14O6	194.18	244581
29	10.906	0.10	2-Ethylthio-5-methylimidazoline	C6H12N2S	144.24	575741
30	11.02	6.91	D-Allose	C6H12O6	180.16	439507
31	11.097	0.82	Phenol, 3,5-bis(1,1-dimethylethyl)-	C14H22O	206.32	70825
32	11.19	0.35	Dodecanoic acid, methyl ester	C13H26O2	214.34	8139
33	11.348	0.12	Benzenepentanoic acid, .beta.-methyl-, ethyl ester	C14H20O2	220.31	563065
34	11.53	0.36	(3R,3aS,6aR)-Hexahydrofuro[2,3b]furan-3-ol, TMS	C6H10O3	130.139	9942150
35	11.715	1.72	.alpha.-D-Galactopyranoside, methyl	C7H14O6	194.18	76935
36	11.798	0.57	2-(1,2,3,4-Tetrahydronaphthalen-1yl)ethan-1-ol, TMS	C12H16O	176.25	98036
37	11.955	3.89	1,6-Anhydro-.beta.-D-glucofuranose	C6H10O5	162.14	13037722
38	12.074	0.49	Cyclooctasiloxane, hexadecamethyl-	C16H48O8Si8	593.2	11170
39	13.312	0.53	Ethanol, 2-(dodecyloxy)-	C14H30O2	230.39	24750
40	14.86	1.39	Neophytadiene	C20H38	278.5	10446
41	15.211	0.61	3,7,11,15-Tetramethyl-2-hexadecen-1ol	C20H40O	296.5	5366244
42	15.323	7.16	1,2-Benzenedicarboxylic acid, bis(2methylpropyl) ester	C16H22O4	278.34	6782
43	16.177	5.39	Hexadecanoic acid, methyl ester	C17H34O2	270.5	8181

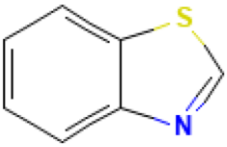
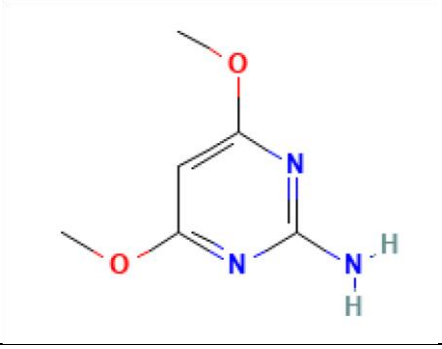
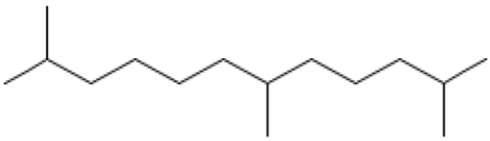
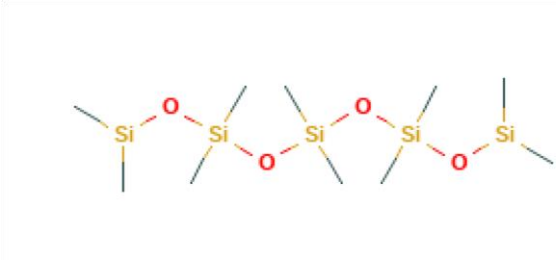
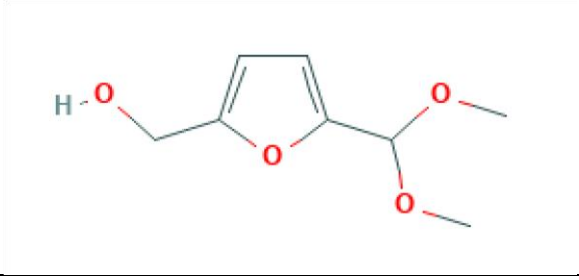
44	16.765	0.26	Sulfurous acid, isohexyl hexyl ester	C12H26O3S	250.4	6421370
45	17.316	7.61	Ricinine	C8H8N2O2	164.16	10666
46	18.349	0.18	Tetrahydropyranyl ether of citronellol	C15H28O2	240.38	558921
47	18.91	1.68	9,12-Octadecadienoic acid, methyl ester	C19H34O2	294.5	5284421
48	19.017	15.49	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C19H32O2	292.5	5319706
49	19.166	2.07	Phytol	C20H40O	296.5	5280435
50	19.294	0.46	Tetrapentacontane	C54H110	759.4	521846
51	19.42	1.02	Methyl stearate	C19H38O2	298.5	8201
52	20.358	0.40	Hexadecanamide	C16H33NO	255.44	69421
53	20.481	0.65	Heptasiloxane, hexadecamethyl-	C16H48O6Si7	533.1	10912
54	22.265	0.38	1-Decanol, 2-hexyl-	C16H34O	242.44	95337
55	23.31	0.55	Methyl 5,12-octadecadienoate	C19H34O2	294.5	12255536
56	23.4	18.48	9-Octadecenamide, (Z)-	C18H35NO	281.5	5283387
57	23.798	0.29	3-Hexadecanol	C16H34O	242.44	79052
58	23.887	0.31	11-Methyltricosane	C24H50	338.7	530326
59	25.251	0.38	cis,cis,cis-7,10,13-Hexadecatrienal	C16H26O	234.38	5367366
60	34.898	0.91	alpha-Tocopheryl acetate	C31H52O3	472.7	86472
61	38.449	0.74	beta-Sitosterol	C29H50O	414.7	222284

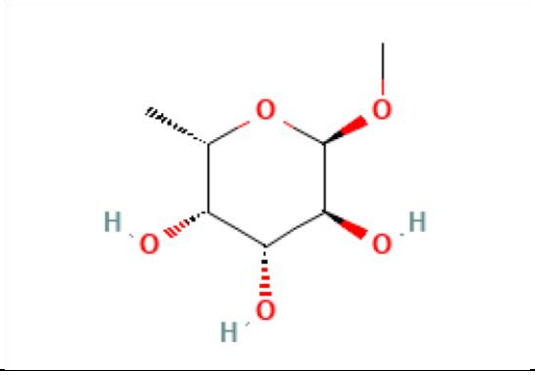
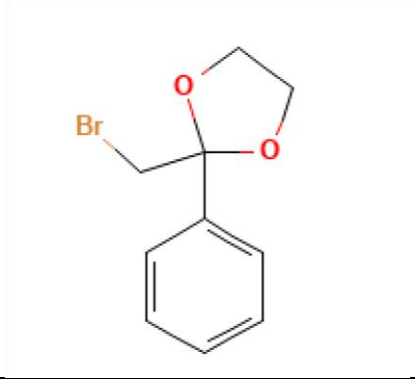
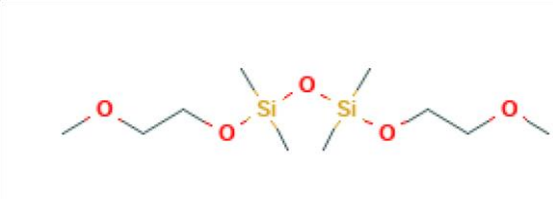
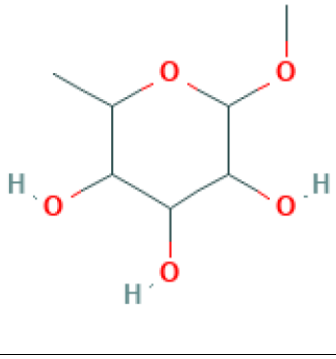
Table 5: Chemical structures of constituents of e ethanolic leaf extract of Ricinus Communis L identified by GC–MS analysis

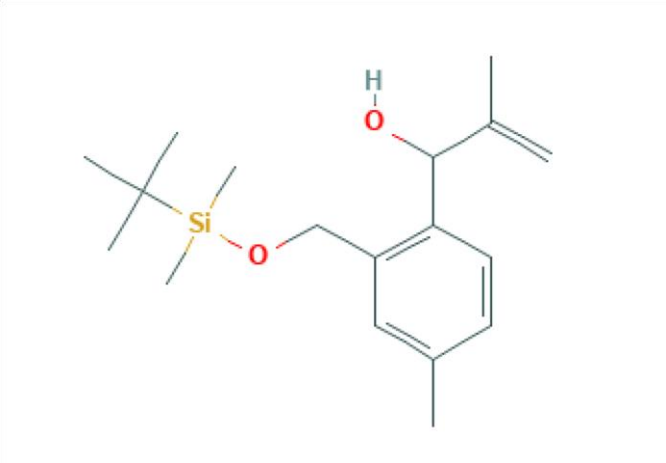
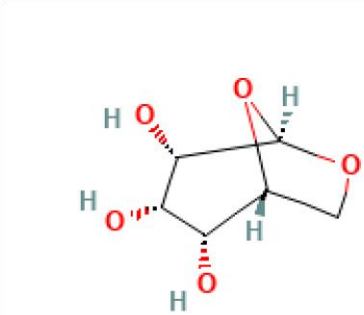
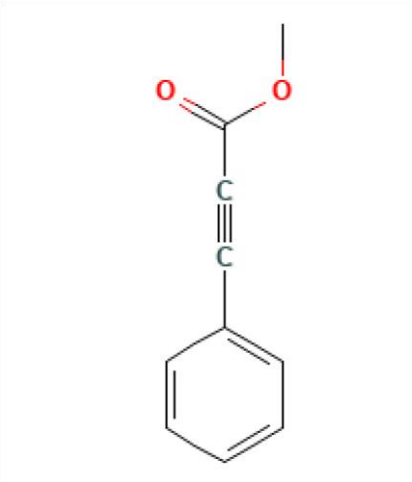
Sl. No.	Name of constituent	PubChem CID	Chemical Structure
1.	d-Mannitol, 1-decylsulfonyl-	568528	
2.	3,3-Dichloropropyne	33035	
3.	Furan, 2,3-dihydro-5-methyl-	15145	
4.	Phenol, 4-(methoxymethyl)-	79310	

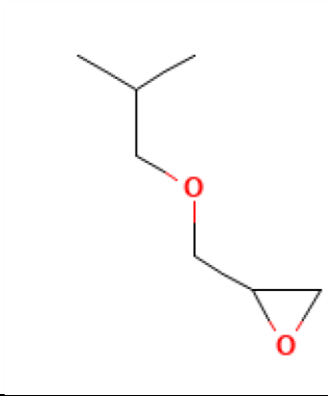
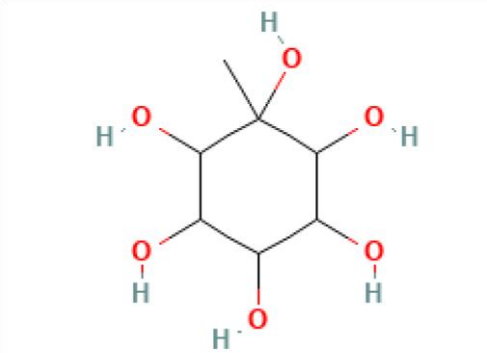
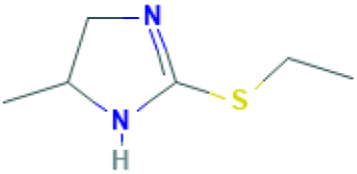
5.	Furfural	7362	
6.	Isobenzofuran, 1,3-dihydro-1,3-dimethoxy-	11629690	
7.	Sulfuric acid, dimethyl ester	6497	
8.	2,6-Dihydroxybenzoic acid, 3TMS derivative	520869	
9.	Hepta-2,4-dienoic acid,		

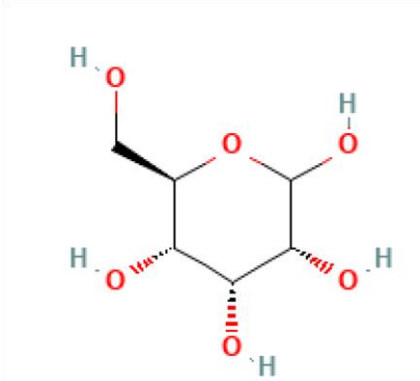
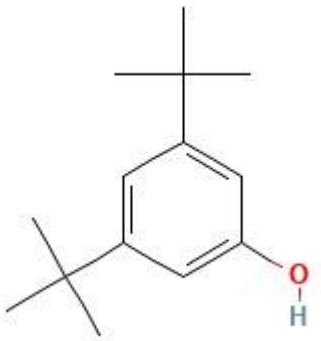
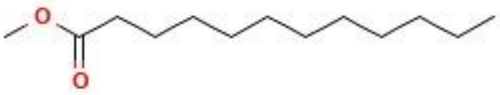
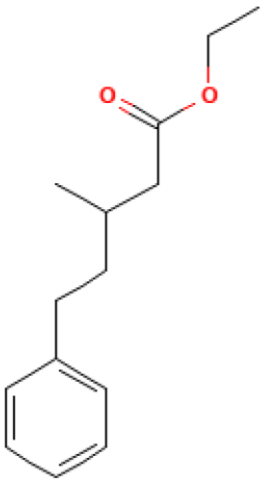
	methyl ester	5370266	
10.	4-Penten-1-ol, TMS derivative	10866616	
11.	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	22213879	
12.	Benzaldehyde, 3,5-dimethyl-	34225	
13.	Cyclohexanol, 2-(1-methylethyl)-	95331	

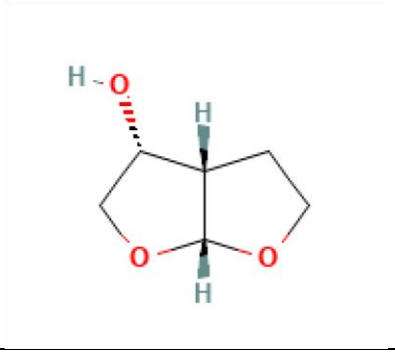
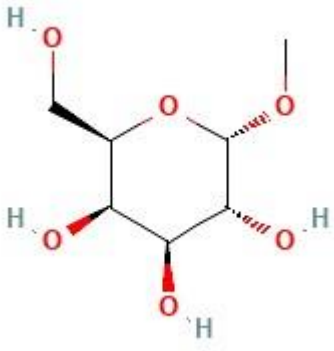
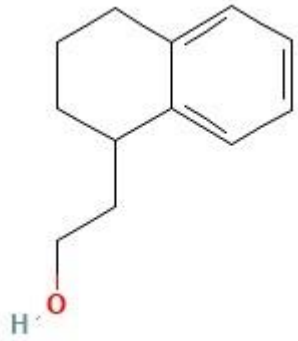
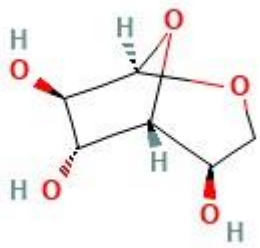
14.	Benzothiazole	7222	
15.	2-Amino-4,6-dimethoxypyrimidine	118946	
16.	Dodecane, 2,6,11-trimethyl-	35768	
17.	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl-	6329089	
18.	5-(Hydroxymethyl)-2-(dimethoxymethyl)furan	588127	

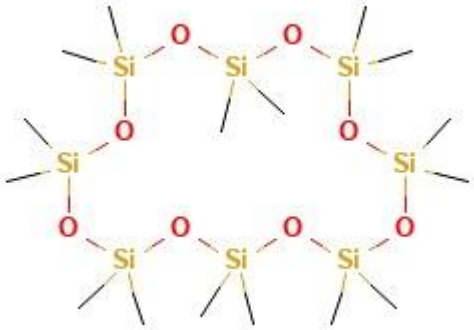
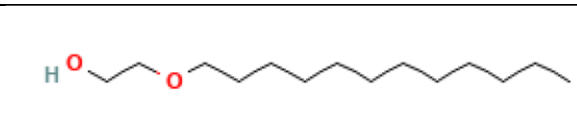
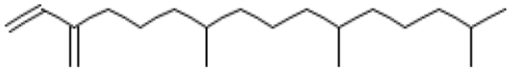
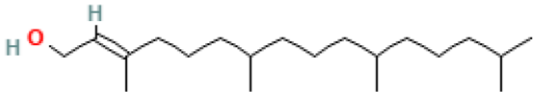
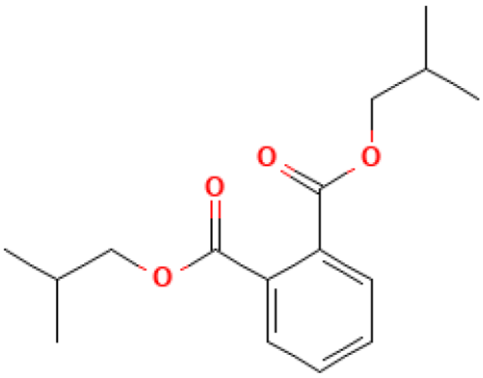
19.	.alpha.-L-Galactopyranoside, methyl 6-deoxy-	446578	
20.	2-Bromomethyl-2-phenyl[1,3]dioxolane	291574	
21.	6,6,8,8-Tetramethyl-2,5,7,9,12-pentaoxa-6,8-disilatridecane	91724015	
22.	2H-Pyran-3,4,5-triol, tetrahydro-2-methoxy-6-methyl-	242517	

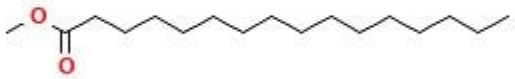
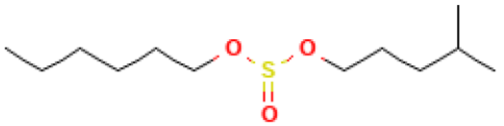
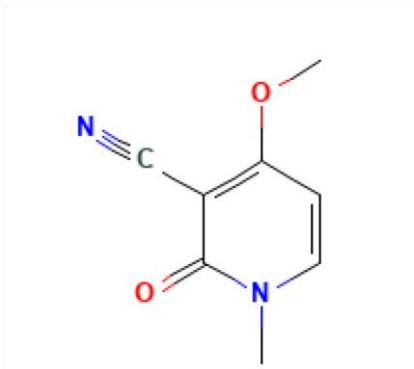
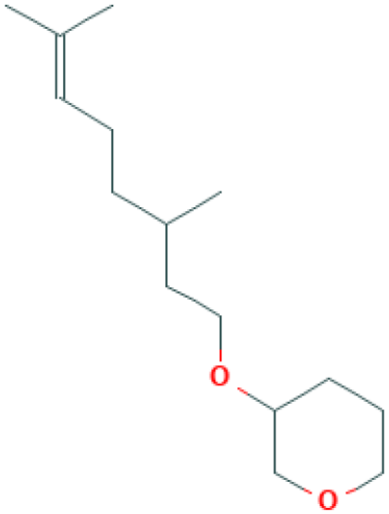
23.	3-Hydroxymethyl-4-(1hydroxy-2-methylprop-2enyl)toluene, TBDMS derivative	554606	
24.	1,6-Anhydro-.beta.-d-talopyranose	1777528	
25.	Propiolic acid, 3-phenyl-, methyl ester	138378	
26.	2-		

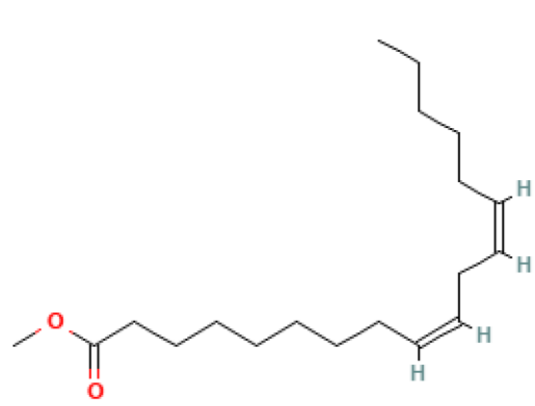
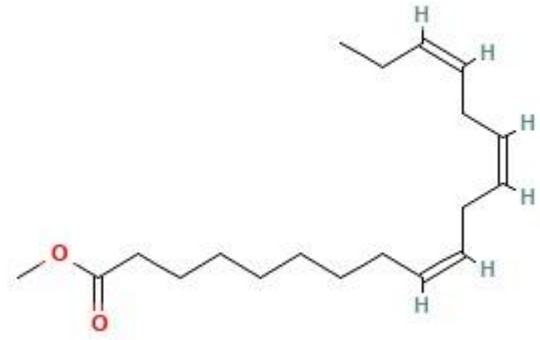
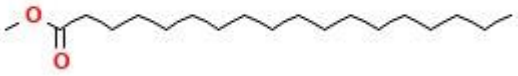
	(Isobutoxymethyl)oxirane	98155	
27.	1-Dodecanol	8193	
28.	Scyllo-Inositol, 1-C-methyl-	244581	
29.	2-Ethylthio-5-methylimidazoline	575741	

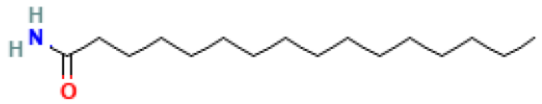
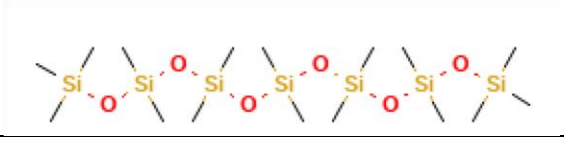
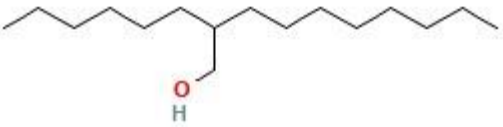
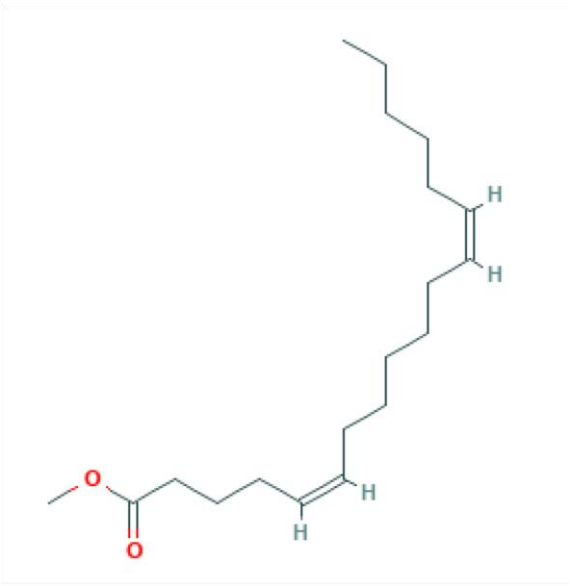
30.	D-Allose	439507	
31.	Phenol, 3,5-bis(1,1-dimethylethyl)-	70825	
32.	Dodecanoic acid, methyl ester	8139	
33.	Benzenepentanoic acid, .beta.-methyl-, ethyl ester	563065	
34.	(3R,3aS,6aR)-Hexahydrofuro[2,3-		

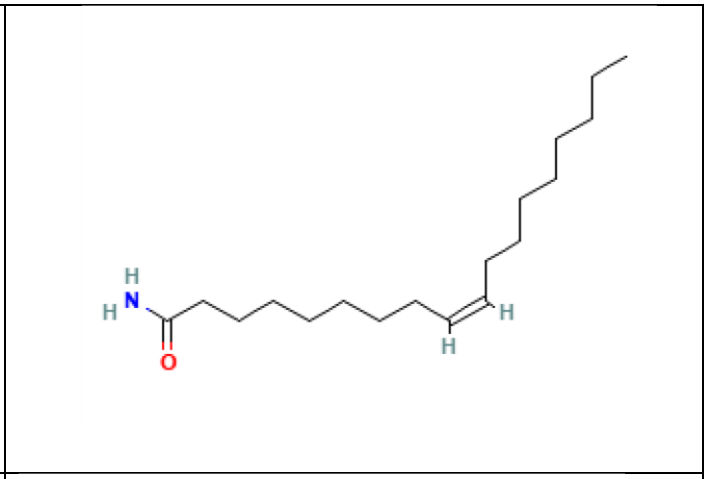
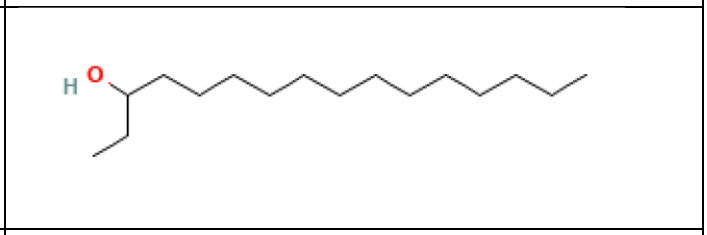
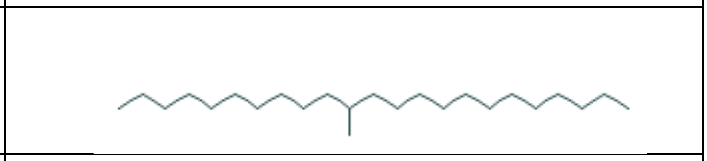
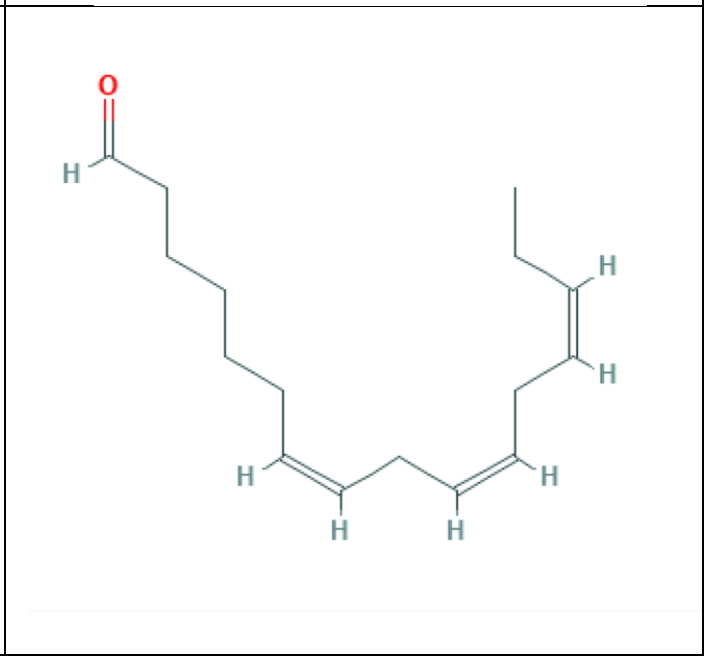
	b]furan-3-ol, TMS	9942150	
35.	.alpha.-D-Galactopyranoside, methyl	76935	
36.	2-(1,2,3,4-Tetrahydronaphthalen-1-yl)ethan-1-ol, TMS	98036	
37.	1,6-Anhydro-.beta.-D-glucofuranose	13037722	
38.	Cyclooctasiloxane,		

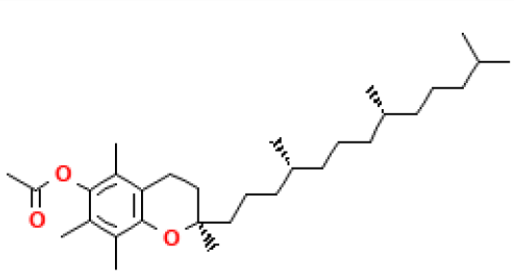
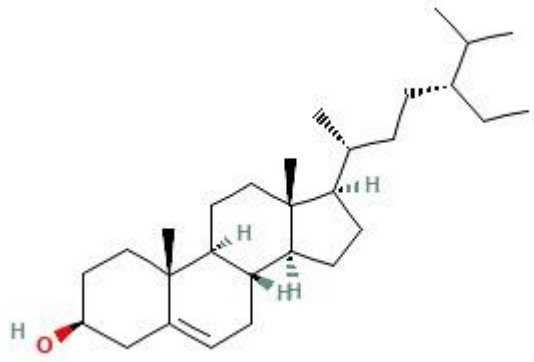
	hexadecamethyl-	11170	
39.	Ethanol, 2-(dodecyloxy)-	24750	
40.	Neophytadiene	10446	
41.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	5366244	
42.	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	6782	

43.	Hexadecanoic acid, methyl ester	8181	
44.	Sulfurous acid, isohexyl hexyl ester	6421370	
45.	Ricinine	10666	
46.	Tetrahydropyranyl ether of citronellol	558921	
47.	9,12-Octadecadienoic		

	acid, methyl ester	5284421	
48.	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	5319706	
49.	Phytol	5280435	
50.	Tetrapentacontane	521846	
51.	Methyl stearate	8201	

52.	Hexadecanamide	69421	
53.	Heptasiloxane, hexadecamethyl-	10912	
54.	1-Decanol, 2-hexyl-	95337	
55.	Methyl 5,12-octadecadienoate	12255536	

56.	9-Octadecenamide, (Z)-	5283387	
57.	3-Hexadecanol	79052	
58.	11-Methyltricosane	530326	
59.	cis,cis,cis-7,10,13-Hexadecatrienal	5367366	

60.	alpha-Tocopheryl acetate	86472	
61.	beta-Sitosterol	222284	

4.3 Thrombolytic Activity Test

Solution	Blank Tube Weight gm	1st clot+ Tube Weight gm	1st clot Weight gm	2nd clot+ Tube Weight gm	2nd clot Weight gm	Lysis Weight gm	% Of lysis
Standard	0.778	1.615	0.846	0.964	0.189	0.612	76%
Control	0.776	1.607	0.831	1.356	0.58	0.251	30.20%
Ricinus Communis L	0.779	1.628	0.849	0.974	0.195	0.654	77.03%

Figure 10: Thrombolytic Activity Test

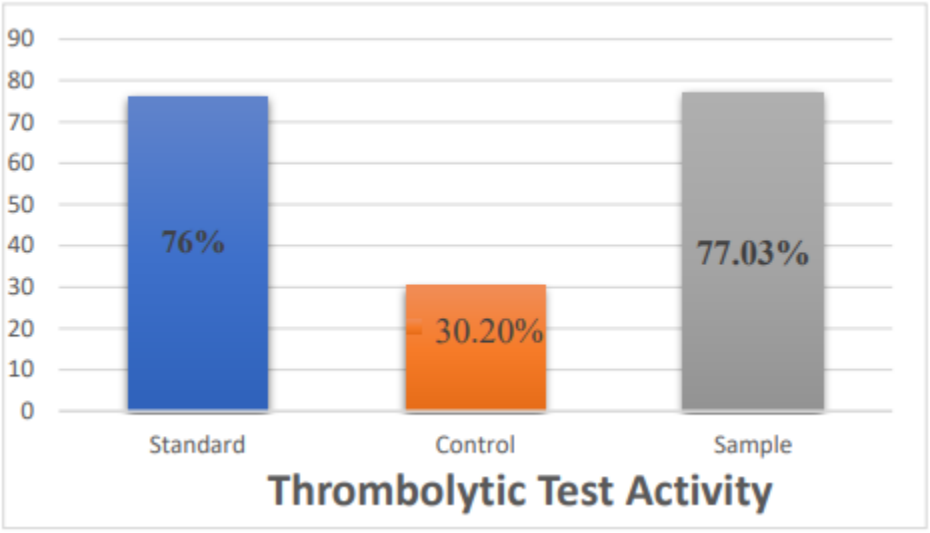


Figure 11: Graphical representation of Thrombolytic Activity Test

Discussion about Thrombolytic Activity Test

The standard was streptokinase, which had a 76% clot lysis rate in a previous study. In distilled water (Control), the clot lysis rate was 30.20 percent. In a study, methanol extract from *Ricinus Communis L* demonstrated 77.03% clot lysis. Comparing the *Ricinus Communis L* methanol extract to the standard (Streptokinase), the thrombolytic activity finding is positive.

4.4 Result of Antioxidant evaluation

IC50 for methanol extract of *Ricinus Communis L:*

Concentration (µg/ml)	% of Inhibition	IC_{50} (µg/ml)
500	78.93%	**17.937**
250	74.34%	
125	66.82%	
62.5	59.78%	
31.25	55.67%	

Table 6: IC50 for methanol extract of *Ricinus Communis L*

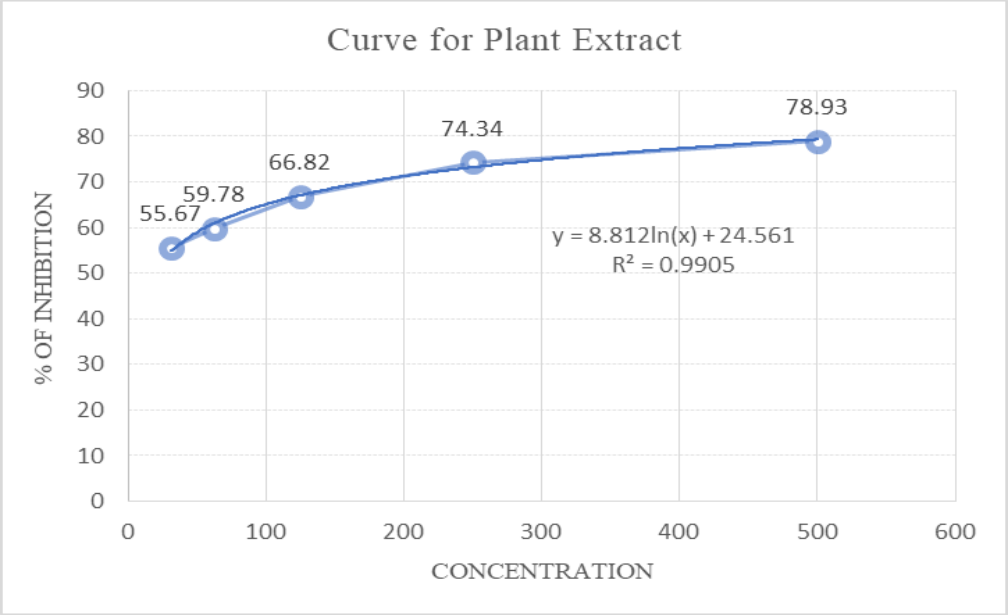


Figure 12: Graphical representation of % of Inhibition and concentration for Sample

IC50 for ascorbic acid **(standard):**

Concentration (µg/ml)	**% of Inhibition**	**IC_{50} (µg/ml)**
500	92.48%	**9.072**
250	85.79%	
125	78.57%	
62.5	69.33%	
31.25	63.74%	

Table 7: IC50 for ascorbic acid (standard)

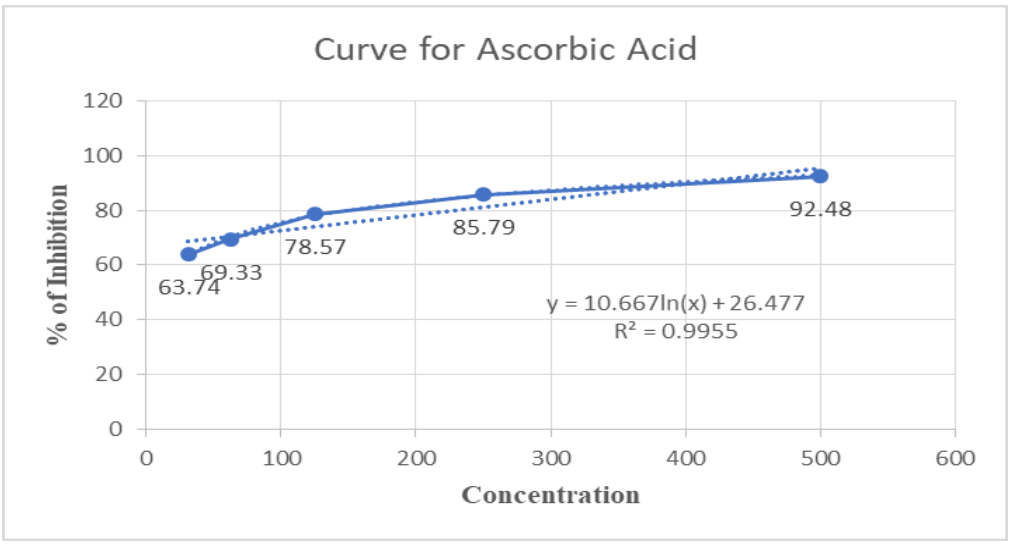


Figure 13: Graphical representation of % of Inhibition and concentration for Ascorbic Acid Test

Discussion on the antioxidant test

The IC50 value of the antioxidant activity was determined using the DPPH technique. The results of statistical testing for the antioxidant activity of ascorbic acid and the *Ricinus Communis L*. methanol extract are shown in the above figure. Lastly, we found that the solvent employed during the extraction process may have an effect on the material concentration in the extract. The present study illustrates the methanol extract of *Ricinus Communis L* antioxidant properties.

4.5 Determination of total flavonoid content (TFC)

Concentration	Concentration (µg/mL)	Mean Absorbance at 765 nm	SD
6.5	7.812	0.135	0.003
12.5	15.625	0.202	0.012
25	31.25	0.355	0.002
50	62.5	0.645	0.052
100	125	1.133	0.026
200	250	1.752	0.002
500	500	2.860	0.063

Table 8: Mean absorbance at different concentration (flavonoid)

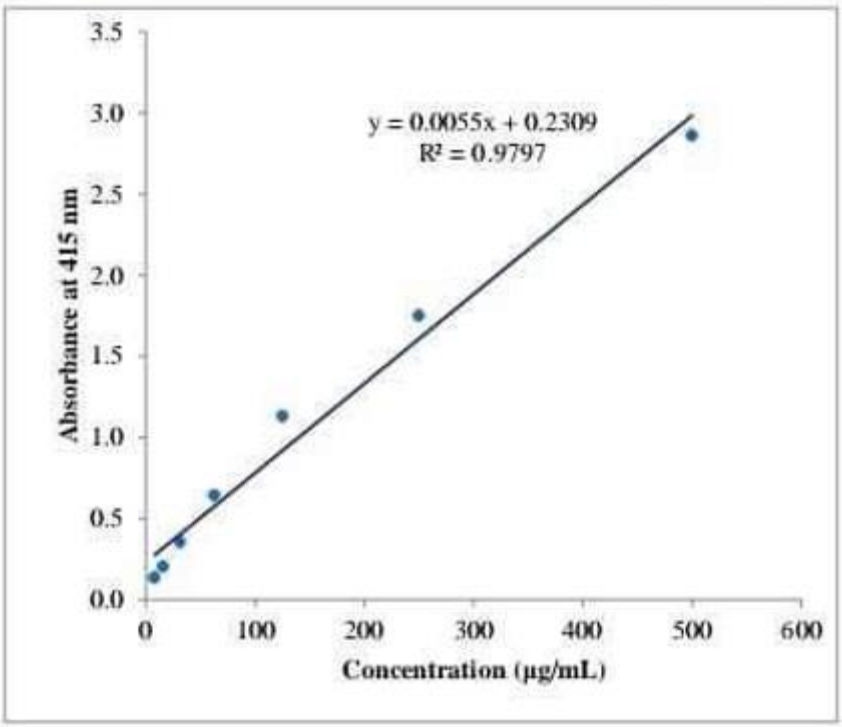


Figure 14: Standard curve for the different concentration (flavonoid)

Extract solution (µg/mL)	Mass of dry extract per mL m (g)	Absorbance	QE conc. c (µg/mL)	QE conc. c (mg/mL)	TFC as QE, $C = \frac{cxv}{m}$ (mg/g)	Mean (mg/g)	SD	Mean ± SD
200	0.0002	0.57	61.655	0.062	341.27	366.65	22.98	366.65 ± 22.98
200	0.0002	0.562	81.814	0.082	386.07			
200	0.0002	0.578	84.525	0.085	372.63			

Table 9: Mean SD value of flavonoid

4.6 Determination of total tannin content (TNC)

Concentration (µg/mL)	Mean Absorbance at 725 nm	SD
6.5	0.054	0.003
12.5	0.065	0.009
25	0.074	0.002
50	0.116	0.062
100	0.191	0.003
200	0.342	0.057
500	1.078	0.001

Table 10: Mean absorbance at different concentration (tannin)

Extract solution (µg/mL)	Mass of dry extract per mL m (g)	Absorbance	GAE conc. c (µg/mL)	GAE conc. c (mg/mL)	TTC as GAE, $C = \frac{cxv}{m}$ (mg/g)	Mean (mg/g)	SD	Mean ± SD
200	0.0002	0.056	21.476	0.021	127.38	128.01	6.21	128.01 ± 6.21
200	0.0002	0.058	22.429	0.022	122.14			
200	0.0002	0.059	22.905	0.023	134.52			

Table 11: Mean SD value of tannin

4.7 Determination of total phenol content (TPC)

Concentration (µg/mL)	Mean Absorbance at 765 nm	SD
7.812	0.038	0.003
15.625	0.084	0.005
31.25	0.142	0.012
62.5	0.572	0.021
125	0.968	0.127
250	1.684	0.096
500	2.928	0.069

Table 12: Mean absorbance at different concentration (Phenol)

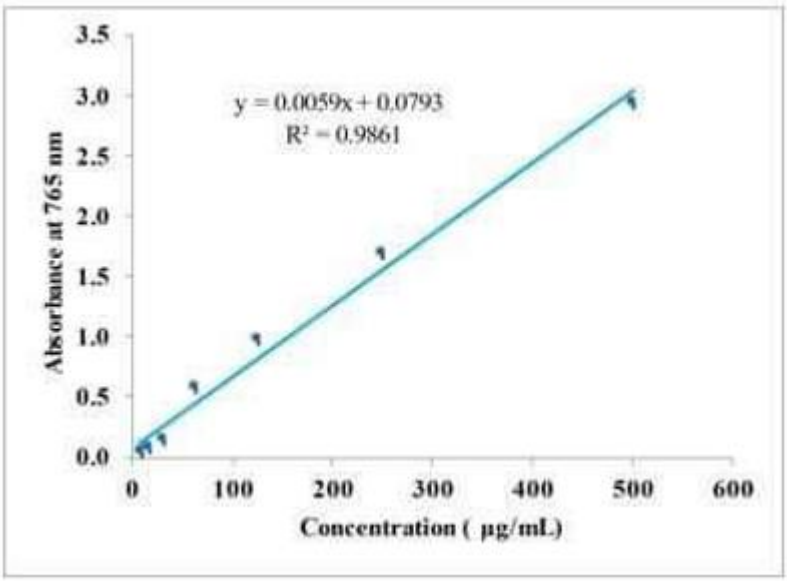


Figure 15: Standard curve for the different concentration (phenol)

Chapter five
Conclusion

5.1 Conclusion

In conclusion, the GC-MS profiling and evaluation of antioxidant and thrombolytic activity of methanolic leaf extract of *Ricinus communis L*. provide valuable insights into the potential therapeutic applications of this plant. The GC-MS analysis revealed the presence of various bioactive compounds, suggesting its pharmacological significance. The results of this study's research demonstrated that the methanol extract of *Ricinus communis L* contained organic components such as carbohydrates, alkaloids, glycosides, tannins, and diterpenes that may be implicated in pharmacological activity. Additionally, the results of the biological studies show that the drug has potential pharmacological effects, including **thrombolytic and antioxidant** qualities. However, further studies are warranted to elucidate its mechanisms of action and assess its safety profile for potential therapeutic use. Overall, this research contributes to the growing body of knowledge on medicinal plants and highlights the importance of harnessing natural resources for the development of novel pharmaceuticals.

Chapter six
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