ESSENTIAL OIL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE RHIZOME OF *Curcuma longa* L. {Fam. *Zingiberaceae*} USING HYDRO-DISTILLATION METHOD

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CERTIFICATION

This is to certify that this research project was carried out by **SYLVESTER EBIBOTE KOWEI**, of the Department of Chemistry, Faculty of Physical Sciences, University of Benin

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Sylvester Ebibote Kowei (Student)	Date
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Dr I.G. Osigbemhe (Project Supervisor)	Date
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Prof. E.E.I Irabor (Head of Department)	Date

DEDICATION

This work is dedicated to God Almighty for making this project research a success and seeing me throughout the university of Benin and to my beloved parents, **Barr Benjamin Kowei and Mrs. Roseline Kowei** for their unconditional love and support in my life.

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ABSTRACT

This project was carried out to extract essential oil from the rhizome of *Curcuma longa* using the hydro-distillation method with a Clevenger apparatus. *Curcuma longa* is widely recognized for its medicinal uses, especially in India and other Asian cultures. The rhizomes are reported to contain essential oils with antimicrobial and antioxidant properties. However, while much attention has been given to the medicinal value of turmeric, comparatively less focus has been placed on the processes of extracting its essential oil and systematically evaluating their outcomes. In this study, dried rhizomes were ground into powder and hydro-distilled. The essential oil obtained was yellowish with a strong aromatic odor. The GC-MS analysis revealed a variety of compounds in the oil, with phenylbutazone (19.34%) and 6-theothiophylline (19.01%) being the most significant constituents, along with some other minor compounds. The antimicrobial efficacy of the oil was evaluated against selected pathogenic fungal and bacterial isolates. The *curcuma longa* extract stopped the growth of several fungi (*Aspergillus Niger*, *Trichophyton rubrum*, *Penicillium sp*.) and bacteria (*Klebsiella pneumoniae* and *Staphylococcus aureus*). However, it did not kill them at the tested concentration. Some microbes, like the bacteria Escherichia *coli* and *Pseudomonas sp*., and the fungus *Aspergillus flavus*, were not affected at all. Overall, this study shows that hydro-distillation method is a simple and effective method for obtaining turmeric essential oil at the laboratory scale. Characterizing its chemical profile and biological properties provides insight into its value and supports its potential applications in medicine, cosmetics, and food preservation. These findings validate the medicinal use of *curcuma longa* and highlight its potential as a source of natural antimicrobial agents. The oil shows particular promise for applications targeting both gram-positive and gram-negative bacteria and fungi. This study provides a foundation for the further development of *curcuma longa* based natural preservatives.

CHAPTER ONE

1.1 Introduction

The genus *Curcuma L*. (*Zingiberaceae*) consist of perennial plants with rhizome, mostly found in tropical and subtropical regions. These plants are cultivated widely in Asia and also in parts of Australia and South American. Researchers estimate that they are around 93 to 100 species, although the exact figure is still debated. *Curcuma* species are valued for their natural pigments and flavor, making them important in Asian cooking, herbal medicine, spices, dyes, perfumes, cosmetics, and even as ornamentals. In herbal medicine, different species are used in countries like India, Bangladesh, Nepal, Malaysia, and Thailand for treating ailments such as bronchial problems, pneumonia, diarrhea, dysentery, wounds, abscesses, and insect bites. The rhizome is the part most often used, as it contains two main groups of active compounds: curcuminoids (like curcumin, desmethoxycurcumin, and bisdemethoxycurcumin) and volatile essential oils. The curcuminoids are polyphenolic in nature, non-toxic, and are known for their wide range of biological activities (Doskey & Setzer, 2018).

1.1.1 Background of Study

According to (Sahoo *et al*., 2021) *curcuma longa* is widely studied by researchers because of its many health related benefits. It has shown strong antioxidant and anti-inflammatory effects, and may also help prevent or treat cancer, Metabolic disorder, Arthritis, and infections caused by bacteria and viruses. In addition, *curcuma longa* has potential benefits for protecting the brain, heart, and liver as well as managing obesity, asthma, diabetes, depression, and anxiety. Medicinal plants have long been a corner stone of health care with over 35,000 species used worldwide for therapeutic purpose (Wong-Yee ching *et al*., 2014). Plants extract and essential oil are not only valued for their for their flavor and aroma but also for their medicinal properties, offering compound with with significant therapeutic potential. In many developing countries, plant-based healing practices still forms the basis of primary healthcare, with herbs such as *curcuma longa*, ginger, and galangal widely utilized for its healing effect (Wong-Yee ching *et al*., 2014). *Curcuma longa* is commonly used in kitchen as natural colorant, preservative and and spice. Its long history in Ayurda reflects its broad medicinal application, including its role as an antiseptic, wound healing agent and anti-inflammatory remedy (Wong-Yee ching *et al*., 2014).

Essential oils are highly concentrated, volatile mixtures that plant produce as secondary metabolites. They can be found in in different parts of the plant such as flowers, leaves, roots, rhizome, seed. And obtain by method like hydro distillation, steam distillation, or even supercritical CO_2 extraction. Because of their aroma and different biological effects, essential oils are widely used in cosmetics, perfumery, aromatherapy and also in food application. *Curcuma longa* which belongs to the ginger family (zingiberaceae), is one of the most widely studied plant in this regard. It is a perennial herb that is not only used in cooking but also in herbal medicine, especially in many Asian countries (Stanojevic *et al*., 2015). India is the main producer and exporter of *curcuma longa*, followed by countries like China, Myanmar, and Bangladesh. *Curcuma longa* is a very important spice in many households, but apart from cooking, it also plays a role in cosmetics, pharmaceutical, and food preservation (Jaiswal & Naik, 2021). One of the key component found in curcuma longa is curcumin, which gives curcuma longa its yellow color. Curcumin is well known for its anti-inflammatory, anti-oxidant, and anti-microbial properties. In addition to curcumin, the turmeric rhizome also contains volatile essential oil, which are mostly made up of compounds like aromatic-turmerone, α-turmerone, β-turmerone and zingiberene, these are classified as sesquiterpenes and have shown strong biological activity, especially in fighting microbes and neutralizing harmful oxidant (Stanojevic *et al*., 2015).

According to (Bhowmik *et al*., 2009) *Curcumin* is believed to help stimulate bile production, and also curcuma longa has been noted for its use in managing measles. The dried rhizomes are usually ground into fine powder and then mixed with a little honey and the juice bitter gourd leaves can be taken by people affected by measles as part of natural practice.

1.1.2 Statement of Problem

Curcuma longa has long been valued both in the kitchen and in herbal medicine. In Ayurveda and other indigenous healing systems, it has been used in treating inflammation, infections, and a range of other health conditions (Wong-Yee ching *et al*., 2014). Studies have shown that *curcuma longa* rhizomes, whether fresh or dried, contain compounds with strong antioxidant properties, confirming its medicinal potential (Wong-yee ching *et al*., 2014). Much of its activity comes from curcuminoids particularly curcumin as well as essential oil rich in turmerones (Wong-Yee ching., 2014).

Medicinal plants provide an invaluable source of bioactive compounds with potential therapeutic uses. According to world health organization (WHO) more than 80% of the world's population relies on plant derived medicine for primary health care, underscoring the importance of plant-based remedies (Sasidharan *et al.,*2011). Plants used in these healing practices are known to contain wide range of substance that can treat both chronic and infectious diseases, and many of these remedies are reported to have anti-cancer, anti-microbial, antioxidant, anti-diarrheal, analgesics, and wound healing activities (Sasidharan *et al*., 2011). Yet despite their promise, these natural products require require scientific evaluation through extraction, isolation and characterization of the compounds responsible for their biological effect. The first problem lies in extraction, which is described as the most important step in the analysis of plant constituents (Sasisdharan *et al*.,2011) plant extract are complex mixtures, and poor extraction methods may result in in the loss, distortion, or destruction of of bioactive compound. While conventional method such as Soxhlet extraction are mostly used, they are time consuming and can be inefficient. Although modern methods such as supercritical fluid and micro wave assisted extraction reduce solvent use and improve efficacy, they remain technically demanding and costly (Sasidharan *et al*., 2011)

Another limitation lies in in identification and characterization. Plant extract contains many compound with different polarities, which makes separation difficult. Also, *curcuma longa* naturally gives a small quantity of oil, especially when dried, which makes extraction a bit challenging. So it is important to look into how the oil can be properly extracted, and also to analyze the components inside it to know which one are actually active, this is where gc-ms (gas

chromatography-mass spectrometry) comes in. it helps in identifying the chemical compounds present in the oil. In addition, doing an anti-microbial test on the extracted oil can help show how effective it is against certain bacteria or fungi.

This project focuses on extracting essential oil from from dried *curcuma longa* using hydro-distillation with Clevenger apparatus, analyzing the oil using gc-ms, and also testing its anti-microbial activity. The aim is to find out not just how much oil can be gotten, but also what it contains and whether it can really fight microorganism, so it can be a natural alternative to chemical preservatives.

1.1.3 Justification of The Study

Curcuma longa has been used for many years, especially for treating wounds, skin condition, minor infection as well as homes for cooking. Even though *curcuma longa* is commonly used in its powered or raw form, not more attention has been given to its essential oil, especially in scienctific research. Many people are still unaware of the potential benefit of the oil or the chemical components it contains.

In today's world, there are many health problems caused by bacteria, and some medicine are no longer working as well as before. Because of these people are now looking for natural solutions that may have fewer sides effect. Essential oils from plant are being studied more, and *curcuma longa* would be one of those with good potential.

Also, many people still use curcuma longa based on tradition, without knowing its scientific value. This project is important because it helps to study the oil in a proper way using extraction and analysis method like hydro-distillation and gc-ms. The oil is also being tested to check if it can fight bacteria, which can help prove if it really works as people believe. This study may also help connect empirical knowledge with modern science by showing what's really inside the oil and whether it can be useful in real life application, such as medicine, food storage, or even skincare. It also creates more awareness about how *curcuma longa* can be used even beyond cooking and home remedies. This kind of research can open more ways to use curcuma longa instead of just hearing that curcuma longa is good.

1.1.4 Scope of The Work

This research mainly focuses on the extraction of essential from *curcuma longa* using hydro-distillation method with Clevenger apparatus. The aim was to extract the oil, also observe its antimicrobial properties. The study also involves analyzing the chemical component of the oil using gas chromatography-mass spectrometry to identify the major compounds present in it.

Apart from just extracting and analyzing this oil, this study also includes anti-microbial testing to check if the oil can act against certain bacteria. This part help to give us an idea whether the oil has any effect in terms of fighting germs or preventing growth of micro-organism

This study is limited to *curcuma longa* and does not cover other plants or spices. Also, this experiment was carried out in the lab using available materials and equipment. It does not include large scale production, but the outcome may be useful for future research or possible development of natural product

1.1.5 Limitations

During the course of this project, they were few limitations that affected how everything went. One of the issues was the low amount of essential oil gotten from the dried curcuma longa even though a reasonable amount of curcuma longa was used, and this is because *curcuma longa* naturally yields very little oil. Also, there were times the hydro-distillation process took longer than expected, and sometimes we were not sure if the heat was too much or too low. We also had issues maintaining a consistent temperature, we tried our best to monitor it closely because the set wasn't fully digital or automatic as there might have been some small fluctuation that could have affect the oil quality. Lastly another challenge was electricity supply. There were moments when we had to pause the whole processes due to power failure, especially during the hydro-distillation process since the Clevenger apparatus depends on constant or consistent heating. The equipment used was quite basic, we had no tempeture control so we had to monitor the boiling manually and this made the whole process slower and a bit stressful

1.1.6 Aim and Objectives

The aim of this research is to determine the chemical composition and biological activity of *curcuma long* using hydro-distillation method.

Objectives of the study:

1. To extract the essential oil from turmeric using hydro-distillation process.
2. To analyze the chemical composition of the oil using gc-ms (Gas Chromatography-Mass Spectrometry).
3. To test the antimicrobial activity of the oil using selected microorganisms.
4. To observe and record the effectiveness of the oil based on zone of inhibition from the microbial test.

1.2 Literature Review

1.2.1 History of Turmeric (*Curcuma longa)*

Over a century, *curcuma* species have also been used and grown in countries like India, Pakistan, Indonesia, Malaysia, Nepal and Thailand where they play an important role in both herbal medicine and local culture (Dosoky *et al*., 2019). The exact origin of turmeric is not clearly known, but it has been used for thousands of years in many cultures. In India, turmeric is strongly connected with Ayurveda, an ancient healing system that means "science of life" (Ayur = life, Veda = knowledge). In ancient practice, *curcuma longa* was burned and the fumes inhaled to relieve congestion, its juice was used to heal wounds, and pastes made from it were applied on skin conditions like chickenpox, blemishes, and rashes. In Ayurvedic texts, turmeric is given more than 100 names, such as jayanti meaning "one who is victorious over diseases," and matrimanika meaning "as beautiful as moonlight."

Plate 1: Dried *curcuma longa* rhizome

Curcuma longa has also been seen as sacred in both Aryan and Dravidian cultures, with its importance stretching back to ancient indigenous beliefs. In northern India it is called haldi (from the Sanskrit word haridra), while in the south it is known as manjal, a name often found in old Tamil writings. Its medicinal uses were recorded in Sanskrit medical treatises and both Ayurvedic and Unani systems of medicine. For example, Susruta's Ayurvedic text from around 250 B.C. suggested using turmeric ointments to treat the effects of poisoned food.

Beyond medicine, turmeric has a spiritual role in India. It was used in worship of the Sun God and mentioned in the Atharvaveda. People also wore it for purification rituals. Buddhists later used turmeric as a dye for their robes while traveling abroad, spreading its use further. Records show curcuma longa being used in Chinese medicine as far back as the 7th century, especially for treating spleen, stomach, and liver problems.

Curcuma longa only became known in the western world much later. Marco Polo described it in 1280 AD as "Indian saffron," noting that it looked like saffron but came from a root. By around 700 AD turmeric had reached China, by 800 AD East Africa, by 1200 AD West Africa, and gradually it spread across the globe. In China it was also used as an antibiotic, antiviral, and pain reliever.

Today, India remains the largest producer and consumer of *curcuma longa*, though it is also grown in countries like Bangladesh, Pakistan, Sri Lanka, Myanmar, Indonesia, and parts of Latin America and the Caribbean such as Jamaica, Haiti, Peru, and Brazil. Portuguese traders, including Vasco da Gama in the 15th century, also helped introduce turmeric to the West.

Turmeric is now considered one of the most important spices in the world. Apart from its cooking value, it is also widely used as a natural medicine in countries like India, Pakistan, and Bangladesh, and still holds a central place in Ayurveda, Unani, and Siddha healing systems. (Kaur, 2019).

1.2.2 Extraction Method of *Curcuma Longa* Essential Oil

1.2.2.1 Hydro-Distillation Method

Hydro-distillation method is one of the oldest methods of *extracting* essential oil from plant. The plant material is boiled in water, the steam that come out carries the oil which is then cooled in the condenser so that it turns back into liquid. The oil and the water separate naturally because of their difference in densities.

The Clevenger apparatus used for this set-up is designed so that the oil can be separated continuously during distillation. This makes the whole process easier since the oil separates on his own and you don't have to keep stopping the distillation to remove it manually.

The main advantage of hydro-distillation is that it is easy to use, with low set up and running cost. Another benefit is that it often gives a higher yield of essential oils compared to steam distillation, this is because in hydro distillation, the plant material is soaked and heated directly in water which allows for better penetration and release of the oil. However, heat sensitive material may degrade because the plant material is in direct contact with the boiling water. In steam distillation, the steam sometimes causes the plant material to clump together making it harder for the steam to pass through evenly, which can reduce extraction efficiency (Sethunga *et al*., 2022).

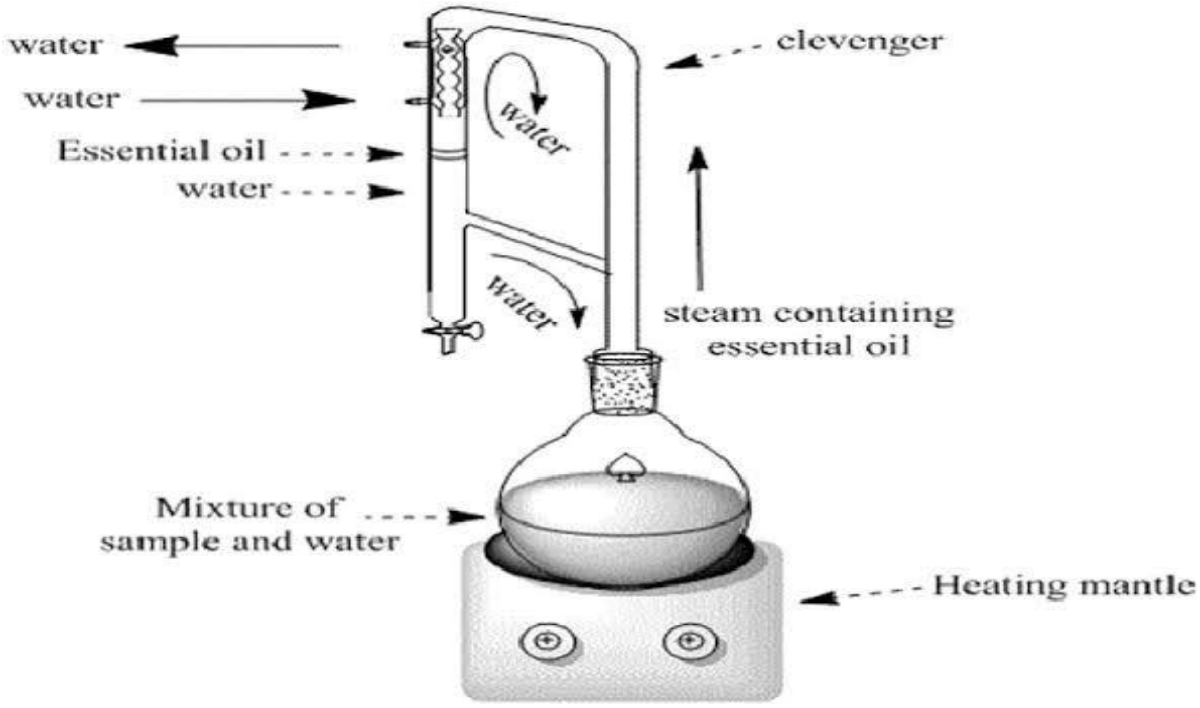

Plate 2: clevenger apparatus set-up

1.2.3 Biological Activities of *Curcuma Longa* Essential Oil

1.2.3.1 Antimicrobial Properties

One of the most well-known activities of *curcuma longa* essential oil is its ability to fight against bacteria and fungi. Research have shown it that it can slow down or stop the growth of harmful micro-organism such as *Escherichia coli Staphylococcus aureus.* These bacteria are known to cause food poisoning and other infection, so having a natural product that can control them is important.

1.2.3.2 Antioxidant Activity

Curcumin, the main active compound in turmeric, is well known for its strong antioxidant properties. It works by neutralizing oxygen free radicals, which are harmful molecules that can damage cells. Infact, its antioxidant effect has been reported to be almost as strong as that of vitamin C and E. Curcumin helps protect important biomolecules such as lipids and hemoglobin from getting oxidized. It can also reduce the production of reactive oxygen species (ROS) like

hydrogen peroxide (H_2O_2), superoxide anions, and nitrite radicals that are usually produced by activated macrophages during stress. Interestingly, curcumin is not the only compound with this effect, its related forms, bis-demethoxycurcumin and demethoxycurcumin, also show similar antioxidant activity. Studies have even shown that pre-treatment with curcumin lowers oxidative stress in the heart caused by ischemia (restricted blood supply). In vitro experiments further revealed thar curcumin can increase the expression of stress-response proteins, which improves the ability of cells to resist oxidative damage (Dada khalander *et al.,* 2018).

1.2.3.3 Anti-Inflammatory Activity

Joint inflammation is a common cause of rheumatic pain. *Curcuma longa* which considered a healing spice is known for its anti- Inflammatory properties. It's thought to treat both the root causes and the physical effect of inflammatory. Furthermore, a study on animals showed that taking curcumin (the active ingredient in curcuma longa) orally was effective at reducing inflammation in both short term and long-term cases (Dada khalander *et al.,* 2018).

1.2.3.4 Anti-Cancer Agent

Cancer is responsible for about one-fifth of deaths worldwide. It is mainly caused by changes in the body's genes that make cells grow out of control, spread to other parts of the body, and form new blood vessels to support tumors. Normally, the body uses a process called apoptosis (programmed cell death) to remove damaged or unnecessary cells, but in cancer this process fails.

Curcumin, the active compound in turmeric, has been widely studied for its anticancer effects. Research has shown that it can help in cancers such as those of the stomach, breast, and lungs. Experiments in the lab and in animals also suggest that curcumin can slow down or prevent cancer by stopping tumor growth and blocking the blood supply that tumors need. In addition, special curcumin-based compounds (curcumin aptamers with sulfone) have shown strong effects against prostate, colon, lung, and pancreatic cancers (Sahoo *et al*., 2021).

1.2.4 Health Benefit of *curcuma longa*

Turmeric provides a wide range of health benefits in daily life. It acts as a natural antiseptic and antibacterial agent, making it useful for disinfecting cuts and burns. Research has shown that when combined with cauliflower, it can help prevent prostate cancer and even slow the growth of

existing cancer cells. In animal studies, turmeric has been observed to prevent breast cancer from spreading to the lungs and to induce self-destruction in melanoma cells. It also lowers the risk of childhood leukemia and serves as a natural detoxifier for the liver.

Turmeric has been linked to improved brain health, with evidence suggesting it may prevent or slow the progression of Alzheimer's disease by reducing amyloid plaque buildup. It shows potential in preventing cancer metastasis and offers strong natural anti-inflammatory effects comparable to conventional drugs, but without harmful side effects. Studies in mice also indicate that turmeric may help slow the progression of multiple sclerosis.

Beyond its anti-inflammatory role, turmeric works as a natural painkiller and COX-2 inhibitor, supports fat metabolism, and aids in weight management. It has long been valued in traditional Chinese medicine as a treatment for depression. Because of its ability to ease inflammation, it is also widely used to relieve arthritis and rheumatoid arthritis. In cancer care, turmeric has been found to boost the effects of chemotherapy drugs such as paclitaxel while reducing their side effects. Promising studies are underway to explore its role in treating pancreatic cancer, multiple myeloma, and other cancers by inhibiting the growth of new blood vessels in tumors (Bhowmik *et al*., 2009).

1.2.5 *Curcuma longa* Medicinal Uses

For many years, awareness of turmeric and its medicinal applications has continued to grow. Turmeric is a flowering plant from the ginger family, widely used as a food coloring and as one of the main ingredients in curry powder. Traditionally, it has been employed to treat health issues such as liver problems, digestive disorders, skin diseases, and wounds, largely due to its strong anti-inflammatory properties. Its active compound, curcumin, has been shown to possess a wide range of therapeutic effects (Bhowmik *et al*., 2009).

1.2.5.1 Digestive Disorders

Turmeric is regarded as both a digestive bitter and a carminative. When added to foods such as rice and beans, it helps improve digestion while reducing gas and bloating. It acts as a cholagogue, stimulating bile production in the liver and promoting bile release through the gallbladder. This enhances the body's ability to digest fats. For individuals with chronic digestive weakness or congestion, turmeric is often recommended. It can be taken alone as an extract or combined with other herbs in digestive bitters. Turmeric is particularly beneficial for people who feel sluggish or experience discomfort such as bloating after meals. In all forms, it supports both the digestive system and the liver (Bhowmik *et al*., 2009).

1.2.5.2 Liver Diseases

Turmeric also provides protective effects for the liver. It contains compounds similar to those found in milk thistle and artichoke leaves, which are known for liver support. It is believed to reduce swelling in the hepatic ducts, making it potentially useful in the treatment of conditions such as hepatitis, cirrhosis, and jaundice. Consuming turmeric, particularly in the spring, is thought to help strengthen liver function (Bhowmik *et al*., 2009).

1.2.5.3 Cancer

Recent scientific studies indicate that *curcuma longa* has the potential to treat a wide range of diseases and can inhibit the growth of several types of cancer. It has been particularly noted for its role in managing skin cancer and precancerous skin conditions. Both topical application and oral consumption of turmeric have shown beneficial effects in this regard (Bhowmik *et al*., 2009).

1.2.5.4 Menstrual Problems in Women

Turmeric may also help women who experience monthly menstrual cramps. Taking turmeric extract or bitters twice daily for two weeks before menstruation has been suggested to reduce pain. Its antispasmodic properties relax smooth muscles, thereby easing both digestive and menstrual cramps. While overall diet and lifestyle strongly influence the menstrual cycle, turmeric can serve as a valuable natural aid in reducing the severity of menstrual discomfort (Bhowmik *et al*., 2009).

1.2.6 Uses Of *Curcuma Longa*

1.2.6.1 Food Addictive

Turmeric has been valued since ancient times for both medicinal and cosmetic purposes. In the Ayurvedic system of Indian medicine, it is regarded as an important herbal remedy prescribed for a variety of health conditions. Interestingly, turmeric has even been applied in non-medical uses, such as plugging leaks in water-cooled radiators. Its wide range of applications can be grouped into food, medicine, and cosmetics (Bhowmik *et al*., 2009).

1.2.6.2 Cosmetic Uses

Turmeric has long been applied in skincare. A paste made from raw turmeric juice is traditionally applied to the skin to enhance its glow. It is also used in Indian wedding rituals, where it is combined with sandalwood paste and applied before bathing. Regular bathing with turmeric-infused water is believed to slow the growth of body hair, while consistent use is said to promote fairer, softer, and smoother skin. Furthermore, turmeric is used to treat pigmentation spots, blotches, and certain skin conditions such as eczema (Bhowmik *et al*., 2009).

CHAPTER TWO

MATERIALS AND METHODS

2.0 Materials

- Condenser
- 1000ml flat bottom flask
- Clevenger Extractor
- Retort stand
- Petri dish

2.1 Instruments

- Heating Mantle
- Analytical Balance

2.2 Reagents and Chemicals

- Water

2.3 Methodology for Hydro distillation and Bacterial Activity

2.3.1 Collection of Sample

Dried turmeric rhizomes (*Curcuma longa*) were purchased from retailers along Mission Road market Benin city, Edo State on April 29th, 2025. The sample was then taken to the department of Plant Biology and Biotechnology (PBB), University of Benin (UNIBEN) where it was identified and authenticated with the herbarium voucher number "UBH-C397 of May 2, 2025. After authentication, the rhizomes were thoroughly cleaned to remove soil and other unwanted particles. The outer coverings were carefully removed, and the rhizomes were sliced into smaller pieces. They were then ground into powder using a milling machine at the Faculty of Pharmacy, University of Benin. The powdered sample was packed in a clean polythene bag and stored in a cool, dry place until further analysis.

2.3.2 Preparation of *Curcuma Longa* for Extraction Using Hydro Distillation

About 100g of the powdered turmeric rhizome was carefully weighed and transferred into a 1000ml round bottom flask. Then, 700ml of distilled water was added to the flask containing the sample. The flask was them placed on a heating mantle and connected to the hydro distillation apparatus (Clevenger setup). The temperature of the mantle was adjusted to 80^0C to allow steady boiling and generation of steam. As the process continued, steam passed through the plant material, carrying the volatile oil components along with it. The vapor mixture moved into the condenser, where it was cooled and converted back into liquid form. The distillate collected contained both and water layers, which gradually separated based on their density differences. The essential oil was carefully collected, dried, and stored im a clean, airtight sample bottle for further characterization and antimicrobial studies.

2.3.3 GC-MS Analysis

Gas chromatography–mass spectrometry (GC–MS) analysis of the extracted turmeric oil (0.20 mL) was conducted on a PHYTOSCAN 2.M GC–MS system fitted with an HP-5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness). The injector oven temperature was initially set at 60 °C for 2.5 minutes, then increased at a rate of 10 °C/min to 180 °C, where it was held for 1 minute. The temperature was further raised at 20 °C/min to 280 °C and maintained for 15 minutes. A 1 µL aliquot of the oil sample, diluted in 1% hexane, was introduced in split mode with a ratio of 1:10. Helium served as the carrier gas at a constant flow of 1.0 mL/min, with the column pressure maintained at 100 kPa. Identification of the constituents was achieved by comparing the mass spectra obtained with those in the NIST 2020.L GC–MS library.

2.3.4. Antimicrobial Activity

2.3.4.1 Inoculation of Plates

The antibacterial test was carried out using a modified method of Acar and Goldstein with the flood-inoculation technique. A bacterial suspension was prepared to match the 0.5 McFarland turbidity standard. From this, 2 mL was transferred onto the surface of Mueller-Hinton agar plates and spread gently by rocking the plate. Any excess fluid was carefully drained, and the plates were incubated at 37 °C for 30 minutes to dry before the test discs were applied.

2..3.4.2 Agar Well Diffusion Method

The antimicrobial activity of the extract was evaluated following the modified method of Bauer *et al*. (2012). Mueller-Hinton agar was prepared, sterilized, and poured into Petri dishes to solidify. A sterile cork borer (6 mm diameter, 4 mm depth) was used to punch wells into the agar. Different concentrations of the extract were then pipetted aseptically into the wells, and the agar plates were inoculated with bacterial isolates. The plates were incubated at 37 °C for 24 hours. After incubation, the diameters of the inhibition zones around each well were measured in millimeters using a ruler.

2.3.4.3 Determination of Minimum Inhibitory Concentration (MIC) And Minimum Bactericidal Concentration (MBC) Using Both Dilution Method

The Minimum Inhibitory Concentration (MIC) of the extract was checked using the broth dilution method described by Nagalakshmi *et al*., (2019). Different concentrations of the extract (50 µg/ml and 100 µg/ml) were prepared in sterile nutrient broth inside test tubes. With the help of a sterile wire loop (Hi-media), a loopful of the bacterial culture was added into each test tube. The tubes were then incubated at 37 °C for 24 hours. After incubation, the tubes were observed to see if there was bacterial growth or if the broth remained clear

2.3.4.4 Antifungal Activity

Antifungal testing was performed using the agar well method. Potato Dextrose Agar (PDA) plates were prepared and inoculated with fungal isolates. About 0.1 mL of the fungal suspension was spread evenly over the surface of each plate with a sterile glass rod and allowed to dry. Wells were made in the agar, and different concentrations of the extract (50 g/mL and 100 µg/mL) were introduced. The plates were left for 1 hour to allow proper diffusion of the extract, then incubated at 37 °C for 48 hours. Fluconazole was used as the positive control. Zones of inhibition were measured after incubation.

2.3.4.5 Determination of Minimum Inhibitory Concentration (MIC) and MFC Using The Broth Dilution Method

The broth dilution method described by Nagalakshmi *et al*. (2019) was used to determine the Minimum Inhibitory Concentration (MIC) of the extract against fungi. Different concentrations of the oil extract (50 mg/mL and 100 mg/mL) were prepared in sterile Tryptone Soy Broth inside test tubes. Using a sterile wire loop (Hi-media), a loopful of fungal culture was added into each tube. The tubes were incubated at 37 °C for 72 hours and later checked for turbidity or visible growth.

The MIC was recorded as the lowest concentration of the extract that showed no visible growth. To determine the Minimum Fungicidal Concentration (MFC), the tubes that showed no growth at the MIC stage were sub-cultured onto fresh Potato Dextrose Agar plates and incubated again at 37 °C for 48 hours. After incubation, the plates were examined for colonies. The lowest concentration of the extract that showed no fungal growth was taken as the MFC (Borman *et al*., 2017).

2..3.4.6 Minimum Fungicidal Concentration

First, a standardized fungal suspension was prepared by adjusting the turbidity of the inoculum so that the number of spores per milliliter was the same, using a hemocytometer. Each dilution tube or well was then inoculated with a fixed volume of the fungal suspension and incubated at the proper temperature (25–30 °C for molds and 35–37 °C for yeasts) for 48 hours, depending on the growth rate of the organism. After incubation, the Minimum Inhibitory Concentration (MIC) was taken as the lowest concentration of the extract that completely stopped visible fungal growth.

To determine the Minimum Fungicidal Concentration (MFC), 10 µL aliquots were taken from tubes or wells with no visible growth and sub-cultured onto fresh Sabouraud Dextrose Agar (SDA) plates without any antifungal agent. These plates were incubated again under the same conditions. The MFC was recorded as the lowest concentration of the extract where no fungal colonies appeared after incubation, meaning that 99.9% of the original inoculum had been killed rather than just inhibited.

CHAPTER THREE

3.0 RESULT AND DISCUSSION

Table 1: chemical composition of *Curcuma longa* essential oil

S/N	Compound Name	RT (min)	Conc %
1	1-Butanethiol	4.146	0.38
2	Butane, 2,2'-thiobis	4.466	1.33
3	Diglycerol	4.592	1.68
4	Mequinol	4.947	0.74
5	Butanoic acid, 2-methyl-3-oxo-, methyl ester	5.347	0.37
6	3,4-Dimethyldihydrofuran-2,5-dione	5.553	0.36
7	3,4-Dimethyldihydrofuran-2,5-dione	6.126	0.50
8	Dianhydromannitol	6.681	1.02
9	2-methoxy-4-vinylphenol	7.814	0.45
10	2-methy-2,3-divinyloxirane	8.432	0.32
11	5-Benzofuranacetic acid	8.586	0.32
12	Trans-linalool oxide (furanoid) Propanedioic acid	8.798	0.42
13	Caryophyllene	8.958	0.54
14	1,4-Anhydro-d-galactitol	10.572	3.03
15	1,4-Anhydro-d-galactitol	10.738	3.27
16	1,5-Anhydroglucitol	11.029	3.97
17	1-Propanamine, N-(2-furanylmethylene)-2-methyl-2-Cyclohexen-1-one	11.390	3.97
18	1,5-Anhydroglucitol	11.579	0.47
19	1-Deoxy-d-altritol	11.956	4.20
20	. beta.-D-Glucopyranose, 4-O-. beta. -D-galactopyranosyl-Heptanoic acid	12.288	0.32
21	Octadecanoic acid	12.460	0.31
22	2-Pentyne	12.712	0.93
23	1,4-Benzenediol, 2-methyl-Orcinol	12.849	1.42

24	Isopulegol2-(Heptadecyl) furan trans-2-Hexenoic acid	12.998	2.00
25	Geranyl linalool	13.352	0.61
26	Hexadecanoic acid, methyl ester	13.782	0.58
27	n-Hexadecanoic acid	14.222	3.82
28	2-Methyl-3-phenylpyridine	14.405	0.95
29	Phytol	15.315	1.23
30	Phenylbutazone	15.824	19.34
31	(Z)-3-(Heptadec-10-en-1-yl) phenol	16.213	2.11
32	Ajmalan-17-ol, 19,20-didehydro-cetate (ester)	16.728	8.76
33	Phenylbutazone	17.655	8.55
34	1-(4-Chlorophenyl)-4-(morpholine-4 -carbonyl)-2-pyrrolidone	18.056	1.79
35	1,3-Benzodioxole-5-propanal	18.834	0.36
36	6-Thiotheophylline	19.853	19.01
37	Acetamide, 2,2-diphenyl-N-(3,3,5-trimethylcyclohexyl)	20.253	4.06

The GC-MS analysis shows that the extract contains a complex mixture of compounds as outlined in table 1 and confirmed in appendix, but only a few of them are present in higher amounts. These compounds found can be arranged into different chemical classes to better understand the nature of the sample. Fatty acids and their derivatives were identified in the extract, represented by n-hexadecanoic acid (palmitic acid, 3.82%). This is a saturated fatty acid normally found in almost all plants and animals. It is often used in making soaps, cosmetics, and also as a base material in many organic extracts. Terpenoids were also present, which are natural compounds often linked with aroma and biological effects. Phytol (1.23%) is a diterpene alcohol that comes from chlorophyll. Apart from being common in plant extracts, it is used in the production of vitamin E and vitamin K. It also shows antimicrobial and anti-inflammatory properties. Isopulegol (2.00%) is a monoterpenoid with a minty odor. It is widely used in perfumes and flavoring. Research also shows it can work as an insect repellent and has antimicrobial activity. Phenolic compounds were detected as well, which are well known for their antioxidant activity. 1,4-Benzenediol, 2-methyl- (1.42%) is a derivative of hydroquinone.

Compounds of this type are often used in skincare products (like skin-lightening creams) because of their antioxidant nature. (Z)-3-(Heptadec-10-en-1-yl) phenol (2.11%) is a long-chain alkylphenol. Such compounds sometimes act like surfactants and can also show antimicrobial and antioxidant activities. Sugar anhydrides and polyols were also dominant in the extract. These compounds are usually derived from sugars through processes like dehydration (for anhydrides) or reduction/deoxygenation (for polyols). for instance, 1,5-Anhydroglucitol (3.97%) is a sugar anhydride, while 1-deoxy-altritol (4.20%) is a polyol. 1,4-Anhydro-D-galactitol (3.27%), diglycerol (1.68%) and dianhydromannitol (1.02%) were detected. Compounds like these are common in carbohydrate-rich materials. Some of them, like anhydro glucitol, are even used as markers in medical research, for example, to monitor blood sugar levels in diabetes patients. Notable synthetic or pharmaceutical compounds were also detected in the extract in very high amounts. Phenylbutazone (19.34%) is a synthetic anti-inflammatory drug (NSAID) commonly used in veterinary medicine to reduce pain and swelling. 6-Thiotheophylline (19.01%) is similar to theophylline, which is used in treating asthma and chronic obstructive pulmonary disease (COPD). Being the most abundant compound in the sample, its presence is very significant. Ajmalan-17-ol, 19,20-didehydro-, acetate (8.76%) belongs to the indole alkaloid family, similar to reserpine, which is used for lowering blood pressure. Alkaloids of this type are known to have strong pharmacological effects.

Table 3.1 Antimicrobial test of bacterial isolates

The results indicate that the turmeric extract exhibited varying degrees of antimicrobial activity

		ZONES OF INHIBITIONS (mm)	
Test organism	Extract (mg/ml)	100	50
Escherichia coli	Tumeric	15	11
Klebsiella pneumonia	Tumeric	16	9
Pseudomonas sp	Tumeric	10	0
Staphylococcus aureus	Tumeric	11	9

against the tested bacteria. The effect was concentration-dependent, with larger zones of inhibition observed at the higher concentration (100 mg/ml) for *Escherichia coli*, *klebsiella pneumoniae*, and *Staphylococcus aureus*. *klebsiella pneumoniae* was the most susceptible organism, while *Pseudomonas sp*. showed significant resistance, with no inhibition at the lower concentration.

Table 3.2 Antimicrobial result of fungi isolates

		ZONES OF INHIBITIONS (mm)	
Test organism	Extract (mg/ml)	100	50
Aspergillus niger	Tumeric	17	10
Trichophyton rubrum	Tumeric	16	14
Penicillium sp	Tumeric	12	0
Aspergillus flavus	Tumeric	14	9

The *curcuma longa* extract demonstrated antifungal activity against all tested fungi in a concentration-dependent manner, with the largest zone of inhibition (17 mm) observed against *Aspergillus niger* at 100 mg/ml. *Penicillium sp*. exhibited the highest resistance, showing no inhibition at the 50 mg/ml concentration.

Table 3.3 Minimum inhibitory concentration of bacterial isolates

ISOLATES	Tumeric (extract)
Escherichia coli	
Klebsiella pneumoniae	100 mg/ml
Pseudomonas sp	-
Staphylococcus aureus	100 mg/ml

The minimum inhibitory concentration (MIC) is the lowest concentration of an extract that prevents visible growth. The *curcuma longa* extract successfully stopped the growth of *Klebsiella pneumoniae* and *Staphylococcus* aureus at a concentration of 100 mg/ml. However, it did not stop the growth of *Escherichia coli* and *Pseudomonas* sp., even at the highest concentration tested.

Table 3.4 Minimum inhibitory concentration of fungal isolate

ISOLATES	Turmeric (extract)
Aspergillus niger	100mg/ml
Trichophyton rubrum	100mg/ml
Penicillium sp	100mg/ml
Aspergillus flavus	-

The *curcuma longa* extract was able to stop the growth of three fungi-*Aspergillus niger*, *Trichophyton rubrum*, and *Penicillium sp*. at a concentration of 100 mg/ml. However, it did not stop the growth of *Aspergillus flavus*, even at the highest concentration tested.

Table 3.5 Minimum bactericidal concentration

ISOLATES	Tumeric (extract)
Escherichia coli	Static
Klebsiella pneumoniae	Static
Pseudomonas sp	Static
Staphylococcus aureus	Static

The results show that the turmeric extract was bacteriostatic against all tested bacteria. This means it was able to stop them from growing and multiplying, but it did not kill them. This is indicated by the result "Static" for all isolates, including *Escherichia coli, Klebsiella pneumoniae, Pseudomonas sp., and Staphylococcus aureus.*

Table 3.6 Minimum fungicidal concentration

ISOLATES	alligator (extract)	Turmeric (extract)
Aspergillus niger	Static	Static
Trichophyton rubrum	Static	Static
Penicillium sp	Static	Static
Aspergillus flavus	Static	Static

The results from the Minimum Fungicidal Concentration (MFC) indicate that the turmeric extract exhibited a fungistatic effect against all tested fungal isolates. The designation "Static" for *Aspergillus niger, Trichophyton rubrum, Penicillium sp.,* and *Aspergillus flavus* signifies that the extract was effective at inhibiting the growth and reproduction of the fungi at the tested concentrations, but it was not fungicidal (i.e., it did not kill the fungal cells).

Conclusion

This project successfully extracted essential oil from *curcuma longa* using the hydro-distillation method. The oil was able to stop the growth of several harmful microbes, including the fungi *Aspergillus niger*, *Trichophyton rubrum*, and *Penicillium* sp., and the bacteria *Klebsiella pneumoniae* and *Staphylococcus aureus*. However, the oil did not kill these germs; it only prevented them from multiplying. Some microbes, like the bacteria *Escherichia coli* and *Pseudomonas sp*., and the fungus *Aspergillus flavus*, were not affected by the oil at all. This study shows that turmeric oil can work as a natural product to stop some germs from growing. This supports its traditional use and suggests it could be useful in creating natural medicines or preservatives in the future.

Recommendations

From the findings of this research, it is recommended that more work should be done to improve the yield and quality of turmeric essential oil. Although hydro-distillation proved effective, other advanced methods such as supercritical CO_2 extraction or microwave-assisted extraction could be compared in future studies to see which gives better recovery of active compounds. It is also advised that more bacterial and fungal species should be tested, since this study only considered a limited number of organisms. Doing so would give a broader understanding of the antimicrobial potential of turmeric oil. Furthermore, researchers should look into formulating the oil into stable products such as creams, capsules, or natural preservatives for food. This will help in finding practical applications of the oil in medicine, cosmetics, and food industries. Lastly, there is a need for more detailed toxicity and safety studies on *curcuma longa* oil before it can be widely adopted for therapeutic use.

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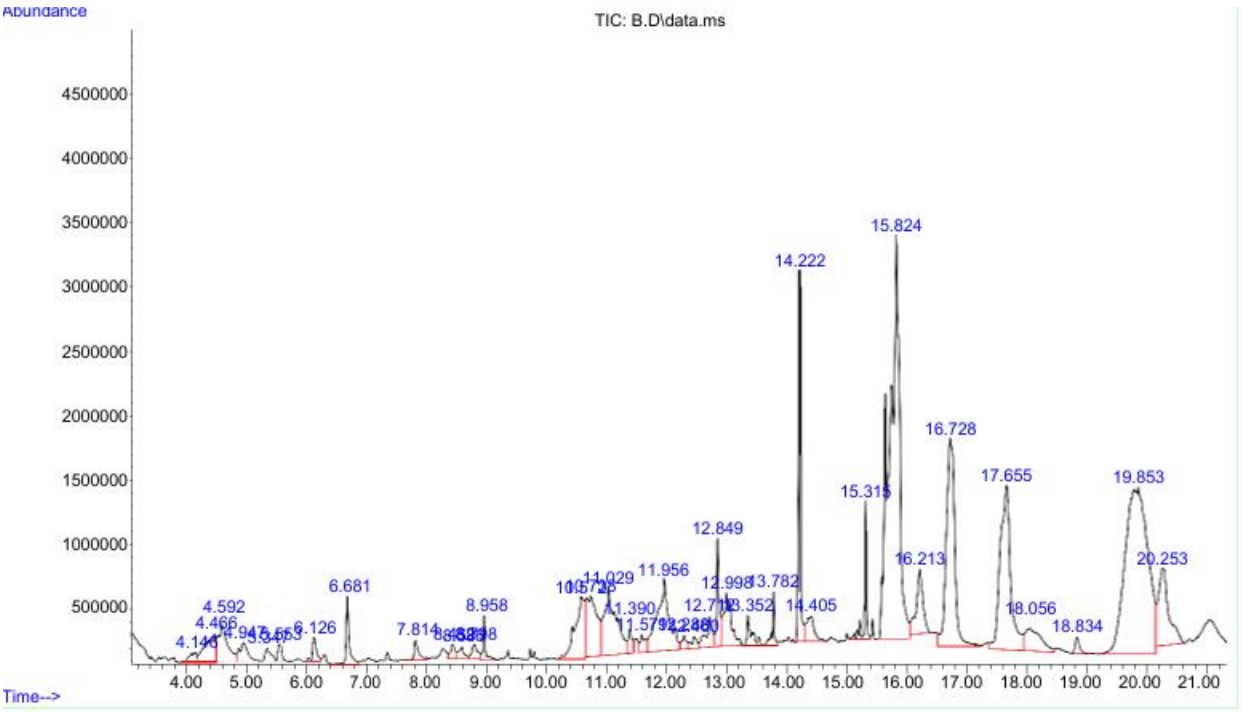
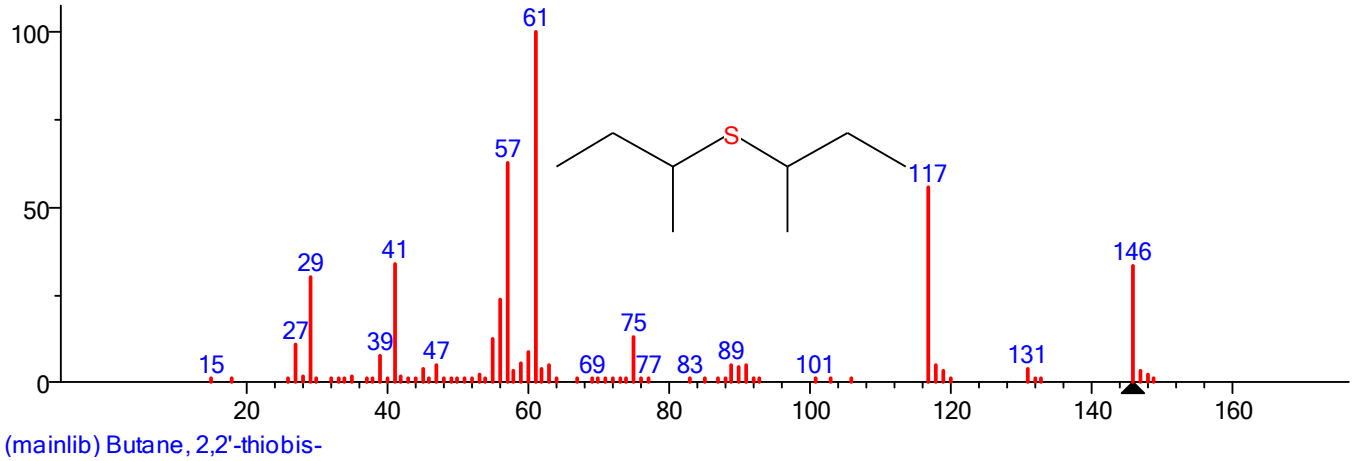
APPENDIX 1

FIG 3.1 CHROMATOGRAM OF THE ESSENTIAL OIL FROM *CURCUMA LONGA*



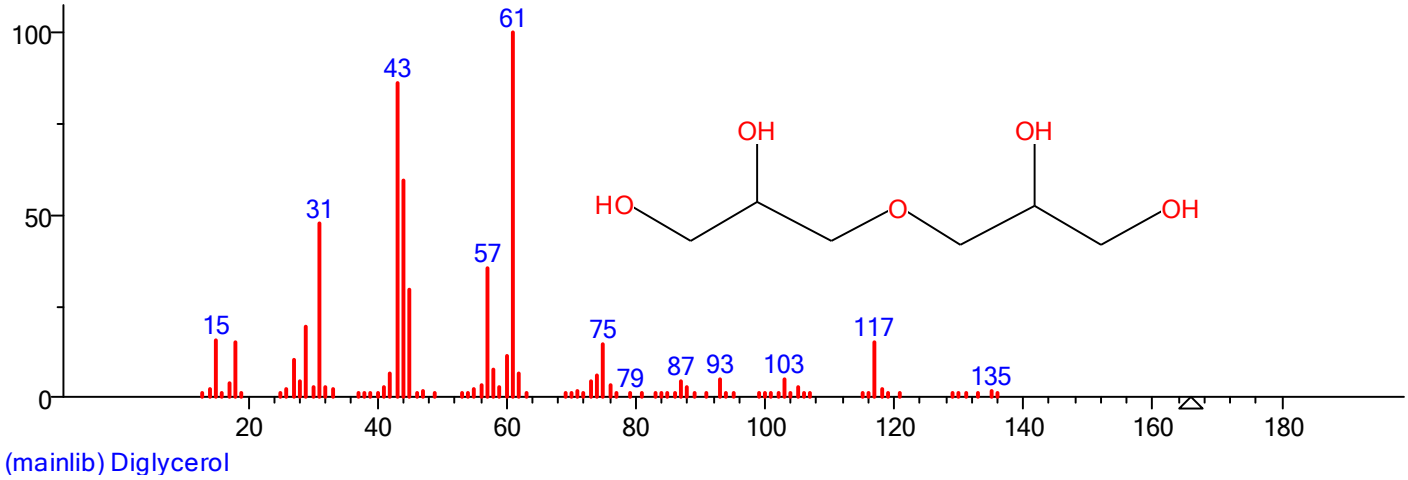
APPENDIX 2

MS SPECTRA OF MOST ABUNDANT COMPOUNDS



(mainlib) Butane, 2,2'-thiobis-

APPENDIX 3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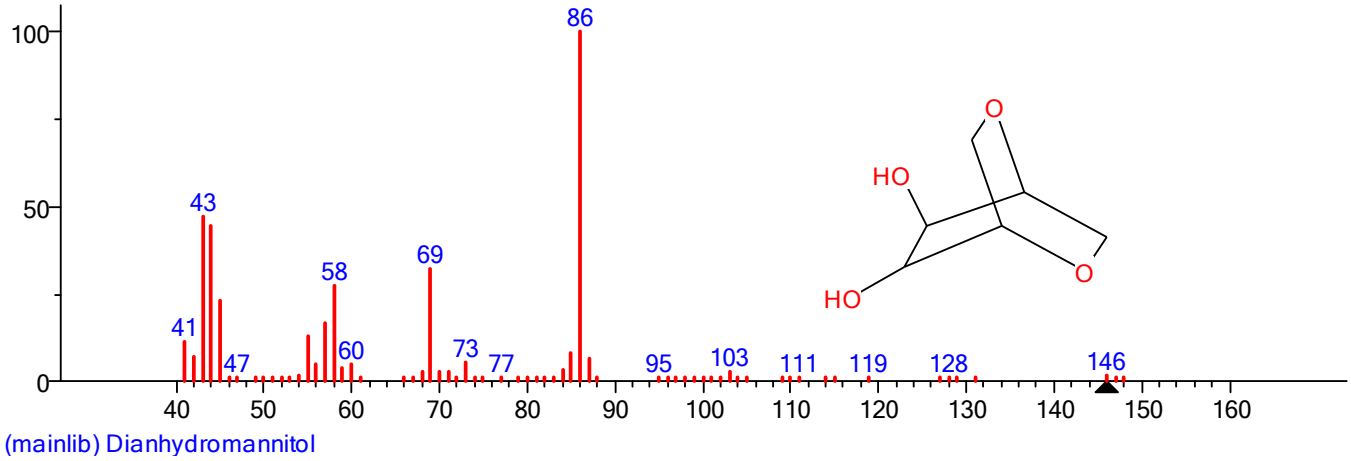
(mainlib) Diglycerol

APPENDIX 4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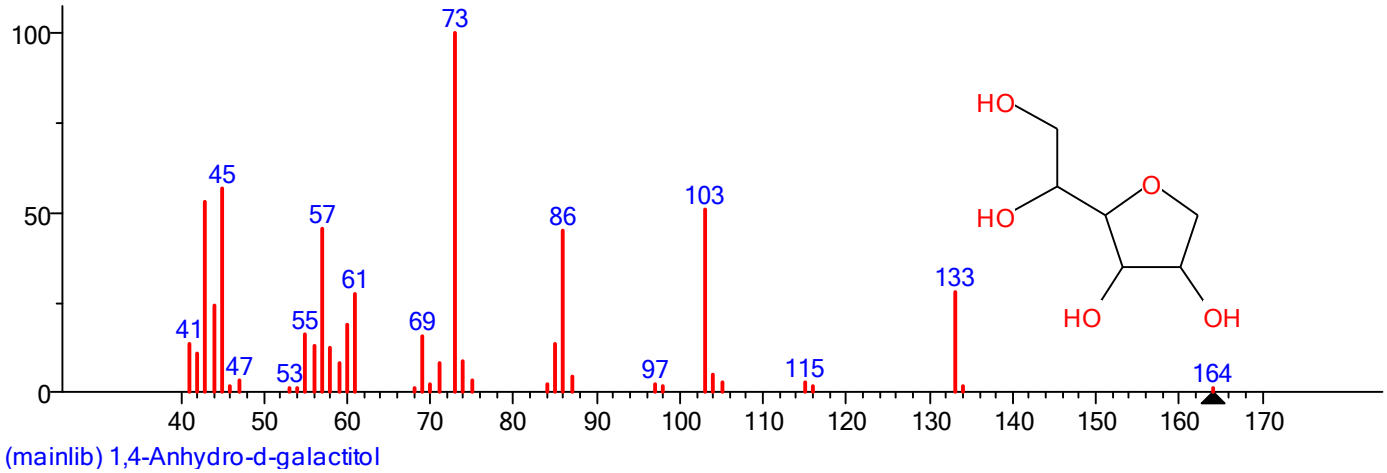
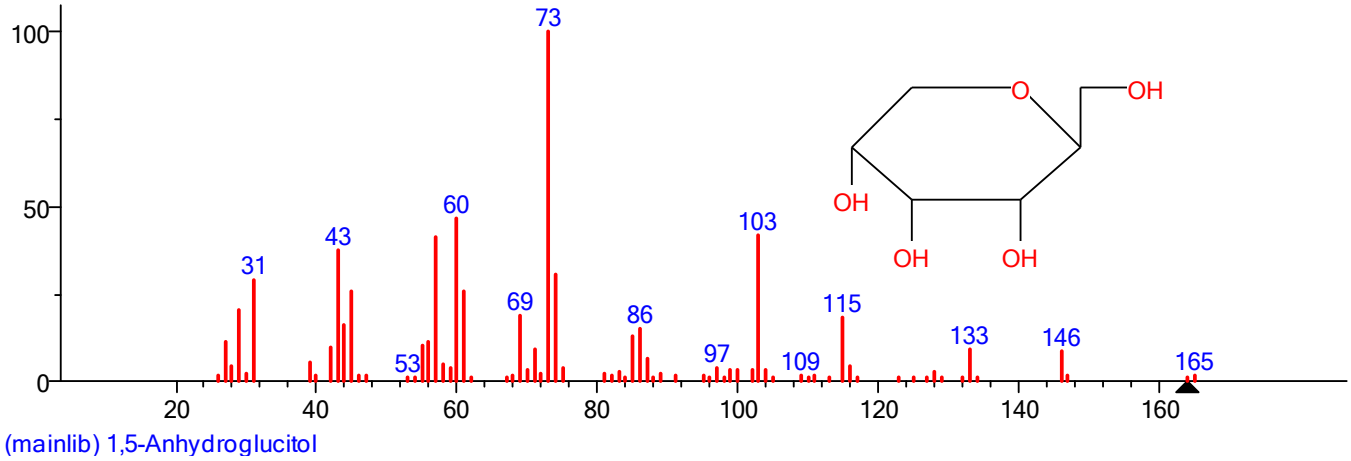
(mainlib) Dianhydromannitol

APPENDIX 5



(mainlib) 1,4-Anhydro-d-galactitol

APPENDIX 6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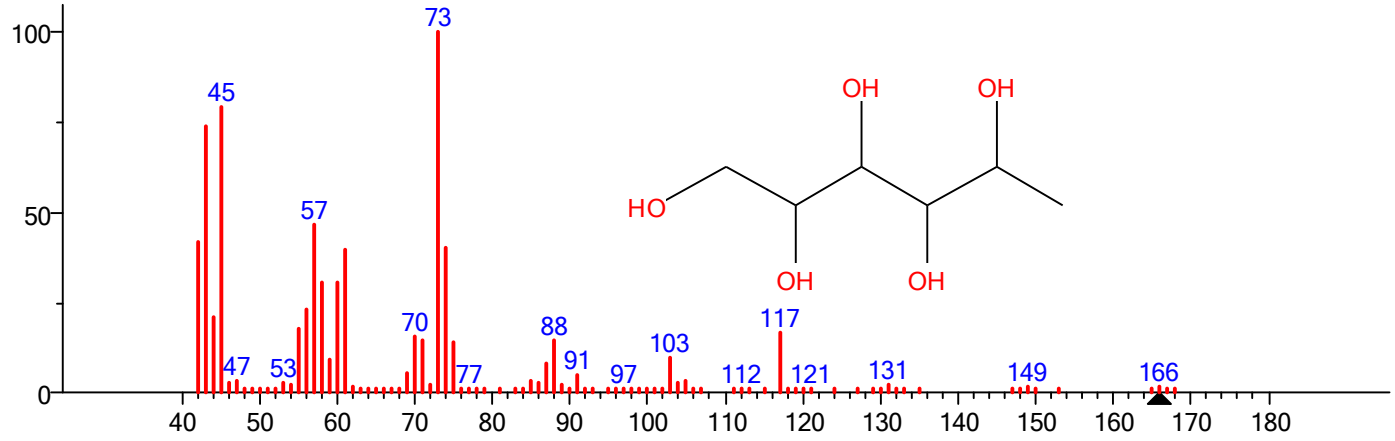
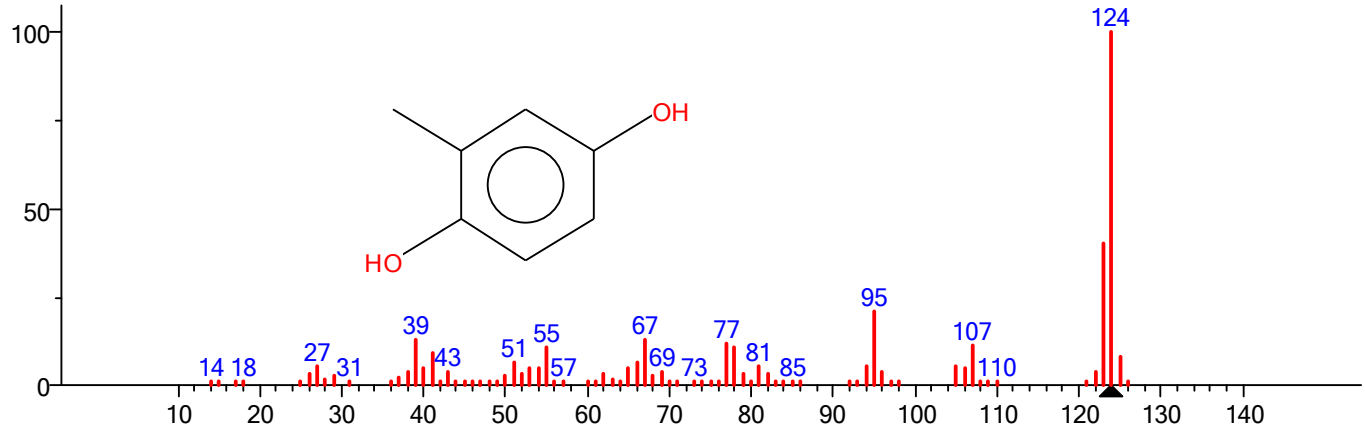
APPENDIX 7



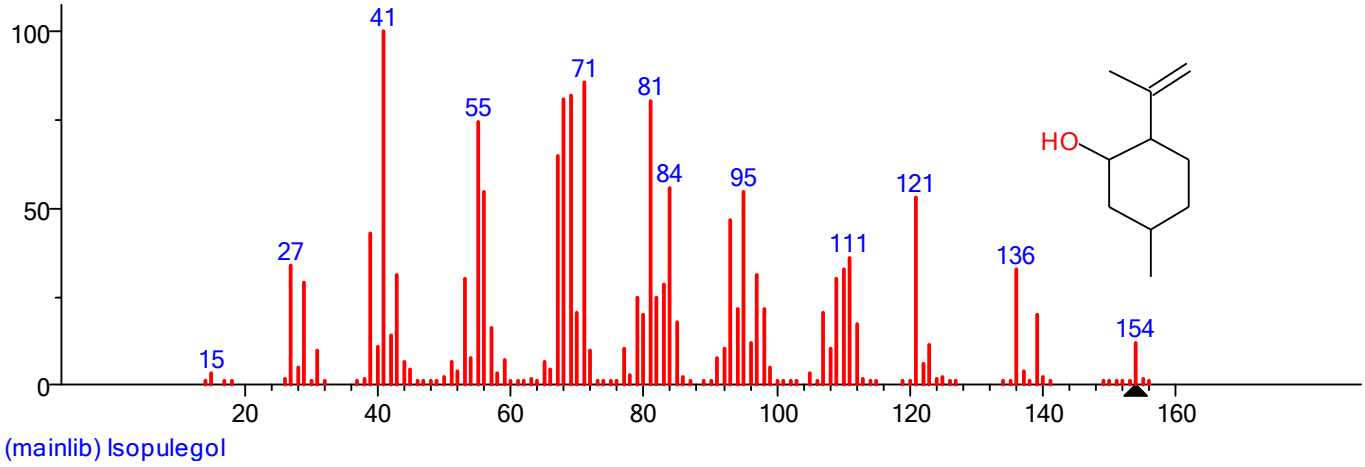
(mainlib) 1-Deoxy-d-altritol

APPENDIX 8



(mainlib) 1,4-Benzenediol, 2-methyl-

APPENDIX 9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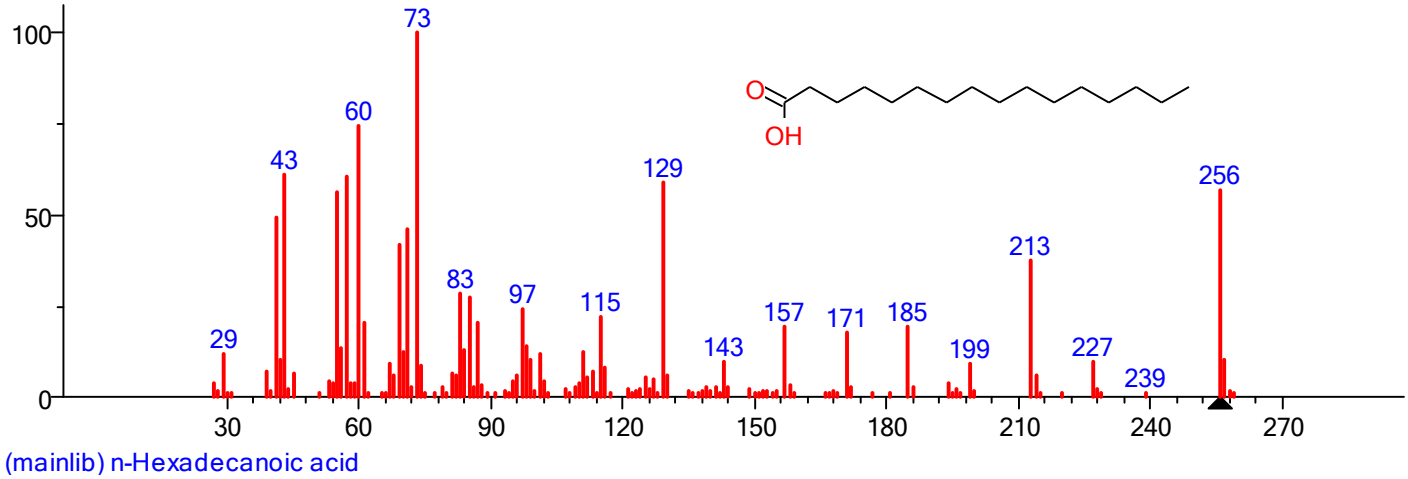
(mainlib) Isopulegol

APPENDIX 10



(mainlib) n-Hexadecanoic acid

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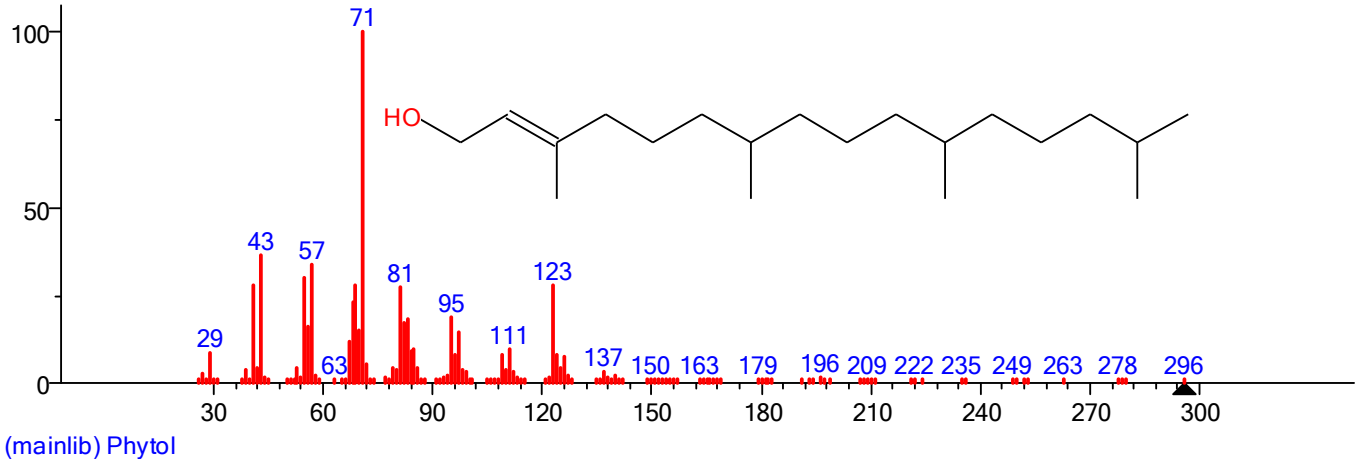
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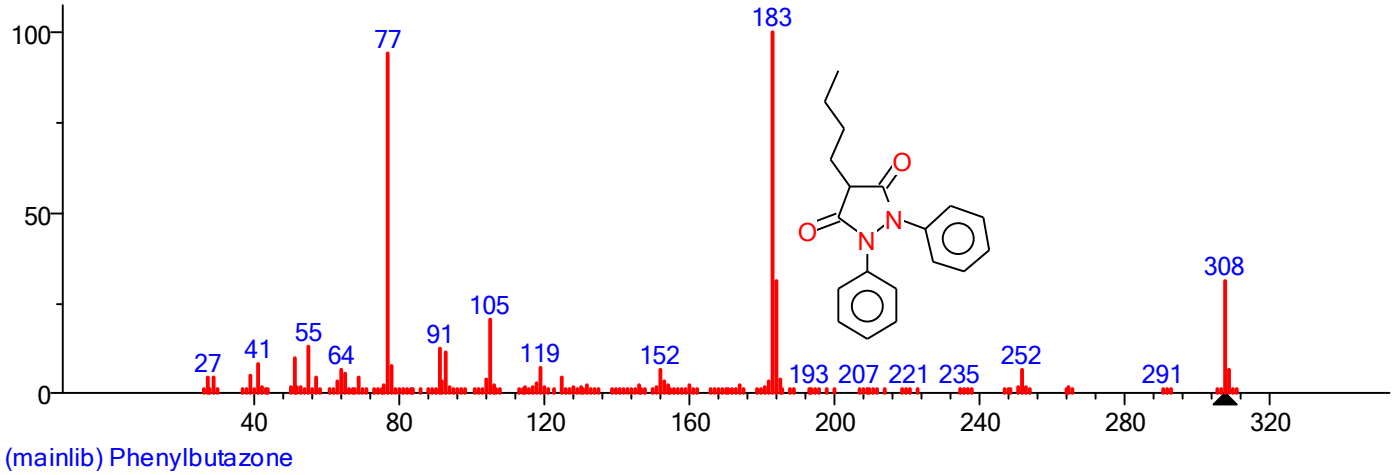
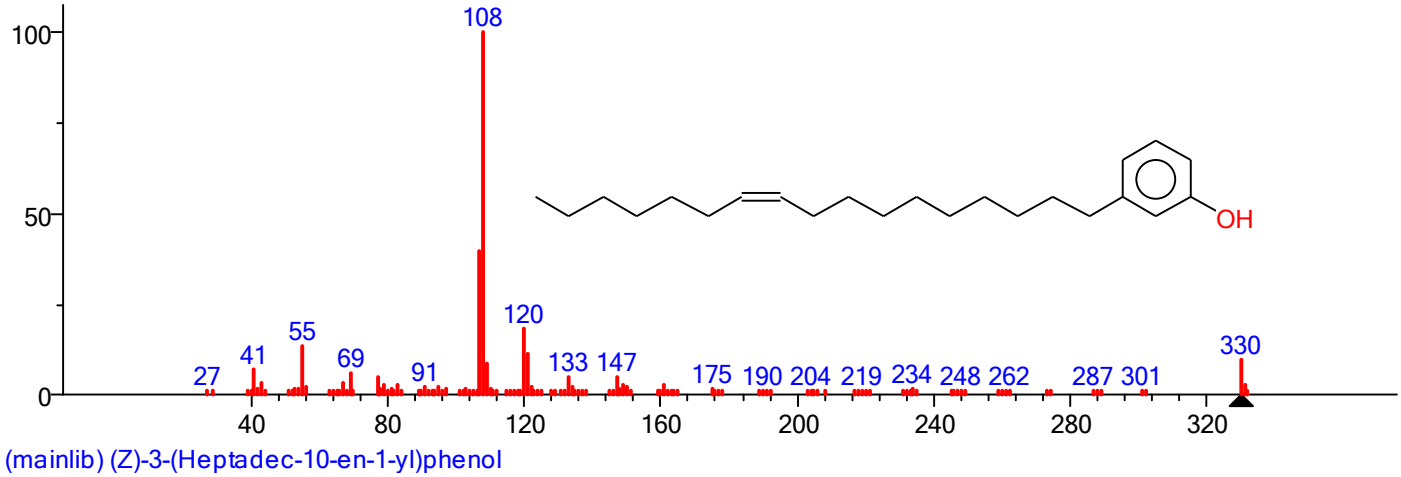
(mainlib) Phytol

APPENDIX 12



(mainlib) Phenylbutazone

APPENDIX 13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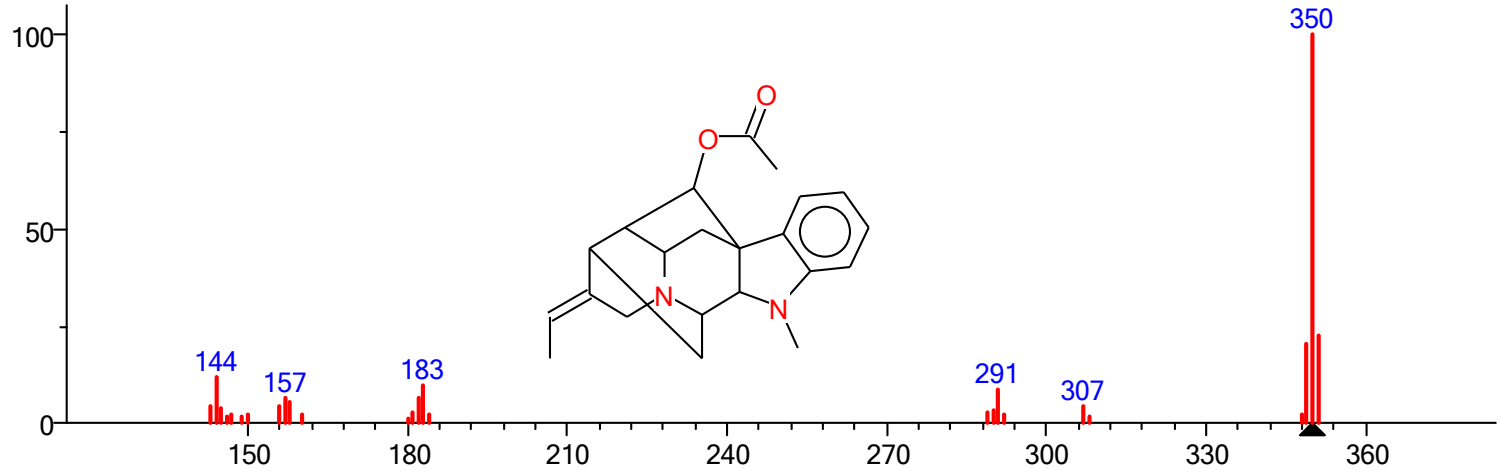
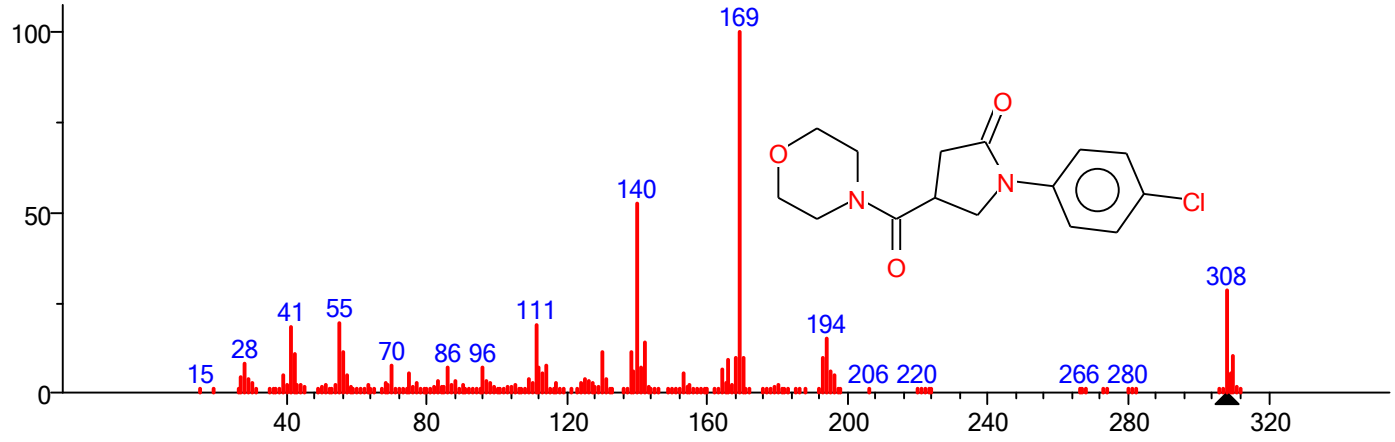
(mainlib) (Z)-3-(Heptadec-10-en-1-yl)phenol

APPENDIX 14



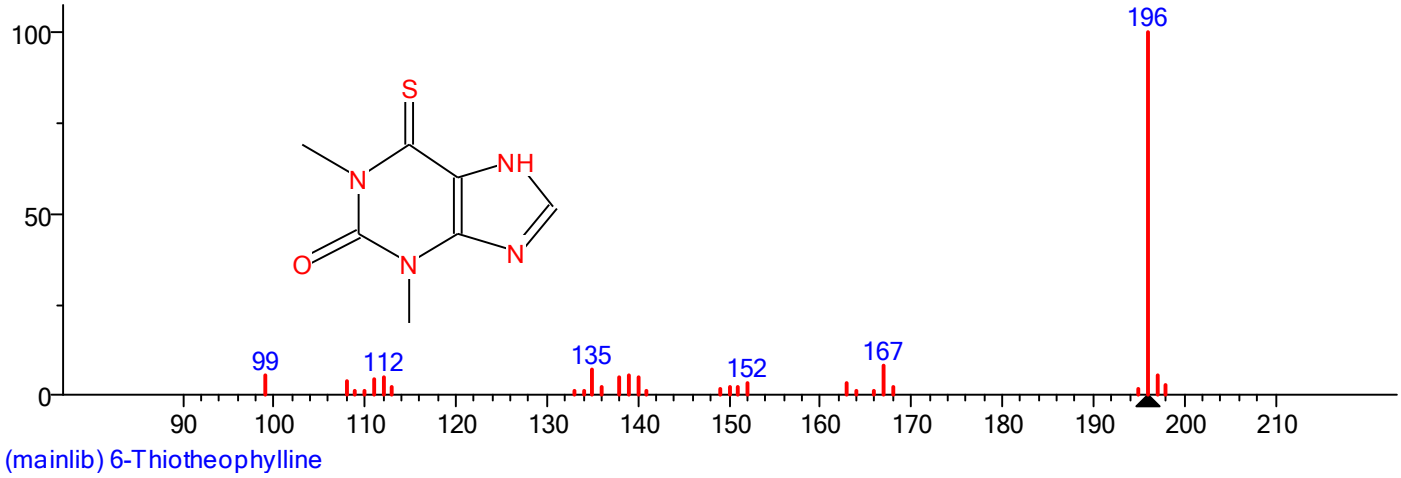
(mainlib) Ajmalan-17-ol, 19,20-didehydro-, acetate (ester), (17R,19E)-

APPENDIX 15



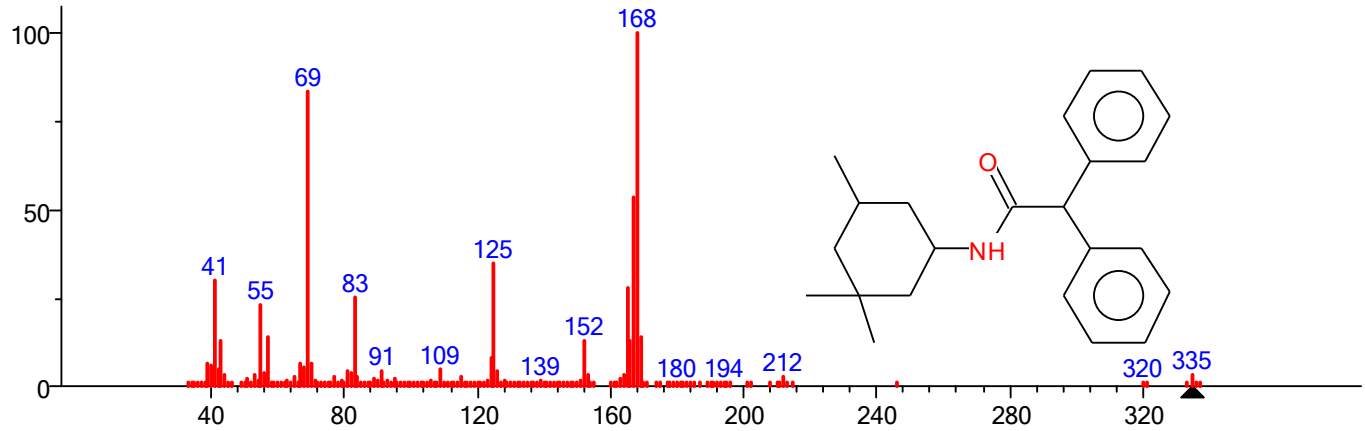
(mainlib) 1-(4-Chlorophenyl)-4-(morpholine-4-carbonyl)-2-pyrrolidone

APPENDIX 16



(mainlib) 6-Thiotheophylline

APPENDIX 17



(mainlib) Acetamide, 2,2-diphenyl-N-(3,3,5-trimethylcyclohexyl)-