Project on

# Phytochemical screening, GC-MS Profiling, Antioxidant, Thrombolytic, Analgesic and Antidiarrheal Activity Assessment of Ethanolic Extract of *Acmella oleracea:* An *in vitro* and *in vivo* approach.

A project report turned in to the Department of Pharmacy and faculty of Health and life Science at Daffodil International University as a requirement toward a Bachelor of Pharmacy (B. Pharm) degree.

**Submitted To**

Department of Pharmacy

Daffodil International University


**Submitted By**

ID: 203-29-404

Batch: 24th

Department of Pharmacy

Daffodil International University


**Date of submission:** October,2024

# *Approval*

I get great satisfaction in knowing that studies are being conducted on **Phytochemical screening, GC-MS Profiling, Antioxidant, Thrombolytic, Analgesic and Antidiarrheal Activity Assessment of Ethanolic Extract of *Acmella oleracea:* An *in vitro, in vivo* approach.**

The study that I completed and managed under my supervision is the basis for this project, which was submitted in with ID: 203-29-404 to the Department of Pharmacy at Daffodil International University. After completion, the exposition was acknowledged as partially meeting the requirements for a Bachelor of Pharmacy degree and accepted in terms of style and substance.

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**Board of Examiners**:

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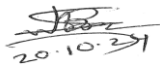
# *Declaration*

This research project, titled **" Phytochemical screening, GC-MS Profiling, Antioxidant, Thrombolytic, Analgesic and Antidiarrheal Activity Assessment of Ethanolic Extract of *Acmella oleracea:* An *in vitro, in vivo* approach**," is an original work conducted to fulfill the requirements for the Degree of Bachelor of Pharmacy (B. Pharm). This work has not been submitted elsewhere for consideration toward any other degree under the supervision of **-Mr. Subrato Kumar Barman** sir, **Senior Lecturer** in the Department of Pharmacy, Daffodil International University's Faculty of Health and Life Science, I successfully finished this assignment.

**Supervised By:**



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# *Acknowledgement*

In order to complete the requirements for the Bachelor of Pharmacy (B. Pharm), I must first give thanks to Allah, the creator, for giving me the opportunity to conduct research on the subject, the capacity to finish my dissertation for my bachelor's degree, and the last one, the willingness to compile the research paper's findings.

I am pleased and feel proud to express my deep appreciation, indebtedness and profound respect to my honorable supervisor sir **Mr. Subrato Kumar Barman**, Senior Lecturer, Department of Pharmacy at Daffodil International University, Faculty of Health and Life Science for his outstanding support, direction, constant supervision and support, counseling and continuous backup to carry out the research work as well as to prepare this dissertation.

I extend a hearty greeting to my respected sir Professor **Dr. Muniruddin Ahmed**, the chairman of the pharmacy department of Daffodil International University. I want to express my gratitude to everyone who helped me finish my research, write my dissertation, and put this project together in any way. Additionally, I would want to express my gratitude to all of the instructors at the Daffodil International University Pharmacy Department as well as other participants for their superb collaboration with us. I express my gratitude to the office personnel of Daffodil International University's Department of Pharmacy.
Lastly, I would want to express my gratitude to my parents and other family members for their constant encouragement and support during this effort.

Author

**Shibly Noman**

## *Dedication*

Dedicated to my beloved family and respected teacher.

# *Abstract*

*Acmella oleracea* commonly referred to as the toothache effect, has been shown to provide therapeutic benefits by researches. The purpose of the study was to perform phytochemical screening and specific compounds present in the ethanolic extract of *A. oleracea* (EAO). This study was also designed for performing in vitro antioxidant, thrombolytic and in vivo analgesic and antidiarrheal activity of EAO. Phytochemical screening was done by following the standard method. Compound profiling was executed by GC-MS analysis. The antioxidant potential of EAO was performed by DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay with determination of secondary metabolites (TPC, TFC, TTC). Thrombolytic activity was performed using an *in vitro* streptokinase assay. Castor oil-induced antidiarrheal assay in mice was performed for evaluation of antidiarrheal potential of EAO and acetic acid-induced writhing assay in mice was done for assessment of its analgesic activity. Numerous secondary metabolites, including alkaloids, flavonoids, phenols, tannins, saponins, and terpenoids, were found in EAO. GC-MS profiling revealed the presence of a total 84 compounds. TPC, TFC and TTC were 159.33 ± 6.89 mg GAE/g, 139.42 ± 4.50 mg QE/g and 124.11 ± 5.46 mg GAE/g, respectively. EAO showed comparative DPPH free radical scavenging potential with standard ascorbic acid. The calculated IC50 value was 62.01 µg/mL for EAO and 32.33 µg/mL for ascorbic acid. The thrombolytic potential of standard streptokinase and EAO were found 69.00 % and 43.87 %. Significant ($p$<0.05) analgesic and antidiarrheal activity of EAO were found in a dose-dependent fashion. EAO showed promising antioxidant, thrombolytic, antidiarrheal and analgesic potential. The findings of the study highlight the diverse array of phytochemicals present in *A. oleracea* as well as their potential pharmacological effects, including thrombolytic, analgesic, antioxidant, and antidiarrheal properties. More thorough study is needed to determine the fundamental processes by which the plant extract works and to evaluate its potential as therapy for different diseases.

Keywords: *Acmella oleracea*, phytochemical screening, GC-MS profiling, antioxidant activity, antidiarrheal activity, thrombolytic activity, medicinal plants

# Table of contents

# Chapter 01

# Introduction

# *Chapter: One*

# *Introduction*

## 1.1 Background [01]

The genus Acmella, part of the Asteraceae family, includes 30 species along with nine additional subspecies. Among these, one of the most notable is Acmella oleracea (L.) R.K. Jansen, easily recognized by its large, cylindrical discoid flower heads that exhibit a distinctive golden-yellow color with red tips. Acmella oleracea is an annual herb distributed globally, found in places like Sri Lanka, India, Nepal, China, Taiwan, Mexico, Bolivia, Brazil, and regions of Africa, America, and Asia. It is cultivated for various purposes, including horticulture, medicine, insecticides, and culinary use. Nutritionally, raw Acmella (commonly known as jambu) is rich in vital components. Studies show that raw Acmella leaves contain approximately 24.01% protein, 1.54% lipids, 63.38% carbohydrates, 62.61% fiber, and 10.92% ash (on a dry basis). The plant is also rich in minerals like calcium (Ca), magnesium (Mg), copper (Cu), and amino acids such as asparagine, glutamic acid, valine, and isoleucine. The primary alkamide found in Acmella, spilanthol, is responsible for the plant's distinctive sensory effects. In vitro studies have shown that Acmella oleracea possesses a variety of health benefits, including anti-inflammatory, antimicrobial, anesthetic, antipyretic, antioxidant, insecticidal, antiseptic, immune-boosting, anti-obesity, and anticancer properties. Over time, significant multidisciplinary research on Acmella oleracea has led to a rise in its use in commercial products, including personal care, healthcare, and culinary items. Often referred to as the "toothache plant," Acmella is well-known for its ability to stimulate saliva production (sialagogue) and its numbing and tingling effects. Other common names for Acmella oleracea L. include jambu, agrião do Pará, akmaella, spot flower, brede mafane, and paracress. The plant is particularly popular in Brazilian cuisine due to its unique anesthetic and spicy sensory qualities.

## 1.2 Chemical Processing routes for bulk drug [02]

The processing of APIs, or bulk medicines, varies based on the drug molecule and synthesis pathway. To manufacture bulk APIs, each medicine may require a unique set of chemical processes and purifying methods. I'll summarize bulk drug chemical processing approaches and provide a reference:

Synthesis Planning: Chemists plan a synthetic route to the desired API, considering reaction efficiency, yield, safety, and environmental impact. For synthesis, chemists can use biocatalysts, transition metal catalysis, and traditional organic chemistry processes.

Chemical Reactions: Bulk drug production includes gradual chemical reactions. The reactions may include functional group transformations, coupling reactions, cyclizations, and reductions. Optimizing reaction parameters like temperature, pressure, and catalysts maximizes yield and selectivity.

Purification: We remove impurities and byproducts from the synthesized API. Recrystallization, distillation, column or preparative HPLC, and filtration are purification methods.

Characterization: To verify the purified API's chemical identity and purity, we use NMR, MS, IR, and HPLC.

Formulation: we can formulate pure bulk drugs into tablets, capsules, injections, or creams.

### 1.3 Natural source of medicine [03]

Many studies have focused on plants as a natural source of medicine due to their abundance of bioactive chemicals. A lot of traditional herbal medicines and current pharmaceutical drugs come from plants. Below, you will find a summary and a link to additional resources:

Plants make many different secondary metabolites, such as alkaloids, flavonoids, terpenoids, phenolics, and glycosides. These all have different drug-like effects. You can separate these bioactive substances from leaves, stems, roots, flowers, seeds, and fruits, among other parts of the plant. Traditional herbal medicine systems, like Traditional Chinese Medicine (TCM), Ayurveda, and Indigenous medicine, have used plant-based medicines to treat a wide range of illnesses for a long time. Finding and making new drugs from natural sources has also been a big part of modern pharmaceutical studies. Many drugs used in medicine today, like aspirin, morphine, quinine, and paclitaxel, come from natural sources. Researchers have also studied many compounds produced by plants to explore their potential as medicines for illnesses such as cancer, heart disease, infections, and neurological problems.

### 1.4 Plant as source of bulk drugs [04]

Because plants are rich in bioactive chemicals, which are used as active pharmaceutical ingredients (APIs) in medicine formulations, people have long recognized plants as a dependable source of bulk drugs. A synopsis and a link to more resources are provided below:

Many medications used in modern medicine are derived from plants. The opium poppy yields morphine, quinine from the cinchona tree, Artemisia annua yields artemisinin, and the Pacific yew tree yields paclitaxel, among other compounds. Many secondary compounds with various chemical structures and medicinal applications are produced by plants. Alkaloids, phenolics, glycosides, terpenoids, and flavonoids are a few of them. These bioactive substances have been used by humans for hundreds of years due to their therapeutic properties, and plants employ them for a number of ecological reasons. Many medications have been discovered and developed by researchers using chemicals obtained from plants. Pharmaceutical scientists frequently conduct tests on plant products or extract pure chemicals from plants in order to find substances that may have medicinal use. After then, scientists can investigate these compounds in more detail to learn about their pharmacological characteristics, efficacy, and safety. Certain plants that are used to make bulk pharmaceuticals are grown or extracted on a big scale. For example, cultivate opium plants to produce opioids, or extract taxins from yew trees to produce anti-cancer medications.

### 1.5 Medicinal plants [5-8]

Since the beginning of human history, medicinal plants have been used for healing. Written documents, historical monuments, and even the first plant medicines provide strong evidence of

the long-standing human connection to the search for pharmaceuticals in nature. For thousands of years, plants have served as the cornerstone of elaborate traditional medical systems, and they continue to offer people novel avenues for self-healing. Even though some of the therapeutic benefits attributed to plants have been disproven, the empirical discoveries of thousands or even hundreds of years form the basis of medicinal plant therapy. Searching the natural world for potential chemotherapeutic drugs continues to be of interest. In global clinical trials, natural substances or their derivatives account for more than half of all medications. Among the total, higher plants account for at least 25%. Within the last four decades, at least a dozen potent drugs have been derived from flowers, including reserpine and other anti-hypertensive and tranquillizing alkaloids from Rauwolfia species, pilocarpine, which treats glaucoma and "dry mouth," which comes from a family of Citrus-derived South American trees (Podocarpus spp. ), two powerful anti-cancer agents from the Rosy Periwinkle (Catharanthus roseus), and laxatives from Cassia sp. It is necessary to establish certain general principles in order to investigate the antibacterial capabilities of plant extracts, essential oils, and the compounds extracted from them. It is crucial to establish common characteristics, such as the type of plant material, the techniques employed, the growth medium, and the microorganisms that are tested. Even while medicinal plants have been used for thousands of years to prevent and treat a wide range of disorders, the usage of herbal medicines has increased significantly in the last fifteen years in particular. At the start of the new century, the U.S.P. and N.F. officially recognized about 170 herbal remedies. According to a 1993 estimate by the Director of WHO Traditional Medicine, 80% of the world's population predominantly uses traditional medicine, which is based primarily on plants, for their basic healthcare needs.

### 1.6 History of medicinal plants [9-12]

Medicines were created by man as a result of his serendipitous discovery of pharmaceuticals in nature. Plants have been used by humans daily for as long as there have been humans, dating back to the early days of human civilization. Many countries view herbs or pharmaceuticals as the primary means of treatment. The use of therapeutic herbs was documented by Indo-Aryans in the Rig-Veda between 4500 and 1600 BC. Bangladesh, an Indian nation, is home to an extensive range of plants. In Bangladesh, there are around 2,000 medicinal plants, 449 of which are listed. Bangladesh has many medicinal plants that are threatened by habitat loss, overharvesting, climate change, and unsustainable harvesting methods. The natural environments in which these plants occur are becoming more and more degraded due to deforestation, urbanization, and agricultural growth. In Bangladesh, efforts are being made to grow therapeutic plants to lessen the strain on natural populations. Through various programs and activities, organizations, governmental agencies, and non-governmental organizations are encouraging the growth of medicinal plants. To evaluate the state of medicinal plants in Bangladesh and to record their quantity, distribution, and conservation requirements, numerous surveys and research have been carried out. These investigations offer important new perspectives on the nation's medicinal plant resources as of right now.

## 1.7 Importance of medicinal plant materials [13]

The pharmacological and therapeutic properties of medicinal plant components are valued in both traditional and modern medicine. Medicinal plants have been used for a variety of ailments by many cultures. Medicinal plants are used in Indigenous medicine, Ayurveda, and Traditional Chinese Medicine (TCM). Source of Bioactive Compound: Medicinal plants contain bioactive compounds such as glycosides, phenolics, terpenoids, flavonoids, and alkaloids. These substances function as analgesics, anti-inflammatory, antioxidants, and anti-cancer agents. Medicinal plants provide lead compounds for medication research and development. Many contemporary medications come from plant sources. Researchers in pharmaceuticals analyze plant extracts or isolate pure compounds in quest of medicinal medicines. Natural and comprehensive health is promoted by complementary and alternative medicine through the use of medicinal plant components. Typical complementary therapies include plant extracts, herbal supplements, and phytotherapy. Healthcare sustainability is promoted by medicinal plant treatments, which are affordable, widely available, and culturally appropriate. Cultivating, gathering, and processing medicinal plants helps to sustain rural lives and economic growth in many places.

## 1.8 Economic importance of medicinal plants [14]

Because medicinal plants are used in so many different fields—traditional medicine, pharmaceuticals, dietary supplements, cosmetics, and other industries—they are commercially valuable. What follows is a brief synopsis with a reference for more information:

1. Traditional medical: For thousands of years, medicinal plants have been an integral part of traditional medical systems all across the world. They still play a vital role in primary healthcare in many places, especially in developing nations where access to modern medicine may be limited.

2. Medicinal plants are particularly beneficial to the pharmaceutical industry since they give important lead chemicals for the research and discovery of new medications. Aspirin, morphine, quinine, and artemisinin are just a few of the pharmaceutical drugs that are developed using natural substances derived from plants. Medicinal plant components are used by the pharmaceutical industry to create active pharmaceutical ingredients (APIs), which are then incorporated into medicine formulations.

3. The growing consumer desire for natural and alternative healthcare choices is driving a major expansion of the global market for herbal items and dietary supplements. Manufacturers employ therapeutic plant resources to create a wide range of herbal remedies, nutritional supplements, and nutraceuticals, marketing them for a number of health benefits.

4. Cosmetics and Personal Care: Medicinal plant extracts and essential oils are commonly used in cosmetics and personal care products because of their alleged therapeutic and skincare

benefits. Plant-based ingredients such as aloe vera, tea tree oil, and chamomile are frequently found in skincare, haircare, and aromatherapy products.

5. Because of their economic value, the horticultural and agricultural sectors grow some therapeutic plants. In the agricultural and horticultural industries, the production, gathering, and processing of medicinal plants are important activities that create jobs and revenue for local farmers and communities.

### 1.9 Medicinal plant use scenario in Bangladesh [15]

Herbal remedies have long been employed in traditional medicinal systems, such as Ayurveda, Unani, and folk medicine in Bangladesh. Particularly in remote locations without access to contemporary facilities, these therapeutic plants are indispensable to basic treatment. The scenario of medicinal plant use in Bangladesh is outlined below, along with a source for more information:

Herbal remedies are an integral part of traditional Bangladeshi medicine. Herbal remedies are utilized by traditional healers such as Kabirajes, Hakims, and Vaidyas to treat ailments related to the digestive, respiratory, skin, and reproductive systems. Medicinal plants play a major role in Bangladeshi culture, rituals, and beliefs. Numerous medicinal plants that are employed in rites and ceremonies are passed down through traditional knowledge systems. In Bangladesh, plant-based treatments like Ayurveda and phytotherapy are widely used. The Indian branch of Ayurveda medicine, which emphasizes natural healing and holistic health, has an influence on traditional medicine in Bangladesh. Studies by scientists and traditional healers on the medicinal plants of Bangladesh are being recorded. Plant species are cataloged, bioactive compounds are identified, and medicinal plant pharmacology is evaluated by the Bangladesh National Herbarium and BCSIR. Bangladesh has expressed a great deal of interest in combining modern healthcare with traditional medicine. Government initiatives certify and accredit conventional therapies while preparing traditional healers to deliver high-quality, safe healthcare.

### 1.10 Traditional medicine [16]

Traditional medicine, which includes herbal remedies and native healing techniques, has long been used by numerous communities. Below are a synopsis and citation: Traditional medicine is the body of information, abilities, and procedures used to prevent, diagnose, treat, and improve physical and mental ailments. It is based on the theories, beliefs, and experiences of certain cultures and can be explained or not. Ayurvedic, herbal, TCM, Unani, indigenous healing practices, spiritual therapies, mind-body interventions, and acupuncture are all considered forms of traditional medicine. These methods typically make use of natural resources including plants, animals, minerals, and other elements. Traditional medicine is strongly ingrained in the traditions of many cultures and religions. It employs generational healing techniques, ceremonies, and rituals. Herbalists, shamans, and other traditional healers play a critical role in community health care. Many nations still value and practice traditional medicine, particularly those with strong cultural traditions or limited access to modern medical care. In order to enhance overall health

and lessen healthcare disparities, traditional medicine and contemporary healthcare systems are combining more and more.

### 1.10.1 Definition of traditional medicine [17]

Conventional medicine includes the body of knowledge, skills, and practices that are developed from culturally particular theories, beliefs, and experiences. Individuals use it to prevent, diagnose, treat, or otherwise deal with physical and mental health issues.

### 1.10.2 Ayurveda

The three doshas (energies) of Vata, Pitta, and Kapha are the basis of Ayurveda. The physiological processes that take place within the body are regulated by these doshas. It's said that one can attain health when these doshas are in balance with each other; when they're out of balance, illnesses may arise. The core of Ayurvedic medicines, which aim to restore equilibrium among the doshas, is prakti, the term used to characterize distinct constitutional sorts.

### 1.10.3 Unani medicine

The core of Unani medicine is the theory of the four humors. The four elements (earth, water, fire, and air) and the four qualities (hot, cold, wet, and dry) are corresponding to these four humors. It is thought that health is kept when the humors are in balance; when they are out of balance, diseases develop. Curing humoral imbalances by nutrition, lifestyle, herbal medicine, and other therapeutic methods is the main objective of Unani treatment.

### 1.10.4 Traditional Chinese Medicine (TCM)

Traditional Chinese medicine (TCM) is based on a number of core principles. These concepts include blood and Qi, the Five Elements theory, the theory of Zang-Fu organs, and the philosophy of Yin-Yang. It is thought that the body's vital energy, or Qi, is maintained when it flows freely along meridians, or energy channels. On the other hand, imbalances or blockages in the meridians are thought to be the source of illnesses. Treatments based on Traditional Chinese Medicine (TCM) strive to restore health by balancing Yin and Yang, nourishing blood, and controlling Qi.

### 1.10.5 Traditional Japanese Medicine (Kampo) [18]

The use of traditional herbal remedies made from plants, minerals, and animal products is the main focus of kampo medicine, a vital aspect of Traditional Japanese Medicine (TJM). Since each patient has a different pattern of disharmony, or Sho, the Japanese government usually standardizes and regulates Kampo formulations and recommends them to patients.

## 1.11 Contribution of plants in modern drugs [19]

Bioactive substances obtained from plants are essential to modern medicine. Many of the medications used in modern medicine are derived from natural chemicals that are taken from plants. Numerous secondary metabolites with varying pharmacological characteristics, including glycosides, alkaloids, flavonoids, terpenoids, and phenolics, are produced by plants. In order to identify compounds with potential applications in medicine, pharmaceutical research often involves the analysis of plant extracts or the separation of pure chemicals from plants.

## 1.12 Drug development process from medicinal plants [20]

Medicinal plant drug development includes finding, isolating, characterizing, doing preclinical research, conducting clinical trials, and getting regulatory approval. Further details are given in this summary and reference:
1. Finding: Drug discovery begins with the identification of medicinal plants having established pharmacological activity or traditional uses. Lead compounds are frequently found by ethnobotanical surveys, literature reviews, and biological activity assessment of plant extracts.

2. Isolation and Characterization: The active ingredients in medicinal plant extracts are separated by chromatography, extraction, and purification. The separated compounds are examined using analytical techniques such as NMR, MS, and IR to determine their chemical structures and purity.

3.Preclinical Studies: In preclinical research, isolated substances are evaluated for their pharmacological properties, safety, and efficacy in animal models. In order to guide future development, this study evaluates toxicity, pharmacokinetics, pharmacodynamics, and mechanism of action.
4. Clinical Trials: Individual drugs or plant extracts may go through human clinical trials if preclinical research is positive. Three stages comprise clinical studies:
Phase 1: In a small group of healthy volunteers, safety, dosage, and pharmacokinetics are tested.
Phase 2: A larger population of target ailment patients is evaluated for effectiveness and safety.
Phase 3: Verifies safety, effectiveness, and the right dosage in a more extensive multicenter study.
5. Regulatory Approval: After clinical trials, regulatory agencies such as the FDA or EMA review the information and provide marketing license to a drug if its advantages outweigh its disadvantages.

## 1.13 *Acmella Oleracea* [21]

Acmella is a flowering herb species in Asteraceae o Compositae's family. Its native distribution is unclear, but it is likely derived from Brazil, where it is called jambu. It is grown as an ornamental or medicinal plant and attracts fireflies when in bloom. A small, erect plant, it grows quickly and bears gold or red inflorescences. It is frost-sensitive but perennial in warmer

Climates. *acmella* flower is also known as the “Toothache plant”. In the Northern parts of Brazil, this flower is added to vegetables during cooking. In many regions of the world, the entire plant is employed as a medical treatment. Even in modern dentistry, pain is one of the most prevalent symptoms. Pain and its relief were the subject of numerous studies. The analgesic qualities of acmella, known by its traditional name Jambu, have been utilized for millennia to relieve mouth discomfort. In Brazil's northern region, oral and throat ailments are treated with the leaves and flowers as a common household medication. When used to treat toothaches, it enhances the effects of local anesthetic.

### 1.14 General botanical information about *Acmella Oleracea* [21]

A blooming plant species in the Asteraceae family is called Acmella oleracea. Common names for this plant include buzz buttons, tingflowers, paracress, Szechuan buttons, electric daisy, and toothache plant. Its original range is unknown, although it most likely originated from a Brazilian species of Acmella.

#### 1.14.1 Scientific Classification [22]

Kingdom: Plantae (Plants)

Clade: Tracheophytes (Vascular plants)

Clade: Angiosperms (Flowering plants)

Clade: Eudicots

Clade: Asteroids

Order: Asterales

Family: Asteraceae (Composite) - Aster family

Genus: *Acmella*

Species: *Acmella Oleracea*

### 1.14.2 Local common name [23]

Acmella oleracea is a species of flowering herb in the family Asteraceae. Common names include toothache plant, Szechuan buttons, paracress, jambu, buzz buttons, tingflowers and electric daisy.

### 1.14.3 Plant description [24-25]

The genus Acmella which belongs to the family Asteracea comprises 30 species and nine additional infraspecific taxa.One of the most distinguished members of the genus is Acmella oleracea (L.) R.K.Jansen due to its large cylindrical discoid capitula which owe aunique golden yellow color with the red tip. Acmella oleracea (R.K. Jansen) is an annual herb that is occurring around the world including Sri Lanta, India, Nepal, China, Taiwan, Mexico, Bolivia, Brazil and regions such as Africa, America, and Asia. It has been widely cultivated for horticultural, medicinal, insecticidal, and culinary purposes. Raw Acmella (jambu) have substantial amounts of nutrients.Recent studies revealed that raw Acmella leaves are comprised with approximately 24.01% of protein, 1.54% of lipid, 63.38% carbohydrate, 62.61% fiber,10.92% of ash (dry basis), high amount of some mineral such as Ca,Mg and Cu, and amino acids such as asparagine, glutamic acid, valine and isoleucine. There have been remarkable promote in Acmella oleracea herb by multidisciplinary studies, and an intensified number of commercial products have appeared in the market as personal care products, health care products, and for culinary use over years. Acmella is generally called as “toothache plant” because of its ability to stimulate saliva production (sialagogue), numbing and tingling properties.The other common names for Acmella oleracea L.include jambu, agrião do pará, akmaella, spot flower, brede mafane and paracress.This plant is popular among Brazilian (jambu) dishes due to their sensorial anesthetic and spicy properties.There is some doubt in the literature over the name of the genus and species of Acmella plant.The monographs on Acmella mentioned of false synonyms for A oleracea that appear on various websites. Acmella oleracea flower pod is generally 3.5–0.5mm in diameter, occasionally two per capitulum, apex acute; palea stramineous, often with apurplered tinged when young but other species are having apod without ared tip. The genus is attributed to anumber of medicinal, antimicrobial, larvicidal, and insecticidal properties due to the presence of bioactive compounds, one of them is isobutyl amides, which is used in folk medicine for the treatment of several disorders including stomatitis and colds. However, there is aserious constraint of recurrent availability of the material in mass scale which in turn is needed for the extraction of secondary metabolites.Moreover, there is a complete nutritional composition analysis for each part of the plant. Over the past few decades, many products have been developed by extracting the active ingredient (spilanthol) in the fields of pharmacological products, cosmetics, and food.

Figure 01: *Acmella Oleracea* plant

### 1.14.4 Global distribution [26-27]

The "toothache plant," Acmella Oleracea. This flower is cooked with vegetables in the northern regions of Brazil. Around the world, the entire plant is used as a medicinal cure. One of the most prevalent symptoms in modern dentistry is pain. Numerous studies examined pain and its management. Because of its analgesic qualities, acmella, also known by its traditional name, jambu, has been used for generations to relieve mouth discomfort. In northern Brazil, the leaves and flowers are used as a common folk medication to cure throat and oral ailments. In order to relieve the toothache, it encourages local anesthetic action.

**Asia**: The Philippines, Malaysia, Indonesia, Bangladesh, Burma, Thailand, Vietnam, and China are home to Acmella oleracea.

**Africa:** South Africa, Kenya, Tanzania, Uganda, Ethiopia, Nigeria, and Ghana are home to it.

**Oceania:** Australia, Papua New Guinea, Fiji, and Samoa were all colonized by Acmella oleracea.

**Americas:** Acmella oleracea is indigenous to Central and South America, but it has spread to the Caribbean, Central America, and the United States, particularly Florida and Hawaii.

**Pacific Islands:** Hawaii, Guam, and the Marshall Islands are affected.

### 1.15 Preview of Acmella Oleracea [29]

1. **Sesquiterpenes:** Acmella Oleracea includes terpenoids that are anti-inflammatory and antioxidant.

2. **Flavonoids:** Flavonoids are polyphenolic compounds that have antioxidant, anti-inflammatory, and anticancer properties. Quercetin, kaempferol, and rutin are all found in Acmella Oleracea.

3. **Phenolic Compound**: Acmella Oleracea has high levels of phenolic acids and tannins. Their antioxidant activity boosts the plant's medicinal benefits.

4. **Alkaloids:** Acmella Oleracea contains nitrogenous alkaloids that have antibacterial and analgesic properties.

5. **Saponins:** Acmella Oleracea includes saponins, which are foamy glycosides. They have both anti-inflammatory and immunomodulatory effects.

6. **Triterpenoids**: Acmella Oleracea includes triterpenoids that are anti-inflammatory, anti-cancer, and antibacterial.

7. **Steroids:** Acmella Oleracea may produce pharmacological effects similar to steroids.

### 1.16 Principle of antioxidant activity [32-33]

Antioxidants are defined as any substance that can postpone or prevent oxidative damage caused by free radicals. The antioxidant prevents free radicals from causing oxidative damage through a variety of processes. Antioxidant sources are numerous in nature and can be found in the daily diet, including fruit, vegetables, seeds, nuts, leaves, roots, and bark. Polyphenols, vitamins, and carotenoids are among the key substances identified as antioxidants. Antioxidants are vital for food preservation because they inhibit oxidation. They are also an important component of many dietary supplements, nutraceuticals, and functional food ingredients that enhance health. To assess antioxidant activity, a variety of assays can be performed, including metal chelation, hydrogen atom transfer (HAT), single electron transfer (ET), reducing power, and others. It is critical to understand the underlying workings, benefits, and limitations of the measurement assays in order to select the best approach or procedures for accurately assessing antioxidant

potential in the intended applications. This study provides a summary of current and wide methodologies for evaluating antioxidant activity, including the chemistry involved.

### 1.16.1 Antioxidant effects of natural bioactive compounds [34-39]

Reactive nitrogen species (RNS) and reactive oxygen species (ROS) can be beneficial or harmful to biological systems. ROS has favorable effects on various cellular signaling pathways as well as cellular defenses against pathogenic diseases. High quantities of ROS can disrupt biomolecules such as lipids, proteins, and nucleic acids. Antioxidant enzymes and non-enzymatic antioxidants are both antioxidants that counteract the harmful effects of reactive oxygen species (ROS). Several research have shown that various plants have an abundance of antioxidants. Antioxidants include vitamins A, C, and E, as well as phenolic compounds found in plants such as tannins, lignins, and flavonoids. Because of their high nutritional value and therapeutic properties, eating fruits and vegetables has been linked to a variety of health benefits. Antioxidants regulate and limit oxidative damage in food by postponing or avoiding oxidation caused by reactive oxygen species (ROS), hence extending its shelf life and quality. Ascorbic acid, beta-carotene, and numerous phenolics all have key roles in reducing inflammation, slowing the beginning of aging, and avoiding some types of cancer. Throughout the world, several institutions and healthcare systems have urged individuals to eat more fruits and vegetables.

### 1.16.2 Antioxidant Activity [40]

Several substances were isolated after fractionating the ethanol extract of the Acmella Oleracea plant, including flavonoids, sesquiterpenes, phenolic compounds, alkaloids, saponins, triterpenoids, and steroids. These chemicals were obtained by fractional chromatography. The free radical scavenging assay known as DPPH [1,1-diphenyl-2-picrylhydrazyl] is used to demonstrate the presence of these compounds. According to the study's findings, the flavonoids quercetin, kaempferol, and rutin demonstrated the highest levels of antioxidant activity.

## 1.7 Thrombolytic activity [41]

Thrombolytic activity is the ability of certain chemicals, typically drugs, to dissolve or disintegrate blood clots. These compounds are referred to as thrombolytics or fibrinolytics. Blood clots, also known as thrombi, can form inside blood vessels due to a variety of causes. These diseases include atherosclerosis, deep vein thrombosis (DVT), and atrial fibrillation. Dissolving or eliminating these clots can block blood flow and lead to serious problems such as heart attacks, strokes, or pulmonary emboli. Thrombolytic drugs activate the fibrinolytic system, which dissolves blood clots. Tissue plasminogen activator is one of the most often used thrombolytic drugs. It can convert plasminogen into plasmin, an enzyme that degrades fibrin, the protein meshwork that makes up blood clots.

## 1.18 Antidiarrheal Activity [42]

Diarrhea is defined by frequent bowel movements, moist stool, and stomach discomfort. It is currently the greatest cause of malnutrition and mortality among children in impoverished nations throughout the world. Oral rehydration therapy and chemotherapy are both appropriate therapeutic treatments for diarrhea. Medicinal herbs contribute significantly to the creation and improvement of contemporary studies on anti-diarrheal properties of compounds. In its Diarrheal Disease Control Program, the World Health Organization (WHO) places a special focus on the use of traditional folkloric medicine in the prevention and treatment of diarrhea. Traditional healers commonly utilize a variety of herbs with anti-diarrheal qualities. However, the efficacy of many of these anti-diarrheal traditional treatments has not been clinically proven. The study's goal was to assess the anti-diarrheal properties of an ethanolic extract from Acmella Oleracea leaves.

### 1.19 Analgesic Activity [61]

The central analgesic activity was assessed using the writhing technique, with induced Acetic acid serving as a positive control. This approach measures changes in test animal sensitivity as a result of medication analgesic action. A continual peritonial injection was given to the mice, which functions as a pain stimuli. When the stimulation surpasses the threshold, the rat writhes quickly. Counts obtained by writhing mice. This response time is prolonged by an analgesic substance. This test discriminated between centrally acting acetic acid-like analgesics and non-opiate analgesics.

# *Chapter: Two*

# *Purpose of the study*

## 2.1 purpose of the study

To further understand the chemical composition and bioactivities of Acmella Oleracea ethanolic extract, we apply phytochemical screening, GC-MS profiling, and antioxidant, antidiarrheal, analgesic, and thrombolytic activity. Each component is broken out separately. This includes identifying phytochemicals such as alkaloids, flavonoids, tannins, saponins, and so on in plant extract. These compounds may have biological effects that increase the plant's medicinal properties. Gas chromatography-mass spectrometry (GC-MS) is a sophisticated technology for analysing a sample's complicated components. Researchers can better comprehend the chemical makeup and pharmacological effects of an extract by identifying its elements. Antioxidants shield cells from oxidative stress by neutralizing free radicals. The plant extract's antioxidant properties can be used to combat oxidative stress-related illnesses and enhance health. Antidiarrheal medicine is used to treat acute diarrhea (such as traveler's diarrhea). It works by reducing the movement of the intestines. This reduces the amount of bowel motions and leaves the feces less liquid. The study's goal was to assess the anti-diarrheal properties of an ethanolic extract from Acmella Oleracea leaves.

The purpose of this study was to assess the analgesic efficacy of an ethanolic extract of Acmella Oleracea leaves.

Blood clots are dissolved by a substance having thrombolytic activity. The thrombolytic action of the plant extract may aid in the prevention or treatment of blood clot-related conditions such as deep vein thrombosis and myocardial infarction.

These constituents of Acmella Oleracea ethanolic extract can be studied to identify its therapeutic potential as well as healthcare and pharmaceutical uses.

# *Chapter: Three*

# *Literature Review*

### 3.1 Biological activities of *Acmella Oleracea*

According to phytochemical investigations, Acmella Oleracea principal components are alkaloids and their derivatives. These compounds have been shown to exhibit a variety of pharmacological activity, including antioxidant, analgesic, antidiarrheal, and thrombolytic properties.

#### 3.1.1 Antioxidants Activity [50-53]

Aerobic metabolic processes can create reactive oxygen species (ROS) that cause cell damage and death. Antioxidants reduce oxidative stress in cells by eliminating free radicals. Many plant-derived compounds have free radical scavenging activity. In the ferric-reducing antioxidant potential (FRAP) assay, the ethanolic extract of *Acmella Oleracea* exhibited greater antioxidant activity. The FRAP test uses antioxidant phytochemicals to convert iron from ferric ($Fe^{3+}$) to ferrous ($Fe^{2+}$) form. Furthermore, *Acmella Oleracea's* phenolic compounds outperform L-ascorbic acid in the removal of 2,2-diphenyl-1-picrylhydrazyl (2,2-DPPH) radicals (Table 1).
Phenolics work as antioxidants by transferring the hydrogen (H) atom from the hydroxyl (OH) group to the ROO• radical transport chain. *Acmella Oleracea's* ethanolic extract exhibited high antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl (1,1-DPPH). This is possibly because it contains phenolic chemicals.

#### 3.1.2 Thrombolytic Activity [54-57]

To measure thrombolytic activity, we tested two different concentrations of the standard and two different concentrations of the extract. Clot lysis rates for extract (200mg) and extract (100mg) were 86.87% and 84.15%, respectively, when compared to the clot lysis rates of conventional streptokinase (30,000 IU) and streptokinase (15,000 IU), which are 87.3 percent and 82.1 percent, respectively. The control group shows a relatively low degree of lysis, ranging from 2.22% to 3.30%. The p-value was less than 0.05, indicating statistical significance at $p < 0.00002$. We proved beyond a reasonable doubt that the extract had significant thrombolytic activity since the extract solution displayed substantial clot lysis when compared to the standard.

#### 3.1.3 Analgesic Activity [58]

*Acmella oleracea* (L.) shows great potential in pain relief thanks to its anti-inflammatory, antioxidant, and pain-relieving properties. These benefits are mainly due to its bioactive compounds, such as phytosterols, phenolic compounds, and N-alkylamides (particularly spilanthol), with spilanthol recognized for its anesthetic effects.

Studies reveal that Acmella's anti-inflammatory action works by inhibiting crucial inflammatory mediators like iNOS, COX-2, IL-6, IL-1β, and TNF-α, controlled by NF-κB. Its analgesic effects

come from suppressing prostaglandin production and activating the opioidergic, serotoninergic, and GABAergic systems, as well as blocking voltage-gated sodium channels.

Overall, *Acmella oleracea* presents itself as a promising natural remedy for pain management, especially in long-term conditions.

### 3.1.4 Antidiarrheal activity [59]

Diarrheais characterized by increased frequency of bowel movement, wet stool and abdominal pain (Ezekwesili *et al.*, 2004). It is a leading cause of malnutrition and death among children in the developing countries of the world today. Appropriate clinical management of diarrhea includes oral rehydrate therapy and chemotherapy. Medicinal plants play a key role in the development and advancement of modern studies on anti-diarrheal activities of substances. The World Health Organization (WHO) in its Diarrheal Disease Control Program has given a special emphasis on the use of traditional folklore medicine in the control and management of diarrhea.
A wide range of plants with anti-diarrheal properties is widely used by traditional healers. However, the effectiveness of many of these anti-diarrheal traditional medicines has not been scientifically evaluated.
The aim of the study was to evaluate the ethanolic extract of plant of *Acmella Oleracea* as anti-diarrheal agents.

# *Chapter: Four*

# *Materials and Method*

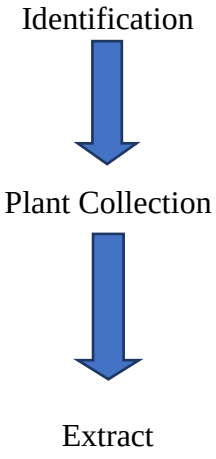
### 4.1 Method

The major objective was to investigate a wide range of plant species. After that, I moved on to the next level and suggested a theory on Acmella Oleracea.This method consists of two steps.

Preparation of ⟶ plant different types of tests.

### 4.1.1Preparation of plant for the test:

Acmella, the chosen plant, is a distinct and characteristic living creature with a specific position in its scientific categorization. Furthermore, it was important to ensure that this facility met the criteria for proceeding with a new type of test, which involved several sub-stages.



### 4.1.2 Plant Collection

*Acmella Oleracea* and other plant specimens were obtained from neighboring areas, including farmed areas. To minimize chemical degradation of the leaves, we took extreme attention during the leaf-gathering procedure. After tenderly removing the leaves, we meticulously cleansed the plants with dry cotton, ensuring that all dust and contaminants were eliminated.

### 4.2 Identification

*Acmella Oleracea* plant species may be found on Earth in abundance. As a result, proving that the plant peel was made from *Acmella Oleracea* was a simple procedure. This tusk was made in the Bangladesh National Herbarium in Mirpur, Bangladesh. *Acmella Oleracea* was the plant's name.

### 4.3 Preparation of the plant leaves

Because of the chilly weather, we dehydrated the leaves in the morning from 9 to 11 a.m. using a sheltered drying procedure. The leaves were then crushed to a fine powder using a grinder. The powder was then placed in a hermetically sealed container to ensure its preservation while keeping it dry, cold, and away from light.

### 4.3 Extraction

Soxhlet extraction is a popular method for obtaining important bioactive compounds from a variety of natural sources. Maintaining a high level of cleanliness before to use is critical for preventing subsequent contamination. In this extraction method, around 500g of dry leaf powder is utilized, and the thimble is then put within a distillation flask holding the suitable solvent. Place the thimble inside the Soxhlet extractor's main chamber. When heated, ethanol functions as a solvent for the *Acmella Oleracea* plant powder. When the Soxhlet chamber is almost filled, the syphon empties. The solvent is then added to the distillation flask once more. The thimble's fast action prevents the solvent from passing any solid material to the still pot. This cycle might last for hours, if not days.

### 4.4 Filtration

We used precision procedures to create a pure extract from an ethanol and leaf powder combination. Because there is little remaining solvent (ethanol) after the extraction procedure, it is advised that *Acmella Oleracea* plant extract be filtered via a rotary filter. The leaf extract was then filtered using sterile cotton and filter paper. We soaked the cotton and paper in the proper

solvent before placing them in a funnel. We collected the filter in a glass container with the proper solvent before transferring it to a funnel. We placed the filter in a glass jar once it was collected.

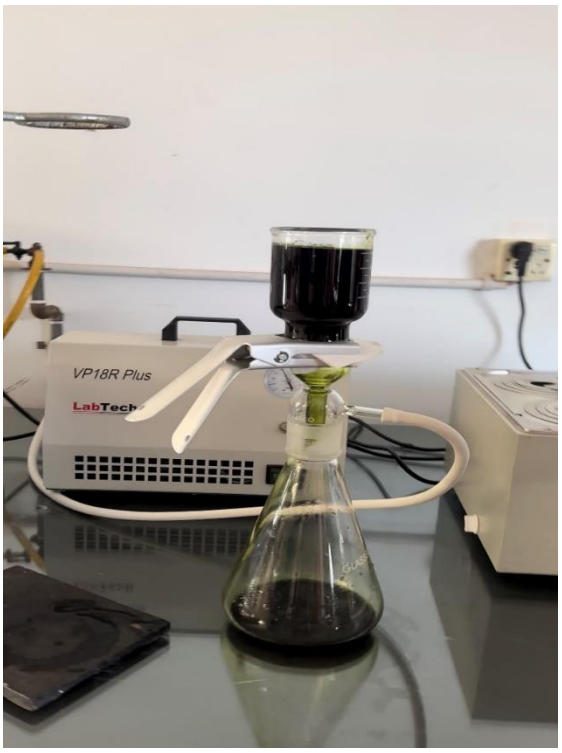



Figure 02: Vaccum filtration of *Acmella Oleracea*

Figure 03: paper filter

### 4.5 Evaporation

A rotary evaporator was used to evaporate the liquid extract. The ethanol was evaporated at 65-67 °C with a rotational speed of 34-38 m/s (rpm). The condensed material was collected using a 50-mL beaker. After separating the concentrated solution, wrap the beakers in aluminum foil and place them immediately beneath the fan. Ultimately, the water content of the leftover extract was reduced using a cold hair drier, allowing the remaining solvent to evaporate.

Figure 04: Rotary Evaporation of *Acmella Oleracea*

### 4.6 Dried extract

The non-polar dry extract is refrigerated until subsequent usage.

Figure 05: plant extract of EAO

## 4.7 Equipment's

1.Electronic Balance.
2. Rotary evaporator.
3. Mortar/Pestle
4. ultraviolet spectrophotometer
5. Magnetically sterile.
6. Water bath.

## 4.8 Apparatus

1. Beaker (1000,50) ml

2. Funnel

3. Filter paper

4. Glass rod

5. Stand with clamp

6. Cotton

7. Measuring Cylinder

8. Test-tube

9. Spatula

1.0 Syringe

11. Measuring cylinder

12. Amber glass bottle (2500 ml)

13. Pipette

14. Micropipette

15. Amber reagent bottle

16. Light-proof box

**4.9 Phytochemical screening**

The amount was unclear because of the qualitative character of the phytochemical screening approach utilized in scientific research. The plant extract was dissolved in distilled water to a sufficient level. Following that, we did several chemical studies.

Phytochemical screening is the process of identifying a medicinal plant's pharmacologically active or inactive component molecules that contribute to its therapeutic success. As a result, photochemical screening is more important in modern physiology. Because of the qualitative character of the phytochemical screening approach used in scientific study, an assay to detect phytochemicals in precise amounts was unknown. Initially, a little quantity of plant extract was completely dissolved in filtered water. Then, precise chemical tests were carried out. Phytochemical screening is a technique for identifying particular molecules in a medicinal plant that have pharmacological action and contribute to its therapeutic success. As a result, photochemical screening has become increasingly important in modern physiology. This study will use a screening technique to determine the presence of phytochemicals in the *Acmella Oleracea* plant. *Acmella Oleracea* plants contain a variety of chemicals, including as alkaloids,

tannins, gums, and flavonoids. Compounds include glycosides, sugars, phenols, steroids, saponins, and other chemicals. This compound is mostly found on plant stems, leaves, crowns, and bark. To separate these mixes, we use an alternate method. The process includes extracting medicinal components from plants in today's technologically advanced environment. The data has been subjected to public scrutiny and verification. Tannins, carotenoids, flavonoids, and alkaloids are among the substances used in cell fortifications. Phenolic chemicals are among the bioactive molecules extracted from plants. It is desirable to find experts and uncover new sources of key ingredients such as gums, oils, tannins, and precursors for the synthesis of complex chemical compounds. Understanding the components of plant material can also help you determine its future worth.

### 4.10.1 Test for tannins (Lead acetate test)

Lead acetate test: In the lead acetate test, a few drops of a 1% solution of lead acetate were added to 5 ml of the crude extract of plant material in a test tube. A yellow or crimson precipitate indicates the presence of tannins.

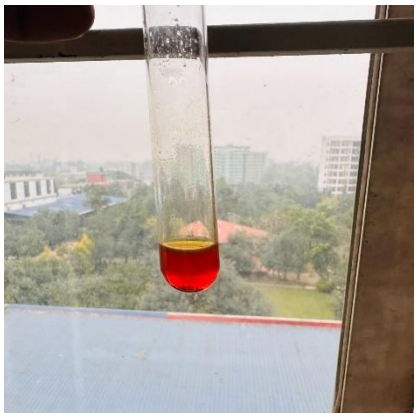


Figure 06: Test for tannins

### 4.10.2 Test for flavonoids (Hydrochloric acid test)

Mixed a little quantity of strong hydrochloric acid with 0.5 gram of plant extract. The quick emergence of a red color indicates the presence of flavonoids.

Figure 07: Test for flavonoid

### 4.10.3 Test for saponin

Frothing test: Foaming experiment: Heat around 0.1 g of finely crushed plant material in 10 ml of filtered water for 3-5 minutes before straining. After cooling the filtrate, add 5 ml of water to dilute it and shake vigorously. Saponins can be identified by the formation of a persistent froth that remains even when heated.

Figure 08: Test for saponin

### 4.11 Test for alkaloid

### 4.11.1 Mayer´s test

In a test tube, we mixed 2 ml of extract solution and 0.2 ml of diluted hydrochloric acid. Next, a milliliter of potassium mercuric iodide, also known as Mayer's reagent, was added. A yellow precipitate suggests the presence of alkaloids.

Figure 09: Test of Mayer

### 4.11.2 Dandruffs test

In a test tube, two milliliters of the extract solution and two milliliters of diluted hydrochloric acid were mixed. Next, a milliliter of the Dandruffs' reagent—also known as the Potassium Bismuth Iodide Solution—was added. The formation of an orange-brown precipitate indicates the presence of alkaloids.

Figure 10: Dandruff’s test

### 4.11.3 Wagner's Test

One milliliter of Wagner's reagent (potassium iodide) and two milliliters of the extract solution. When a reddish or brown precipitation forms, alkaloids are present.

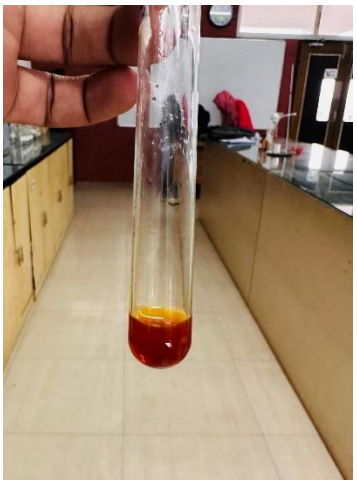


Figure 11: Wagner test

### 4.12 Test for Carbohydrate

A common technique for determining if carbs are present is the Molisch's test. Two milliliters of plant extract were mixed with two drops of Molisch's reagent, a 10% alcoholic solution of α-naphthol. Make sure that the acid forms a layer underneath the water solution by carefully pouring 2 milliliters of concentrated sulfuric acid along the side of the angled test tube. If there are carbohydrates, a reddish-purple ring forms where the two layers converge. A dark purple solution is produced by shaking or agitation. Give it a good shake and give it a two-minute break. To lower the concentration, stir in 5 cc of distilled water next. The precipitate forms instantly into a dull violet color.

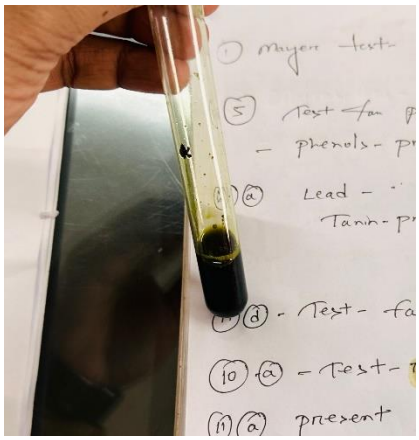



Figure 12: Molisch’s reagent

#### 4.12.1 Barfoed’s test (General test for Monosaccharides)

Place 1 milliliter of plant extract in a test tube and fill it with boiling water in a beaker. Next, let the tube warm up in the hot water. In two minutes, if there is a monosaccharide, a red cuprous oxide residue develops.

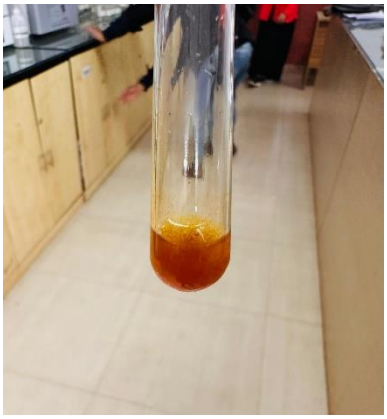


Figure 13: Barfoed test

### 4.12.2. Benedict's test (test for reducing sugars)

A test tube is filled with approximately 0.5 milliliters of the plant's alcoholic extract. Benedict's solution, an alkaline solution containing a cupric citrate complex, constitutes 5 milliliters in the test tube. Following a five-minute boil, it is allowed to cool naturally. There is a reducing sugar present when there is a red cuprous oxide deposit.

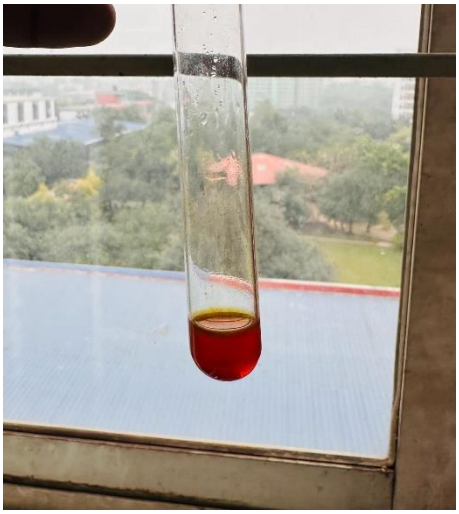


Figure 14: Benedict test

### 4.13 Test of steroids

Salkowski's test: Carefully pouring 1 ml of concentrated sulfuric acid from the test tube's side to a 2 ml chloroform extract is the steps involved in this experiment. An increase in redness in the chloroform layer indicates the presence of phytosterol.

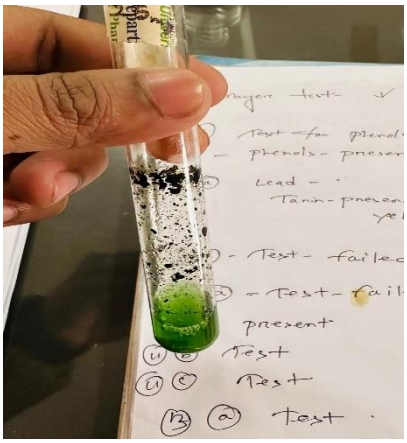



Figure 15: Salkowski’s test

### 4.14 Test for Phytosterols

To a test tube holding two milliliters of extract total, we added one milliliter of chloroform and concentrated H2SO4 until the required volume was attained. Golden yellow or golden red hue.4.15 Examine Glycoside Content.

#### 4.14.1 General test

We prepared an aqueous solution of sodium hydroxide by diluting a tiny amount of an alcoholic extract from either fresh or dried plant material in one milliliter of water and mixing it with a few drops. When there are glycosides present, a yellow color develops.

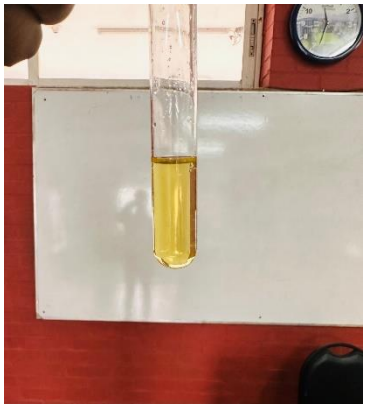


Figure 16: General test

### 4.15 Detection of Reducing Sugar

### 4.15 Detection of Reducing Sugar

Put 0.5 ml of the plant material's alcoholic extract into a test tube. In the test tube, 5 milliliters of Benedict's solution—an alkaline solution with a complex of cupric citrate—are introduced. Allow the liquid to naturally cool after heating it to a boiling point for five minutes. If there is a reducing sugar present, it will show up as a red cuprous oxide precipitate.

Figure 17: Benedict test

### 4.15.2 Molisch's test (General test for Carbohydrates)

We added two drops of Molisch's reagent, a 10% alcoholic naphthol solution, to a 2 ml sample of plant extract. Pour 2 mL of concentrated sulfuric acid down the side of the inclined test tube, making sure it creates a layer under the water solution. If carbohydrate is present, a crimson or purplish-red ring will form where the two layers meet. Agitation or shaking causes a dark purple solution. Agitate vigorously and then let it rest for two minutes. Mix in 5 milliliters of distilled water. A dismal violet precipitate is formed immediately.

Figure 18: Molisch's test

### 4.16 Test for Phenols

Use a filter and 5–10 ml of distilled water to dissolve 0.5 g of crude extract for the ferric chloride test. Drops of a 5% ferric chloride solution should be added to the filtrate. There are phenols (tannins) present when a precipitation or color turns blue, green, blue-black, or blue-green. The color vanishes and a yellowish-brown precipitate forms when a few milliliters of diluted H2SO4 are added.

Figure 19: Phenol test

### 4.17 Test for gum

Molisch reagent, sulfuric acid, and a 5 ml extract solution were mixed. Gum is present when two liquids combine because a reddish-violet ring form.

### 4.18 Antioxidant test

Total antioxidant capacity, total phenolic content, reducing power assay, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, and reduction of ferric ions by the Orth phenanthroline color procedure were the methods used to evaluate the antioxidant properties of the extracts under investigation.

### 4.18.1 Reagent for antioxidant test

1. Folin-Ciocalteu reagent
2. Sodium carbonate
3. Methanol
4. Gallic acid
5. Aluminum Chloride
6. Potassium Acetate
7. Ethanol
8. Quercetin
9. Concentrated $H_2So_4$ (98%)
10. Sodium phosphate ($Na_3PO_4$)
11. Ammonium molybdate
12. 1,1-diphenyl-2picrylhydrazyl (DPPH)
13. Ascorbic acid

### 4.18.2 Equipment for antioxidant test

1. UV-Visible Spectrophotometer:
2. Analytical Balance
3. Vortex Mixer or Shaker
4. Centrifuge
5. Micropipettes and Pipette Tips
6. Cuvettes
7. Glassware
8. DPPH reagent
9. Solvents
10. Antioxidant Standards
11. Control Samples
12. Water Bath or Incubator
13. Laboratory Supplies.

## 4.19 Determination of Total Phenolic Content (TPC)

### Principle

Using the same techniques as previously described, the total phenolic components in plant extracts of Acmella oleracea were measured. Folin-Ciocalteu Reagent (FCR) should be used. The reagent Folin-Ciocalteu (FCR) Phosphomolybdate and phosphor tungstate make up the Folin-phenol reagent, commonly known as the Folin-Denis's reagent. It is employed in the colorimetric assessment of antioxidants that are phenolic and polyphenolic. The process works by calculating the quantity of material under test required to prevent the reagent from oxidizing. All molecules having reducing properties, not simply complete phenols, can be readily reacted with by this reagent. Rather than focusing just on phenolic component content, the reagent assesses a sample's overall reducing ability, taking into account all reducing chemicals. One or two electrons are involved in a series of reversible reduction reactions that lead to the synthesis of blue species, which may include PMoW (11, 40). The prevailing consensus is that the complex facilitates molybdenum reduction more readily, causing an electron-transfer process to occur between Mo and the reducing agents.

### 4.19.1 Procedure for preparing a 7.5% sodium carbonate solution:

Using pure water, we changed the capacity of a 100-ml volumetric flask containing 7.5 grams of sodium carbonate (Na2CO3).

### 4.19.2 Standard solution preparation:

We prepared a stock solution of gallic acid by dissolving it in 95% methanol at a concentration of 1 mg/mL (1000 g/mL). We diluted the stock solution to obtain the experimental concentrations, which ranged from 1000 to 5 ug/ml.

### 4.19.3 Method for Preparing the Extract Solution:

A concentration of 5 milligrams per milliliter was achieved for each plant extract solution by dissolving 0.025 grams of each extract in 5 milliliters of ethanol. Many considered this to be the standard solution.

### 4.19.4 Working procedure

1. A test tube was filled with either a reference solution at different concentrations or 1.0 mL of plant extract (200 g/ml).

2. After being diluted by a factor of 10, 5 mL of the Folin-Ciocalteu reagent solution was added to the test tube.

3. 4 milliliters of a 7.5% sodium carbonate solution were added to the test tube and properly mixed after a 5-minute break.

4. To get a complete reaction, the test tubes containing extract solution were incubated for one

hour at 20°C, whereas the test tubes containing standard solutions were incubated for thirty minutes at 20°C to fully catalyze the reaction.

5. Next, using the blank as a reference, the spectrophotometer was used to measure the absorbance of the solution at a wavelength of 765 mm. "Efland" is what the user entered.

6.The solvent that was used to dissolve the plant extract was published in the blank solution, which was made up of 1 mL of methanol, 5 mL of diluted Folin-Ciocalteu reagent, and 4 mL of a 7.5% sodium carbonate solution.

7. The total quantity of compounds that are phenolic in plant extracts expressed as Gallic acid equivalents (GAE).

### 4.20 Determination of antioxidant activities (DAA)

DPPH free radical scavenging assay

Free radicals that are reactive Other compounds oxidize as a result of DPPH's action as an oxidant, oxidizing agent, or electron acceptor. However, antioxidants also serve as reducing agents or reductants, which means they provide electrons. Antioxidants counteract DPPH because they are oxidizing substances. Comprising stable free-radical molecules, DPPH is a dark-colored, crystalline powder that takes the shape of a solution with a deep violet hue. Neutralization, or the scavenging of the DPPH free radical, causes the deep violet tone to change to a pale yellow or clear condition.

#### 4.20.1 DPPH Solution:

One milligram is equal to 0.004 g. DPPH is dissolved in 100 milliliters of ethanol solvent to get a 0.004% solution.

#### 4.20.2 Standard/Extract Solution Preparation:

Five milliliters of ethanol were used to dissolve 0.025 g of ascorbic acid or extract that had been obtained. 5 mg/mL of ascorbic acid/extract was present in the solution.

#### 4.20.3 Working procedure

1.Test tubes were filled with one milliliter of plant extract or solution each. We put liquids in different concentrations into a set of test tubes.

2. To get a final volume of 3 ml, we added 2 ml of a 0.004% DPPH solution to each test tube. Because DPPH is light-sensitive, it is essential to handle the solution and add it to the test tubes with as little light exposure as possible.

3. No extract or reference ascorbic acid was added to the control sample, which was created by mixing 1 mL of ethanol and 2 mL of 0.004% DPPH.

4. Let the mixture sit for 30 minutes at room temperature in a place that isn't lit.5. Next, we use

95% ethanol as the blank solution and measure the absorbance at a wavelength of 517 nm in proportion to it.

The following formula was used to calculate the percentage of the DPPH free radical scavenged:

$$\% \text{ Scavenging Activity} = \frac{\text{Absorbance the control} - \text{Absorbance the test sample}}{\text{Absorbance the control}}$$

### 4.21 Determination of total flavonoids content (TFC)

The aluminum chloride colorimetric method, as described by, was used to quantify the total flavonoid concentration. The production of acid-stable complexes between aluminum chloride and the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavanols is the foundation of the aluminum chloride colorimetric technique. Moreover, the ortho-dihydroxyl groups in the A- or B-ring of flavonoids can form complexes with aluminum chloride that are acid sensitive. To measure absorbance, we use a wavelength of 415 nm.

#### 4.21.1 Procedure for preparing a 10% solution of aluminum chloride (AICI):

Ten grams of aluminum chloride (AlCl3) were added to a volumetric flask that held one hundred milliliters. We then added distilled water to regulate the volume.

#### 4.21.2 Procedure for preparing a 1M potassium acetate solution:

A 100 milliliters volumetric flask containing 9.815 grams of potassium was filled, and the volume was subsequently tampered with using pure water.

#### 4.21.3 Standard solution preparation:

Quercetin 0.025 grams was dissolved in 5 milliliters of ethanol to create the standard solution, which had a concentration of 5 milligrams per milliliter.

#### 4.21.4 Extract Solution Preparation:

For each plant extract solution, we created a concentration of 5 mg/mL by dissolving 0.025 g of the extract in 5 mL of methanol. These answers were regarded as conventional solutions by us.

#### 4.21.5 Working Procedure

A test tube was filled with one milliliter of a plant extract (200 ug/mL) or a reference solution in different concentrations. To the test tube, we added 3 mL of methanol. Next, 200 milliliters of an

aluminum chloride 10% solution were added to the same test tube. Next, fill the test tube with 200 L of the IM potassium acetate solution. The reaction mixture was then mixed with 5.6 milliliters (mL) of distilled water. We next let the reaction mixture sit at room temperature for 30 minutes to make sure the reaction has finished. The absorbance of the solution at 415 nm was then measured using a spectrophotometer and compared to a blank sample.8. Methanol served as a stand-in. The following formula was used to calculate the total flavonoid content in plant methanol extracts, expressed in quercetin equivalents.

## 4.22 Total tannins contents (TTC)

### 4.22.1 Preparation of a 35% sodium carbonate (Na2CO3) solution:

A 50 ml volumetric flask containing 17.5 grams of sodium carbonate (Na2CO3) was filled with the appropriate volume using pure water.

### 4.22.2 Standard solution preparation:

A stock solution of 1 mg/mL (1000 mg/mL) of gallic acid was created by diluting the acid in 95% ethanol. The stock solution was diluted to generate the experimental concentrations, which ranged from 500 to 6.25 g/mL.

### 4.22.3 Extract Solution Preparation:

0.025g The concentration of each plant extract solution was 5 mg/mL after each extract was diluted in 5 mL of ethanol. These answers were accepted as conventional ones.

### 4.22.4 Working procedure

1. One milliliter of either a reference solution with different concentrations or plant extract (200 g/mL) was put into a test tube.

2. Distilled water (7.5 cc) was added to each test tube.

3. A 0.5 mL tenfold-diluted Folin-Ciocalteu reagent supplement was added to each tube.

4. A 1 mL solution of 35% NaCO3 was added to each tube as an addition.

5. After agitating the solution thoroughly, it was allowed to remain at room temperature for half an hour.

6. Using a UV/Visible spectrophotometer, the absorbance of the test and standard solutions was measured at a wavelength of 725 nm. The blank was used as a reference.

7. 2.58 percent A blank was created by combining 2.5 mL of methanol and 7.5 mL of water.

8.In plant methanol extracts, the total concentration of tannin components was calculated using the following formula equation, and was expressed in Gallic acid equivalent (GAE) per gramme of extract.

### 4.23.1 Thrombolytic test [28]

4.24.1 Reagent for thrombolytic activity test

1. Streptokinase

2. Blood

3. Distilled water

4. Streptokinase

### 4.23.2 Equipment for thrombolytic activity test

1. Test tube

2. Glass Rod.

3. Vortex Mixture

4. Eppendorf tube

5. Eppendorf tube rack

6.Incubator

7. Cotton

8. Beaker

9. Syringe

10. Syringe Filter

11. Micropipette

### 4.24.3 Preparation of test Sample

To 2.5 milliliters of distilled water, a total of 25 milligrams of extract were added. It was then taken out of the vortex mixer after being shook for a while. It was possible to separate the soluble supernatant from the solution by leaving it alone for a full day and then filtering it using syringe filter paper.

### 4.23.4 Preparation of Eppendorf

Three Eppendorf tubes were disinfected, weighed, and cleaned with distilled water. They were then tagged with a permanent marker to distinguish them as individual tubes.

### 4.23.5 Streptokinase

A vial containing commercially available lyophilized streptokinase (15,00,000 IU) under the name S-Kinase, produced in Bangladesh by Popular Pharmaceuticals Ltd., was added along with five milliliters of phosphate buffered saline and well mixed. The concentration of streptokinase was changed to 30,000 IU and 15,000 IU due to its frequent use as a thrombolytic medication. The thrombolytic activity was measured using these concentrations as the reference point.

### 4.23.6 Study design [29-30]

Five milliliters of blood were drawn into individual sterile, pre-weighed vials, each holding one milliliter of blood at first. For forty-five minutes, the tubes were kept in an incubator with the temperature adjusted to 37°C. The tubes were allowed to coagulate after incubation, and then they were weighed again. Once a clot had formed, the translucent portion was carefully removed. The weights of the preceding two measurements were subtracted to get the clot weight. Each vial holding a clot received a single addition of 100 microliters of test samples (1 milligram for 100 microliters of water). One hundred microliters of streptokinase (30,000 I.U.) served as the positive control, while one hundred microliters of distilled water served as the negative control. 90 minutes of incubation at 37°C resulted in the appearance of clot lysis. After extracting the ejected clear plasma, the vials were weighed once more to ascertain the weight change. This formula was used to find the clot lysis percentage.

% Of thrombolytic activity = (wt. of clot after treatment / wt. of clot before treatment) × 100

## 4.24 GC-MS Analysis

### 4.24.1 Introduction

Gas chromatography (GC) and mass spectrometry (MS) are two distinct forms of analysis that are combined in a process known as gas chromatography-mass spectrometry (GC-MS). The material is initially divided into groups according to its chemical properties using gas chromatography (GC). The separated components are then subjected to mass spectrometry (MS) analysis to ascertain their mass-to-charge ratio and get structural information. A fixed phase and a moving phase are used in gas chromatography to separate the components of a mixture of samples. As the stationary phase, a thin film covers a capillary column. Different interactions between the analytes and this film lead to their separation. The mobile phase, also known as the carrier gas, makes it easier for the sample to pass through the column. Ionization, separation, and detection of ions based on their mass-to-charge ratio (m/z) are all steps in the mass spectrometry technique. The molecules in the sample create ions when exposed to an ionization source, such as electron ionization (EI). The ions are separated based on their mass/mass ratios using a mass analyzer, such as a time-of-flight (TOF) analyzer or quadrupole. In the end, the ions are located and their frequency is recorded to produce a mass spectrum that provides information on the chemical composition and structure of the materials under study.

In GC-MS, the separated individual components may be continuously analyzed since the mass spectrometer and gas chromatograph are directly connected. After being extracted from the gas chromatography column, each chemical is sent into the mass spectrometer to undergo ionization and have its mass measured.

### 4.24.2 Experimental procedure

The GC-MS electron impact ionization (EI) technique was utilized to evaluate the leaf extract of Acmella Oleracea that was based on ethanol. An analysis was conducted using a Shimadzu GC-2010 gas chromatograph and GCMS-TQ8040 mass spectrometer, both provided by the Bangladesh Reference Institute for Chemical Measurements (BRiCM). We equipped the gas chromatograph with a DB-5MS column that contained 5% phenyl-95% methyl polysiloxane. The dimensions of the column were 30 m in length, 0.25 mm in diameter, and 0.25 m in film thickness.

**The parameters of GC were programmed as follows:** A temperature of 250 °C was assigned to the input. The oven was first set for one minute at 50 degrees, then for two minutes at a pace of 15 degrees Celsius per minute, and for the last seven minutes at a rate of five degrees Celsius per minute, we increased the temperature to 300 degrees Celsius. At a constant pressure of 53.5 kPa, helium was utilized as the carrier gas. 11.0 milliliters of gas were flowed per minute overall, and 1.0 milliliters per minute were flowed via the column. An 5.0 split ratio was employed. A minute and a half was spent sampling. Utilizing methanol as a solvent allowed the substance to dissolve. 40 minutes was the overall retention period for the chromatographic analysis.

**The MS parameters were set as follows:** The temperature of the ion source is 230 degrees Celsius, whereas the interface is 250 degrees Celsius. With a scan speed of 2000, we have selected Q3 scan mode as the acquisition option. The mass range for scanning is 50 to 600 m/z. The "NIST-MS Library" was used to analyze mass spectra and identify different compounds. Based on the peak areas of the total ionic chromatogram (TIC), we computed the fraction of separated chemicals. Olecea Acmella.

### 4.25 Analgesic Activity

### 4.25.1 Reagent for Analgesic activity test[63]

1. Extract
2. Diclofenac
3. CMC
4. Acetic acid

### 4.25.2 Equipment for Analgesic activity test

1. syringes
2. Petri dishes or microplates:
3. Vortex mixture
4. Pipettes
5. Timer or stopwatch
6. Balance
7. pH meter
8. Water bath or heating block
9. Centrifuge
10. Analytical balance

Acetic acid injected intraperitoneally

Mice writhing

### 4.25.3 Preparation of test Sample

### 4.25.4 Preparation of drug & extract solutions [63]

**Writhing test:**

The mice's writhing test was conducted using the publication's methodology. Twenty mice were split into four groups at random and given various dosages of CMC-Na orally, with five mice in each group, using the identical procedures as in 2.6.1.2. Following the last dosage, 1% acetic acid (w/v, 0.1 mL/10 g body weight) was intraperitoneally given into each and every animal for 30 minutes. Next, during the course of the following 20 minutes, the number of writhing mice was promptly tallied. An indicator was determined by calculating the average number of writhing mice in a group.

## 4.26Antidiarrheal activity

### 4.26.1 Reagent for Antidiarrheal activity test

1. Extract
2. Loperamide
3. Tween 80
4. Castor Oil

Administered Castor Oil

### 4.26.2 Equipment for Antidiarrheal activity test

1. Microscopes
2. Petri dishes or microplates:
3. Incubator
4. Pipettes
5. Timer or stopwatch
6. Balance
7. pH meter
8. Water bath or heating block
9. Centrifuge
10. Analytical balance

### 4.26.3 Preparation of test Sample

### 4.26.4 Preparation of drug & extract solutions [62]

**Castor oil induced diarrhea**

Four groups—groups I and II for test samples, standard samples, and control mice—were created by randomly selecting experimental animals. Each group included three mice. According to Shoba and Thomas (9, 10), this experiment. Before the test, the mice were given a free pass to water and fasted for 18 hours. The test samples, which included *Acmella olecea* at 100 and 200 mg/kg and 250 and 500 mg/kg, were given orally together with the control (Twin80 5 ml/kg) and standard (loperamide 3 mg/kg). Subsequently, each mouse was given 0.3ml of castor oil orally to induce diarrhea one hour later. Every animal was housed in a separate cage with white blotting paper covering the floor. Every hour, more documents were distributed. For a duration of four hours, the total amount of dry and moist feces that the animals expelled was counted every hour. The control group's total number of diarrheal feces was regarded as 100%. The percentage of defecation inhibition was

# *Chapter five*

# *Results & discussion*

## 5.1 Result of chemical group test

The presence of fixed oils, alkaloids, carbohydrates, flavonoids, tannins, saponins, phenols, and steroids was shown by the phytochemical examination of the aqueous extracts. Glycosides, proteins, amino acids, and resins were not present.

The qualitative chemical analysis of extract of ***Acmella Oleracea,*** **(+ Present; - Absent)**

| SI. No. | Phytochemical | Types of tests | Observation |
|---|---|---|---|
| 01 | Alkaloids | Mayers test<br>Wagner's test<br>Hagger's test<br>Dandruff's test | -<br>-<br>-<br>- |
| 02 | Glycosides | General test | ++ |
| 03 | Carbohydrates | Fehling's test<br>Molisch's test<br>Benedict's test | -<br>+<br>+ |
| 04 | Flavonoids | Alkaline Reagent Test | + |
| 05 | Tannins | Lead acetate test | -- |
| 06 | Saponin | Frothing test | ++ |
| 07 | Phenols | Ferric chloride test | + |
| 08 | Steroids | Liebermann-Burchard's test<br>Salkowski's test | + |
| 09 | Proteins and amino acids | Biuret's test | - |
| 10 | Resins | Resin test | - |
| 11 | Fats & Fixed Oils | Stain test | ++ |
| 12 | Terpenoids | Salkowski test | ++ |

Table 01: Results of phytochemical test.

## 5.2 Antioxidant activity test results:

### 5.2.1 Determination of total phenolic content (TPC) -

| Concentration (µg/mL) | Mean Absorbance at 765 nm | SD |
|---|---|---|
| 6.25 | 0.008 | **#DIV/0!** |
| 12.5 | 0.028 | **#DIV/0!** |
| 25 | 0.055 | **#DIV/0!** |
| 50 | 0.068 | **#DIV/0!** |
| 100 | 0.331 | **#DIV/0!** |
| 200 | 1.027 | **#DIV/0!** |
| 400 | 2.557 | **#DIV/0!** |

Table 02: Results of Total phenolic content test

| Extract solution (µg/mL) | Mass of dry extract per Ml m (g) | Absorbance | GAE conc. c (µg/mL) | GAE conc. c (mg/mL) | TPC as QE, $C=\frac{c \times v}{m}$ | Mean (mg/g) | SD | Mean ± SD |
|---|---|---|---|---|---|---|---|---|
| 200 | 0.0002 | 0.039 | 30.277 | 0.030 | 151.38 | 159.33 | 6.89 | 159.33 ± 6.89 |
| 200 | 0.0002 | 0.054 | 32.585 | 0.033 | 162.92 | | | |
| 200 | 0.0002 | 0.055 | 32.738 | 0.033 | 163.69 | | | |

Table 03: Plant extract Total phenolic contents

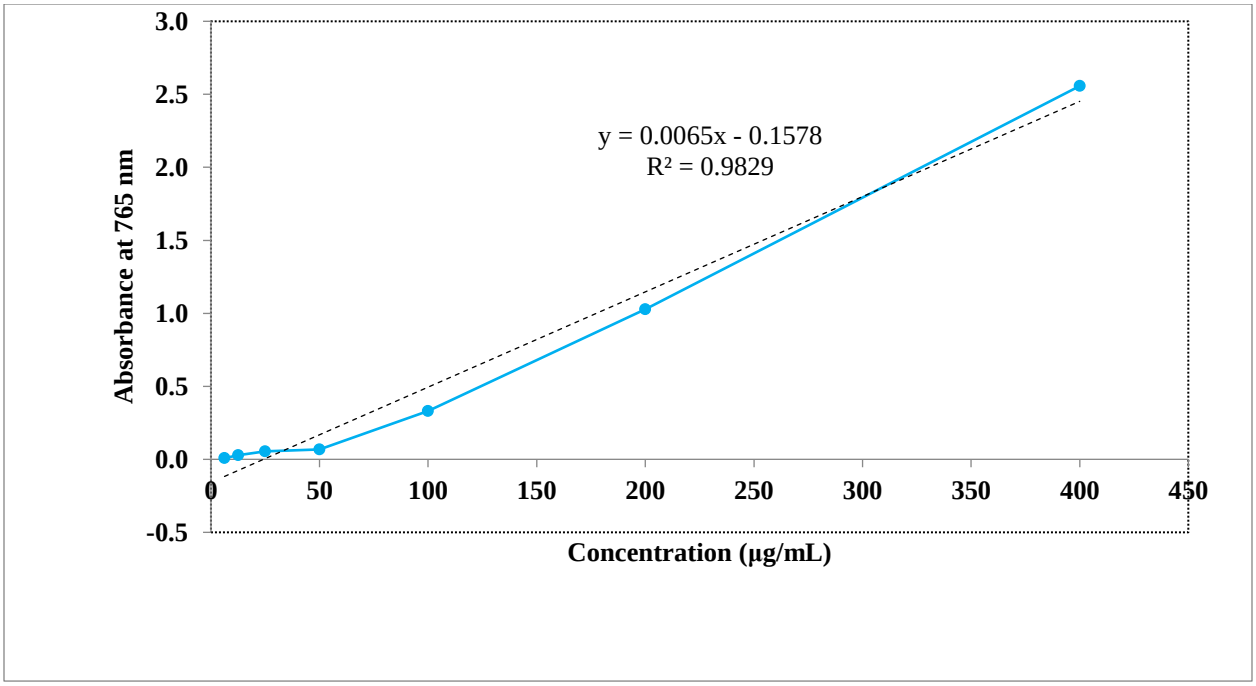



Figure 20: Activity of *Acmella Oleracea*

**Discussion:**

The objective of the current study was to measure the total phenolic content (TPC) in leaf extracts made with ethanolic solvent. It was discovered that the mean value ± standard deviation was 159.33 ± 6.89. With a standard deviation of 6.89, the mean concentration is 159.33 mg/g.

### 5.2.2 Total flavonoids contents (TFC)

| Concentration (µg/mL) | Mean Absorbance at 765 nm | SD |
|---|---|---|
| 6.25 | 0.060 | #DIV/0! |
| 12.5 | 0.080 | #DIV/0! |
| 25 | 0.093 | #DIV/0! |
| 50 | 0.250 | #DIV/0! |
| 100 | 0.521 | #DIV/0! |
| 200 | 0.991 | #DIV/0! |
| 400 | 2.325 | #DIV/0! |

Table 04: Results of Total flavonoids content test

| 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, $C=\frac{c \times v}{m}$ | Mean (mg/g) | SD | Mean ± SD |
|---|---|---|---|---|---|---|---|---|
| 200 | 0.0002 | 0.125 | 24.649 | 0.028 | 138.25 | 139.42 | 4.50 | 139.42 ± 4.50 |
| 200 | 0.0002 | 0.122 | 27.123 | 0.027 | 135.61 | | | |
| 200 | 0.0002 | 0.132 | 28.877 | 0.029 | 144.39 | | | |

Table 05: Plant extract Total flavonoids contents

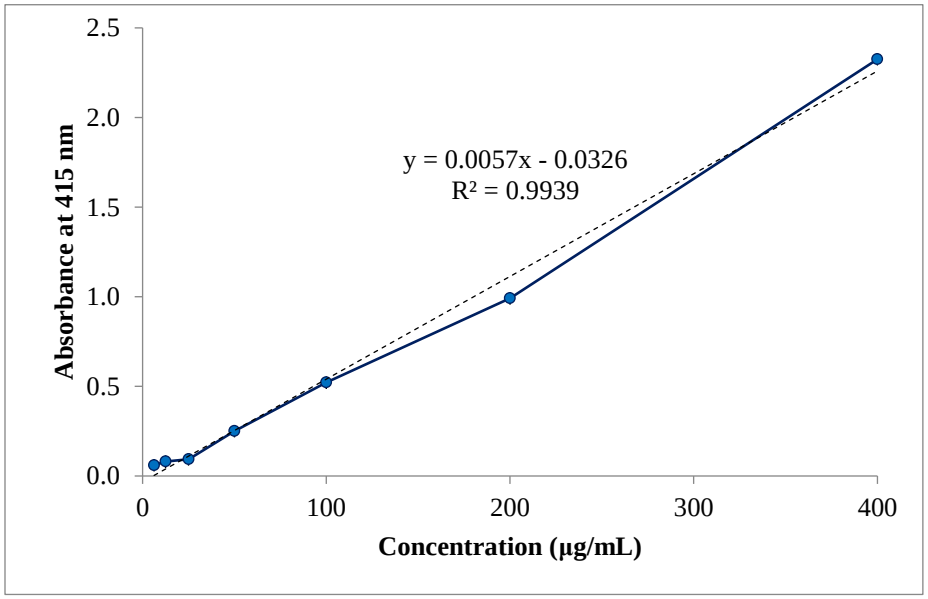



Figure 21: Total Flavonoids Contents (TFC)

**Discussion:**

Quantifying the total flavonoid content (TFC) in leaf sample extracts made in ethanolic solvent was the goal of the current investigation. After calculating the standard deviation, the TFC was determined to be 4.50 mg/g, with a mean value of 139.42 mg/g.

### 5.2.3 Determination of total tannins contents (TTC)

| Concentration (µg/mL) | Mean Absorbance at 725 nm | SD |
|---|---|---|
| 6.5 | 0.023 | #DIV/0! |
| 12.5 | 0.099 | #DIV/0! |
| 25 | 0.046 | #DIV/0! |
| 50 | 0.167 | #DIV/0! |
| 100 | 0.501 | #DIV/0! |
| 200 | 1.240 | #DIV/0! |
| 400 | 2.276 | #DIV/0! |

Table 06: Results of Total tannins content test

| 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 QE, $C=\frac{c \times v}{m}$ | Mean (mg/g) | SD | Mean ± SD |
|---|---|---|---|---|---|---|---|---|
| 200 | 0.0002 | 0.141 | 25.745 | 0.026 | 128.72 | 124.11 | 5.46 | 124.11± 5.46 |
| 200 | 0.0002 | 0.131 | 23.617 | 0.024 | 118.09 | | | |
| 200 | 0.0002 | 0.138 | 25.106 | 0.025 | 125.53 | | | |

Table 07: Plant extract Total tannins contents

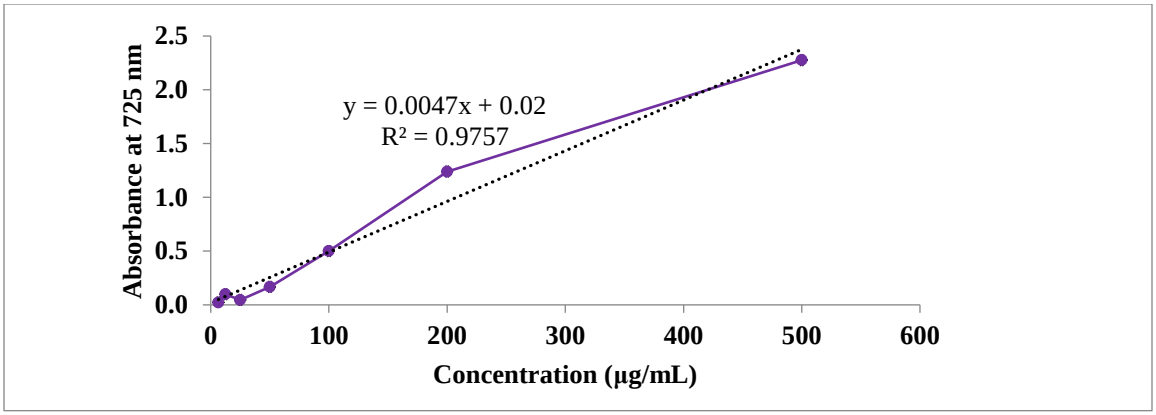



Figure 22: Activity of *Acmella Oleracea*

**Discussion:**

The total flavonoid content (TFC) of leaf extracts made in ethanolic solvents was the study's main objective. The TFC (mean ± standard deviation) was determined to be 124.11± 5.46. With a standard deviation of 5.46, the mean concentration is 124.11 mg/g.

### 5.2.4 Determination of Antioxidant Activities

| Concentration (µg/mL) | % inhibition | |
|---|---|---|
| | Ascorbic Acid | EAO |
| 6.25 | 2.86 | 1.63 |
| 12.25 | 36.73 | 17.36 |
| 25 | 55.10 | 35.51 |
| 50 | 58.78 | 44.49 |
| 100 | 77.14 | 58.37 |
| 200 | 88.16 | 73.03 |
| 400 | 92.65 | 88.16 |

Table 08: Results of antioxidant test

| Group | ln(x) = (y-c)/m | IC50 (µg/mL) |
|---|---|---|
| Ascorbic Acid | 3.47606581 | 32.33 |
| EAO | 4.127253814 | 62.01 |

Table 09: Plant extract Total tannins contents

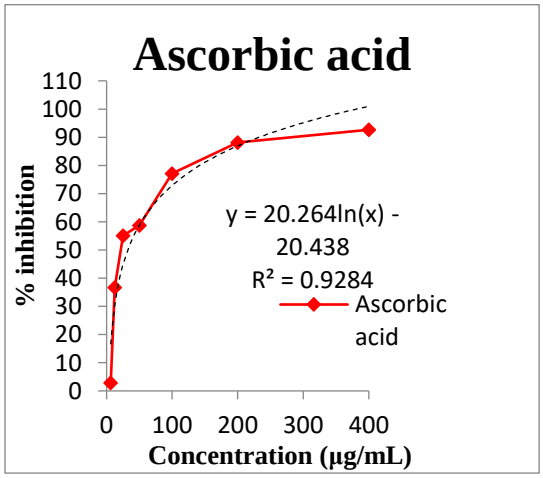


Figure 23: Free radical inhibition activity of *Mikania Micrantha*

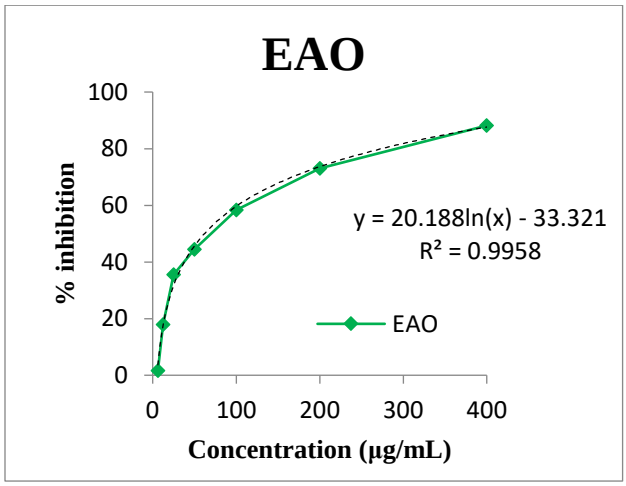



Figure 24: Free radical inhibition activity of of *Acmella Oleracea*

**Discussion:**

The standard medication used in the DPPH test to assess antioxidant activity was ascorbic acid. Ascorbic acid was found to have an IC50 value of 32.33 µg/mL. It was found that Acmella Oleracea has an IC50 value of 65.01 µg/mL. The DPPH radical scavenging activity of the extracts was substantial.

## 5.4 Thrombolytic Activity test results

| Solution | Blank tube weight (gm.) | 1st clot + tube weight (gm.) | 1st clot weight (gm.) | 2nd clot + tube weight (gm.) | 2nd weight clot (gm.) | Weight of lysis (gm.) | % Of Lysis |
|---|---|---|---|---|---|---|---|
| Group - I [Standard] | 0.83 | 1.85 | 1.02 | 1.16 | 0.33 | 0.69 | 69% |
| Group - II [Control] | 0.77 | 1.58 | 0.81 | 1.52 | 0.75 | 0.06 | 6% |
| Group - III [Sample] | 0.81 | 1.79 | 0.98 | 1.36 | 0.55 | 0.4387 | 43.87% |

Table 11: % of clot lysis in different concentrations of standard, sample, and control

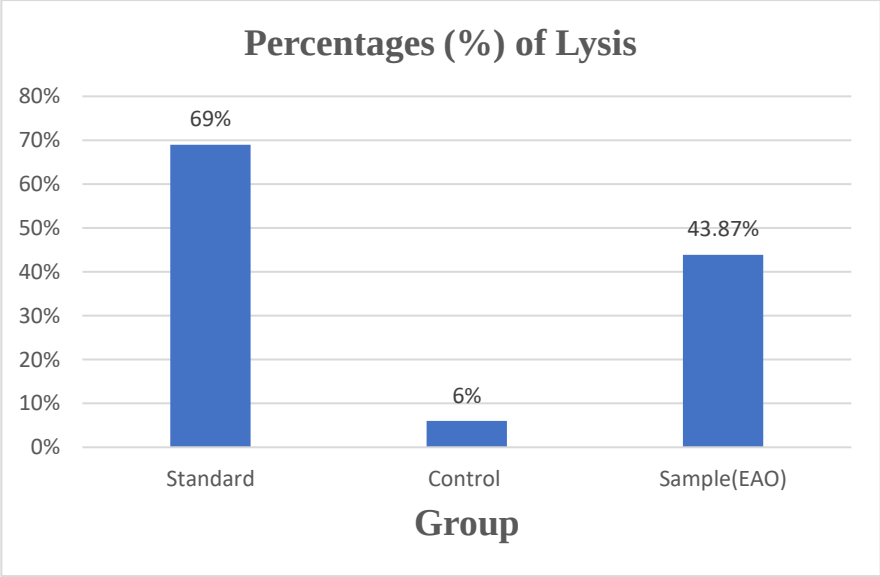



Figure 25: Thrombolytic activity by *Acmella Oleracea*

### 5.5 GC-MS Analysis

The National Institute of Standards and Technology (NIST) provided a database with approximately 62,000 patterns, which were interpreted to examine the mass spectra of the GC-MS equipment. We contrasted the NIST collection's stated component's spectrum with that of the unidentified chemical. To identify the components, we compared the spectra of the known components from the NIST collection with the spectra of the extracts. We identified and quantified thirty-nine (84) distinct components in the ethanolic extract of *Acmella Oleracea* using GC-MS analysis.

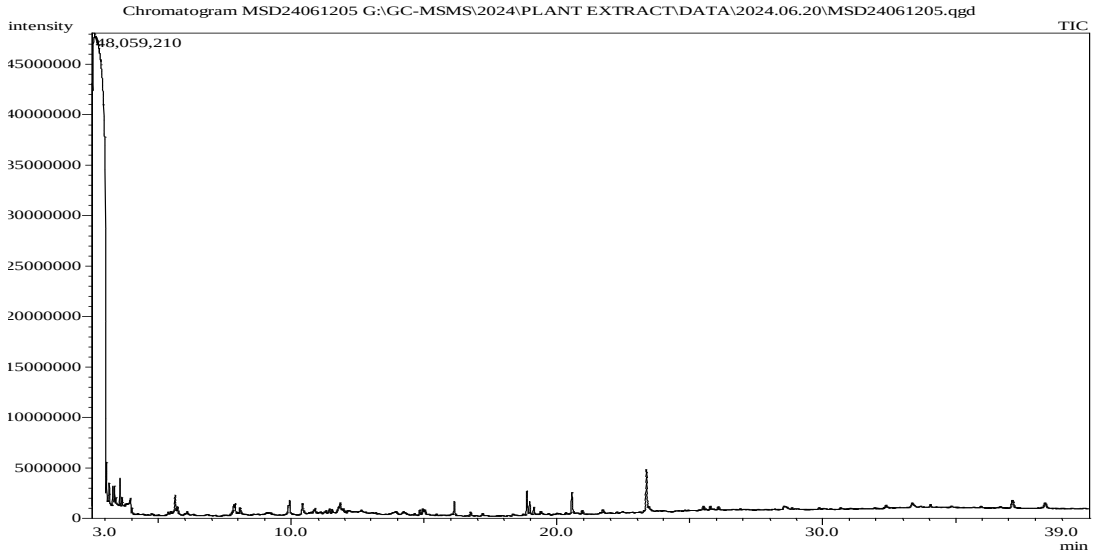



Figure 26: Chromatograph (GC/MS) of ethanolic extract of *Acmella Oleracea*

#### 5.5.1: Constituent of *Acmella Oleracea*

| Sl. No. | Name of constituent | Retention Time | Peak Area (%) | Molecular Formula | Molecular Weight | PubChem CID |
|---|---|---|---|---|---|---|
| 1. | 1,1,1-Trichloro-2-methylpropan-2-ol, O-trimethylsilyl- | 2.575 | 13.45 | $C_8H_{17}Cl_3OSi$ | 263.67 | |
| 2. | 1,3-Butadiyne | 2.597 | 3.68 | $C_4H_2$ | 50.06 | 9997 |
| 3. | Carbonic acid, ethyl-, methyl ester | 2.62 | 3.67 | $C_4H_8O_3$ | 104.1 | 522046 |
| 4. | (E,E,E)-2,4,6-Octatriene | 2.64 | 66.53 | $C_8H_{12}$ | 108.18 | 5368766 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 5. | Butanoic acid, 2-hydroxy-, methyl ester | 3.053 | 0.31 | $C_5H_{10}O_3$ | 118.13 | 520445 |
| 6. | Methyl formate | 3.153 | 0.26 | $C_2H_4O_2$ | 60.05 | 7865 |
| 7. | Silanol, dimethyl- | 3.29 | 0.21 | $C_2H_8OSi$ | 76.17 | 521864 |
| 8. | Trimethylsilyl ethaneperoxoate | 3.357 | 0.23 | $C_5H_{12}O_3Si$ | 148.23 | 12571375 |
| 9. | Trimethylsilyl fluoride | 3.402 | 0.12 | $C_3H_9FSi$ | 92.19 | 9869 |
| 10. | 1-Butanol, 3-methyl- | 3.633 | 0.16 | $C_5H_{12}O$ | 88.15 | 31260 |
| 11. | 3,3-Dimethyl-1,2-epoxybutane | 3.755 | 0.12 | $C_6H_{12}O$ | 100.16 | 92174 |
| 12. | Butanoic acid, 3-hydroxy- | 3.795 | 0.09 | $C_4H_8O_3$ | 104.1 | 441 |
| 13. | Butanoic acid, ethyl ester | 3.952 | 0.58 | $C_6H_{12}O_2$ | 116.16 | 7762 |
| 14. | Ethylene glycol, TMS derivative | 4.014 | 0.04 | $C_5H_{14}O_2Si$ | 134.25 | 554455 |
| 15. | (S)-(+)-2-Amino-3-methyl-1-butanol | 4.736 | 0.03 | $C_5H_{13}NO$ | 103.16 | 640993 |
| 16. | Mesitylene | 5.55 | 0.08 | $C_9H_{12}$ | 120.19 | 7947 |
| 17. | Cyclohexanamine, N,N-dimethyl- | 5.617 | 0.44 | $C_8H_{17}N$ | 127.23 | 7415 |
| 18. | Benzene, 1,2,4-trimethyl- | 5.714 | 0.16 | $C_9H_{12}$ | 120.19 | 7247 |
| 19. | D-Limonene | 6.074 | 0.05 | $C_{10}H_{16}$ | 136.23 | 440917 |
| 20. | 1-Dodecene | 7.835 | 0.3 | $C_{12}H_{24}$ | 168.32 | 8183 |
| 21. | 3-Tetradecene, (E)- | 7.894 | 0.19 | $C_{14}H_{28}$ | 196.37 | 5352802 |
| 22. | 4-Hydroxy-4-methylhex-5-enoic acid, tert.-butyl ester | 7.982 | 0.04 | $C_{11}H_{20}O_3$ | 200.27 | 545523 |
| 23. | 1-Butanol, 3-methyl-, formate | 8.064 | 0.16 | $C_6H_{12}O_2$ | 116.16 | 8052 |
| 24. | Benzocycloheptatriene | 9.157 | 0.06 | $C_{11}H_6O$ | 154.16 | 129656441 |
| 25. | 1-Undecanol | 9.905 | 0.26 | $C_{11}H_{24}O$ | 172.31 | 8184 |
| 26. | 9-Octadecene, (E)- | 9.95 | 0.21 | $C_{18}H_{36}$ | 252.5 | 5364599 |
| 27. | 2-(Isobutoxymethyl)oxirane | 10.419 | 0.27 | $C_7H_{14}O_2$ | 130.18 | 98155 |
| 28. | 2-Methyltetracosane | 10.844 | 0.09 | $C_{25}H_{52}$ | 352.7 | 527459 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 29. | 7-Tetradecene | 10.889 | 0.09 | $C_{14}H_{28}$ | 196.37 | 5364651 |
| 30. | 1-Penten-3-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)- | 11.086 | 0.05 | $C_{14}H_{22}O$ | 206.32 | 5375218 |
| 31. | Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]- | 11.23 | 0.06 | $C_{15}H_{24}$ | 204.35 | 519764 |
| 32. | (1S,5S)-4-Methylene-1-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hexane | 11.301 | 0.05 | | | |
| 33. | Tetraethyleneglycol monomethylether | 11.425 | 0.09 | $C_9H_{20}O_5$ | 208.25 | 90263 |
| 34. | Ethanol, 2-[2-(2-methoxyethoxy)ethoxy]- | 11.522 | 0.09 | $C_7H_{16}O_4$ | 164.2 | 8178 |
| 35. | 5-Aza-4,5,6,7(3H)-tetrahydrobenzimidazole-6-carboxylic acid | 11.675 | 0.06 | $C_7H_9N_3O_2$ | 167.17 | 565071 |
| 36. | Methyl 3,5-tetradecadiynoate | 11.765 | 0.19 | $C_{15}H_{22}O_2$ | 234.33 | 14957556 |
| 37. | 9-Eicosene, (E)- | 11.839 | 0.3 | $C_{20}H_{40}$ | 280.5 | 5365037 |
| 38. | Diethyl Phthalate | 11.91 | 0.09 | | | |
| 39. | 10-12-Pentacosadiynoic acid | 11.96 | 0.1 | $C_{25}H_{42}O_2$ | 374.6 | 538433 |
| 40. | Caryophyllene oxide | 12.051 | 0.06 | $C_{15}H_{24}O$ | 220.35 | 1742210 |
| 41. | Ethyl-1-thio-.beta.-d-glucopyranoside | 12.168 | 0.08 | $C_8H_{16}O_5S$ | 224.28 | 71774698 |
| 42. | 1,5:2,4-Dimethanopentalene-3,6-diol, octahydro- | 12.24 | 0.05 | $C_{10}H_{14}O_2$ | 166.22 | 556428 |
| 43. | Retinal | 12.353 | 0.1 | $C_{20}H_{28}O$ | 284.4 | 638015 |
| 44. | Tricyclo[3.2.2.0]nonane-2-carboxylic acid | 12.446 | 0.08 | $C_{10}H_{14}O_2$ | 166.22 | 564376 |
| 45. | Bicyclo[3.1.1]heptan-2-one, 6,6-dimethyl-, (1R)- | 12.54 | 0.11 | $C_9H_{14}O$ | 138.21 | 32735 |
| 46. | Bicyclo[6.1.0]nonane, 9-(1-methylethylidene)- | 12.628 | 0.08 | $C_{12}H_{20}$ | 164.29 | 534869 |
| 47. | 5-[(1E,3E)-Hepta-1,3-dienyl]cyclohexane-1,2,3-triol | 12.695 | 0.06 | $C_{13}H_{22}O_3$ | 226.31 | 128964467 |
| 48. | Cholestan-3,22,26-triol 16-[2-[formylthio]ethyl]- | 12.805 | 0.05 | $C_{30}H_{52}O_4S$ | 508.8 | 543370 |
| 49. | 3-Methyl-cis-3a,4,7,7a-tetrahydroindan | 13.68 | 0.04 | $C_{10}H_{16}$ | 136.23 | 549177 |
| 50. | 1,2,3,4-Cyclopentanetetrol, (1.alpha.,2.beta.,3.beta.,4.alpha.)- | 13.84 | 0.09 | $C_5H_{10}O_4$ | 134.13 | 552302 |

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|---|---|---|---|---|---|---|
| 51. | 1,2,3,4,5-Cyclopentanepentol | 13.93 | 0.07 | $C_5H_{10}O_5$ | 150.13 | 552295 |
| 52. | 1-Hexadecanol | 14.22 | 0.06 | $C_{16}H_{34}O$ | 242.44 | 2682 |
| 53. | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 14.831 | 0.1 | $C_{20}H_{40}O$ | 296.5 | 5366244 |
| 54. | CH3SiH2SiH(CH3)2 | 14.945 | 0.18 | | | |
| 55. | Hexaethylene glycol dimethyl ether | 15.013 | 0.14 | $C_{14}H_{30}O_7$ | 310.38 | 70621 |
| 56. | Undecanoic acid, 10-methyl-, methyl ester | 16.01 | 0.06 | $C_{13}H_{26}O_2$ | 214.34 | 554144 |
| 57. | Hexadecanoic acid, methyl ester | 16.144 | 0.26 | $C_{17}H_{34}O_2$ | 270.5 | 8181 |
| 58. | Dibutyl phthalate | 16.735 | 0.07 | $C_{16}H_{22}O_4$ | 278.34 | 3026 |
| 59. | Ethyl 14-methyl-hexadecanoate | 17.2 | 0.04 | $C_{19}H_{38}O_2$ | 298.5 | 13530407 |
| 60. | N-Isobutylundeca-(2E,4E)-diene-8,10-diynamide | 18.334 | 0.03 | $C_{15}H_{19}NO$ | 229.32 | 5373537 |
| 61. | 9,12-Octadecadienoic acid, methyl ester | 18.869 | 0.46 | $C_{19}H_{34}O_2$ | 294.5 | 5284421 |
| 62. | 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- | 18.973 | 0.28 | $C_{19}H_{32}O_2$ | 292.5 | 5319706 |
| 63. | Phytol | 19.127 | 0.15 | $C_{20}H_{40}O$ | 296.5 | 5280435 |
| 64. | Methyl stearate | 19.378 | 0.08 | $C_{19}H_{38}O_2$ | 298.5 | 8201 |
| 65. | Z,Z,Z-1,4,6,9-Nonadecatetraene | 19.672 | 0.03 | $C_{19}H_{32}$ | 260.5 | 5362676 |
| 66. | 8-Methyl-6-nonenamide | 19.991 | 0.04 | $C_{10}H_{19}NO$ | 169.26 | 5365367 |
| 67. | Hexadecanamide | 20.326 | 0.04 | $C_{16}H_{33}NO$ | 255.44 | 69421 |
| 68. | N-Isobutyl-(2E,4E,8Z,10Z)-dodecatetraenamide | 20.559 | 0.5 | $C_{16}H_{25}NO$ | 247.38 | 11413953 |
| 69. | (4-Methyl-1-methylenepent-4-enyl)benzene | 21.718 | 0.11 | $C_{13}H_{16}$ | 172.27 | 575493 |
| 70. | 6,9-Octadecadienoic acid, methyl ester | 23.263 | 0.05 | $C_{19}H_{34}O_2$ | 294.5 | 5365662 |
| 71. | 9-Octadecenamide, (Z)- | 23.351 | 1 | $C_{18}H_{35}NO$ | 281.5 | 5283387 |
| 72. | 2,5-Octadecadiynoic acid, methyl ester | 23.443 | 0.21 | $C_{19}H_{30}O_2$ | 290.4 | 42151 |
| 73. | 5,5-Diethylheptadecane | 25.33 | 0.04 | $C_{21}H_{44}$ | 296.6 | 85977275 |
| 74. | 2-Methylhexacosane | 25.5 | 0.1 | $C_{27}H_{56}$ | 380.7 | 150931 |

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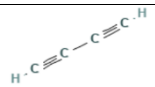
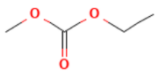
|---|---|---|---|---|---|---|
| 75. | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | 25.761 | 0.07 | $C_{19}H_{38}O_4$ | 330.5 | 123409 |
| 76. | Methyl 7,11,14-eicosatrienoate | 28.536 | 0.08 | $C_{21}H_{36}O_2$ | 320.5 | 91694374 |
| 77. | 14-Methyl-14-(3-oxobutyryloxy)-hexadec-15-enoic acid, methyl ester | 28.6 | 0.07 | $C_{22}H_{38}O_5$ | 382.5 | 538299 |
| 78. | cis-11-Eicosenamide | 29.849 | 0.05 | $C_{20}H_{39}NO$ | 309.5 | 5365374 |
| 79. | 3-(1,3-Dihydroxyisopropyl)-1,5,8,11,14,17-hexaoxacyclononadecane | 30.025 | 0.04 | | | |
| 80. | Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester | 33.353 | 0.14 | $C_{35}H_{62}O_3$ | 530.9 | 16386 |
| 81. | D-Streptamine, O-2-amino-2-deoxy-.alpha.-D-glucopyranosyl-(1-4)-O-(3-deoxy-4-C-methyl-3-(methylamino)-.beta.-L-arabinopyranosyl | 33.42 | 0.03 | $C_{11}H_{10}O_3$ | 190.19 | 35703 |
| 82. | .alpha.-Tocopheryl acetate | 34.836 | 0.03 | $C_{31}H_{52}O_3$ | 472.7 | 86472 |
| 83. | Stigmasterol | 37.133 | 0.3 | $C_{29}H_{48}O$ | 412.7 | 5280794 |
| 84. | .beta.-Sitosterol | 38.351 | 0.22 | $C_{29}H_{50}O$ | 414.7 | 222284 |

Table 12: Constituents of ethanolic leaf extract of Mikania micrantha (EMM) identified by GC–MS analysis

### 5.5.2: Chemical structures of constituents

| Sl. No. | Name of constituent | PubChem CID | Chemical Structure |
|---|---|---|---|
| **1** | 1,1,1-Trichloro-2-methylpropan-2-ol, O-trimethylsilyl- | | |
| **2** | 1,3-Butadiyne | 9997 |  |
| **3** | Carbonic acid, ethyl-, methyl ester | 522046 |  |

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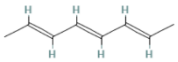
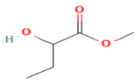
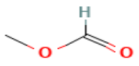
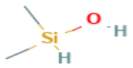
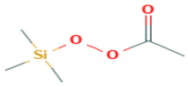
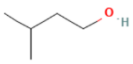
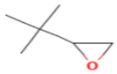
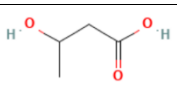
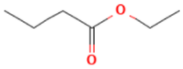
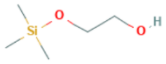
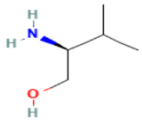
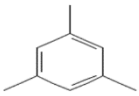
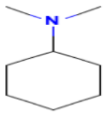
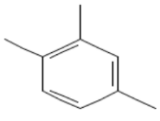
|---|---|---|---|
| **4** | (E,E,E)-2,4,6-Octatriene | 5368766 |  |
| **5** | Butanoic acid, 2-hydroxy-, methyl ester | 520445 |  |
| **6** | Methyl formate | 7865 |  |
| **7** | Silanol, dimethyl- | 521864 |  |
| **8** | Trimethylsilyl ethaneperoxoate | 12571375 |  |
| **9** | Trimethylsilyl fluoride | 9869 |  |
| **10** | 1-Butanol, 3-methyl- | 31260 |  |
| **11** | 3,3-Dimethyl-1,2-epoxybutane | 92174 |  |
| **12** | Butanoic acid, 3-hydroxy- | 441 |  |
| **13** | Butanoic acid, ethyl ester | 7762 |  |
| **14** | Ethylene glycol, TMS derivative | 554455 |  |
| **15** | (S)-(+)-2-Amino-3-methyl-1-butanol | 640993 |  |
| **16** | Mesitylene | 7947 |  |
| **17** | Cyclohexanamine, N,N-dimethyl- | 7415 |  |
| **18** | Benzene, 1,2,4-trimethyl- | 7247 |  |

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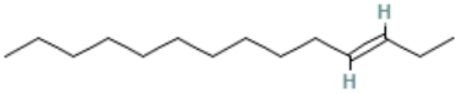
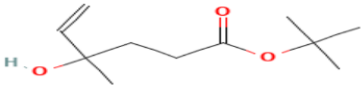
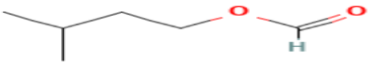
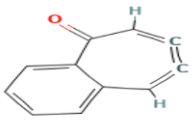
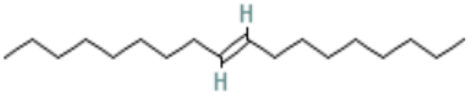
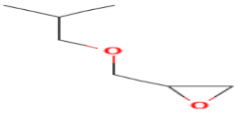
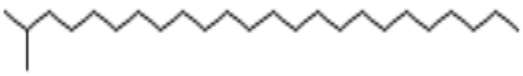
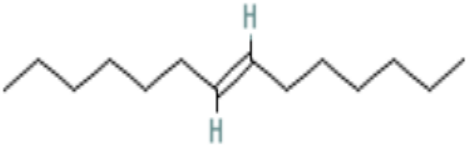
|---|---|---|---|
| **19** | D-Limonene | 440917 |  |
| **20** | 1-Dodecene | 8183 |  |
| **21** | 3-Tetradecene, (E)- | 5352802 |  |
| **22** | 4-Hydroxy-4-methylhex-5-enoic acid, tert.-butyl ester | 545523 |  |
| **23** | 1-Butanol, 3-methyl-, formate | 8052 |  |
| **24** | Benzocycloheptatriene | 129656441 |  |
| **25** | 1-Undecanol | 8184 |  |
| **26** | 9-Octadecene, (E)- | 5364599 |  |
| **27** | 2-(Isobutoxymethyl)oxirane | 98155 |  |
| **28** | 2-Methyltetracosane | 527459 |  |
| **29** | 7-Tetradecene | 5364651 |  |

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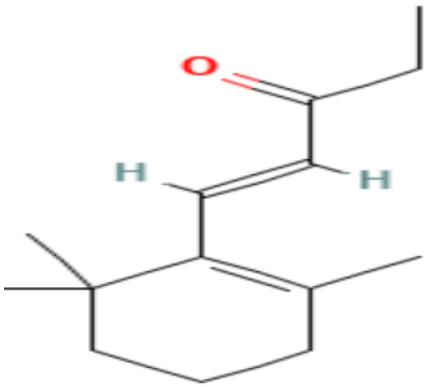
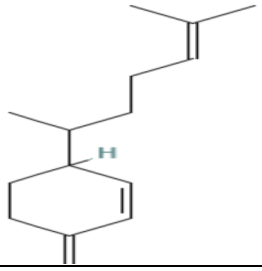
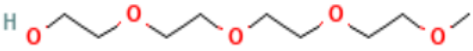
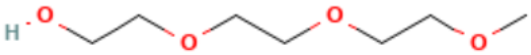
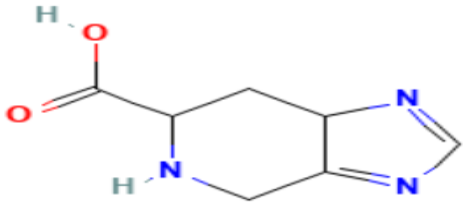
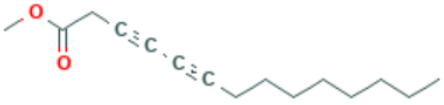
|---|---|---|---|
| **30** | 1-Penten-3-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)- | 5375218 |  |
| **31** | Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]- | 519764 |  |
| **32** | (1S,5S)-4-Methylene-1-((R)-6-methylhept-5-en-2-yl)bicyclo[3.1.0]hexane | | |
| **33** | Tetraethyleneglycol monomethylether | 90263 |  |
| **34** | Ethanol, 2-[2-(2-methoxyethoxy)ethoxy]- | 8178 |  |
| **35** | 5-Aza-4,5,6,7(3H)-tetrahydrobenzimidazole-6-carboxylic acid | 565071 |  |
| **36** | Methyl 3,5-tetradecadiynoate | 14957556 |  |

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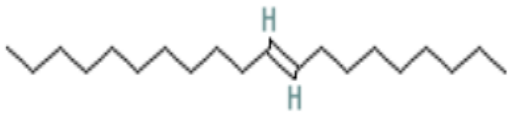
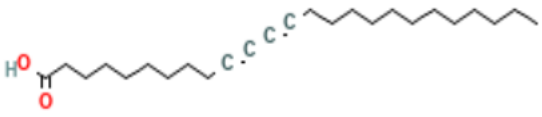
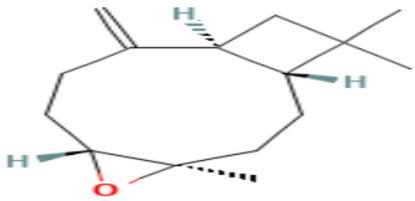
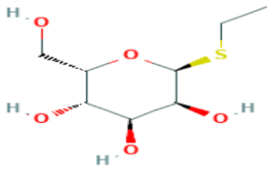
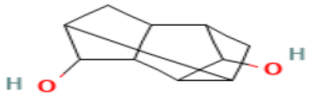
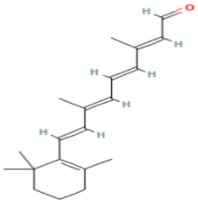
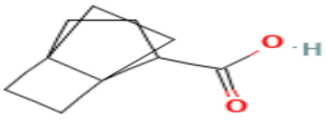
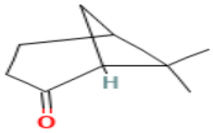
|---|---|---|---|
| **37** | 9-Eicosene, (E)- | 5365037 |  |
| **38** | Diethyl Phthalate | | |
| **39** | 10-12-Pentacosadiynoic acid | 538433 |  |
| **40** | Caryophyllene oxide | 1742210 |  |
| **41** | Ethyl-1-thio-.beta.-d-glucopyranoside | 71774698 |  |
| **42** | 1,5:2,4-Dimethanopentalene-3,6-diol, octahydro- | 556428 |  |
| **43** | Retinal | 638015 |  |
| **44** | Tricyclo[3.2.2.0]nonane-2-carboxylic acid | 564376 |  |
| **45** | Bicyclo[3.1.1]heptan-2-one, 6,6-dimethyl-, (1R)- | 32735 |  |
| **46** | Bicyclo[6.1.0]nonane, 9-(1-methylethylidene)- | 534869 | |

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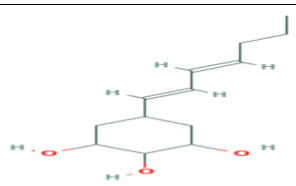
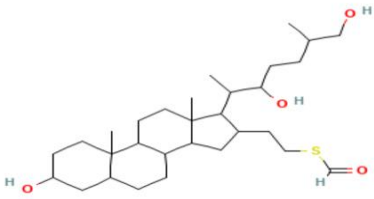
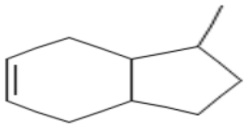
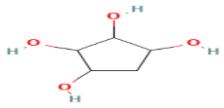
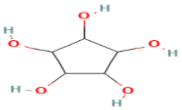
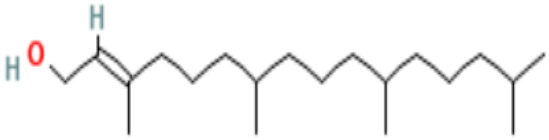
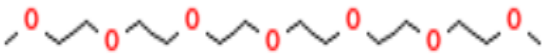
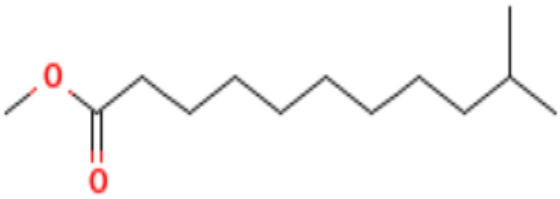
|---|---|---|---|
| **47** | 5-[(1E,3E)-Hepta-1,3-dienyl]cyclohexane-1,2,3-triol | 128964467 |  |
| **48** | Cholestan-3,22,26-triol 16-[2-[formylthio]ethyl]- | 543370 |  |
| **49** | 3-Methyl-cis-3a,4,7,7a-tetrahydroindan | 549177 |  |
| **50** | 1,2,3,4-Cyclopentanetetrol, (1.alpha.,2.beta.,3.beta.,4.alpha.)- | 552302 |  |
| | 1,2,3,4,5-Cyclopentanepentol | 552295 |  |
| | 1-Hexadecanol | 2682 |  |
| | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 5366244 |  |
| | CH3SiH2SiH(CH3)2 | | |
| | Hexaethylene glycol dimethyl ether | 70621 |  |
| | Undecanoic acid, 10-methyl-, methyl ester | 554144 |  |

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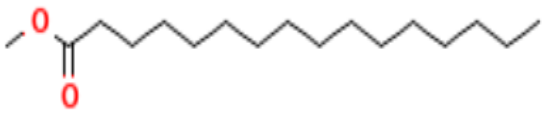
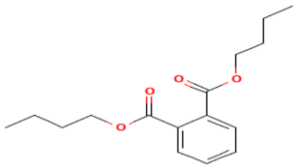
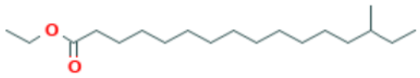
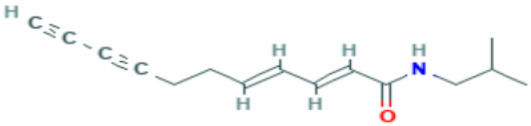
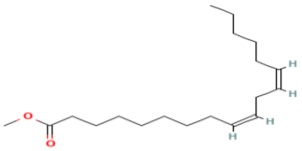
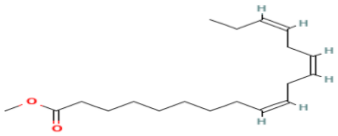
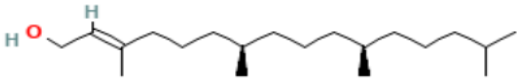
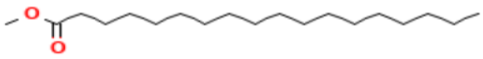
|---|---|---|---|
| | Hexadecanoic acid, methyl ester | 8181 |  |
| | Dibutyl phthalate | 3026 |  |
| | Ethyl 14-methyl-hexadecanoate | 13530407 |  |
| | N-Isobutylundeca-(2E,4E)-diene-8,10-diynamide | 5373537 |  |
| | 9,12-Octadecadienoic acid, methyl ester | 5284421 |  |
| | 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- | 5319706 |  |
| | Phytol | 5280435 |  |
| | Methyl stearate | 8201 |  |
| | Z,Z,Z-1,4,6,9-Nonadecatetraene | 5362676 |  |

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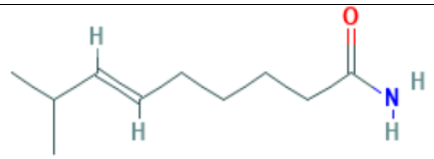
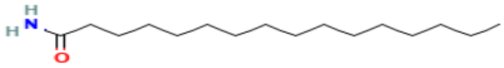
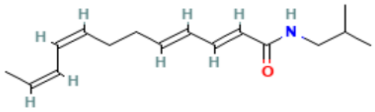
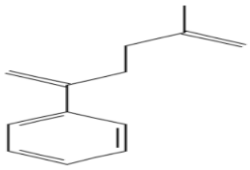
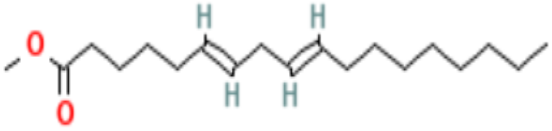
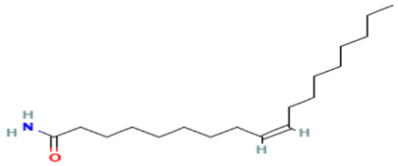
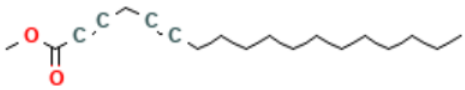
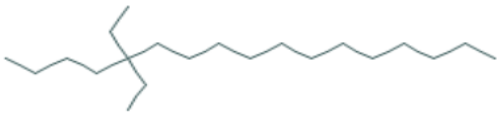
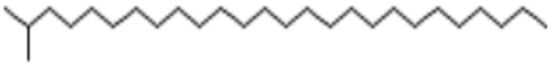
|---|---|---|---|
| | 8-Methyl-6-nonenamide | 5365367 |  |
| | Hexadecanamide | 69421 |  |
| | N-Isobutyl-(2E,4E,8Z,10Z)-dodecatetraenamide | 11413953 |  |
| | (4-Methyl-1-methylenepent-4-enyl)benzene | 575493 |  |
| | 6,9-Octadecadienoic acid, methyl ester | 5365662 |  |
| | 9-Octadecenamide, (Z)- | 5283387 |  |
| | 2,5-Octadecadiynoic acid, methyl ester | 42151 |  |
| | 5,5-Diethylheptadecane | 85977275 |  |
| | 2-Methylhexacosane | 150931 |  |

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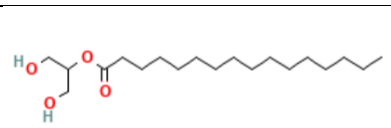
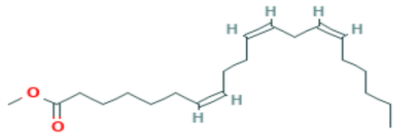
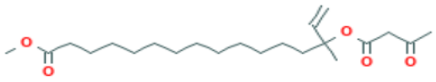
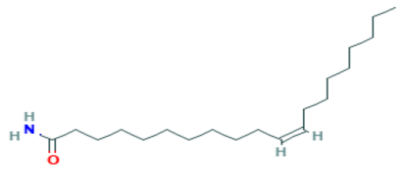
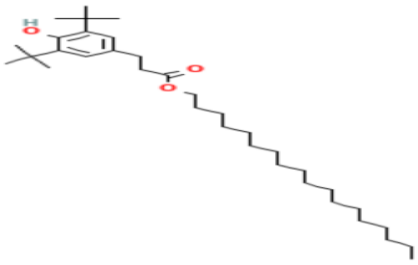
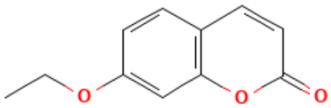
|---|---|---|---|
| | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | 123409 |  |
| | Methyl 7,11,14-eicosatrienoate | 91694374 |  |
| | 14-Methyl-14-(3-oxobutyryloxy)-hexadec-15-enoic acid, methyl ester | 538299 |  |
| | cis-11-Eicosenamide | 5365374 |  |
| | 3-(1,3-Dihydroxyisopropyl)-1,5,8,11,14,17-hexaoxacyclononadecane | | |
| | Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester | 16386 |  |
| | D-Streptamine, O-2-amino-2-deoxy-.alpha.-D-glucopyranosyl-(1-4)-O-(3-deoxy-4-C-methyl-3-(methylamino)-.beta.-L-arabinopyranosyl | 35703 |  |

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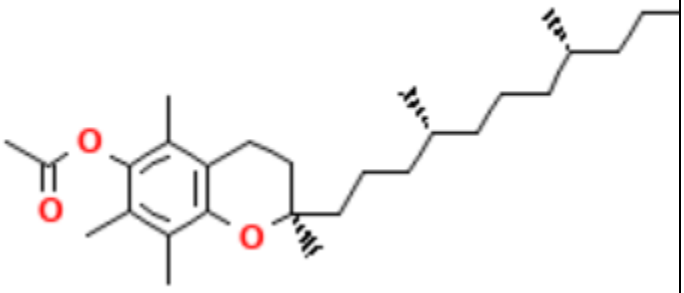
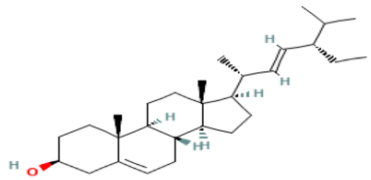
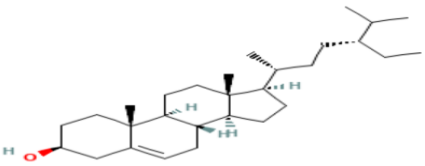
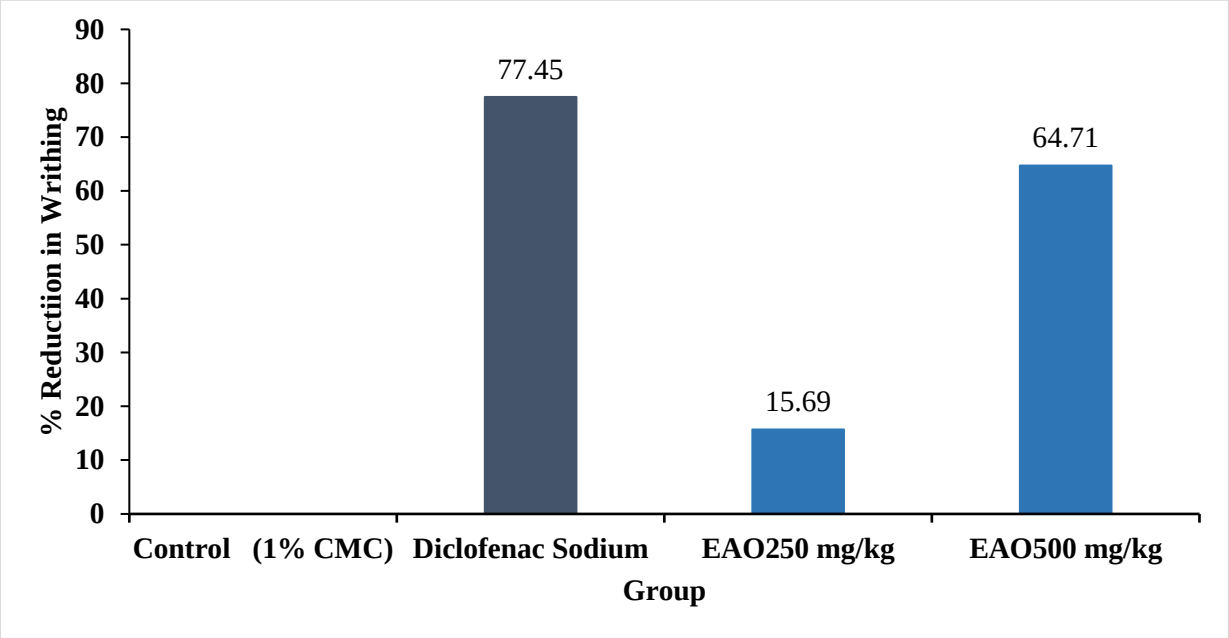
|---|---|---|---|
| | .alpha.-Tocopheryl acetate | 86472 |  |
| | Stigmasterol | 5280794 |  |
| | .beta.-Sitosterol | 222284 |  |

Table 13: Chemical structures of constituents of e ethanolic extract of Acmella Oleracea (EAO) identified by GC–MS analysis.

### 5.6 Analgesic Activity test results:

| Group | First Phase Writhing (0-10 minutes) | Second Phase Writhing (11-20 minutes) | Total Writhing (0-20 minutes) | % Reduction in Writhing |
|---|---|---|---|---|
| Control (1% CMC) | 15.40 | 25.40 | 40.80 | 0.00 |
| Diclofenac Sodium | 1.20 | 7.80 | 9.20 | 77.45 |
| EAO250 mg/kg | 9.40 | 25.00 | 34.40 | 15.69 |
| EAO500 mg/kg | 3.80 | 10.60 | 14.40 | 64.71 |




**Analgesic Activity And % of Reduction of writhing**

**5.3 Antidiarrheal activity test results:**

| Code no. | Mice No. | Total no. of diarrheal faeces | | | | Total faeces | Avg. | MEAN | SD |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 hour | 2 hour | 3 hour | 4 hour | | | | |
| CTL (1% tween 80) | 1 | 1 | 1 | 2 | 2 | 6 | 4 | 4 | 1.7 |
| | 2 | 0 | 1 | 1 | 1 | 3 | | | |
| | 3 | 0 | 0 | 2 | 1 | 3 | | | |
| STD (Loperamide) | 1 | 0 | 0 | 1 | 1 | 2 | 2 | 2 | 0.0 |
| | 2 | 0 | 0 | 1 | 1 | 2 | | | |
| | 3 | 0 | 0 | 0 | 2 | 2 | | | |
| EAO (250) | 1 | 0 | 0 | 1 | 1 | 1 | 3 | 3 | 0 |
| | 2 | 0 | 0 | 0 | 1 | 2 | | | |
| | 3 | 0 | 0 | 1 | 1 | 1 | | | |
| EAO (500) | 1 | 0 | 1 | 1 | 1 | 3 | 2.33 | 2.33 | 0.6 |
| | 2 | 0 | 1 | 1 | 0 | 2 | | | |
| | 3 | 0 | 0 | 1 | 1 | 2 | | | |

| Group | Total Diarrhea | % Of Reduction Diarrhea |
|---|---|---|
| Control | 12 | 00% |
| Standard | 06 | 50% |
| EAO 250 | 09 | 25% |
| EAO 500 | 07 | 41.67% |

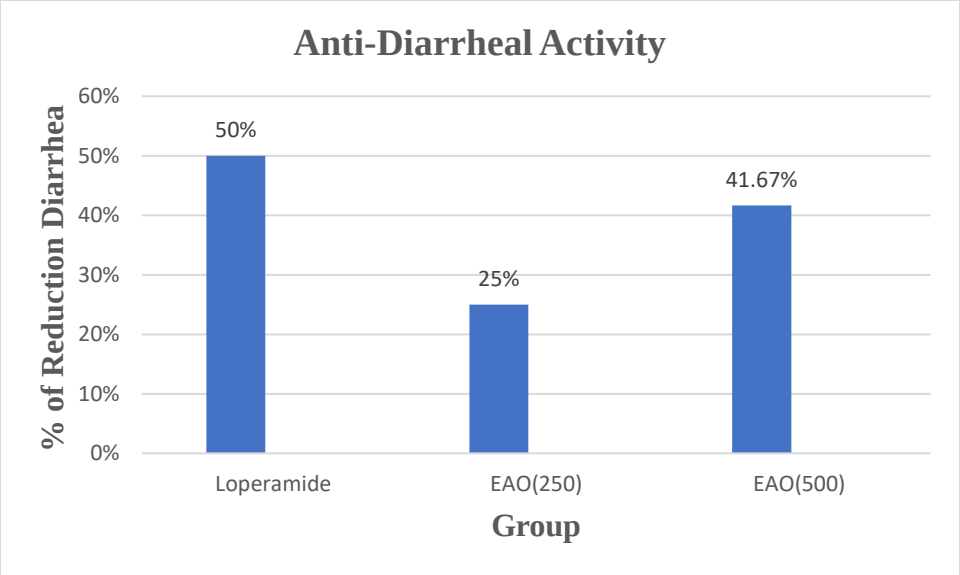



Table 10: Antidiarrheal Activity And % of Reduction Diarrhea

# *Chapter Six*

# *Conclusion*

Finally, as demonstrated by the identification of many secondary metabolites by GC-MS profiling, the ethanolic extract of *Acmella Oleracea* displayed a noteworthy spectrum of phytochemical variety." Its therapeutic benefits could be facilitated by certain plant components. The extract showed a notable capacity for antioxidants, suggesting that illnesses linked to oxidative stress may benefit from its application. Additionally, the treatment's proven capacity to suppress diarrhea suggests that it may be a viable alternative to antidiarrheal medication. Additionally, the extract demonstrated thrombolytic activity, suggesting that it may be used to treat thrombotic illnesses. To understand the processes behind these effects and assess their safety profile for potential therapeutic applications, more study is yet required.

# *Chapter Seven*

# *Reference*

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