Antifungal Activity of the Senna Alata Ultrasonicated crude extracts

SITI NUR AFIQAH BINTI MOHD ZABIDI

J20A0697

A report submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Science Bioindustry Technology) with Honours

FACULTY OF BIOENGINEERING AND TECHNOLOGY

UMK

2023

DECLARATION

I declare that this thesis entitled "Antifungal of the Senna Alata Ultrasonicated crude extracts" is the results of my own research except as cited in the references.

Signature : _____

Student's Name : Siti Nur Afiqah Bt Mohd Zabidi

Date : 28/02/2024

Verified by:

Signature : _____

Supervisor's Name : DR. WAN SURIYANI FALIQ ADEEBA WAN IBRAHIM
Pensyarah Kanan
Fakulti Biokejuruteraan & Teknologi
Universiti Malaysia Kelantan
Kampus Jeli

Stamp : _____

Date : 3 March 2024

ACKNOWLEDGEMENT

In the name of Allah, the Most Gracious, the Most Merciful. Praise be to Allah, the Lord of all worlds, and peace and blessings be upon His noble messenger, Muhammad, and upon his family and companions.

I would like to express my deepest gratitude to my supervisor, Dr Wan Suriyani Faliq Adeeba Binti Wan Ibrahim, for her invaluable guidance, encouragement, and unwavering support throughout the research process. Their expertise, patience, and insightful feedback have been instrumental in shaping this thesis. I am immensely grateful to University Malaysia Kelantan , Jeli , for providing me with the resources and facilities necessary to conduct this research. Special thanks to the Faculty of Bioengineering and Technology (FBKT) for fostering an intellectually stimulating environment that nurtured my academic growth.

I extend my heartfelt appreciation to my family for their endless love, understanding, and encouragement. Their unwavering belief in my abilities has been a constant source of motivation. I would like to thank my friends and colleagues for their encouragement, support, and camaraderie. Their discussions and insights have enriched my understanding and enhanced the quality of this thesis. I am thankful to all the participants who generously shared their time and insights for this study. Without their contributions, this research would not have been possible.

In conclusion, I extend my deepest appreciation to myself for the dedication, hard work, and resilience demonstrated throughout the journey of writing this thesis. This accomplishment stands as a testament to my capabilities, determination, and unwavering commitment to academic excellence.

ABSTRACT

Senna Alata, a plant with a long history of use in traditional medicine, has shown promise in treating a variety of ailments ranging from typhoid to skin infections. With approximately 80% of the global population relying on plant-derived drugs for healthcare needs, there is growing interest in evaluating herbal products from a pharmacological perspective to discover new medicines. Fungal infections pose a significant public health challenge, particularly in tropical and subtropical regions. Current antifungal medications may have adverse effects on human health and the environment, necessitating exploration of alternative sources for antifungal drugs. *S. alata* contains secondary metabolites such as anthraquinones, flavonoids, tannins, and terpenoids, which have potential antifungal properties, this study aims to investigates the yield, antifungal activity, phytochemical composition, and functional groups of *S. alata* crude extracts obtained using different solvents (methanol, ethyl acetate, n-hexane and distilled water) and extraction methods (ultrasonic bath and decoction). The yield of crude extracts varied with the solvent used, with methanol showing the highest yield (24.48%) and n-hexane the lowest (3.62%). Antifungal analysis against *Candida Albicans* revealed no activity at a concentration of 500 mg/mL for all extracts. Gas chromatography-mass spectrometry (GC-MS) analysis identified various compounds in each extract, with methanol extracts containing the largest number of phytocomponents with reported antifungal properties (Phenol, 2,4-bis (1,1-dimethylethyl), Benzene propanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester, and Oleic Acid). Fourier transform infrared (FT-IR) spectroscopy highlighted functional groups consistent with known antifungal compounds in methanol and ethyl acetate extracts. Overall, methanol and ethyl acetate extracts showed potential for antifungal activity due to their phytochemical composition and functional groups, suggesting their suitability for further investigation as natural antifungal agents. However, the choice of solvent significantly influenced the yield, phytochemical composition, and potential antifungal activity of *S. alata* extracts, highlighting the importance of solvent selection in plant extraction processes.

Keywords: *S. alata*, GCMS, FTIR

ABSTRAK

Senna Alata, tumbuhan yang mempunyai sejarah penggunaan yang panjang dalam perubatan tradisional, telah menunjukkan janji dalam merawat pelbagai jenis penyakit daripada kepialu hingga jangkitan kulit. Dengan kira-kira 80% daripada populasi global bergantung pada ubat-ubatan yang berasal dari tumbuhan untuk keperluan penjagaan kesihatan, terdapat minat yang semakin meningkat untuk menilai produk herba dari perspektif farmakologi untuk menemui ubat-ubatan baharu. Jangkitan kulat menimbulkan cabaran kesihatan awam yang ketara, terutamanya di kawasan tropika dan subtropika. Ubat antikulat semasa mungkin mempunyai kesan buruk terhadap kesihatan manusia dan alam sekitar, yang memerlukan penerokaan sumber alternatif untuk ubat antikulat. *S. alata* mengandungi metabolit sekunder seperti antrakuinon, flavonoid, tanin, dan terpenoid, yang mempunyai potensi sifat antikulat, kajian ini bertujuan untuk menyiasat hasil, aktiviti antikulat, komposisi fitokimia, dan kumpulan berfungsi ekstrak mentah *S. alata* yang diperoleh menggunakan pelarut yang berbeza. (metanol, etil asetat, n-heksana dan air suling) dan kaedah pengekstrakan (ultrasonik dan merebus). Hasil ekstrak mentah berbeza-beza mengikut pelarut yang digunakan, dengan metanol menunjukkan hasil tertinggi (24.48%) dan n-heksana paling rendah (3.62%). Analisis antikulat terhadap *Candida Albicans* mendedahkan tiada aktiviti pada kepekatan 500 mg/mL untuk semua ekstrak. Analisis kromatografi gas-spektrometri jisim (GC-MS) mengenal pasti pelbagai sebatian dalam setiap ekstrak, dengan ekstrak metanol mengandungi bilangan fitokomponen terbesar dengan sifat antikulat yang dilaporkan (Phenol, 2,4-bis (1,1-dimethylethyl), asid Benzene propanoik , 3,5-bis(1,1-dimetiletil)-4-hidroksi-, metil ester, dan Asid Oleik). Spektroskopi inframerah transformasi Fourier (FT-IR) menyerlahkan kumpulan berfungsi selaras dengan sebatian antikulat yang diketahui dalam ekstrak metanol dan etil asetat. Secara keseluruhannya, ekstrak metanol dan etil asetat menunjukkan potensi untuk aktiviti antikulat disebabkan oleh komposisi fitokimia dan kumpulan berfungsi, mencadangkan kesesuaiannya untuk penyiasatan lanjut sebagai agen antikulat semulajadi. Walau bagaimanapun, pilihan pelarut secara signifikan mempengaruhi hasil, komposisi fitokimia, dan potensi aktiviti antikulat ekstrak *S. alata*, menonjolkan kepentingan pemilihan pelarut dalam proses pengekstrakan tumbuhan.

Kata kunci: *S. alata*, GCMS, FTIR

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

S. Alata	*Senna Alata*
UAE	Ultra Assisted Extraction
FTIR	Fourier Transform Infrared
GC-MS	Gas Chromatography Mass Spectrometry
C14H8O2	Anthraquinone
C. Albicans	Candida Albicans
SDA	Sabouraud Dextrose Agar
MAE	Microwave Assisted Extraction
ATR	Attenuated Total Reflectance
RT	Retention Time
NMR	nuclear magnetic resonance
MW	Molecular Weight

LIST OF SYMBOLS

%	Percentage
cm	Centimetres
m	Metres
$C°$	Celsius
ml	Millimetre
Hz	Hertz

g	Gram
min	Minutes
hrs	Hours
µ	Micro
mg/ml	Milligram per millimetre
µL	Micro Litre

CHAPTER 1

INTRODUCTION

1.1 Background study

Research into medicinal plants has gained a lot of attention around the world in recent years. There is a growing body of data supporting the use of medicinal plants in complementary, alternative, and traditional medicine to treat a variety of human ailments (Sher, 2009) . As antifungal resistance increases and sustainable alternatives are urgently needed, research into antifungal medicines derived from plants has received a lot of attention. *Senna alata* is a promising example of a plant with antifungal effects. Argentina is the origin of S. alata(Gilman & Watson, 1993) The herb S. alata, commonly known as *Cassia alata*, is a member of the Leguminosae family and grows all over the world. Among its many names, this plant is often referred to as a candle bush, craw-craw plant, acapulo, ringworm bush, or ringworm plant. The plant is widespread in Asia and Africa and is known by a wide variety of indigenous names (A. Kumar, Shukla, Singh, Prasad, & Dubey, 2008). *Gelenggang* (Malaysia) and *Ketepeng China* (Indonesia) are other names for the plant *Senna alata*. In Malaysia, it is used to make medicines to treat various disorders such as skin diseases, rheumatism, constipation, diabetes, and gonorrhoea (Hazni, Ahmad, Hitotsuyanagi, Takeya, & Choo, 2008).

Base on Figure 1, *S. alata* plant three to four meters tall, *S. alata* is a tropical shrub. The yellow blossom blooms from the bottom to the top. The petals are placed vertically in a column. Each winged seed pod has four sides and is dark purple to black in colour. The pod measures 25 cm long and 2.8 cm wide. It has a capacity of 50-60 triangular to square-shaped seeds. When unripe, the seeds are green, but when ripe, they turn black(Yon et al., 2023). In India, seeds and leaves are highly effective as fungicides and eczema treatments (Shiddamallayya, Yasmeen, & Gopakumar, 2010) . *S. alata* leaves have traditionally been used in Indonesia, particularly in South Sulawesi, to remove fungus on the skin that can cause hives and other symptoms by grinding or rubbing directly on the affected skin. *S. alata* leaves extract has been shown to have antifungal efficacy against Trichophyton verrucosum, Epidermophyton floccose, and other

microorganisms (Sule et al., 2010). The root or leaves are effective purgatives, while the leaves, blossoms, and stems are antifungal medicines used to treat skin infections

Medicinal plants are recognized to have healing properties due to biological active chemicals found in plant components. Many phytochemicals, sometimes called active principles or phytochemical substances, have been shown to be effective against fungal infections. These chemicals include phenols, flavonoids, coumarins, quinones, saponins, xanthones, alkaloids, lectins, polypeptides, and terpenoids. It was found that these chemicals, either directly or with minor modifications, are effective against a variety of fungal illnesses (Arif et al., 2009) (Senthilkumar, Madhanraj, & Panneerselvam, 2011). Because of its antifungal qualities, it is a common ingredient in soaps, shampoos, and lotions. These chemicals not only have antifungal properties, but also antibacterial, antidiabetic, cardioprotective, anticancer, and anti-inflammatory properties. Because natural goods have been shown to be less hazardous, synthetic medicines should be replaced with these natural products. Natural goods have been shown to be more cost effective than pharmaceuticals. Natural products have little negative effects and are widely available (Kooti & Daraei, 2017).

Previously, researchers extracted bioactive components from *S. alata* using maceration and Soxhlet extraction. Both maceration and Soxhlet extraction are older methods that necessitate a lengthier extraction period. Furthermore, thermal degradation of bioactive components may occur as a result of the standard extraction method's inherent extended exposure to higher temperatures. Ultrasonic extraction is a novel technology that improves extraction by lowering the internal and external mass transfer limitations of bioactive components in the plant matrix and solution. Sonication disrupts the plant's cell membrane, allowing bioactive components to be released into the bulk liquid.

Figure 1 : *Senna Alata* plant

(Chew et al., 2022)

1.2 Problem statement

Senna Alata has been used for centuries to treat typhoid, diabetes, malaria, asthma, ringworms, tinea infections, scabies, urticaria, herpes, and eczema (Oladeji, Adelowo, Oluyori, & Bankole, 2020) A variety of ailments and infections, according to folk medicine, can be treated with chemicals extracted from various plant sections. Currently, herbs are not just essential components of our sustenance, but the vast majority of the population also uses them as medicine (Nagi, 2015) . It has been stated that about 80% of people worldwide rely on plant-derived drugs to meet their fundamental health care needs (El Kabbaoui et al., 2017). So, herbal products need to be evaluated from a pharmacological point of view to encourage the search for new and useful medicines to treat different diseases.

Fungal infections are a major public health concern, especially in tropical and subtropical countries where they are prevalent (Gadre, Enbiale, Andersen, & Coates, 2022). These infections can be challenging to cure, and standard antifungal medicines can have negative consequences on human health and the environment. Therefore, it is important to look into other potential

antifungal drug sources. Anthraquinones, flavonoids, tannins, and terpenoids are just some of the secondary metabolites discovered in *S. alata*.

1.3 Objectives

This study will serve several objectives listed:

1. This study aims to estimate the total yield of crude extracted using ultrasonic extraction and decoction method.
2. To observe the antifungal activities on fungal *Candida Albicans*.
3. To investigate the phytochemical analysis of antifungal bioactive compounds of S. *alata* crude extracted by GC-MS and FT-IR.

1.4 Scope of study

Study demonstrates that the antifungal activity of crude *S. alata* extract is affected by the extraction solvent used. The study will also optimize the ultrasonic extraction and distinguish it against the decoction technique. FT-IR and GC-MS analysis had performed on the compounds that are bioactive. The disc diffusion method then be used to assess the antifungal activity of *S. alata* crude extracts against *Candida Albicans*.

1.5 Significance of study

The results of this research shed light on the process of obtaining crude leaf extracts from *S. alata*. Using the ultrasonic extraction method, not only is it possible to boost yield, but also purity, efficiency, and cost. Due to the demand for eco-friendly extraction technologies, this alternative extraction method, such as ultrasound water bath, is acquiring popularity. This environmentally friendly technique reduces the amount of solvent utilized in the extraction process. The investigation will also highlight the contrast between ultrasonic extraction and the traditional extraction method of decoction. Furthermore, this work provided light on the significance of *S. alata* for future investigation. *S. alata* provides a more realistic market for the pharmaceutical sector, encouraging additional research on *S. alata* for good effect for future great success. In addition, the antibacterial, analgesic, antimutagenic, antioxidant, antiviral, antidiabetic, and anti-inflammatory characteristics of *S. alata* have been the primary focus of research.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to *Senna alata* plant

The Fabaceae family (subfamily *Caesalpinioideae*) includes *Senna alata*, also known as *Cassia alata.* It originated in Central America and is now widely distributed throughout the Caribbean and other tropical regions of the world. Its inflorescences are shaped like candles, therefore the plant gets the name "candle bush." Another common name is "ringworm tree," after its historic medicinal usage. (Abo, Fred-Jaiyesimi, & Jaiyesimi, 2008). It grows between 1 and 4 meters tall, blooms once a year or twice, and thrives in sunny, moist environments. The leaves have a broad, yellowish green color, and are divided into 5-14 leaflet pairs (each 5-21 2-13 cm). The most distal leaflets are often bigger and have a notched apex. Flowers of the zygomorphic genus are often a vivid yellow and grow in an upright raceme, giving the impression of a thick, golden rod. They have a hairy ovary and seven stamens, two of which are significantly longer than the others. Fruit is a brown, winged, tetragonal pod that measures 10–16 1.5 cm and contains dozens of diamond-shaped brown seeds. Seeds and leaves in India are highly effective fungicides and treatments. (Shiddamallayya et al., 2010) . *S. alata* leaves have traditionally been used in Indonesia, particularly in South Sulawesi, to remove fungus on the skin that can cause hives and other symptoms by grinding or rubbing directly on the affected skin. *S. alata* leaves extract has been shown to have antifungal efficacy against Trichophyton verrucous, Epidermophyton floccose, and other microorganisms (Sule et al., 2010). The root or leaves are effective purgatives, while the leaves, blossoms, and stems are antifungal medicines used to treat skin infections

2.2 Bioactive Compounds in *Senna Alata*

Many different types of phytochemicals, including carotenoids, four phenolic acids, twenty flavonoids, two alkaloids, seven terpenoids, seventeen anthraquinones, four glycosides, twenty-eight fatty acids, and two phytosterols, have been identified in *S. alata*. Some phytochemicals are only found in specific plant tissues. Leaves are the most commonly reported source, while seeds, roots, and flowers also contain small amounts. As a result, the leaves are the primary plant component utilized in therapeutic applications. Particular phytochemicals that have been shown to have positive pharmacological effects are identified and explored in this article.

2.2.1Anthraquinone

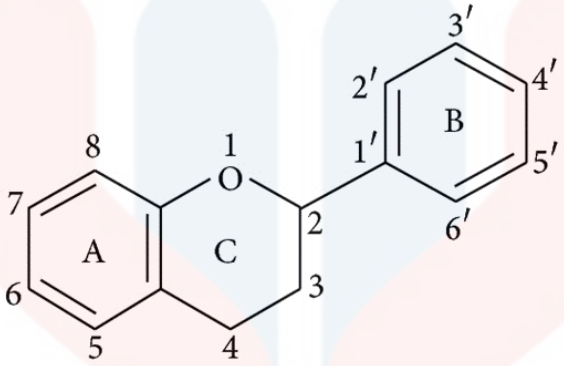


Figure 2: Anthraquinone structure

(National Center for Biotechnology Information ,2023)

Based on figure 2, anthraquinone is a derivative of the aromatic chemical molecule anthracene. It's a solid crystalline powder that can be anywhere from yellow to light grey or even green. C14H8O2 is its chemical formula. It boils at 379.8°C and melts at 286°C. It dissolves in nitrobenzene and aniline but not water or alcohol. Under typical conditions, it maintains a high degree of chemical stability. Some plants (such as aloe, senna, rhubarb, and cascara), fungi, lichens, and insects have anthraquinone as a structural component of their colors. Laxative effects are common among natural anthraquinone derivatives. The Borntrager test is a chemical analysis used to identify these glycosides due to their characteristic microsublimation. The hydrolysis of the glycosides yields derivatives of 1,8-dihydroxy anthraquinone, anthranol, anthrone, or dianthrone; the majority of the glycosides are O-glycosides and S-glycosides. Anthraquinones, anthranols, and anthrones are all possible forms of the common aglycones, which include aloe-emodin, emodin, rhein, chrysophanol, and physician. Glucose, arabinose, and rhamnose are the most common sugars found. Glycosides or dimers, which are reduced derivatives, were previously included in the medication. Hydrolysis and oxidation create free anthraquinones during drying and storage.

2.2.2 Flavonoids

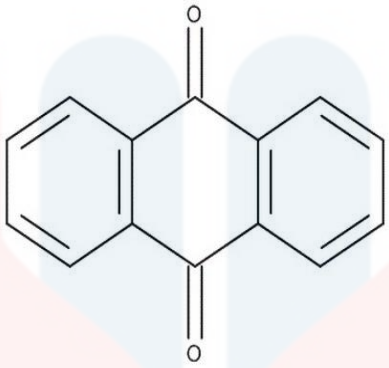


Figure 3: Flavonoids basic structure

(S. Kumar & Pandey, 2013)

In the plant kingdom, flavonoids are abundant and ubiquitous. They serve as plant pigments and cause numerous floral and fruit colors. From figure 3, flavonoids which are found in high concentrations in plant foods, are a regular part of the human diet, especially in fruit and vegetable-rich diets. Flavonoids are a type of polyphenol found primarily in plants; they are a secondary metabolite. These organic compounds can be found in abundance in a wide variety of foods and drinks, such as red wine, black tea, green tea, cider, and berries.(Cassidy & Minihane, 2017; Oteiza, Fraga, Mills, & Taft, 2018). Flavonoids have a 15-carbon structure made up of two phenyl rings joined by a 3-carbonated heterocyclic ring (C6-C3-C6). Flavanols, flavanones, isoflavones, flavones, flavan, and anthocyanidins are all subclasses of polyphenols that can be further subdivided based on structural alterations to the core carbon ring (Cassidy & Minihane, 2017). Chalcones are formed when the joints in the C3-portion don't cyclize (Seleem, Pardi, & Murata, 2017) They are part of a unique numbering scheme that also includes an isoflavonoid (Felice et al., 2019). There are around 6,000 different types of flavonoids available in plants, many of which are found in combination with glycosylated derivatives or acylated with phenolic acids. Most of the time, flavanol's and anthocyanidins are called "condensed tannins." These are very complicated groups of flavonoids that make up most of the flavonoids.

2.3 Fungus

Unlike prokaryotic bacteria, fungi are eukaryotic. A fungal infection can take the shape of yeasts, molds, or a hybrid of the two. Diseases at various levels of the body, including allergies,

can be caused by certain fungi. Yeasts are tiny fungus made up of single-celled organisms that divide by budding. In contrast, molds develop into new colonies by sending out new hyphae from their tips. Hyphae can have anywhere from a few nuclei to many, and their degree of septation can range from sparse to regular. All fungi, regardless of size or form, are heterotrophs that digest their food by secreting hydrolytic enzymes into the surrounding environment (absorptive nutrition). Fungi also have chitinous cell walls, plasma membranes with the sterol ergosterol, 80S rRNA, and microtubules made of tubulin, and they can produce lysine via the L--adipic acid biosynthetic route(McGinnis & Tyring, 1996).

2.3.1 *Candida Albicans*

Candida albicans is categorized as an opportunistic fungus since it typically only causes disease in immunocompromised individuals or those with altered flora. Yeast-like fungus belong to the genus Candida. When it comes to the Candida genus, the most prevalent pathogen is candida albicans. (Garber, 2001) *C. albicans* gets its name from the white patches that form when the fungus infects the skin or a mucous membrane. *Candida glabrata*, *Candida guilliermondii*, *Candida krusei*, *Candida parapsilosis*, and *Candida tropicalis* are a few of the other species in this genus that can cause infection. (Dowd, 2014). *C. albicans* can be either a yeast or a mycelium, depending on the conditions of its growth. candidiasis, which may usually be treated with little trouble (Lee & Chee, 2010) . Compared to the mycelial cell wall, which contains only a trace quantity of this sugar, mannan makes up around 20% of the cell wall in *C. albicans* yeast. There are three different forms of *C. albicans*, or serotypes: A, B, and C. Differences between these can be seen in their mannans. For serotype A, the mannoheptaose side chain is the antigenic determinant. It is the mannohexose side chain in serotype B. The resistance of serotype B to 5-fluorocytosine is often higher than that of serotype A. Approximately 50–70% of the yeast cell wall is made up of glucans with (1-3) - and (1-6)-linked groups. These glucans may prevent amphotericin B from crossing the cell membrane, it has been hypothesized (McGinnis & Tyring, 1996).

2.4 Antimicrobial Properties of *Senna Alata* Crude Extract

2.4.1 Antifungal Activity

Microbroth dilution testing was used to determine antifungal efficacy against four prevalent human pathogenic fungi: Trichophyton rubrum, Trichophyton mentagrophytes,

Aspergillus fumigatus, and *C. albicans*. (Da et al., 2019). After 18 to 24 hours of incubation at 37°C, the growth inhibition zones around the disks were assessed. The sensitivity of the microorganism species to the plant extracts was measured by the size of the inhibitory zones, which included the width of the disks on the agar surface around the disks. Values less than 8 mm were considered not active against microorganisms. (Bhalodia & Shukla, 2011).

2.5 Extraction Method

2.5.1 Extraction using Ultrasonic

Extraction is the most important step in getting back bioactive substances. This can be done in a number of ways, including liquid extraction, supercritical extraction, microwave-assisted extraction, and more.(K. Kumar, Srivastav, & Sharanagat, 2021). Some of the relative drawbacks of these mechanisms include a high demand for solvents, poor yields, a capital-intensive process, and numerous more (Anxo Carreira-Casais et al., 2021). Several analytical chemistry applications have been shown to benefit from ultrasound energy, including slurry dispersion, homogenization, and the extraction of inorganic and organic chemicals (Nascentes, Korn, S, & Arruda, 2001). Using ultrasound for processing and manufacturing purposes has become more prevalent in the food industry in the past few years. The process by which ultrasound destroys microbes involves stimulating the formation of cavitation bubbles in aqueous solutions and then spreading these bubbles throughout the cellular architecture (Gao, Lewis, Ashokkumar, & Hemar, 2014).

Ultrasonic baths and probes are the most common tools used in sonochemistry. Ultrasonic probe has demonstrated that the ultrasonic bath allows for simultaneous repeat extractions, while the ultrasonic probe provides a reduced extraction time (Stanisic et al., 2012). Ultrasonic generators convert mechanical energy from electricity into ultrasonic energy. For phenolic extraction, the most used instrument is the ultrasonic bath since it is accessible, cheap, and can extract multiple samples at once. On the other hand, it is impractical for use on a large scale in industry due to its poor repeatability and production rate as well as its weak ultrasonic power when applied directly to the sample. (Farid Chemat et al., 2017; Nascentes et al., 2001) . In addition to the equipment specifics, the ultrasound application conditions also impact the efficacy of the energy conversion.

In order to extract certain bioactive components, ultrasonic extraction makes use of ultrasonic energy and the right solvents. Cavitation bubbles are formed when ultrasonic waves move through a medium (solid, liquid, or gas) and through a series of compression and rarefaction cycles. The coalescence and collapse of these bubbles during the compression phase might create a hot spot, which can further accelerate the metabolic reactions in the area. In this way, acoustic cavitation plays a significant role in the source extraction. Fragmentation, pore creation, and shearing in the cellular matrix of the plant are just a few of the numerous phenomena that occur individually or in concert as cavitation bubbles and sound waves collapse, resulting in the solubilization of the bioactive principle in the extraction liquid. Similarly, ultrasonic-induced erosion may facilitate the release of bioactive substances. Sonoporation, or the formation of pores from cavitation, is another way that bioactive substances are released from cellular structures. In order to facilitate extraction, ultrasound is used to increase the material's ability to absorb water. The active component can be more evenly dispersed in water, leading to a higher yield, because water functions as a solvent. Therefore, ultrasound increases extraction yield while decreasing bioactivity loss (A. Carreira-Casais et al., 2021).

2.5.2 Decoction

Previously, water-soluble and heat-stable components have been extracted using the decoction method. The unprocessed material from plants is cooked in an open-type extractor with a certain amount of water for a given amount of time (Manousi, Sarakatsianos, & Samanidou, 2019). Decoction, which is also called "boiling," is the liquid made by boiling the hard parts of a plant in water. Filtering the heated extractive solution, washing the residue with water, and refilling the solution to the starting volume are all part of the extraction process. Within 24 hours after preparation, the decoction solution can be used directly from the refrigerator(Rodino & Butu, 2019).

This method comprises a constant hot extraction with a controlled amount of water. A plant material that has been dried, ground, and powdered is stored in a sterile container. The next step is to add water and mix it up. The extraction is then sped up by repeatedly applying heat. The entire procedure takes no more than 15 minutes. Typically, a 4:1 or 16:1 ratio of solvent to crude medication is used. It is employed in the removal of plant constituents that are both heat-

and water-stable. (Azwanida, 2015; Ingle et al., 2017; Majekodunmi, 2015; Pandey & Tripathi, 2014)

2.6 Phytochemical analysis

The medication, lead compounds, and components are displayed and isolated from the parts of medicinal plants by an analysis of their phytochemical qualities. Phytochemical characteristics are a reliable indicator of a plant's specific biological activity. The majority of plants employed for testing phytochemical qualities came from these four sources: leaves, roots, stem barks, and fruits. (Agidew, 2022).

2.6.1 Fourier transform infrared (FTIR)

FTIR is an effective tool for characterizing and identifying chemicals or functional groups (chemical bonds) in an uncharacterized plant extract combination. In addition, the FTIR spectra of purified compounds are typically so distinctive as to be analogous to a molecular "fingerprint." The spectra of unknown plant chemicals may usually be identified by comparing them to a large database of known compounds.

2.6.2 Gas Chromatography–Mass Spectrometry (GC-MS)

The range of uses for gas chromatography is extensive. However, its primary application is in the analysis and separation of complex mixtures including several components, including hydrocarbons, solvents, and essential oils. One of the most fundamental instruments in chemistry is gas chromatography due to its ease of use, sensitivity, and efficacy in separating mixture components. Its many applications include qualitative and quantitative mixture analysis, compound purification, and the calculation of a variety of thermochemical constants, including vapour pressure, activity coefficients, temperatures of solution and vaporization, and many more. Since gas chromatography–mass spectrometry (GC–MS) is a useful tool for analyzing volatile essential oils, lipids, alkaloids, and non-polar components, it has been used more and more in medicinal plant investigation in recent years. (Altameme, Hadi, & Hameed, 2015; Hussein, Mohammad, Hadi, & Hameed, 2016) .

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials and Preparation

In this study, the materials that have been used is *Senna Alata* plant. The part of the plant that being used in this study are only the leaves which estimated 100 grams of leaves. The plant was collected at Jeli, Kelantan.

3.2 Chemicals

The chemicals used in this study are DMSO, n-hexane, methanol, ethyl acetate and ethanol

3.3 Apparatus and Equipment

The apparatus that being used in this study were beaker (250 mL ,500ml), aluminum foil, filter paper, (10mL, 20 mL) test tube, spatula, aluminum foil, petri dish, sieve, hot plate. The equipment used in this extraction process is the blender, autoclave, GC-MS, FTIR, Ultrasonic bath Branson 2800 with frequency of 20Hz and rotary evaporator.

3.3 Preparation of samples

The first step is to wash the *S. alata* leaves in running water and let it dry in the sun for four or five days. The dried leaves were blend using a blender to get a powder form. The leaves are then pulverized to the point where they can pass through a sieve with a 0.5 mm mesh. Because of their greater surface area and higher solubility, smaller leaves yield a greater concentration of the chemical constituent during extraction. (Zubairi, Sarmidi, & Aziz, 2014). After that, 100 g leaves of *S. alata* were weight and 20g to be extracted in each form of extract. The samples were kept in a small bottle container at room temperature for future use. Keep the sample in a Ziplock bag and store it in a place with a temperature room.

3.4 Preparation of Media Sabouraud Dextrose Agar (SDA)

Suspended 65 g of the SDA into 1000 mL of distilled water. Mixed it well the content and heated it with continuous agitation to dissolved the constituents. Autoclave at 121° C for 15 minutes. Allowed it to cool for 45 to 50°C and pour into petri dishes for slants.

3.5 Preparation of Sabouraud Dextrose Broth (SDB)

Suspended 3 g of SDB into 100 mL of distilled water. Mixed it well the content by swirling the flask and heated it with continuous agitation to dissolve the constituents. Then pour about 5 to 10mL of the broth medium in test tubes and apply non-absorbent cotton plug to all the tubes. Autoclave the content at 121° C for 15 minutes

3.6 Inoculation of *Candida Albicans*

Using *C. albicans* ATCC 10231 glycerol stock to streak on the SDA plate using sterile cotton swab in the biosafety cabinet. Incubate at 37°C overnight in the incubator to grow the fungal. After the culture had grown, pick the fungal colony from culture plate and inoculate it with fresh SDB using inoculating loop then incubated it at 28 °C for 48 hours.

3.6 Extraction Method

3.6.1 Extraction method using ultrasonic extraction with different solvents

The extraction was performed using ultrasonic bath Branson 2800, ultrasonic cleaner 3/4-gallon tank, 20 khz, Available with heat, degas and digital controls. The value of operating parameters of ultrasonic water bath are summarized in Table 1. By using a solid-solvent ratio of 1:20, 400 mL of solvents (methanol, ethyl acetate, n-hexane) were added ,20g of *S. alata* leaf sample. The temperature of the ultrasonic water bath was set to be 30℃. The sonication was applied in continuous mode at frequency of 20 kHz. The extraction was performed in triplicate.

Table 1: Operating parameters of ultrasonic extraction

Parameters	Values
Temperature (℃)	30
Operating frequency (kHz)	20
Sonication time (min)	45

3.6.2 Decoction

This method requires the use of a constant flow of hot water as the solvent throughout the extraction procedure. A plant material that has been dried, ground, and powdered is stored in a sterile container. The next step is to add water and mix it up. The extraction is then sped up by repeatedly applying heat. The entire procedure takes no more than 15 minutes. Typically, the

proportion of solvent to crude medicine is 4:1 or 16:1. It is employed in the removal of plant constituents that are both heat- and water-stable (Azwanida, 2015; Ingle et al., 2017; Majekodunmi, 2015; Pandey & Tripathi, 2014).

3.6.3 Rotary Evaporator

In this research, a rotary evaporator (rotavap) was used after extraction by ultrasonic extraction and decoction. By lowering the pressure within the flask with a vacuum, rotating the sample to maximize its effective surface area, and heat the solution to appropriate temperatures for each solvent, the solvent can be removed using the rotavap method, as shown in the table 2 below. The percentage of *S. alata* crude leaf extracts yield (%) calculated with the formula of y (%) = C/W x 100, where the c is the content of the *S. alata* and W represent weight of dried sample.

Table 2: Solvent temperature for rotary evaporator

Solvent	**(℃)**
Methanol	64.7
Ethyl Acetate	77.1
n-Hexane	68.7
Distilled water	60

3.7 Disc Diffusion Method of Antifungal Activity

Filter paper discs were prepared by cutting paper discs of 6.0 mm in diameter from Whatman No. 1 filter paper using a punch. Using the 6 mm diameter sterile paper discs were impregnated of the crude extracts. The discs were then air-dried in laminar flow for few minutes before transferred onto the inoculated test microbial plates using sterile forceps**.** The test was performed by the disc diffusion method. The discs contained 10 µL of methanol, ethyl acetate, n-hexane and distilled water extracts with the concentration of 500mg/mL, which were placed on the agar that contains microorganisms *C. Albicans*. The plates containing the impregnated disks were incubated overnight at 37 ºC and the diameters of the inhibition zones were measured. *C. albicans* assay plates were incubated at 25 and 28 °C for 2 days. The formations of inhibition zones were observed daily and recorded.

3.8 Phytochemical Analysis

3.8.1 Fourier Transform Infrared (FT-IR)

The FTIR analysis will first examine the standard, which has already been prepared, and then the sample itself. In this investigation, an ATR model FTIR was used. The range of permitted wavenumbers is 3500 to 800 cm (-1). The Attenuated total reflection (ATR) diamond is cleaned with 70% ethanol before analysis. The unprocessed extracts found on the stone's surface. The sample analysis was performed in accordance with established protocols. Prior to FT-IR analysis, the sample was prepared by diluting the crude extracts (distilled water, methanol, ethyl acetate, and n-hexane) with ethanol at a concentration of 1 mg/ml.

3.8.2 Gas Chromatography- Mass Spectrometry (GC-MS)

The GC-MS analysis was done on a Shimadzu GC-MS-QP2010 device that has a gas chromatograph connected to a mass spectrometer. The following methods were used. these conditions: The VF-SMS fussed silica capillary column (30.0m x 0.25mm x 0.25im, made of 5% phenyl and 95% dimethylpolysiloxane) worked in electron impact mode at 70ev with helium (99.999%) as the carrier gas at a steady flow rate of 1 ml/min and an injection volume of 0.51l (split ratio of 10:1). The injector is heated to 240°C and the ton source is heated to 200°C. From 70°C (Isothermal for 3 min) to 240°C, with a 10°C/min rise, the oven temperature was set to end with a 9mm isothermal at 2809C. At 70cv, scans were done every 0.5 seconds, and pieces from 40 to 440Da were used. The whole time GiC ran was 40 minutes. (Oluwadun, 2015). Before performing GC-MS analysis, the sample was prepared by diluting the crude extracts (distilled water, methanol, ethyl acetate, and n-hexane) with ethanol at a concentration of 1 mg/ml.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Yield of *Senna Alata* crude extracts

Table 3, displays the weights of four distinct crude extracts of *S. alata*. One was obtained by decoction with distilled water, while the other three were obtained by ultrasonication with methanol, hexane, and ethyl acetate. It also showed how much of each extract was made as a portion of the 20 g of dried *S. alata* plant material. The yield of the n-hexane extract is the lowest at 3.62%. The yield of the methanol extract is the highest at 24.48%. The amounts of the ethyl acetate and distilled water extracts are 5.12% and 21.17%, respectively, less than the methanol extract but more than the n-hexane extract.

According to the results, methanol is the best solvent for ultrasonicating *S. alata* to extract its crude components. This is because the polarity of the solvents varies; n-hexane is non-polar, methanol and ethyl acetate are at least polar, and distilled water is most polar. The extraction of polar compounds is enhanced by polar solvents, whereas the extraction of non-polar compounds is enhanced by non-polar solvents. Variation in solvent polarity has a significant impact on plant extract antibacterial activity, phytochemical component percentage, and yield. (Padalia & Ch, 2015). It is important to keep in mind that many variables, including temperature, solvent sample ratio, solvent type and concentration, plant material size, extraction time, agitation speed, and extraction pH, can impact the extraction effectiveness while using this approach (Chirinos, Rogez, Campos, Pedreschi, & Larondelle, 2007) .

This result is consistent with previous reports that different extraction processes using different solvents yielded varying amounts of extract. Moreover, this is also in line with the earlier study that said the percentage of yield is affected by the polarity of the solvent and the way of extraction (Altemimi, Lakhssassi, Baharlouei, Watson, & Lightfoot, 2017; Chandrapala, Oliver, Kentish, & Ashokkumar, 2013).

Based on the findings presented in Table 3, and the accompanying discussion, it can be concluded that the ultrasonicated method with methanol solvent is the most effective extraction technique for obtaining *S. alata* crude compounds. According to the results, methanol produced

the highest yield of crude extract when compared to the other solvents and extraction techniques that were examined (Awad et al., 2021). The methanol extract yielded 24.48%, demonstrating its superior efficiency in extracting bioactive compounds from S. alata.

Table 3: Total yield (%) of S. alata crude extracts

Method	**Extraction solvent**	**Weight of dried sample (g)**	**Weight of Extract (g)**	**Yield (%)**
Ultrasonic Extraction	Methanol	20	4.90	24.48
	Ethyl Acetate	20	1.02	5.12
	n-Hexane	20	0.72	3.62
Decoction	Distilled water	20	4.23	21.17

4.2 Antifungal Analysis

The antifungal activities of *S. alata* leaves were assessed according to the diameter zone of inhibition against *C. albicans* at concentrations 500 mg/mL (Figure 4). All *S. alata* crude extract had impregnated disc with 10 µL at concentration of 500 mg/mL, negative control Dimethyl sulfoxide (DMSO) and positive control Fluconazole (150mg/mL) each extract was run in triplicate n=3. The negative control DMSO did not exert any inhibition on the strains tested. In contrast, the positive control (Fluconazole) of *C. albicans* has shown zone of inhibition. Besides that, which all crude extracts (methanol, ethyl acetate, n-hexane and distilled water) did not show any antifungal activity against *C. albicans* at concentration 500mg/ml.

This might be due to insufficient ultrasonic power of ultrasonic bath extraction which only 20khz to extract bioactive compound. The ultrasonic bath-type method has disadvantage as it generally operates at a single frequency (20kHz or 40kHz) while the probe-type develop a power up to 100 times more than the provided ultrasonic cleaner (jeevan kumar, Garlapati, Dash, Scholz, & Banerjee, 2017). On top of that, the ultrasonic irradiation of low power ultrasound only managed to irradiate through the walls where the sample is contained (indirectly), while the ultrasonic probe system is more powerful due to an ultrasonic intensity delivered through a smaller surface (only the tip of the probe) resulting in a direct delivery of ultrasound in the extraction media (F. Chemat et al., 2017) .Additionally, the ultrasonic probe has been reported to

be a more powerful tool for compared to ultrasonic baths (Dickson, Liu, Li, Tachiev, & Cai, 2012).

The ultrasonic probe is known for its ability to focus energy on a localized sample zone, providing more efficient cavitation in the liquid, and has been observed to result in higher extraction yields in certain studies (Abedi, Sahari, & Hashemi, 2016; Han et al., 2018) Furthermore, the ultrasonic probe has been found to be effective in accelerating the extraction process and achieving better yields for certain compounds compared to traditional heating baths(Gong et al., 2019). The ultrasound probe system is more powerful than the ultrasonic bath because the ultrasound delivery is direct and there is minor energy loss (Sukor, Jusoh, Rahim, & Kamarudin, 2018).

The study by (Yeong, Pang, Chong, & Gimbun, 2018) also compared microwave, microwave assisted extraction (MAE), and ultrasonic-assisted extraction (UAE) of kaempferol from *Cassia Alata*. It showed that the extraction method affected the quality, recovery, and yield of bioactive components in the extract. This showed that there may be ways to improve extraction techniques to get higher yield of bioactive compound.

It has been observed that *Candida albicans* can acquire resistance to several antimicrobial drugs, including those derived from plants. Without an inhibitory zone, the *C. albicans* strain utilized in the experiment may be resistant to the compounds found in the *S. alata* extract. However, as shown in a study it appears that *S.alata* leaf extract may be beneficial against Trichophyton verrucosum and Epidermophyton floccosum (Fatmawati, Yuliana, Purnomo, & Bakar, 2020).

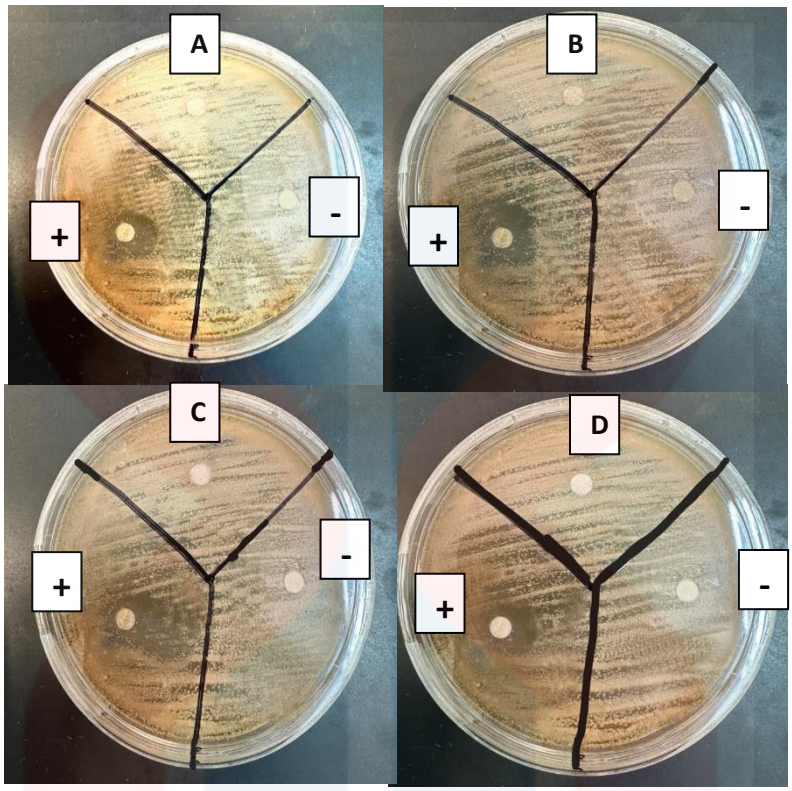


Figure 4 : No inhibition zones observed on assay plates of *S.Alata* leaves extracts against *C.albicans* at concentration 5mg/ml respectively

A : distilled water S.alata leaves extract B : methanol S.alata leaves extract C : ethyl acetate S.alata leaves extract D: n-hexane S.alata leaves extract Positive control: (+) Negative Control: (-)

4.3 Gas Chromatography Mass Spectrophotometry Analysis (GC-MS)

A GC-MS analysis was performed to identify the main secondary metabolites in the extract. Base on the GCMS analysis, 19 compounds were identified in methanol *S. alata* extracts, 8 in ethyl acetate extracts ,38 in n-hexane extracts and 16 in distilled water extracts. The identification of the phytochemical compounds was confirmed based on the peak area, retention time (RT) and molecular formula (MW).

GC-MS chromatogram of the methanol leaf extract of Senna Alata (Table 4) showed the presence of 19 compounds indicating with the retention time range between 4.69 and 33.81. The most abundant Ethyl iso-allocholate (0.45%), Pterin-6-carboxylic acid (0.43%), 2-Myristynoyl

pantetheine (0.34%). There are compounds such as 3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone (0.29%), Estra-1,3,5(10)-trien-17. beta. -ol (0.29%), Hexadecenoic acid, methyl esther (0.21%), 7-Methyl-Z-tetradecen-1-ol acetate (0.25%), Dodecanoic acid, 3-hydroxy-(0.27%), 2,4,7,9-Tetramethyl-5-decyn-4,7-diol (0.27%), Propanamide, 2-hydroxy-(0.23%) were recognized as moderately abundant ones. Other compounds such as Octadecanoic acid, ethyl ester (0.11%), Oleic Acid (0.12%) , Oxirane undecanoic acid, 3-pentyl-, amethyl ester, trans- (0.14%), 10-Octadecenoic acid, methyl ester (0.16%), Hexadecenoic acid, ethyl ester (0.19%) , Benzene propanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester (0.09%) , Phenol, 2,4-bis(1,1-dimethylethyl- (0.13%) , Propanoic acid, 2-hydroxy-, ethyl ester, (S)- (0.16%), Ethyl Oleate (0.17%) were comparatively least abundant.

Table 4: Phyto-components identified in the methanol extracts of the leaf of *S. alata*

Retention Time	**Name Of Compound**	**Molecular Formula**	**Molecular Weight**	**Peak Area %**
4.692	Propanoic acid, 2-hydroxy-, ethyl ester, (S)-	C5H10O3	118.13	0.16
5.739	Propanamide, 2-hydroxy-	C3H7NO2	89.09	0.23
19.054	2,4,7,9-Tetramethyl-5-decyn-4,7-diol	C14H26O2	226.35	0.27
20.616	Pterin-6-carboxylic acid	C7H5N5O3	207.15	0.43
21.006	Phenol, 2,4-bis(1,1-dimethylethyl)-	C14H22O	206.32	0.13
22.568	Dodecanoic acid, 3-hydroxy-	C12H24O3	216.32	0.27
29.026	7-Methyl-Z-tetradecen-1-ol acetate	C17H32O2	268.4	0.25
30.392	Hexadecanoic acid, methyl esther	C17H34O2	270.5	0.21
30.645	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	C18H28O3	292.4	0.09
31.069	Estra-1,3,5(10)-trien-17. beta.-ol	C18H24O	256.399	0.29
31.354	Hexadecanoic acid, ethyl ester	C18H36O2	284.5	0.19
31.912	2-Myristynoyl pantetheine	C25H44N2O5S	484.7	0.34
32.454	3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	C28H25NO7	487.5	0.29

32.673	10-Octadecenoic acid, methyl ester	C19H36O2	296.5	0.16
32.816	Oleic Acid	C18H34O2	282.5	0.12
32.973	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans-	C19H36O3	312.5	0.14
33.402	Ethyl Oleate	C20H38O2	310.5	0.17
33.683	Octadecanoic acid, ethyl ester	C20H40O2	312.5	0.11
33.807	Ethyl iso-allocholate	C26H44O5	436.6	0.45

GC-MS chromatogram of the ethyl acetate leaf extract of Senna Alata showed the presence of 8 compounds in ethyl acetate extracts of S. Alata (Table 5) indicating with the retention time range between 3.19 and 33.76. The most abundant compound was (E)-9-Octadecenoic acid ethyl ester (1.09%)..Three compounds namely Hexadecanoic acid, ethyl ester (0.69%), Hexadecanoic acid, methyl ester (0.43%) , 1,2-15,16-Diepoxyhexadecane (0.31%) were categorized as moderately abundant compound .Other compounds such as Naphtho[1,2-b] furan-2-one, 2,3,3a,4,5,5a,6,7,9a,9b-decahydro-3,5a,9-trimethyl-7,9a-peroxy- (0.29%), 1,2-Bis(trimethylsilyl)benzene (0.26%), 2-Pentadecanone, 6,10,14-trimethylz (0.22%) and 2-Hexanol, (S)- (0.16%) were comparatively least abundant.

Table 5: Phyto-components identified in the ethyl acetate extracts of the leaf of *S. alata*

Retention Time	**Name Of Compound**	**Molecular Formula**	**Molecular Weight**	**Peak Area %**
3.192	1,2-Bis(trimethylsilyl)benzene	$C_{18}H_{24}SI_2$	280.55	0.26
3.463	2-Hexanol, (S)-	$C_6H_{13}OH$	116.16	0.16
23.468	Naphtho[1,2-b] furan-2-one, 2,3,3a,4,5,5a,6,7,9a,9b-decahydro-3,5a,9-trimethyl-7,9a-peroxy-	$C_{11}H_6O_2$	170.18	0.29
29.045	2-Pentadecanone, 6,10,14-trimethylz	$C_{21}H_{42}O$	326.58	0.22
29.673	1,2-15,16-Diepoxyhexadecane	$C_{16}H_{30}O_4$	290.4	0.31
30.535	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	0.43

31.383	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.48	0.69
33.759	(E)-9-Octadecenoic acid ethyl ester	$C_{20}H_{3}{}^{\wedge}O_2$	322.51	1.09

GC-MS chromatogram of the n-hexane leaf extract of Senna Alata showed the presence of 38 compounds in ethyl acetate extracts of S. Alata (Table 6) indicating with the retention time range between 3.83 and 35.56. The most abundant compounds in n-hexane extracts of S. Alata were Ethyl Oleate (26.27%), Hexadecanoic acid, methyl ester (18.52%) ,2-Pentadecanone, 6,10,14-trimethylz (17.54%) and Octadecanoic acid, ethyl ester (21.04%). Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl- (5.63%), Heptadecanoic acid, 16-methyl-, methyl ester (4.91%),2,4,7,9-Tetramethyl-5-decyn-4,7-diol (4.67%), Octahydrate[b]pyran, 4a-acetoxy-5,5,8a-trimethyl- (4.36%) ,Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate (4.3%) ,Trimethyl(2-octyldodecyloxy) silane (3.36%),1-Heptatriacotanol (3.26%) Ethyl 9-hexadecenoate (2.75%) , Octan-2-one, 3,6-dimethyl- (2.75%),Propanoic acid, 2-hydroxy-, ethyl ester, (S)- (2.68%) ,Oleic Acid (2.44%) and Bicyclo [3.1.1] hept-2-en-4-ol. 2.6.6-trimethyl-. (2.1%) were categorized as moderately abundant .There other compounds such as Linalyl acetate (1.68%), tert-hexadecenoic (1.58%),7-Methyl-Z-tetradecen-1-ol acetate (1.5%),Octan-2-one, 3.6-dimethylz (1.46%),1-Hexyn-3-ol, 3.5-dimethyl- (1.36%),Trimethyl(2-octyldodecyloxy) silane (1.31%),1H-2.8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, la,2.5.5a.6.9,10,10a-octahydro-5.5a.6- (1.26%),1H-Cyclopropa [3.4] benz [1.2-e]azulene-5,7b.9.9a-tetrol, la,1b.4.4a.5.7a.8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 5,9,9a- (1.15%),Z.Z.Z-4.6.9-Nonadecatriene (1.06%),2-Propanone, 1.1-diethoxy- (1.02%) and 2.2-Dimethyl-6-methylene-1-[3.5-dihydroxy-1-pentenyl] cyclohexan-1-perhydrol (1.08%) were comparatively less abundant.

Table 6: Phyto-components identified in the n-hexane extracts of the leaf of *S. Alata*

Retention Time	**Name Of Compound**	**Molecular Formula**	**Molecular Weight**	**Peak Area %**
3.834	2-Pentanol, 3-chloro-4-methyl-, (R*, R*)- (. +/- .)-	C6H13OC	136.620	1.02
5.006	Propanoic acid, 2-hydroxy-, ethyl ester, (S)-	C5H10O3	118.1311	2.68
5.872	1-Hexyn-3-ol, 3,5-dimethyl-	C8H14O	126.1962	1.36
7.396	Linalyl acetate	C12H20O2	196.29	1.68

10.196	Octan-2-one, 3,6-dimethylz	C10H20O	156.26	1.46
11.392	2-Propanone, 1,1-diethoxy-	C7H14O	146.1843	1.71
12.558	Z, Z, Z-4,6,9-Nonadecatriene	C19H34	262.5	1.06
12.996	Trimethyl(2-octyldodecyloxy) silane	C21H46OSi	342.7	3.36
13.796	Bicyclo [3.1.1] hept-2-en-4-ol, 2,6,6-trimethyl-, acetate	$C_{12}H_{18}O_2$	194.2701	2.1
14.001	Octan-2-one, 3,6-dimethyl-	$C_{10}H_{22}$	142.2817	2.75
14.82	7-Methyl-Z-tetradecen-1-ol acetate	C17H32O2	268.4	1.5
19.001	2,4,7,9-Tetramethyl-5-decyn-4,7-diol	C14H26O2	226.35	4.67
21.368	Octahydrobenzo[b]pyran, 4a-acetoxy-5,5,8a-trimethyl-	C14H24O3	240.34	4.36
24.54	Trimethyl(2-octyldodecyloxy) silane	C23H50OSi	370.7	1.31
26.325	tert-Hexadecanethiol	C16H34S	258.51	1.58
27.464	1-Heptatriacotanol	C37H76O	537.0	3.26
28.006	Hexadecanoic acid, ethyl ester	C18H36O2	284.4772	3.64
28.811	2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl] cyclohexan-1-perhydrol	C14H24O4	256.34	1.08
28.902	Z-(13,14-Epoxy) tetradec-11-en-1-ol acetate	C16H28O3	268.39	4.3
29.021	2-Pentadecanone, 6,10,14-trimethylz	$C_{18}H_{36}O$	268.4778	17.54
30.387	Hexadecanoic acid, methyl ester	C17H34O2	270.5	18.52
30.93	Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-	C9H9F3O2	206.16	5.63
31.273	Ethyl 9-hexadecenoate	C18H34O2	282.5	2.75
32.754	Oleic Acid	C18H34O2	282.5	2.44
32.959	Heptadecanoic acid, 16-methyl-, methyl ester (Deepthi et al., 2016)	C19H38O2	298.5	4.91
33.068	1H-Cyclopropa [3,4] benz[1,2-e] azulene-5,7b,9,9a-tetrol, 1a,1b,4,4a,5,7a,8,9-octahydro-3-(hydroxymethyl)-1,1,6,8-tetramethyl-, 5,9,9a-	C26H36O8	476.6	1.15
33.345	Linoleic acid ethyl ester	C20H36O2	308.5	2.24
33.402	Ethyl Oleate	C20H38O2	310.5	26.27

33.673	Octadecanoic acid, ethyl ester	C20H40O2	312.5	21.04
34.692	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-	C20H28O6	364.4	1.26
35.235	4,8,12,16-Tetramethylheptadecan-4-olide	C21H40O2	324.5	8.97
35.564	5H-Cyclopropa [3,4] benz[1,2-e]azulen-5-one, 9,9a-bis(acetyloxy)-3-[(acetyloxy)methyl]-1,1a,1b,2,3,4,4a,7a,7b,8,9,9a-dodecahydro	C26H36O11	524.6	1.8

There were 16 compounds detected in distilled water extracts of S. Alata (Table 7) indicating with the retention time range between 3.76 and 35.56. The most abundant compounds were Ethyl Oleate (11.76%), 1-Pyrrolidinebutanoic acid, 2-[(1,1-dimethylethoxy) carbonyl]-. alpha. -nitro-, 2,6-bis(1,1-dimethylethyl)-4-methoxyphenyl ester (9.34%), Hexadecanoic acid, ethyl ester (8.94%), 2,4,7,9-Tetramethyl-5-decyn-4,7-diol (6.49%). 2-Myristynoyl pantetheine (3.93%), Ethyl iso-allocholate (2.28%), Propanoic acid, 2-hydroxy-, ethyl ester, (S)- (2.06%) were categorized moderate abundant. Oleic Acid (1.97%), 2-Hexanol, (S)-(1.73%), Imidazole, 2-amino-5-[(2-carboxy) vinyl]- (1.6%), Hexadecanoic acid, methyl ester (1.59%), Lactic acid (1.23%) , Octasiloxane (1.22%), Estra-1,3,5(10)-trien-17.beta.-ol (1.2%) , 5,7-Dioxa-1-octene, 1-chloro-4-isopropyl-3-methyl-(1.1%), and Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)- (1.08%) were present in less concentrations .

Table 7: Phyto-components identified in the distilled water extracts of the leaf of *S. Alata*

Retention Time	**Name Of Compound**	**Molecular Formula**	**Molecular Weight**	**Peak Area %**
3.763	Lactic acid	C3H6O3	90.08	1.23
4.935	Propanoic acid, 2-hydroxy-, ethyl ester, (S)-	C5H10O3	118.1311	2.06
5.806	2-Hexanol, (S)-	C6H14O	102.1748	1.73

4.782	Bicyclo [3.1.1] heptane, 6,6-dimethyl-2-methylene-, (1S)-	C10H16	136.2340	1.08
5.806	5,7-Dioxa-1-octene, 1-chloro-4-isopropyl-3-methyl-	C10H19ClO2	206.71	1.1
19.059	2,4,7,9-Tetramethyl-5-decyn-4,7-diol	C14H26O2	226.35	6.49
23.792	Imidazole, 2-amino-5-[(2-carboxy) vinyl]-	C6H7N3O2	153.14	1.6
30.397	Hexadecanoic acid, methyl ester	C17H34O2	270.4507	1.59
31.078	Estra-1,3,5(10)-trien-17. beta. -ol	C18H24O	256.3826	1.2
31.259	Octasiloxane			1.22
31.359	Hexadecanoic acid, ethyl ester	C18H36O2	284.4772	8.94
32.683	Oleic Acid	C18H34O2	282.4614	1.97
33.073	Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	C27H42O4	430.6	1.11
33.407	Ethyl Oleate	C20H38O2	310.5	11.76
34.207	2-Myristynoyl pantetheine	C25H44N2O5S	484.7	3.93
34.559	Ethyl iso-allocholate	C27H48O5	452.7	2.28
35.559	1-Pyrrolidinebutanoic acid, 2-[(1,1-dimethylethoxy) carbonyl]-. alpha. -nitro-, 2,6-bis(1,1-dimethylethyl)-4-methoxyphenyl ester	C28H44N2O7	520.7	9.34

Based on the GC-MS results, potential antifungal properties that have been identified in *S. alata* extracts. There were 6 phytocomponents in *S. alata* methanol extracts which are Phenol, 2,4-bis (1,1-dimethylethyl) (0.13%), Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester (0.09%), Hexadecanoic acid, methyl esther (0.21%),10-Octadecenoic acid, methyl ester (0.61%), Oleic Acid (0.12%) an Ethyl iso-allocholate (0.45%). Only 1 phytocomponents in *S.alata* ethyl acetate extracts which was Hexadecanoic acid, methyl esther (0.43%) while 4 phytocomponents in n-hexane *S. alata* extracts which were Linalyl acetate (1.68%), Hexadecanoic acid, methyl ester(18.52%), Oleic Acid (2.44%)and Heptadecanoic acid,

16-methyl-, methyl ester (4.91%) .There were 4 phytocomponents in *S .alata* distilled water extracts which were Imidazole, 2-amino-5-[(2-carboxy)vinyl]- (1.6%) Hexadecanoic acid, methyl ester (1.59%)Oleic Acid (1.97%) and Ethyl iso-allocholate(2.28%).

Phenol, 2, 4-bis (1, 1- dimethylethyl) (2,4-DTBP) is a naturally occurring chemical with a long list of potential uses in the medical, food, and agricultural industries, according to earlier research. It protects against cognitive damage caused by trimethyltin (TMT), and it also possesses antioxidant, anticancer, antifungal, and antibacterial effects in medicine (Ren, Wang, Karthikeyan, Liu, & Cai, 2019). The references that have been given indicate that 2,4-DTBP may have antifungal effects. In particular, the antifungal activity of 2,4-DTBP was highlighted in the work by as a result of salicylic acid treatment of avocado roots (Rangel-Sánchez, Castro-Mercado, & García-Pineda, 2014).

Benzenepropanoic acid methyl ester (0.09%), also known as 3,5-bis(1,1-dimethylethyl)-4-hydroxybenzenepropanoic acid methyl ester, is another name for this compound. The study by (Nandika et al., 2021) reported that this compound showed antifungal and antioxidant activity. Therefore, it can be inferred that Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester exhibits antifungal properties, as indicated by its antifungal reported in various study.

Hexadecanoic acid and methyl esther are present in every extract of *S. alata*. While hexadecenoic acid methyl ester (or methyl palmitate) has demonstrated antifungal activity in particular studies it is crucial to be aware of the study's limitations and the circumstances in which it was tested. It works as both a plant and a mammalian metabolite (National Center for Biotechnology Information). Base on previous study, hexadecanoic acid, methyl ester, which was found explicitly in the root extracts of *S. Alata*. (Toh, Lihan, Bunya, & Leong, 2023) . In addition, hexadecanoic acid methyl ester was previously found to be one of the main phytocompounds in *Jatropha Curcas* leaf extracts, which may explain why they were effective against *Phaeoisariopsis Personata* fungus (Francis, 2021).

Oleic Acid vesicles can be used as an effective carrier for the delivery of antifungal agent for the treatment of localized fungal infections (S. Verma et al., 2014) . Based on the provided references, there is conflicting evidence regarding the antifungal activity of oleic acid in plant

extracts. The study by highlighted the antifungal activity of naturally occurring oleic acid, further supporting its potential as an antifungal compound (Petrović et al., 2022)

Ethyl iso-allocholate are most effective plant extract which possesses antifungal activity. (Parthasarathy, Jayalakshmi, N, & Varadharaj, 2018). Endophytic Aspergillus Tereus extract included ethyl iso-allocholate, which was found to have antifungal efficacy against mucormycosis fungus in the study (Hashem, Shehabeldine, Abdelaziz, Amin, & Sharaf, 2022).

Linalyl acetate actively inhibits microorganism growth and inhibits the extension of fungal hyphae (D'Auria et al., 2005). Additionally, linalyl acetate, in conjunction with other constituents, plays a role in the antifungal properties of Lavandula angustifolia essential oil as a whole (Mijatovic et al., 2022). The research found heptadecanoic acid, 16-methyl-, methyl ester to be a possible antimicrobial compound. It also showed a minor peak in plant extracts, which suggests it might have antimicrobial qualities, such as antifungal ones (Mohamad et al., 2018).

10-Octadecenoic acid, methyl ester may be responsible for the antifungal activity of Cirsium arvense extracts (Banaras, Javaid, Shoaib, & Ahmed, 2017). Base on the previous study also identified 10-Octadecenoic acid, methyl ester as antibacterial and antifungal properties (Belakhdar, Benjouad, & Abdennebi, 2015) .

Imidazole derivatives exhibit a broad range of biological activities, including antibacterial, anticancer, antitubercular, analgesic, and anti-HIV properties (A. Verma, Joshi, & Singh, 2013) . Imidazole, 2-amino-5-[(2-carboxy) vinyl]-was also found in the methanolic Cassia fistula extract, suggesting that it may have bioactive properties and occurs naturally in the plant (Kadhim, Mohammed, & Hameed, 2016).

The GC-MS analysis identified several phytocomponents in methanol extracts that have been reported to exhibit antifungal properties. These include Phenol, 2,4-bis (1,1-dimethylethyl), Benzene propanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester, and Oleic Acid, among others. Therefore, methanol extracts contained a larger number of phytocomponents with reported antifungal properties in the GC-MS data compared to other extracts.

4.4 Fourier Transform Infrared Analysis (FT-IR)

The bioactive compound of (methanol, ethyl acetate, n-hexane and distilled water) extracts leaves of plant *S. alata* was traced out by FT-IR spectrophotometer (Thermo electron

Scientific). Total peaks were obtained for the FTIR analysis of *S. alata* extracts which were 17 peaks (methanol), 18 peaks (ethyl acetate), 15 peaks (n-hexane) and 17 peaks (distilled water). Base on the table shows the functional groups that can be identified using FT-IR spectroscopy. The wavenumber range (in cm-1) is given along with a description of the bond type and potential compound functional groups.

Base on the Table 8, methanol extracts of *S. alata* which subjected to FT-IR analysis to identify the functional groups of the active components present in extract based on the peak values in the region of IR radiation. The IR spectrum showed that broad O-H stretching peak at 3317.74 cm^{-1} and the C-O stretching peaks at 1327.62 cm^{-1} and 1273.93 cm^{1}, C-H stretching peaks at 2972.93 cm^{-1}, 2926.58 cm^{-1}, and 1455.10 cm^{-1}, and the C-H bending peaks at 1417.15 cm^{-1}, C=O stretching peak at 2362.92 cm^{-1}, C=N stretching peak at 2138.31 cm^{-1}. C=C stretching peak at 1922.33 cm^{-1} and the benzene ring deformation peaks at 879.70 cm^{-1} and 802.97 cm^{-1}, C=O stretching peak at 1652.55 cm^{-1}, C-O stretching peaks at 1087.39 cm^{-1} and 1045.31 cm^{-1}. Base on the IR spectrum which corresponds all wavelength of the alcohol and phenols, alkanes, carbonyl and ketones, nitriles, aromatic rings, carboxylates, amides and polysaccharides.

Table 8: Peak value, bond type and functional group for FT-IR spectra of methanol extract of *S. alata* leaf

Wavenumber (cm^{-1})	**Bond type**	**Potential Functional Groups**
3317.74	O-H stretching (broad)	Alcohols, phenols
2972.93, 2926.58	C-H stretching (asymmetric and symmetric)	Alkanes
2881.49	C-H stretching (aldehyde)	Aldehydes
2362.92	C=O stretching	Carbonyls, ketones
2138.31	C≡N stretching	Nitriles
1922.33	C=C stretching (aromatic)	Aromatic rings
1652.55	C=O stretching (conjugated)	Carboxylates, amides
1455.10, 1417.15	C-H bending	Alkanes
1379.72	N-O stretching	Nitrates
1327.62, 1273.93	C-O stretching	Alcohols, phenols, ethers

1087.39, 1045.31	C-O stretching	Polysaccharides
879.70, 802.97	Benzene ring deformation	Aromatic rings

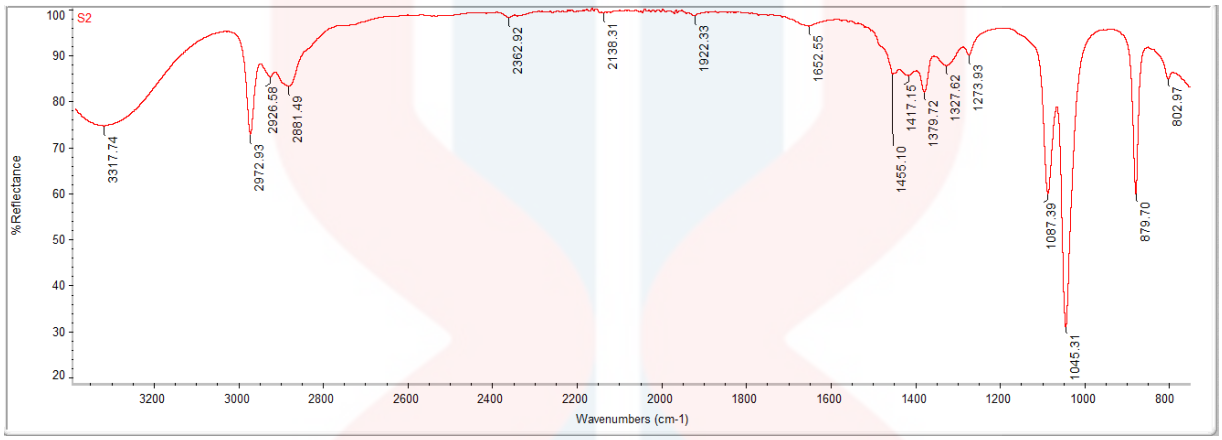


Figure 5: FT-IR spectra of methanol extract of *S. alata* leaf

Base on the Table 9, ethyl acetate extracts of *S. alata* which subjected to FTIR analysis to identify the functional groups of the active components present in extract based on the peak values in the region of IR radiation. The IR spectrum indicated that broad O-H stretching peak at around 3300 cm^{-1} and the C-O stretching peaks at around 1050 cm^{-1} and 1250 cm^{-1}, C-H stretching peaks at around 2900 cm^{-1} and 1450 cm^{-1}, and the C-H bending peaks around 1400 cm^{-1}C=O stretching peak at around 1700 cm^{-1}C=C stretching peak at around 1600 cm^{-1} and the benzene ring deformation peaks at around 800 cm^{-1}, C-N stretching peak at around 1650 cm^{-1} and the N-H bending peaks around 1550 cm^{-1}.Base on the IR spectrum which corresponds all wavelength to a functional groups were alcohol and phenols, alkanes, carbonyls, aromatic rings and amides .

Table 9: Peak value, bond type and functional group for FT-IR spectra of ethyl acetate extract of *S. alata* leaf

Wavenumber (cm^{-1})	**Bond type**	**Potential Functional Groups**
3318.08	O-H stretching (broad)	Alcohols, phenols
2972.80, 2916.76, 2882.26	C-H stretching	Alkanes

2358.47	C=O stretching	Ketones, aldehydes, carboxylic acids
2161.15, 2133.70	C≡C stretching	Alkynes
1919.87	C=N stretching	Amides, amines
1653.06	C=O stretching (conjugated)	Ketones, enones
1456.01, 1418.49	C-H bending	Alkanes
1379.47	C-N stretching	Aromatic amines
1328.51, 1274.11	C-O stretching	Ethers, phenols
1087.48, 1045.42	C-O stretching	Alcohols
879.71, 802.90	C-H bending	Aromatic hydrocarbons

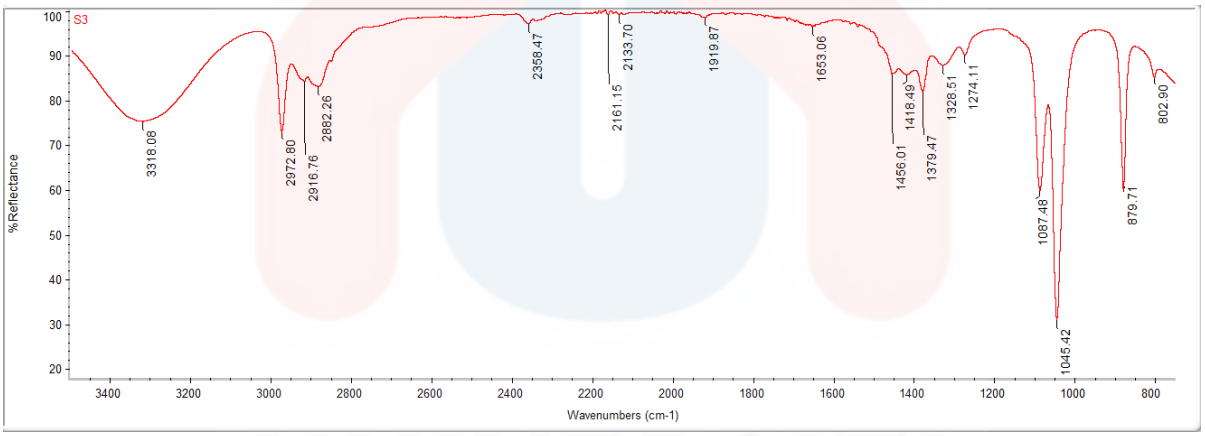


Figure 6: FT-IR spectra of ethyl acetate extract of *S. alata* leaf

Base on the Table 10, n-hexane extracts of *S. alata* which subjected to FTIR analysis to identify the functional groups of the active components present in extract based on the peak values in the region of IR radiation. The IR spectrum indicated that broad O-H stretching peak at 3317.72 cm^{-1} and the C-O stretching peaks at 1087.60 cm^{-1} and 1045.58 cm^{-1}, C-H stretching peaks at 2972.63 cm^{-1}, 2921.03 cm^{-1}, and 1456.01 cm^{-1}, and the C-H bending peaks at 1419.69 cm^{-1}, C-H stretch (asymmetric and symmetric) at 2972.63 cm^{-1} and 2921.03 cm^{-1}. They can also be indicated by the weak C=C stretch in the range of 1640-1680 cm^{-1} C-N stretch peak at 2360.82 cm^{-1}, C=O stretch peak at 1725.35 cm^{-1}, N-O stretching peaks at 1379.35 cm^{-1} and

1330.33 cm^{-1}.Base on the IR spectrum which corresponds all wavelength to functional groups were alcohol and phenols, alkanes, alkenes, nitriles, ketones, ketones and nitrates

Table 10: Peak value, bond type and functional group for FT-IR spectra of n-hexane extract of *S. alata* leaf

Wavenumber (cm^{-1})	**Bond type**	**Potential functional Groups**
3317.72	O-H stretch (broad)	Alcohols, phenols, carboxylic acids
2972.63, 2921.03	C-H stretch (asymmetric and symmetric)	Alkanes, alkenes, alkynes
2853.24	C-H stretch (methylene)	Alkanes
2360.82	C=N stretch	Nitriles, amides
2013.68	C=C=C stretch (alkyne)	Alkynes
1926.86	C=O stretch (aldehyde)	Aldehydes, ketones
1638.18	C=O stretch (conjugated)	Ketones, enones
1456.01, 1419.69	C-H bend (methylene)	Alkanes
1379.35, 1330.33	N-O stretch	Nitrates, nitro compounds
1273.98	C-O stretch (ether)	Ethers
1087.60, 1045.58	C-O stretch (alcohol)	Alcohols, phenols

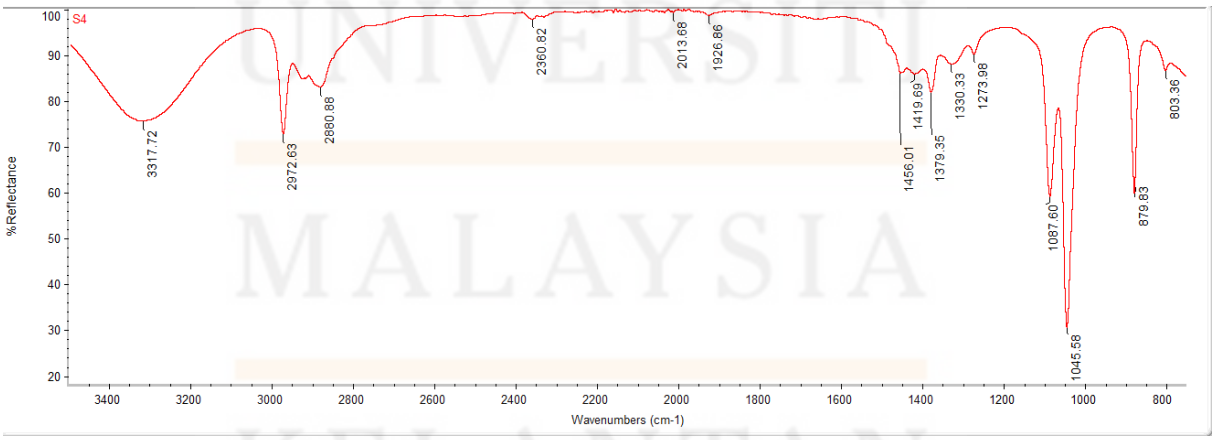


Figure 7: FT-IR spectra of n-hexane extract of *S. alata* leaf

Base on the Table 11, distilled water extracts of *S. alata* which subjected to FTIR analysis to identify the functional groups of the active components present in extract based on the peak values in the region of IR radiation. The IR spectrum indicated that broad O-H stretching peak at 3316.18 cm^{-1} and the C-O stretching peaks at 1087.58 cm^{-1} and 1045.43 cm^{-1},C-H stretching peaks at 2972.72 cm^{-1}, 2927.16 cm^{-1}, and 1455.81 cm^{-1}, and the C-H bending peaks at 1419.08 cm^{-1},N-O stretching peaks at 1379.46 cm^{-1} and 1328.85 cm^{-1},C-H bend peak at 879.74 cm^{-1} and the benzene ring deformation peak at 802.95 cm^{-1}. Base on the IR spectrum which corresponds all wavelength to functional groups were alcohol and phenols, alkanes nitrates and aromatic rings

Table 11: Peak value, bond type and functional group for FT-IR spectra of distilled water extract of *S. alata* leaf

Wavenumber (cm^{-1})	**Bond type**	**Potential Functional Groups**
3316.18	O-H stretch (broad)	Alcohols, phenols, carboxylic acids
2972.72, 2927.16	C-H stretch (asymmetric and symmetric)	Alkanes, alkenes, alkynes
2882.56	C-H stretch	Alkanes
2357.66	C=N stretch	Nitriles, amides
2050.26	C=C=C stretch	Alkynes
1923.22	C=O stretch	Aldehydes, ketones
1653.55	C=O stretch (conjugated)	Ketones, enones
1455.81, 1419.08	C-H bend	Alkanes
1379.46, 1328.85	N-O stretch	Nitrates, nitro compounds
1274.20	C-O stretch	Ethers
1087.58, 1045.43	C-O stretch	Alcohols, phenols
879.74	C-H bend	Aromatic rings
802.95	Benzene ring deformation	Aromatic rings

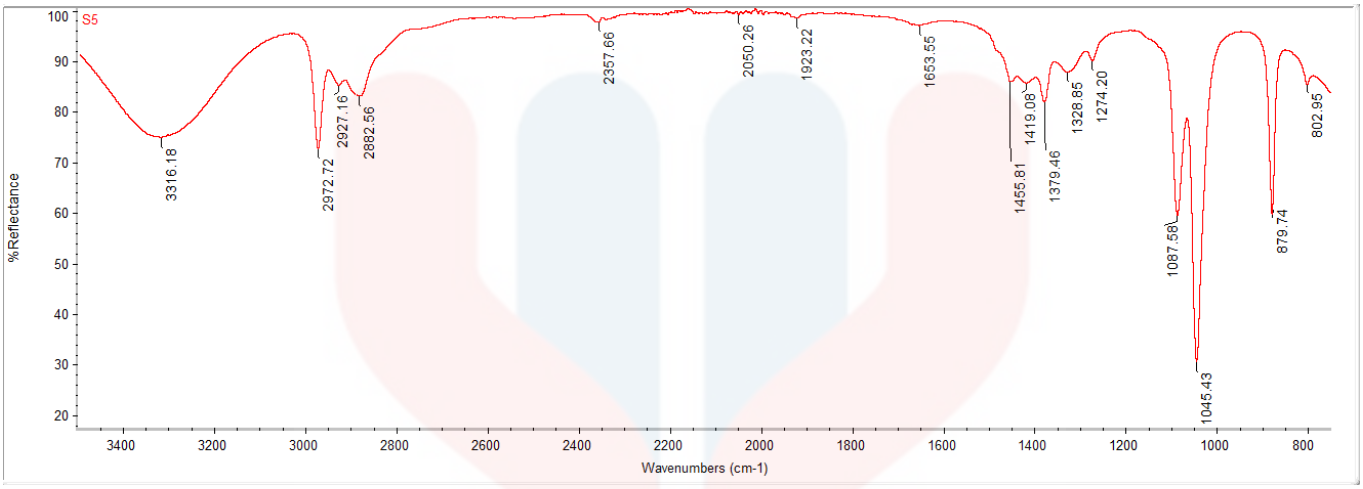


Figure 8: FT-IR spectra of distilled water extract of *S. alata* leaf

Based on the all of FTIR spectra figure and table above of *S. alata* extracts were commonalities between each extract. All extracts exhibit O-H stretching bands around (3316-3318 cm^{-1}), indicating the presence of alcohols, phenols, or carboxylic acid. C-H stretching bands observed in all extracts (2853-2973 cm^{-1}) confirm the presence of alkanes. All *S. alata* extracts show peaks between (1638-1926 cm^{-1}), suggesting the presence of carbonyl groups in aldehydes, ketones, or conjugated systems. There also were a weak band around (802-879 cm^{-1}) in all extracts indicate the presence of aromatic rings.

An additional factor that may significantly impact the outcomes of FTIR research is the solvent used for plant extraction. For example, research has shown that methanol is superior to other solvents in forming hydrogen bonds with lignin's hydroxyl groups, allowing for the extraction of longer macromolecular chains rich in OH (Passoni, Scarica, Levi, Turri, & Griffini, 2016). Methanol extract possesses the most diverse range of functional groups, including nitriles (2362 cm^{-1}), polysaccharides (1045 & 1087 cm^{-1}), and potentially tannins (1327 & 1273 cm^{-1}). The presence of hydroxyl (OH) stretching for hydroxyl group, alkanes (C-H), alkenes (C=C), aromatic rings (C=O), carboxylic (C=O), and amides (aromatic) has also been observed in plant extracts with antifungal potential (Ahmad & Ali, 2013)

Both methanol and ethyl acetate *S. alata* extracts exhibit peaks indicative of functional groups commonly found in antifungal compounds, such as phenols, aldehydes, and aromatic rings. The presence of these functional groups suggests that both methanol and ethyl acetate extracts have the potential to contain compounds with antifungal activity. FTIR analysis of crude compounds and identified functional groups such as aldehyde, acetyl, and cyanide, which may contribute to the antifungal properties of the plant extract (Prajakta, Suvarna, Raghvendra, & Alok, 2019). The presence of functional groups such as carbonyl (C=O) and >CO- groups, which were powerful in separating the populations and could potentially contribute to the antifungal properties of *Cassia Alata* (Sujatha & Asokan, 2017).While n-hexane extracts contain functional groups commonly found in lipids and hydrocarbons, they may not contain as many functional groups associated with known antifungal compounds. Distilled water extracts show peaks associated with aromatic compounds but may not have as diverse a range of functional groups as methanol and ethyl acetate extracts.

The FTIR results show that methanol and ethyl acetate *S. alata* extracts are more likely to have antifungal activity than n-hexane and distilled water *S. alata* extracts. This is because they contain functional groups that are linked to known antifungal compounds. Also, according to the GC-MS data, there were more phytocomponents in the methanol extracts that had antifungal effects than in the other extracts.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Finally, the study investigated the antifungal activity of *Senna Alata* leaf crude extracts utilizing different solvents and extraction methods. The extract yields exhibited variation dependent upon the solvent employed, whereby ultrasonic extraction of methanol produced the highest yield in comparison to the extractions conducted with hexane, ethyl acetate, and distilled water. The bioactive components from *S. alata* leaves were best extracted using methanol, a solvent with intermediate polarity and an ability to extract a broad variety of phytochemicals.

Antifungal analysis against *Candida albicans* revealed that all *S. alata* crude extracts at a concentration of 500 mg/mL did not exhibit significant antifungal activity. This could be attributed to extraction method, solvent polarity, and the solubility of the active compounds. However, previous studies have reported the antifungal activity of *S. alata* extracts against other fungal species, suggesting that further investigation with different method of extraction and fungal strains may be warranted.

Gas Chromatography Mass Spectrophotometry (GC-MS) analysis identified several phytocomponents in the *S. alata* extracts, including Phenol, 2,4-bis(1,1-dimethylethyl), Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester, Hexadecanoic acid, methyl ester, Oleic Acid, and Ethyl iso-allocholate, among others. These compounds have been reported to possess potential antifungal properties, supporting the potential antifungal activity of *S. alata* extracts.

Fourier Transform Infrared (FT-IR) analysis of the *S. alata* extracts revealed the presence of functional groups associated with antifungal compounds, such as phenols, aldehydes, aromatic rings, and carbonyl groups. Methanol and ethyl acetate extracts exhibited a wider range of functional groups compared to n-hexane and distilled water extracts, further supporting their potential antifungal activity. Overall, based on the combined analysis of GC-MS and FT-IR data, methanol extracts of *S. alata* appear to have the most potential antifungal activity.

5.2 Recommendations

There are several recommendations that can be made. Firstly, considering the influence of extraction solvent polarity on yield and phytochemical composition, it would be valuable to explore additional solvents or solvent combinations to optimize the extraction process. Solvents with different polarities could potentially extract different sets of bioactive compounds, leading to enhanced antifungal activity. Additionally, exploring different extraction techniques such as microwave-assisted extraction (MAE), supercritical fluid extraction (SFE) or Soxhlet extraction could provide insights into more efficient extraction methods.

Moreover, conducting bioassays against a broader spectrum of fungal strains, including those known to be resistant to conventional antifungal agents, would provide valuable insights into the spectrum of activity of *S. alata* extracts. This approach would help determine the potential of these extracts as alternative or adjunct therapies for fungal infections, especially against multidrug-resistant strains.

In addition to traditional methods of evaluating antifungal activity, such as agar diffusion assays, employing more advanced techniques like microdilution assays or biofilm inhibition assays could provide more detailed information on the mechanism of action and potential clinical relevance of *S. alata* extracts.

Finally, to further elucidate the antifungal mechanisms of *S. alata* extracts, conducting studies to identify and isolate specific bioactive compounds responsible for the observed activity would be beneficial. This could involve bioassay-guided fractionation followed by structural elucidation using techniques such as nuclear magnetic resonance (NMR) spectroscopy. Understanding the molecular basis of antifungal activity could facilitate the development of novel antifungal agents derived from *S. alata* extracts.

REFERENCES

Abedi, E., Sahari, M. A., & Hashemi, S. M. B. (2016). Accelerating Bleaching of Soybean Oil by Ultrasonic Horn and Bath Under Sparge of Helium, Air, Argon and Nitrogen Gas. *Journal of Food Processing and Preservation*. doi:10.1111/jfpp.12987

Abo, K. A., Fred-Jaiyesimi, A. A., & Jaiyesimi, A. E. (2008). Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. *J Ethnopharmacol, 115*(1), 67-71. doi:10.1016/j.jep.2007.09.005

Agidew, M. G. (2022). Phytochemical analysis of some selected traditional medicinal plants in Ethiopia. *Bulletin of the National Research Centre, 46*(1), 87. doi:10.1186/s42269-022-00770-8

Ahmad, B., & Ali, J. (2013). Physiochemical, Minerals, Phytochemical Contents, Antimicrobial Activities Evaluation and Fourier Transform Infrared (FTIR) Analysis of Hippophae Rhamnoides L. Leaves Extracts. *African Journal of Pharmacy and Pharmacology*. doi:10.5897/ajpp12.1246

Altameme, H., Hadi, M., & Hameed, I. (2015). Phytochemical analysis of Urtica dioica leaves by fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry. *7*, 238-252. doi:10.5897/JPP2015.0361

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Plants (Basel), 6*(4). doi:10.3390/plants6040042

Arif, T., Bhosale, J. D., Kumar, N., Mandal, T. K., Bendre, R. S., Lavekar, G. S., & Dabur, R. (2009). Natural products--antifungal agents derived from plants. *J Asian Nat Prod Res, 11*(7), 621-638. doi:10.1080/10286020902942350

Awad, A. M., Kumar, P., Ismail-Fitry, M. R., Jusoh, S., Ab Aziz, M. F., & Sazili, A. Q. (2021). Green Extraction of Bioactive Compounds from Plant Biomass and Their Application in Meat as Natural Antioxidant. *Antioxidants (Basel), 10*(9). doi:10.3390/antiox10091465

Azwanida, N. (2015). A Review on the extraction methods use in medicinal plants, principle, strength and limitation. Medicinal & Aromatic Plants, 04 (03), 3–8. In.

Banaras, S., Javaid, A., Shoaib, A., & Ahmed, E. (2017). ANTIFUNGAL ACTIVITY OF Cirsium Arvense EXTRACTS AGAINST PHYTOPATHOGENIC FUNGUS Macrophomina Phaseolina. *Planta Daninha*. doi:10.1590/s0100-83582017350100014

Belakhdar, G., Benjouad, A., & Abdennebi, E. L. H. (2015). Determination of some bioactive chemical constituents from Thesium humile Vahl. *6*, 2778-2783.

Bhalodia, N. R., & Shukla, V. J. (2011). Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant. *J Adv Pharm Technol Res, 2*(2), 104-109. doi:10.4103/2231-4040.82956

Carreira-Casais, A., Otero, P., Garcia-Perez, P., Garcia-Oliveira, P., Pereira, A. G., Carpena, M., . . . Prieto, M. A. (2021). Benefits and drawbacks of ultrasound-assisted extraction for the recovery of bioactive compounds from marine algae. *International journal of environmental research and public health, 18*(17), 9153.

Carreira-Casais, A., Otero, P., Garcia-Perez, P., Garcia-Oliveira, P., Pereira, A. G., Carpena, M., . . . Prieto, M. A. (2021). Benefits and Drawbacks of Ultrasound-Assisted Extraction for the Recovery of Bioactive Compounds from Marine Algae. *Int J Environ Res Public Health, 18*(17). doi:10.3390/ijerph18179153

Cassidy, A., & Minihane, A.-M. (2017). The role of metabolism (and the microbiome) in defining the clinical efficacy of dietary flavonoids. *The American journal of clinical nutrition*, *105*(1), 10-22.

Chandrapala, J., Oliver, C. M., Kentish, S., & Ashokkumar, M. (2013). Use of power ultrasound to improve extraction and modify phase transitions in food processing. *Food Reviews International*, *29*(1), 67-91.

Chemat, F., Rombaut, N., Sicaire, A.-G., Meullemiestre, A., Fabiano-Tixier, A.-S., & Abert-Vian, M. (2017). Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics Sonochemistry*, *34*, 540-560.

Chemat, F., Rombaut, N., Sicaire, A. G., Meullemiestre, A., Fabiano-Tixier, A. S., & Abert-Vian, M. (2017). Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrason Sonochem*, *34*, 540-560. doi:10.1016/j.ultsonch.2016.06.035

Chew, Y.-L., Khor, M.-A., Xu, Z., Lee, S.-K., Keng, J.-W., Sang, S.-H., . . . Ming, L. C. (2022). Cassia alata, Coriandrum sativum, Curcuma longa and Azadirachta indica: Food Ingredients as Complementary and Alternative Therapies for Atopic Dermatitis-A Comprehensive Review. *Molecules*, *27*(17), 5475.

Chirinos, R., Rogez, H., Campos, D., Pedreschi, R., & Larondelle, Y. (2007). Optimization of extraction conditions of antioxidant phenolic compounds from mashua (Tropaeolum tuberosum Ruíz & Pavón) tubers. *Separation and Purification Technology*, *55*(2), 217-225. doi:https://doi.org/10.1016/j.seppur.2006.12.005

D'Auria, F. D., Tecca, M., Strippoli, V., Salvatore, G., Battinelli, L., & Mazzanti, G. (2005). Antifungal activity of Lavandula angustifolia essential oil against Candida albicans yeast and mycelial form. *Medical Mycology*, *43*(5), 391-396. doi:10.1080/13693780400004810

Da, X., Nishiyama, Y., Tie, D., Hein, K. Z., Yamamoto, O., & Morita, E. (2019). Antifungal activity and mechanism of action of Ou-gon (Scutellaria root extract) components against pathogenic fungi. *Scientific Reports*, *9*(1), 1683. doi:10.1038/s41598-019-38916-w

Deepthi, B. V., Poornachandra Rao, K., Chennapa, G., Naik, M. K., Chandrashekara, K. T., & Sreenivasa, M. Y. (2016). Antifungal Attributes of Lactobacillus plantarum MYS6 against Fumonisin Producing Fusarium proliferatum Associated with Poultry Feeds. *PLoS One*, *11*(6), e0155122. doi:10.1371/journal.pone.0155122

Dickson, D., Liu, G., Li, C.-Z., Tachiev, G., & Cai, Y. (2012). Dispersion and Stability of Bare Hematite Nanoparticles: Effect of Dispersion Tools, Nanoparticle Concentration, Humic Acid and Ionic Strength. *The Science of the Total Environment*. doi:10.1016/j.scitotenv.2012.01.012

Dowd, F. J. (2014). Candida Albicans Infections☆. In *Reference Module in Biomedical Sciences*: Elsevier.

Fatmawati, S., Yuliana, Y., Purnomo, A. S., & Bakar, M. F. A. (2020). Chemical Constituents, Usage and Pharmacological Activity of Cassia Alata. *Heliyon*. doi:10.1016/j.heliyon.2020.e04396

Felice, M. R., Giuffrè, L., El Aamri, L., Hafidi, M., Criseo, G., Romeo, O., & Scordino, F. (2019). Looking for New Antifungal Drugs from Flavonoids: Impact of the Genetic Diversity of Candida albicans on the in-vitro Response. *Current Medicinal Chemistry*, *26*(27), 5108-5123.

Francis, M. (2021). Antifungal Effects Against Phaeoisariopsis Personata Under Greenhouse Conditions and Phytochemical Analysis of Jatropha Curcas Leaf Extracts. *International Journal of Agriculture and Biology*. doi:10.17957/ijab/15.1829

Gadre, A., Enbiale, W., Andersen, L. K., & Coates, S. J. (2022). The effects of climate change on fungal diseases with cutaneous manifestations: A report from the International Society of Dermatology Climate Change Committee. *The Journal of Climate Change and Health, 6*, 100156. doi:https://doi.org/10.1016/j.joclim.2022.100156

Gao, S., Lewis, G. D., Ashokkumar, M., & Hemar, Y. (2014). Inactivation of microorganisms by low-frequency high-power ultrasound: 1. Effect of growth phase and capsule properties of the bacteria. *Ultrasonics Sonochemistry, 21*(1), 446-453.

Garber, G. (2001). An overview of fungal infections. *Drugs, 61 Suppl 1*, 1-12. doi:10.2165/00003495-200161001-00001

Gilman, E., & Watson, D. (1993). Cassia Alata Candlebrush. *Southern Group of State Foresters*, 1-3.

Gong, K., Pan, Y., Rather, L. J., Wang, W., Zhou, Q., & Zhang, T. (2019). Natural Colorant Extraction From <i>Cinnamomum Camphora</I> Tree Leaves of Different Maturities and Its Ultrasonic-assisted Extraction Process. *Coloration Technology*. doi:10.1111/cote.12406

Han, H., Wang, S., Rakita, M., Wang, Y., Han, Q., & Xu, Q. (2018). Effect of Ultrasound-Assisted Extraction of Phenolic Compounds on the Characteristics of Walnut Shells. *Food and Nutrition Sciences*. doi:10.4236/fns.2018.98076

Hashem, A. H., Shehabeldine, A. M., Abdelaziz, A. M., Amin, B. H., & Sharaf, M. (2022). Antifungal Activity of Endophytic Aspergillus Terreus Extract Against Some Fungi Causing Mucormycosis: Ultrastructural Study. *Applied Biochemistry and Biotechnology*. doi:10.1007/s12010-022-03876-x

Hazni, H., Ahmad, N., Hitotsuyanagi, Y., Takeya, K., & Choo, C. Y. (2008). Phytochemical constituents from Cassia alata with inhibition against methicillin-resistant Staphylococcus aureus (MRSA). *Planta Med, 74*(15), 1802-1805. doi:10.1055/s-0028-1088340

Hussein, A. O., Mohammad, G., Hadi, M., & Hameed, I. (2016). Phytochemical screening of methanolic dried galls extract of Quercus infectoria using gas chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared (FT-IR). *8*, 49-59. doi:10.5897/JPP2015.0368

Ingle, K. P., Deshmukh, A. G., Padole, D. A., Dudhare, M. S., Moharil, M. P., & Khelurkar, V. C. (2017). Phytochemicals: Extraction methods, identification and detection of bioactive compounds from plant extracts. *Journal of Pharmacognosy and Phytochemistry, 6*(1), 32-36.

jeevan kumar, P., Garlapati, V. K., Dash, A., Scholz, P., & Banerjee, R. (2017). Sustainable Green Solvents and Techniques for Lipid Extraction from Microalgae: A Review. *Algal Research, 21*, 138-147. doi:10.1016/j.algal.2016.11.014

Kadhim, M. J., Mohammed, G. J., & Hameed, I. H. (2016). In Vitro Antibacterial, Antifungal and Phytochemical Analysis of Methanolic Extract of Fruit Cassia Fistula. *Oriental Journal of Chemistry*. doi:10.13005/ojc/320307

Kooti, W., & Daraei, N. (2017). A Review of the Antioxidant Activity of Celery (Apium graveolens L). *J Evid Based Complementary Altern Med, 22*(4), 1029-1034. doi:10.1177/2156587217717415

Kumar, A., Shukla, R., Singh, P., Prasad, C. S., & Dubey, N. K. (2008). Assessment of Thymus vulgaris L. essential oil as a safe botanical preservative against post harvest fungal infestation of food commodities. *Innovative food science & emerging technologies, 9*(4), 575-580.

Kumar, K., Srivastav, S., & Sharanagat, V. S. (2021). Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. *Ultrasonics Sonochemistry, 70*, 105325.

Kumar, S., & Pandey, A. K. (2013). Chemistry and Biological Activities of Flavonoids: An Overview. *The Scientific World Journal, 2013*, 162750. doi:10.1155/2013/162750

Lee, J. A., & Chee, H. Y. (2010). In Vitro Antifungal Activity of Equol against Candida albicans. *Mycobiology, 38*(4), 328-330. doi:10.4489/MYCO.2010.38.4.328

Majekodunmi, S. O. (2015). Review of extraction of medicinal plants for pharmaceutical research. *Merit Res J Med, 3*, 521-527.

Manousi, N., Sarakatsianos, I., & Samanidou, V. (2019). 10 - Extraction Techniques of Phenolic Compounds and Other Bioactive Compounds From Medicinal and Aromatic Plants. In A. M. Grumezescu & A. M. Holban (Eds.), *Engineering Tools in the Beverage Industry* (pp. 283-314): Woodhead Publishing.

McGinnis, M. R., & Tyring, S. K. (1996). Introduction to Mycology. In S. Baron (Ed.), *Medical Microbiology*. Galveston (TX): University of Texas Medical Branch at Galveston

Mijatovic, S., Antić-Stanković, J., Čalovski, I. Č., Dubljanin, E., Pljevljakušić, D., Bigović, D., & Džamić, A. (2022). Antifungal Activity of Lavandula Angustifolia Essential Oil Against Candida Albicans: Time-Kill Study on Pediatric Sputum Isolates. *Molecules*. doi:10.3390/molecules27196300

Mohamad, O. A. A., Li, L., Ma, J., Hatab, S., Xu, L., Guo, J.-W., . . . Li, W. (2018). Evaluation of the Antimicrobial Activity of Endophytic Bacterial Populations From Chinese Traditional Medicinal Plant Licorice and Characterization of the Bioactive Secondary Metabolites Produced by Bacillus Atrophaeus Against Verticillium Dahliae. *Frontiers in Microbiology*. doi:10.3389/fmicb.2018.00924

Nagi, R. (2015). *Role of Medicinal Herbs in Management of Oral Diseases – A Review*.

Nandika, D., Karlinasari, L., Arinana, A., Batubara, I., Sitanggang, P. S., Santoso, D., . . . Hertanto, D. M. (2021). Chemical Components of Fungus Comb From Indo-Malayan Termite Macrotermes Gilvus Hagen Mound and Its Bioactivity Against Wood-Staining Fungi. *Forests*. doi:10.3390/f12111591

Nascentes, N., Korn, M., S, S., & Arruda, M. (2001). Use of Ultrasonic Baths for Analytical Applications: A New Approach for Optimisation Conditions. *Journal of the Brazilian Chemical Society, 12*. doi:10.1590/S0103-50532001000100008

Oladeji, O. S., Adelowo, F. E., Oluyori, A. P., & Bankole, D. T. (2020). Ethnobotanical Description and Biological Activities of Senna alata. *Evid Based Complement Alternat Med, 2020*, 2580259. doi:10.1155/2020/2580259

Oluwadun, A. (2015). *GC-MS Analysis and Antifungal Activity of Senna alata Linn*.

Oteiza, P., Fraga, C. G., Mills, D., & Taft, D. (2018). Flavonoids and the gastrointestinal tract: Local and systemic effects. *Molecular aspects of medicine, 61*, 41-49.

Padalia, H., & Ch, S. (2015). Antimicrobial Efficacy of Different Solvent Extracts of Tagetes Erecta L. Flower, Alone and in Combination With Antibiotics. *Applied Microbiology Open Access*. doi:10.4172/2471-9315.1000106

Pandey, A., & Tripathi, S. (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry, 2*(5), 115-119.

Parthasarathy, A., Jayalakshmi, M., N, P., & Varadharaj, V. (2018). Identification of bioactive components in enhalus acoroides seagrass extract by gas chromatography–mass spectrometry. *Asian Journal of Pharmaceutical and Clinical Research, 11*, 313. doi:10.22159/ajpcr.2018.v11i10.25577

Passoni, V., Scarica, C., Levi, M., Turri, S., & Griffini, G. (2016). Fractionation of Industrial Softwood Kraft Lignin: Solvent Selection as a Tool for Tailored Material Properties. *Acs Sustainable Chemistry & Engineering*. doi:10.1021/acssuschemeng.5b01722

Prajakta, B. M., Suvarna, P., Raghvendra, S. P., & Alok, R. (2019). Potential Biocontrol and Superlative Plant Growth Promoting Activity of Indigenous Bacillus Mojavensis PB-35(R11) of Soybean (Glycine Max) Rhizosphere. *Sn Applied Sciences*. doi:10.1007/s42452-019-1149-1

Rangel-Sánchez, G., Castro-Mercado, E., & García-Pineda, E. (2014). Avocado Roots Treated With Salicylic Acid Produce Phenol-2,4-Bis (1,1-Dimethylethyl), a Compound With Antifungal Activity. *Journal of Plant Physiology*. doi:10.1016/j.jplph.2013.07.004

Ren, J., Wang, J., Karthikeyan, S., Liu, H., & Cai, J. (2019). Natural anti-phytopathogenic fungi compound phenol, 2, 4-bis (1, 1-dimethylethyl) from Pseudomonas fluorescens TL-1. *Indian Journal of Biochemistry and Biophysics, 56*, 162-168.

Rodino, S., & Butu, M. (2019). 3 - Herbal Extracts—New Trends in Functional and Medicinal Beverages. In A. M. Grumezescu & A. M. Holban (Eds.), *Functional and Medicinal Beverages* (pp. 73-108): Academic Press.

Seleem, D., Pardi, V., & Murata, R. M. (2017). Review of flavonoids: A diverse group of natural compounds with anti-Candida albicans activity in vitro. *Archives of oral biology, 76*, 76-83.

Senthilkumar, G., Madhanraj, P., & Panneerselvam, A. (2011). Studies on the compounds and its antifungal potentiality of fungi isolated from paddy field soils of Jenbagapuram Village, Thanjavur District, and South India. *Asian J Pharm Res, 1*, 19-21.

Sher, A. (2009). Antimicrobial activity of natural products from medicinal plants.

Shiddamallayya, N., Yasmeen, A., & Gopakumar, K. (2010). Medico-botanical survey of Kumar parvatha Kukke subramanya, Mangalore, Karnataka.

Stanisic, M. S., Ignjatović, L. M., Andjelkovic, I., Stevic, C. M., Tasic, M. A., & Savic-Bisercic, M. (2012). The Ultrasound Assisted Extraction of Matrix Elements and Heavy Metal Fractions Associated With Fe, Al and Mn Oxyhydroxides From Soil. *Journal of the Serbian Chemical Society*. doi:10.2298/jsc110929209s

Sujatha, J., & Asokan, S. (2017). Studies on the Antioxidant Activity of Ethanol Extract of Cassis Alata Using FT-IR, HPLC and GC-MS Analysis. doi:10.22192/ijarbs.2017.04.12.012

Sukor, N., Jusoh, R., Rahim, S. A., & Kamarudin, N. (2018). Ultrasound assisted methods for enhanced extraction of phenolic acids from Quercus Infectoria galls. *Materials Today: Proceedings, 5*(10, Part 2), 21990-21999. doi:https://doi.org/10.1016/j.matpr.2018.07.060

Sule, W., Okonko, I., Joseph, T., Ojezele, M., Nwanze, J., Alli, J., & Adewale, O. (2010). In vitro antifungal activity of Senna alata Linn. crude leaf extract. *Research journal of biological sciences, 5*(3), 275-284.

Toh, S. C., Lihan, S., Bunya, S. R., & Leong, S. S. (2023). In vitro antimicrobial efficacy of Cassia alata (Linn.) leaves, stem, and root extracts against cellulitis causative agent

Staphylococcus aureus. *BMC Complement Med Ther, 23*(1), 85. doi:10.1186/s12906-023-03914-z

Verma, A., Joshi, S., & Singh, D. (2013). Imidazole: Having Versatile Biological Activities. *Journal of Chemistry, 2013*, 329412. doi:10.1155/2013/329412

Verma, S., Bhardwaj, A., Vij, M., Bajpai, P., Goutam, N., & Kumar, L. (2014). Oleic acid vesicles: a new approach for topical delivery of antifungal agent. *Artificial Cells, Nanomedicine, and Biotechnology, 42*(2), 95-101. doi:10.3109/21691401.2013.794351

Yeong, Y. L., Pang, S. F., Chong, S. Y., & Gimbun, J. (2018). Comparison of Microwave and Ultrasonic Assisted Extraction of Kaempferol From Cassia Alata. *International Journal of Engineering & Technology*. doi:10.14419/ijet.v7i3.13.16330

Yon, J.-A.-L., Lee, S.-K., Keng, J.-W., Chow, S.-C., Liew, K.-B., Teo, S.-S., . . . Chew, Y.-L. (2023). Cassia alata (Linnaeus) Roxburgh for Skin: Natural Remedies for Atopic Dermatitis in Asia and Their Pharmacological Activities. *Cosmetics, 10*(1), 5. Retrieved from https://www.mdpi.com/2079-9284/10/1/5

Zubairi, T. D. S., Sarmidi, M., & Aziz, R. (2014). The Effects of Raw Material Particles Size, Types of Solvents and Solvent-to-Solid Ratio on the Yield of Rotenone Extracted from Derris elliptica Roots. *Sains Malaysiana, 43*, 707-713.

APPENDIX A

The process of sample preparation

Collected the leaves

Dry it under the sun

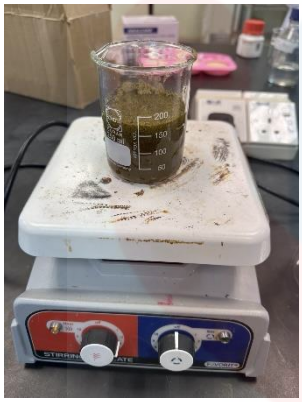
After drying for 4 to 5 days

Blend until get the powdery form

Sieving using steel sieve 0.5mm

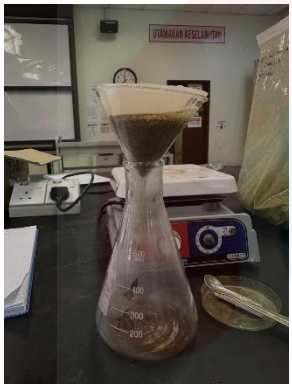
APPENDIX B

The extraction process



Decoction method

Bath ultrasonication



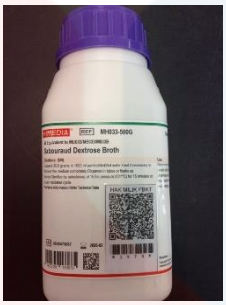
Filter each of the extracts

Rotavapor each of the extracts

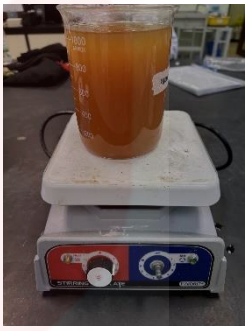
Dried it under the fume hood for a few days until it became crude extracts

APPENDIX C

The preparation of antifungal activity



Sabouraud Dextrose Agar and Sabouraud Dextrose Broth



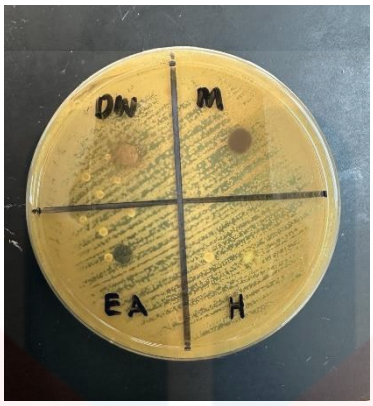
Mix and heat it

Autoclave

Sabouraud dextrose agar & broth

Culture the *C.albicans* on the SDA

Inoculation of *C.albicans*



Disk diffusion method

APPENDIX D

FTIR and GCMS equipment

 FT-IR machine	 GC-MS machine