MICROWAVE-ASSISTED EXTRACTION (MAE) OF PHENOLIC COMPOUNDS FROM LEAVES OF SENNA ALATA (*GELENGGANG*)

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J20A0411

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

FACULTY OF BIOENGINEERING AND TECHNOLOGY
UMK

2023

DECLARATION

I declare that this thesis entitled "**Microwave-Assisted Extraction (MAE) of Phenolic Compounds from leaves *Senna alata (gelenggang)***" is the results of my own research except as cited in the references.

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ACKNOWLEDGEMENT

At the very beginning I would like to express my deepest gratitude to almighty Allah for giving me the strength and the composure to complete this thesis within the scheduled time. I also would like to express my deepest sense of gratitude toward my supervisor, Ts. Dr. Mardawani Binti Mohamad for all the valuable advice, guidance, suggestions and support throughout my final year project as well as thesis writing. Her efforts greatly influenced and motivated me to complete this project work as soon as possible.

Next, I would like to express my infinite gratitude to the laboratory assistants of Universiti Malaysia Kelantan for their advice, offering me infinite help especially in the preparation of materials, and good cooperation throughout my experimental work. All the sacrifices of their time are greatly appreciated. My thanks are also addressed to all my friends who helped me throughout my studies. Each of their views is very helpful for me to improve in continuing this thesis project.

Last but not least, my deepest gratitude to my beloved father Mr. Nordin Bin Ismail, my beloved mother Mrs.Wan Rohana Binti Wan Jaffar and to my dearest siblings for their prayer and encouragement. Special thanks also to all my lovely friends for their support and help especially at times when things were going tough. Also do not forget to those who indirectly contributed in this research, your kindness means a lot for me. Thank you very much.

AINA NADIRAH BINTI NORDIN, 2023.

Pengekstrakan Berbantukan Gelombang Mikro (MAE) bagi Sebatian Fenolik daripada daun *Senna alata (*gelenggang*)*

ABSTRAK

Senna alata atau lebih dikenali sebagai pokok gelenggang adalah ubat-ubatan yang telah digunakan dalam perubatan sejak berkurun lamanya di seluruh dunia. Dalam dunia perubatan tradisional Melayu, daun gelenggang lebih dikenali sebagai alternatif untuk merawat penyakit kulit seperti kurap. Daun gelenggang ubat kurap ini juga banyak digunakan untuk penyakit kulit dan masalah yang lain. Komponen aktif yang diekstraksi dari *Senna alata* bermanfaat untuk mengobati berbagai jenis penyakit seperti kurap, gigitan serangga, julap, tekanan darah tinggi dan juga masalah kecacingan serta berbagai jenis penyakit kulit melalui pengantian bahan kimia tidak aman yang digunakan dalam aplikasi kosmetik dan medis. Tujuan penelitian ini adalah (1) untuk menentukan parameter proses pengekstrakan yang optimal (kuasa gelombang mikro) untuk pengekstrakan sebatian fenolik dari *Senna alata* (L.) (2) untuk menyiasat keberadaan sebatian fenolik dari *Senna alata* (L.) dengan menggunakan uji fitokimia; (3) untuk mencirikan ekstrak menggunakan saringan fitokimia, spektrofotometer Ultraviolet-visible (UV-Vis), dan high-performance liquid chromatography (HPLC). Daun tumbuhan Senna alata diekstrak dengan menggunakan kaedah pengekstrakan berbantukan gelombang mikro (MAE) dengan menggunakan kuasa gelombang mikro yang berbeza. Ekstrak digunakan untuk menguji kehadiran komponen fenolik. Kehadiran komponen fenolik telah ditentukan menggunakan saringan fitokimia. Jumlah kandungan fenolik ditentukan dengan menggunakan spektrofotometer UV-Vis. Jumlah kandungan fenolik ekstrak ditentukan dengan menggunakan asid tanik sebagai standard. Bilangan puncak penyerapan yang diperhatikan dalam ekstrak menggunakan pengekstrakan MAE dengan kuasa 250W (watt) gelombang mikro adalah lebih tinggi berbanding menggunakan 90W dan 180W. Jumlah kandungan fenolik terdapat dalam daun Senna alata 250W kuasa gelombang mikro pada 30 minit berbanding pengekstrakan kuasa gelombang micro yang lain dengan nilai 5.6581 mg/mL. Ekstrak daun *Senna alata* menunjukkan kepekatan kandungan fenolik yang tinggi dan berkemungkinan mempunyai aktiviti antioksidan yang lebih tinggi. Oleh itu, ia boleh mengeksploitasi kepada bidang penyelidikan baharu dalam aplikasi perubatan dan kosmetik pada masa hadapan.

Kata kunci: *Senna alata*, gelenggang, sebatian fenolik, pengekstrakan berbantukan gelombang mikro, komponen aktif

Microwave-Assisted Extraction (MAE) of Phenolic Compounds from leaves *Senna alata* (gelenggang)

ABSTRACT

Senna alata or better known as gelenggang is a medicine that has been used in medicine for centuries all over the world. In the world of traditional Malay medicine, gelenggang leaves are better known as an alternative to treat skin diseases such as ringworm. This ringworm medicinal leaf is also widely used for skin diseases and other problems. Active components extracted from Senna alata are useful for treating various types of diseases such as ringworm, insect bites, laxatives, high blood pressure and also helminth problems as well as various types of skin diseases through the replacement of unsafe chemicals used in cosmetic and medical applications. The purpose of this research is (1) to determine the optimal extraction process parameters (microwave power) for the extraction of phenolic compounds from *Senna alata* (L.) (2) to investigate the presence of phenolic compounds from *Senna alata* (L.) by using phytochemical tests; (3) to characterize the extract using phytochemical screening, Ultraviolet-visible (UV-Vis) spectrophotometer, and high-performance liquid chromatography (HPLC). The leaves of the Senna alata plant were extracted using microwave-assisted extraction (MAE) method with different microwave powers. Extracts were used to test for the presence of phenolic components. The presence of phenolic components was determined using phytochemical screening. Total phenolic content was determined using a UV-Vis spectrophotometer. The total phenolic content of the extract was determined using tannic acid as a standard. The number of absorption peaks observed in the extract using MAE extraction with 250W (watts) of microwave power was higher than using 90W and 180W. The total phenolic content found to be the optional concentration in the leaves of *Senna alata* 250W microwave power at 30 minutes compared to other microwave power extraction with a value of 5.6581 mg/mL. *Senna alata* leaf extract shows a high concentration of phenolic content and is likely to have higher antioxidant activity. Therefore, it can exploit to new research areas in medical and cosmetic applications in the future.

Keywords: *Senna alata*, gelenggang, phenolic compounds, microwave-assisted extraction, active components

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AQs	Anthraquinones
BRs	brassinosteroids
DMAPP	Dimethylallyl diphosphate
DPPH	diphenylpicrylhydrazyl
EE	Enzymatic Extraction
IPP	Isopentenyl diphosphate
MIC	minimum inhibitory concentration
MAE	Microwave-Assisted Extraction
PDA	photodiode array
S. alata	Senna alata
TCM	Traditional Chinese Medicine Herbs
UAEE	Ultrasonic-Assisted Enzymatic Extraction
UV	Ultraviolet
UV-Vis	Ultraviolet-visible
ZOI	Zone of Inhibition

LIST OF SYMBOLS

^{O}C	Celcius
cm	centimetre
g	gram
m	meters
mg	Milligram
ml	Mililiter
µg	microgram
µL	microliters
v/v	volume per volume
W	Watt
w/w	weight by weight
%	Percent

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Herbalism is the practice of traditional medicine or folk medicine based on the use of plants through the characteristics of plant extracts. Herbalism is also known as botanical medicine, herbal medicine, herbology, and phytotherapy. *Senna alata (L.)* is a type of flowering tree from the genus Senna of the Caesalpinioideae branch of the Leguminosae family. This plant is a tropical perennial herb and is native to Southeast Asia, Fiji, Northern Australia, Africa (including Nigeria) and Latin America. This plant is also a tropical and sub-tropical plant. This tree grows wild in the area of bushes and small bushes on the side of the road with a tree height between 1.0 meters to 5.0 meters high. It is also known as cassia, wild senna, candlestick senna, andadasi and palochina. The Indonesian community refers to this flower as Ketepeng Cina, and the Malaysian community refers to it as Gelenggang (Fatmawati, Purnomo, & Bakar, 2020). Studies had been proving that this plant extracts had contains phenolic compounds, alkaloids, anthraquinones, tannins, steroids, flavonoids etc (Adeyi et al., 2022).

Senna alata (L.) species are traditionally used for their medicinal properties in the treatment of enteric fever, diabetes, plasmodium falciparum, asthma, tinea infection, scabies, freckles, herpes, and atopic dermatitis. *Senna alata (L.)* has been utilised by Nigerian herbalists to treat parasite skin conditions and infectious disorders including dermatophytosis (Ugbogu et

al., 2016) and aqueous leaf extract of *Senna alata (L.)* has been used mainly for the treatment of dysentery, wounds, indigestion, respiratory tract infections, stress, odontalgia, scorch, (Alalor et al., 2012) typhoid fever, diabetes, malaria, asthma, hemorrhoids, scabies, pain head and paralysis. Extracts and isolated compounds show significant pharmacological activity. The display of anticonvulsant, antilipogenic, hepatoprotective, antioxidant, antibacterial, anticancer, antidiabetic, antifungal, antimalarial, antihyperlipidemic, dermatophytic, anthelmintic and antiviral activities may be due to the arrangement of secondary metabolites such as flavonoids, phenolic compounds, terpenes, saponins, phenolics, alkaloids cannabinoids, alkaloids (Adelowo & Oladeji, 2017) caryophyllene, germacrene D, reducing sugars, pyrazol-5-ol, isoquinoline, DL-limonene, α-selinene, cinnamic acid, quaalude, quinone, β-caryophyllene, steroids and volatile oils found in plant parts which is different.

Figure 1.1 Flower of *Senna alata*

Figure 1.2 leaves of *Senna alata*

(Source: Manager, n.d.)

Anthraquinones, phytosterols, tannins, alkaloids, flavonoids, terpenes, phenolic compounds, and essential oils are among the bioactive substances that can be extracted. Apart from the leaves and flowers shown in figures 1 and 2, the seeds, fruits, bark, and roots of the wax bush of *Senna alata (L.)* also have bioactive substances according to previous research by Oladeji (O. Oladeji et al., 2016). Anthocyanins were the most major chemical component in this investigation that required extraction. Anthocyanins are a kind of phenolic compound pigment. Phenolic or phenolcarboxylic acids, a kind of phytochemical known as a polyphenol, are one of the most common forms of phenolic compounds found in plants. They may be found in a variety of plant-based foods, with the highest amounts found in seeds, vegetable skins, fruit, and leafy greens. Plant phenolics, in addition to regulating plant colour, frequently have a role in protection against UV radiation or antagonism from pathogens, parasites, and predators. Since they are present in every plant organ, they constitute a crucial component of the human diet.

S. alata was extracted using a chemical approach in earlier investigations (solvent-based extraction method). Soxlet extraction, solvent extraction, and steam distillation are a few

examples of chemical extraction techniques. Traditional techniques for biologically active substances included solvent-based extraction because it was simple to carry out. However, solvent extraction has two drawbacks: first, the solvent will dissolve undesirable pyrolysis by products, maxrix compenents, and other compound; some of them may affect the analysis that follows (La Nasa, Biale, Mattonai, & Modugno, 2021); and second, some of the volatile components in the accelerant residue may also evaporate as a result of the solvent's evaporation. In addition, the use of solvents in conventional procedures for extraction might result in harmful waste and environmental contamination. The waste that produced must undergoes treatment process to reduce the pollution to the environment.

Green extraction is based on analytical techniques that consume less energy, permit the use of various solvents and sustainable natural resources, and provide an extract or product that is superior and safe. In order to tackle the problems of the twenty-first century, green extraction of natural goods may be a novel idea. It would safeguard the environment and customers while also fostering competition among companies to develop more inventive, economical, and ecological ways. According to the green extraction method, a "green extract" is an extract that was obtained with the least amount of environmental impact possible (for example, by using less energy and solvents) and whose eventual recycling was anticipated (Milescu et al., 2020). (coproducts, biodegradability, etc.) Example of green extraction methods were ultrasonic-assisted enzymatic exraction (UAEE), microwave-assisted extraction (MAE), and enzymatic extraction (EE)

Based on this research, MAE was used for the extraction of leaves out of *Senna alata (L.)*. MAE is one of the extraction machines that can extract the fastest samples. By performing the extraction in a sealed container at high temperature, the mass transfer of the target chemical

from the sample matrix can be accelerated. The extraction process normally takes 15 to 30 minutes and requires a minimal amount of solvent (10 to 30 ml). This amount is approximately ten times less than what is required by typical extraction procedures. Furthermore, because multiple samples can be extracted at the same time, sample output is increased. Analyte recovery and reproducibility are frequently enhanced in comparison to older techniques, as demonstrated in a variety of applications. This review provides a brief theoretical foundation of microwave heating as well as the fundamental concepts of extracting microwave energy. It also attempts to synthesise the findings of all prior studies on MAE. Variables such as matrix parameters (including water content), solvent volume, temperature, time, and solvent choice are investigated.

In this study, it will focus on the importance of extracting bioactive compounds that need to be investigated from this plant. Bioactive compounds and extracts from S.alata should be examined and found to have medicinal value for any disease. The MAE method was used in this study to extract and analyze active components from S.alata leaves. The extraction of total bioactive compounds using different microwave power such as 90watt (W), 180W and 250W will be analyzed by phytochemical analysis followed by Ultraviolet-visible (UV-Vis) followed by High Performance Liquid Chromatography (HPLC) to identify the phytoconstituents found in ethanol extract of Senna plant and determine the concentration of certain compounds in the extract solution.

1.2 Problem Statement

Nowday, demand for alternative treatments such as acupuncture, homeopathy, and herbal medicines are gaining attention among the community. According to the opinion of Monika et, al (2022) this situation occurs due to the negative effects of conventional drugs and the high cost of therapy. There are many types of products that can be produced from herbal plants such as herbal teas, energy drinks, as well as health supplements in markets such as pharmacies and shopping centers. However, there are some products that contain ingredients that are prohibited and dangerous for a person to consume. The problem is that products containing these harmful substances can cause after-effects and side effects such as skin inflammation and shortness of breath. Therefore, this study aims to extract the main active compounds of the *Senna alata* plant that are useful for solving health problems and can be used in the medical sector, that is, it can be used as a traditional and modern medicine in disease control, and can be used safely.

The concentration and purification of *Senna alata* (L.) has long been an important procedure. Steam distillation and solvent extraction have been the basis of conventional procedures up to this point. This solvent-based extraction has a problem, which is that the extraction result depends on time, temperature, dielectric properties of the sample mixture, and the type of solvent. Furthermore, the solvents used are potentially toxic during extraction and environmental pollutants. The weakness of this method has prompted researchers to find other alternative extraction methods such as the use of the MAE method. Nevertheless, the need for special equipment, low selectivity, and unavoidable reaction in high temperature are considered as problems in the use of MAE. A study of parameters such as microwave power was investigated to determine the optimal extraction conditions. MAE is expected to produce high anthocyanin content, short extraction time and high quality final product.

1.3 Research Objectives

The main aims for this study was to extract leaves from senna alata by using Microwave-assisted extraction (MAE) methods. To further illustrate and guide the study, the following objectives was set:

1. To determine the optimal extraction process parameters (microwave power) for the extraction of phenolic compounds from *Senna alata* (L.)
2. To investigate the presence of phenolic compounds from *Senna alata* (L.) by using phytochemical tests.
3. To characterize the extract using phytochemical screening, Ultraviolet-visible (UV-Vis) spectrophotometer, and high-performance liquid chromatography (HPLC).

1.4 Scope of Study

In the research of this study, the investigation of the types of compounds from Senna alata using microwave-assisted extraction is a modern and efficient technique used to extract various valuable compounds from various materials, especially from plant samples. It uses microwave irradiation to heat the solvent and sample, speeding up the extraction process and often increasing the yield and purity of the extracted compound. The technique used in this study is to extract active compounds in S.alata such as phenolic compounds.

The second objective in this study is to determine the optimal parameters of different microwave power in the phenolic extraction process. The microwave power that will be used in this study is 90W, 180W and 250W. The use of different power needs to be reviewed because it will affect the extraction efficiency such as process temperature and extraction time.

The final objective is to characterize the extract using phytochemical screening, Ultraviolet-visible (UV-Vis) spectrophotometer, and high-performance liquid chromatography (HPLC). For this objective, qualitative analysis was used in identifying the presence of detection of active compounds in Senna alata extract. Quantitative analysis to measure phenolics in S.alata extract using analytical techniques namely Ultraviolet-visible (UV-Vis) spectrophotometer, and high-performance liquid chromatography (HPLC).

1.5 Significances of study

The MAE technique is anticipated to demonstrate a potential approach to extract the phonetic component from S.alata (L.) in the study. Increased extraction yield, less solvent usage, shorter extraction times, and greater recovery are anticipated as outcomes. By heating the solvent containing the sample with microwave energy and partitioning the analyte from the sample matrix into the solvent, MAE is a traditional method for the extraction of active components from medicinal plants. It has a wide range of applications for quick extraction of analytes, including thermally unstable compounds, because to its capacity to heat sample solvent mixtures quickly. The solvent type, sample material, and extracted component, in particular its dielectric constant, are all aspects that affect how effective MAE is. Several MAE parameters, such as sample size, extraction temperature and duration, solvent polarity and volume, and microwave power, should be optimised when designing procedures for extracting medicines produced from plants.

The knowledge gained from this research study is expected to confirm a good extraction technique to extract phenolic compounds from Senna alata, as well as characterization and optimization in the extraction process for development and application to produce phenolic-rich products, thus providing medicinal materials that can be used directly, such as in cream form, in drinkable liquid or powder form such as herbal tea products.

CHAPTER 2

LITERATURE REVIEW

2.1 Senna alata species

The *S.alata (L.)*, sometimes known as the candle bush, belongs to the subfamily Caesalpinioideae and is a significant medicinal tree. It is also called as a candletree, ringworm tree, emperor candle plant, and candelabra bush. It was an unusual species of Senna that was occasionally placed in the Herpetica genus on its own. The shrub has 50–80 cm long leaves and is 3–4 m tall. The inflorescence resembles a candle-like golden object. Up to 25 cm long, the fruit is formed like a straight pod. Its seeds are dispersed by animals or by water. In the evening, the leaves fall.

Figure 2.1 Senna Species, Candle Bush, Candelabra Bush, Empress Candle Plant, Golden Candlestick (Senna alata) (by Kell Apr 16, 2019)

Senna alata (L.) is known by various local names according to different countries including "Ath thora" and "Eth thora" (Sri Lanka), candlestick, Carrion Crow Bush, Winged Senna, Empress Candle Plant, Dad mardan (India) and is also called ringworm bush. In Malaysia it also has a special name which is bush/candle tree. In the previous case, there is a traditional use of plants among the community to treat ringworm infection. The plant is native to Central and South America but it is now known to have been introduced to almost every continent except Europe. Many pharmacological effects (Hennebelle, Weniger, Joseph, Sahpaz, & Bailleul, 2009) and uses for antidiabetic treatment in Africa, the Caribbean (O. S. Oladeji, Adelowo, Oluyori, & Bankole, 2020), as well as in India (Laha, Lalhriatpuia, Ralte, & Lalremruata, 2016) have been reported.

Senna alata (L.), often known as ringworm bush, is a well-liked traditional remedy, particularly for skin conditions like ringworm and scabies. Research has a tendency to support the efficacy of this conventional therapy. Several derivatives of anthracene have been isolated from the leaves, such as rhein, isochrysophanol, chrysophanic acid, and aloe emodin, as well as the common steroid beta-sitosterol and the alkaloid tyramine (Agbagwa, 2014). Crude leaf extracts have demonstrated antimicrobial efficacy against a variety of microorganisms, including Dermatophilus congolensis, which is responsible for significant skin diseases in cattle, antifungal capabilities (against Pityriasis versicolor in humans), and even anticancer activity. The petals contain anthraquinones, glycosides, steroids, tannins and volatile oils. Petal extract has antibacterial activity against gram-positive bacteria but not against gram-negative bacteria. The plant is laxative, antibacterial, antitumor, anti-inflammatory, diuretic, analgesic, susceptible, weak antifungal, hypoglycemic, and antispasmodic. The leaves are laxative. They are used

internally to treat constipation and cleanse the blood. To cure irritation and high blood pressure, the leaves are either cooked with Tripogandra serrulata and Persea americana, or they are not.

The leaves are frequently used to treat skin conditions. They are an efficient therapy for skin blemishes, scabies, ringworm, and other fungal skin diseases and can be used as a tincture, poultice, powdered, then combined with oil as an ointment, or the sap can be smeared over the afflicted region (ALATA, 2018). The leaves may be picked at any time of the year, but the month or two before the plant flowers is thought to be when they are at their finest. The bark is used to cure eczema, scabies, diarrhea, worms, and parasitic skin conditions. Its root is diuretic. Tympanites, uterine issues, and the ejection of filaria worms are all conditions that are treated with an infusion. For the external treatment of skin fungus and sores, the root is used. The blooms have laxative and vermifuge properties. Spleen ailments are treated with an infusion. Grippe is treated using a decoction that contains Zingiber officinale, which is also used as an abortifacient. They are prepared with coconut milk for laxative purposes. Infusions made from the fruit, flowers, and leaves are used to alleviate digestive issues (ALATA, 2018). Both a laxative and an anthelmintic, the seed. As a treatment for intestinal worms, it is boiled. The leaf has modest antibacterial action in addition to containing the purgative anthraquinone. Chrysophanol, emodin, rhein, and aloe emodin are all present in the stem. Aloe emodin and rhein purgative anthracene derivatives are present in the leaf and fruit (Akbar & Akbar, 2020)

2.2 Pharmacological Activities

The pharmacological properties of medicinal plants of the Fabaceae family have been thoroughly and extensively studied. Various secondary metabolites produced by plants contribute to their medicinal properties. The ethnobiological assertion is confirmed by the therapeutic evaluation of *S. alata (L)*, which also shows its pharmacological properties. (Table 2.1). There are many articles that have been published related to the therapeutic activities of S. alata which are mainly related to antifungal, antioxidant, antibacterial, dermatophytic, and anthelmintic activities. Several studies have also reported its antimalarial activity.

Table 2.1 Pharmacological activities of *S. alata (L)*.

(O. S. Oladeji et al., 2020)

Parts used	Country	Ethnomedicinal use	Solvent used	Pharmacological activity	Model used	Phytochemicals
Leaves	Nigeria	Treatment of diarrhoe, upper respiratory tract infection and to hasten labour	Aqueous	Abortifacient	Pragnant rats	Saponins, flavoids, cardiac glycosides, dienolides, phenolics and alkaloids
Leaves	India	To manage diabetes	Ethanolic	Hepato-renal protective effects	Male albino wistar rats	
Leaves	India	Treatment of allergy/ asthma	Hydromethanolic	Antiallergic	Lipoxygenase (LOX) enzyme	Rhein and kaempferol
Leaves	Thailand	To manage diabetes and weight	Aqueous	Antilipogenic	High-fat diet-induced obese mice	
Leaves	India	Treatment of bacterial infections	Methanol	Antibacterial	Pathogenic bacterial strains	
Leaves	Cameroon	Treatment of gonorrhoea, gastrointestinal and skin diseases	Methanol	Antibacterial	Multidrug-resistant (MDR) Vibrio cholera and shigella flexneri	Kaempferol, luteolin and aloe-emodin

2.2.1 Antifungal Activities

Bioactive compounds that have been isolated from *S. alata (L)* show strong in vivo and in vitro antifungal properties. Antifungal activity of cannabinoid alkaloids (4-butylamine 10-methyl 6-hydroxy cannabinoid dronabinol), 11-dimethyl-3-methylidenedodeca-1,6,10-triene, octadecanoic acid methyl ester, cinnamic acid, 3,7-dimethylocta-1 ,6-diene, pyrazol-5-ol, flavonol and gallic acid, methaqualone, and isoquinoline have been explored (S. O. Oladeji, Adelowo, & Odelade, 2016).

Aspergillus and Candida species standard strains and clinical isolates were tested against volatile oils derived from *S. alata* flowers. The examined microorganisms' development was greatly slowed down by the oils (Essien et al., 2011). A. Niger, C. utilis, G. candidum, A. brevipes, and Penicillium species were all strongly inhibited by the methanolic extract, pure n-hexane, and ethanolic fractions *S. alata* of flowers, with a MIC ranging from 0.312 to 5 mg/ml. The purified fractions, as opposed to the methanolic extracts, showed more pronounced inhibitory activity at various doses. Additionally, the purified fractions greatly reduced mycelia development, with a 100% suppression of sporulation for 96 hours at 2 mg/ml as opposed to less sporulation after 48 hours for methanolic extracts.

Numerous in vitro and clinical investigations supported its efficacy, offering a claim based on fact that can help to reinforce and support the use of this plant for the treatment of skin fungal indications. However, further research on the extract's composition is still needed, particularly in situations where fungal strains are continually evolving genetically and phenotypically. In a few years, current formulas could no longer work. Additionally, the plant extract's cellular and molecular modes of action must be determined. Knowing the bioactive substances can help biotechnologists and plant breeders create plant varieties that can

manufacture more of these substances or can grow more of the plant parts taken for extraction, both of which can significantly help in resource conservation. Finally, the outcomes of investigations on the molecular mechanisms of action may be crucial in the development of customised drugs and treatments.

2.2.2 Antioxidant Activities

An essential factor in determining the therapeutic potential of medicinal plants is their antioxidant or scavenging activity. In the current study, the antioxidant screening test and DPPH (diphenylpicrylhydrazyl) radical scavenging technique were used to assess the antioxidant activity of a subset of TCM plants. Free radical scavenging activity has been measured using the stable synthetic free radical known as DPPH. Through the DPPH test, the scavenging abilities of *S. alata (L)* extracts in methanolic and n-hexane were investigated. When compared to the n-hexane extract, the methanolic extract demonstrated a substantial amount of scavenging action. The three primary bioactive components were isolated when the methanol extract was fractionated. (kaempferol, butylated hydroxytoluene, and emodin). In comparison to the hydroxytoluene and butylated emodin fractions, the kaempferol fraction exhibited much greater scavenging efficacy.

There are further reports of testing on yeast in addition to the leaf extract. The antioxidant activity of samples against physiologically important oxidants is found using a biological test based on yeast. To account for the many ways that various antioxidants operate, it is thought that the antioxidant activity of medicinal plants must be assessed using several approaches (at least two methods) (Dudonne, Vitrac, Coutiere, Woillez, & Mérillon, 2009). Therefore, in this work, a

number of assays were used to comprehensively examine the antioxidant and anti-inflammatory effects of selected TCM herbs.

2.2.3 Antibacterial Activities

The effectiveness of an antibacterial treatment on a surface or product can be quickly assessed using the Zone of Inhibition (ZOI) test. Zone of inhibition (ZOI) or minimum inhibitory concentration (MIC) methods are also used to evaluate antibacterial potential, especially in the context of medicinal plants. The effectiveness of an antibacterial treatment on a surface or product can be quickly assessed using the ZOI test. The procedure, formerly known as the Kirby-Bauer test, was developed from research on antibiotics in the pharmaceutical industry and subsequently expanded to be used to assess the antibacterial capacity of polymers and textiles.

A study of five plant species was conducted. Three strains of Gram negative bacteria (E. coli, S. typhi, and P. aeruginosa), as well as two strains of Gram positive bacteria, were employed to test their antibacterial activity using the disc diffusion technique. (B. cereus and S. aureus)(Mostafa et al., 2018). The results of the evaluation of antibacterial activity of plant extracts were recorded as in table 2.2 and illustrated in figure 2.2. The results of the evaluation of antibacterial activity of plant extracts were recorded as in table 2.2 and illustrated in figure 2.2. The findings show that all plant extracts have varying potential effectiveness in preventing the development of the germs that cause poisoning. In all three strains of Gram negative bacteria, P. granatum extract was the most effective in retarding the microbial growth of all pathogenic bacteria tested through a concentration of 10 mg/ml. Compared to C. cyminum extract only effective against *S. aureus*. Other plant extracts show frequent changes in antimicrobial activity.

The pathogenic strains *B. cereus, S. aureus, E. coli, and P. aeruginosa* were all inhibited by S. aromaticum, whereas *B. cereus, S. aureus, and P. aeruginosa* were all inhibited by Z. officinales and S. aureus and P. aeruginosa were all inhibited by T. vulgaris.

Table 2.2 Antimicrobial screening test of ethanolic plants extract (10 mg/ml) against some bacterial strains of food poisoning diseases.

(Mostafa et al., 2018)

Plant species	Inhibition zones (mm)				
	Gram (+ve) pathogenic bacteria		Gram (−ve) pathogenic bacteria		
	B. cereus	*S. aureus*	*E. coli*	*S. typhi*	*P. aeruginosa*
Cuminum cyminum	0.0 ± 0.0	9.5 ± 0.74	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Punica granatum	16.3 ± 0.57	18.5 ± 0.13	14.2 ± 0.61	9.7 ± 0.22	16.1 ± 0.46
Syzygium aromaticum	14.6 ± 0.37	15.8 ± 0.41	11.9 ± 0.34	0.0 ± 0.0	13.4 ± 0.11
Thymus vulgaris	0.0 ± 0.0	17.6 ± 0.31	0.0 ± 0.0	0.0 ± 0.0	14.7 ± 0.25
Zingiber officinales	8.3 ± 0.46	15.4 ± 0.23	0.0 ± 0.0	0.0 ± 0.0	11.2 ± 0.17
Gentamycin (5 µg)	16.8 ± 0.37	20.5 ± 0.24	15.6 ± 0.53	18.7 ± 0.61	13.1 ± 0.35

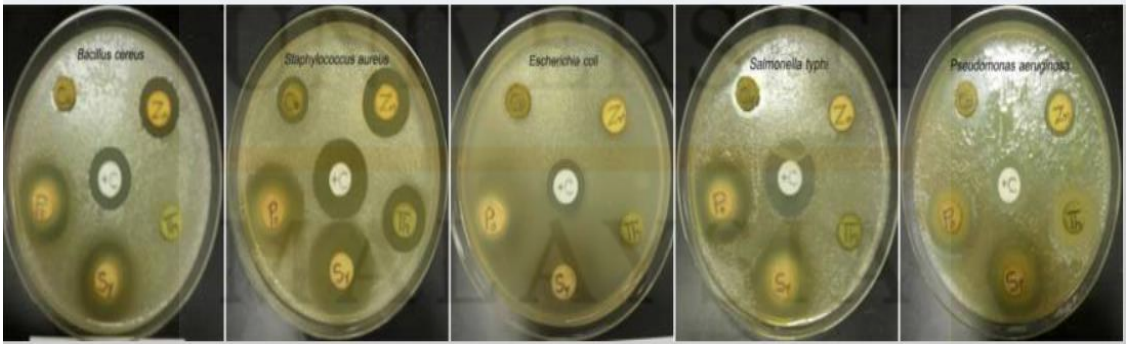


Figure 2.2 show of Growth inhibition in some food poisoning bacterial strains caused by plant extracts. Cu, Cumin; Po, pomegranate; Sy, Clove; Th, Thyme; Zn, Ginger and +C, positive control.

2.2.4 Dermatophytic Activities

Senna alata's bark, flowers, and leaves are now utilised as a remedy for eczema, typhoid, diabetes, malaria, asthma, tinea infection, and other illnesses in addition to skin infections. Nigeria, Malaysia, Australia, Thailand, tropical America, and many more locations across the world are home to this plant. Locally, it is used in Nigeria to cure a variety of ailments, such as ringworm and parasitic skin condition (Umoren, Amusa, & Oladetoun). S. alata's dermatophytic activity is linked to bioactive compounds such tannins, anthrones, phenols,anthranols, flavonoids, and anthracene derivatives (Borah, Baishya, Malick, & Nag, 2022). Strong antibacterial activities were shown in the decoction of the leaves against E. coli, S. aureus, P. aeruginosa, S. rnarcescens, P. cepacia, S. pyogenes, and K. pneurnoniae (Brown, 2011).

A novel cannabinoid alkaloid, 4-butylamine 10-methyl-6-hydroxy cannabinoid dronabinol, was discovered after chemical analysis of the bioactive components from the seeds of the ringworm plant, S. alata. A local antiseptic soap contains apigenin and the cannabinoid alkaloid (4-butylamine 10-methyl-6-hydroxy cannabinoid dronabinol) that was isolated from S. alata seeds. By applying crushed fresh leaves to an affected region, *Senna alata* has been shown in prior research to have great wound-healing qualities and to be helpful in the treatment of eruptive and pustular skin disorders (Felix-Cuencas et al., 2022). Leaves, stem bark, flower exudates, and ethanolic leaf extracts examined against clinical isolates showed strong inhibitory activity against the organism (Sule et al., 2010).

2.2.5 Anthelmintic Activity

Senna alata (L.) and blossoms have historically been used to treat skin conditions including ringworm and ringworm. However, a research revealed that senna alata's leaves and petals may also be utilised to treat stomach problems and intestinal worms. In contrast to the reference medicine piperazine citrate, T. angustifolia leaf extract not only produced paralysis in worms but also their death, especially at concentrations of 100 mg/ml and higher (Anbu, Murali, Sathiya, Saraswathy, & Azamthulla, 2015). The crude extract's phytochemical examination identified tannin as one of the chemical components.

2.2.6 Antimalarial Activities

Malaria is a disease caused by the world's most common protozoan parasite, plasmodium, which accounts for approximately 3 million cases and 1.5 to 2.7 million deaths each year. It is spread by female mosquitoes of the genus Anopheles (mosquitoes), especially Anopheles sundaicus in Asia and An. gambiae in Africa. Malaria is a global threat that contributes to serious health issues in humid and tropical regions (Organization, 2022). Malaria occurs in most parts of sub-Saharan Africa, Southeast and South Asia, Mexico, Haiti, Central and South America, Papua New Guinea and the Solomon Islands.

There are reports where a decoction of the root and alamanda root is used to treat malaria. By using the Desjardin microdilution assay, quinones extracted from S. alata considerably shown antiplasmodial efficacy in vitro against Plasmodium falciparum. In vitro antiplasmodial tests against P. falciparum were demonstrated by terpenes extracted from the leaves of *S. alata* in ethylene glycol water fractions. At doses below 1 g/ml, significant action was seen (Kayembe,

Taba, Ntumba, & Kazadi, 2012). Evaluation of an aqueous leaf extract enables the use of *S. alata* as an ethnomedical treatment for malaria and fever. The 3D7 strain of P. falciparum parasites in Wistar rats were considerably suppressed by the leaf extract (Vigbedor, Osafo-Acquah, Adu-Gyan, & Lotsi, 2015).

2.3 Bioactive Chemical Compounds of Senna alata

Senna alata is one of the traditional plant being studied due to their high content. Various bioactive chemical substances are present in it. According to figure 2.3, phenolic (kaempferol, aloeemodin, rhein, chrysaphanol, and glycosides), fatty acids (oleic, palmitic, and linoleic acids), steroids, anthraquinones (alatinone and alatonal) and terpenoids (campesterol, stigmasterol, and sitosterol) are some of the known chemical ingredients.

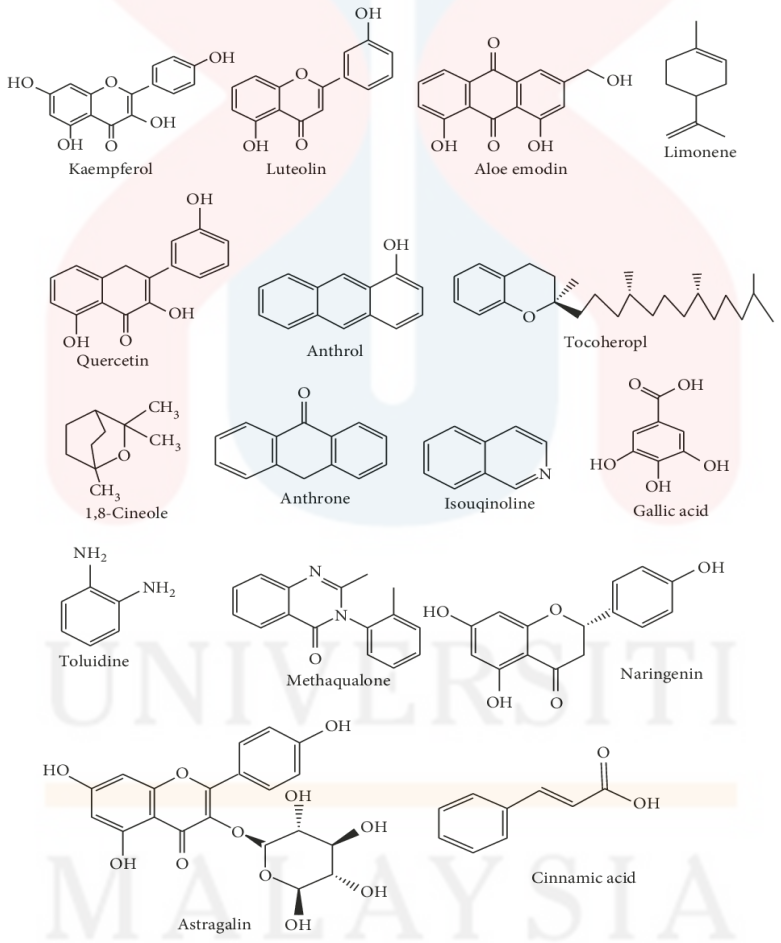


Figure 2.3 Bioactive compounds with therapeutic potencies in *S. alata.*

(O. S. Oladeji et al., 2020)

2.3.1 Phenolics compounds

Due to their organoleptic qualities, phenols, low molecular weight secondary metabolites found in the majority of terrestrial plants, are used in foods and drinks. Anthocyanins, a kind of polyphenol, give food its purple, black, or red hue (Nayak, Liu, & Tang, 2015). The majority of the antioxidant activity in plants or plant-based products is attributed to phenolic compounds, which make up the biggest category of phytochemicals (Aliyu et al., 2009). The antioxidant capabilities of phenolic compounds including flavonol, naringin, apigenin, myricetin, coumarin, and caffeic acid are well recognised and play a significant role in preventing oxidative damage to food, cells, and organs (Sun & Shahrajabian, 2023). Many medicinal plants contain phenols, active components with significant pharmacological actions that affect the function of different enzymes and cell receptors (Middleton, Kandaswami, & Theoharides, 2000).

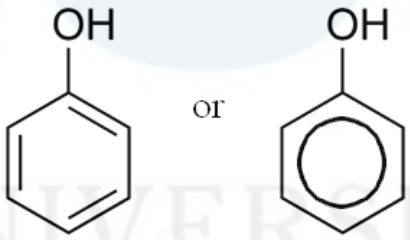


Figure 2.4 Basic structure of Phenol

(Minatel et al., 2017)

According to one study, *S. alata* exhibits antioxidant characteristics as shown by in vitro HPLC experiments, and two phenolic compounds found in Senna alata fractions may be responsible for the plant's antioxidant and other pharmacological effects. Because it has been

utilised in earlier studies to discover a number of bioactive chemicals in plants, the HPLC approach has also been reported to be effective for this kind of research. According Bursal et al. (Bursal, Köksal, Gülçin, Bilsel, & Gören, 2013), this approach is a useful tool for screening phenolic chemicals in plants. Hanachi (Hanachi & Golkho, 2009) also used HPLC experiments to investigate the composition and antioxidant activity of Berberis vulgaris. This technique was also used by Sun et al. (2009) to pinpoint the polyphenol C-glycosides in the Chinese/Tibetan herb Swertia franchetiana. According to the study's findings, naringin and a rise in antioxidant enzymes are two signs that the ethyl acetate fraction has antioxidant characteristics.

2.3.2 Anthraquinones

Some plants contain organic substances called anthraquinones. They are simple anthrones or bianthrones chemically speaking. Anthraquinone is employed in medicine, dyes, and pigments. Anthraquinones are a strong aromatic molecule that, in addition to being utilised commercially, has several medicinal advantages (Ikram et al., 2023). These advantages include reducing constipation, easing bowl movement, and inhibiting the growth of cancer by triggering apoptosis. Senna, buckthorn, yellow dock, and other plants naturally contain anthraquinones, but they may also be made chemically by processes including anthracene oxidation, naphthalene oxidation, and condensation of 1, 4-naphthoquinone with butadiene. However, because it might have some major adverse effects, consumption should be tightly controlled.

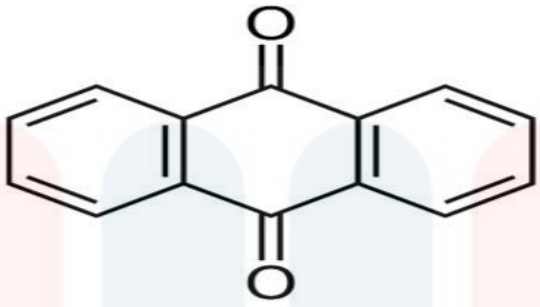


Figure 2.5 Chemical structure of Anthraquinone

(Khan, 2019)

In laxative preparations, Anthraquinones (AQs) are frequently found in Senna leaves, rhubarb root and pods, Cascara, Buckhorn, and Aloe. Physcion, chrysophanol, aloe-emodin, rhein, and sennosides include AQ laxatives. After oral intake, AQ will be converted into an active aglycone, thus affecting the damaged epithelial cells, as well as causing changes in absorption, secretion and intestinal movement directly and indirectly (Gordon, MacDonald, Parker, Akobeng, & Thomas, 2016). The mechanism has two different actions: a change in the motility of the large intestine that causes colonic transit to move faster and reduce fluid absorption, both better fluid absorption due to the influence on the secretory mechanism.

2.3.3 Fatty acids

There are various bioactive chemical compounds in the contents of this plant including fatty acids. Organic substances called fatty acids are important for human health and are involved in several physiological processes. Several fatty acids, including palmitic acid, stearic acid, oleic acid, and linoleic acid, are known to be present in senna alata leaves.

Saturated fatty acids such as palmitic acid are frequently found in plant and animal tissues. It is used as an emollient and thickener in the cosmetic industry and is known to have antibacterial and anti-inflammatory qualities. Another saturated fatty acid detectable in Senna alata leaves is stearic acid. It is frequently used as a stabilizer and emulsifier in the food and cosmetic sectors. In addition, it has been shown to have antibacterial and anti-inflammatory effects. Numerous health advantages of oleic acid, a monounsaturated fatty acid found in large quantities in olive oil, include lowering the risk of heart disease and enhancing insulin sensitivity. Linoleic acid, a necessary polyunsaturated fatty acid for human health, cannot be produced by the body. Vegetable oils frequently include this substance, which is well recognized for its anti-inflammatory and antioxidant qualities.

Senna alata leaves contain a variety of fatty acids that are used in many sectors and have significant health advantages. Some of the fatty acids included in Senna alata leaves are palmitic acid, stearic acid, oleic acid, and linoleic acid. These fatty acids are known to have antibacterial, anti-inflammatory, and antioxidant activities.

2.3.4 Steroids

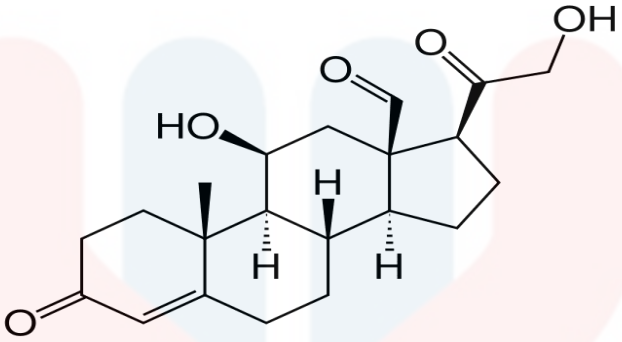


Figure 2.6 Steroid Molecular Structures

(Kolatorova, Vitku, Suchopar, Hill, & Parizek, 2022)

Half a century ago, steroids were only recently recognized as vertebrate hormones of high value and to get that recognition had to take a long and time-consuming (Schaller, 2003). It was discovered in the middle of the 1960s that steroids had hormonal effects on invertebrates as well, particularly on the moulting processes of insects and other arthropods (Higley et al., 1986). Over time, fungal steroid hormones were also discovered. All eukaryotic organisms include sterols, which are membrane constituents that control the fluidity and permeability of the phospholipid bilayer. Plant sterols and the steroid hormones known as brassinosteroids (BRs) have a variety of biological effects. They are crucial for a plant's ability to grow, reproduce, and react to a variety of biotic and abiotic challenges. Brassinosteroids are oxidized steroids that function as growth hormones and are precursors of some sterols seen in small amounts, such as campesterol in Arabidopsis thaliana. Through the study of a set of dwarf mutants, it has been shown how important brassinosteroids are, especially during growth and development, so that they can have an effect during the synthesis or perception of brassinosteroids.

In some recent articles the issue of gene characterization is given the main focus. The role of sterols in biological processes such as embryonic development, cell and plant growth, and fertility has recently come under renewed scrutiny thanks to the characterization of genes encoding sterol biosynthetic enzymes and the isolation of new plant lines affected in the expression of these genes, either by insertional mutagenesis or classical, overexpression or suppression.

2.3.1 Terpenoids

The family of natural organic compounds known as terpenoids is produced from the 5-carbon component of isoprene and its derivatives, such as terpenes and diterpenes. Terpenoids are also known as isoprenoids. Although terpenoids and "terpenes" are frequently used interchangeably, the latter have extra functional groups, typically including oxygen (Leigh, 2011). Although a small number of metabolites are derived from bacteria, some believe that terpenoid compounds are usually found in plant or fungal metabolites. In addition to having biological and pharmacological qualities that are advantageous to people, terpenoids are essential for plant existence. Dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP) can both be produced by two distinct routes in plants.

Plant terpenoids are often used in traditional herbal therapy and are valued for their aromatic properties. The aroma of eucalyptus, the flavor of cinnamon, cloves, and ginger, the yellow color of sunflowers, and the red color of tomatoes are all influenced by terpenoids (Merlet et al., 2014). Citral, menthol, camphor, salvinorin A from Salvia divinorum, ginkgolide

and bilobalide from Ginkgo biloba, and cannabinoids from cannabis are well-known examples of terpenoids. Carotenoids, a type of terpene, are a source of the provitamin beta carotene.

The terpenoid is divided into five, namely monoterpenes, penntpenes, triterpenes, sesquiterpenes, and seserpenes. It all depends on the carbon unit. The majority of its structural variants are biologically active and are used for the treatment of several diseases worldwide. Most of the terpenoids are used by some patients to treat cancer cells and to be used as anticancer drugs such as taxols and their derivatives (Sisodiya, 2013). Because of its exquisite perfume, many pleasant flavours and smells are derived from tastes. Artemisinin and other similar chemicals are used as antimalarial medications, as are its derivatives. Terpenoid, meantime, has a variety of uses in the production of food, drugs, cosmetics, hormones, vitamins, and other products.

2.4 Extraction Methods

The phenolic chemicals in senna alata leaves can be extracted in a variety of methods. There are two types of extraction techniques: conventional and unconventional extraction. Value-added products are created from plant materials using conventional extraction techniques such solvent extraction, steam distillation, and acid or alkali extraction. Microwave-assisted enzyme extraction (MAE) is the technique used for unconventional extraction. While non-traditional technologies are more ecologically friendly, conventional methods are cheaper and simpler to use. Contrarily, non-conventional extraction has substantially greater extraction costs than conventional extraction due to its significant drawbacks, such as low yield, by-product form, and limited stability (Yang, Jiang, Shi, Chen, & Ashraf, 2011).

2.4.1 Conventional Extraction

2.4.1.1 Standardization of ethanolic extracts of *S. alata* leaves

Solvent extraction is very convenient because the solvent provides a physical carrier to transfer molecules between different phases. From the study of the Pharmacognosy Department of Bangkok, Thailand (Gritsanapan & Mangmeesri, 2009), 10.0g of senna alata powder leaves were macerated with 80% ethanol (100 ml). Ethanol (80% v/v) was used as the extraction solvent for *S. alata* leaf samples because both anthraquinone aglycones and glycosides are soluble in this solvent, and ethanol is safe and inexpensive. The extraction method was found to be squeezing produced the extract with the maximum content of total anthraquinone (2.48 ± 0.20% w/w), while the extract from percolation and sochlet extraction contained 2.46 ± 0.31 and 2.13 ± 0.29%w/w anthraquinone, respectively.

All of the *S. alata* leaf extracts at 80% ethanol had a distinctive aroma and were dark brown semi-solid. Rhein and aloe-emodin were identified as key ingredients and their TLC fingerprints all had a similar pattern, with hRf values of 39–44 and 74–79, respectively. The average extract ratio was 3.32:1 (3–4:1) (crude drug: 1g crude extract). From 27.90 to 31.13% w/w, the crude extract yields from the dried leaves ranged (Table 2.3). All extracts included almost similar amounts of total anthraquinones (total aglycones plus total glycosides), were computed as rhein and ranged from 2.41 to 2.48% w/w (on average, 2.45% w/w). *Senna alata* leaf extract had around half to a third as much anthraquinones as standardised senna leaf dry extract (which contains not less than 5.5% and not more than 8.0% of hydroxy anthracene glycosides estimated as sennoside B_{20}).

Table 2.3 Yield, extract ratio, and content of total anthraquinones in the ethanolic extracts and dried powder of S. alata leaves collected from various locations

(Gritsanapan & Mangmeesri, 2009)

Sample	Location/Part	Yield (%w/w)*	Extract ratio	Total antraquinones (%w/w)*	
				In extract	In dried powder
1	Bangkok/C	27.90±0.15	3.58:1	2.41±0.02	0.67±0.08
2	Phichit/C	29.85±0.08	3.35:1	2.47±0.03	0.74±0.02
3	Nongkhai/NE	30.95±0.10	3.23:1	2.48±0.30	0.77±0.04
4	Udonthani/NE	31.20±0.23	3.20:1	2.44±0.10	0.76±0.03
5	Maha sarakham/NE	30.94±0.12	3.23:1	2.43±0.05	0.75±0.12
6	Sukhothai/N	30.30±0.25	3.30:1	2.44±0.10	0.74±0.05
7	Phatthalung/S	29.82±0.17	3.35:1	2.47±0.08	0.74±0.10
8	Nakhon Si-thammarat/S	29.27±0.31	3.42:1	2.48±0.18	0.73±0.12
9	Ron Phibun-Nakhon Si-thammarat/S	31.13±0.29	3.21:1	2.47±0.19	0.77±0.17
10	Surat-Thani/S	29.73±0.17	3.36:1	2.47±0.20	0.73±0.15
average		30.11±0.18	3.32:1 (3-4:1)	2.45±0.12	0.74±0.09

*expressed as mean ±SD (n=3)
C = Central, NE =North-East, N = North, S = South

2.4.1.2 Steam Distillation

The extraction process is a steam distillation method used to recover volatile compounds through a high boiling point from an inert and complex matrix, either solid or liquid, using saturated or superheated steam as a separator and energy source (Cerpa et al., 2009). Usually this method of steam distillation is used in the laboratory to extract essential oils especially from herbal plants. In most cases, plant sample compounds will be soaked in boiling water using high temperature or heated steam. The compounds experience damage and disruption to the cell structure as a result of the use of heat. During volatilization, the separated compounds change to the oil phase during condensation. The boiling point of this extraction usually varies between

200^{O}C to 300^{O}C and is evaporated at a temperature equal to the water temperature while the yield is between 0.005% and 10% (Chemat & Boutekedjiret, 2015).

2.4.2 Non-conventional Extraction

2.4.2.1 Microwave-assisted Extraction (MAE)

Microwave-assisted Extraction (MAE) has the ability to rapidly heat the sample-solvent mixture by dissociating the analyte from the sample matrix into the solvent by heating the solvent in contact with the sample using microwave radiation. This technique offers several advantages over conventional extraction methods, including reduced extraction time, improved extraction efficiency, and the ability to extract a variety of phenolic compounds. Using MAE, the efficiency of the extraction process is affected by many parameters such as solvent choice, solvent volume, temperature, time and matrix characteristics (including water content) (Eskilsson & Björklund, 2000). An extraction operation typically lasts 15 to 30 minutes and employs tiny solvent volumes of 10 to 30 ml. These quantities are around ten times less than those required by standard extraction methods. Additionally, as several samples may be extracted at once, sample throughput is boosted. When compared to traditional procedures, analyte recoveries and repeatability are often better.

Figure 2.7 The apparatus for microwave-assisted extraction

(Source: personal doc)

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

The materials used in this study are leaves of *Senna alata* L. The leaves of *Senna alata* used in this study were collected from a creek in Kampung Lating, Kelantan in July 2023. Approximately 5 kilogram of leaves was collected for this study. The specimen will be identified based on their morphology characteristic of flowers, the colour of petals and leave.

Figure 3.1: Leaves of *Senna alata* L.

(Source: personal doc.)

3.2 Chemicals

The chemicals and reagent needed for extraction of Phenolic components in *Senna alata* using MAE extraction method are ethanol (C_2H_6O), distilled water, tannic acid (C_{76} $H_{52}O_{46}$), hydrocholoric acid (HCI), Iron (III) Chloride ($FeCl_3$), Gallic acid ($C_7H_6O_5$), Dimethyl sulfoxide (DMSO), and HPLC water.

3.3 Apparatus and Equipment

The apparatus and equipment used for extraction of Phenolic components in *Senna alata* using MAE extraction are beaker (50mL,250mL & 500mL), measuring cylinder (10mL & 100mL), test tube, test tube stand, zip lock plastic bag, aluminium foil, disposable dropper, spatula, clear reagent bottle (1000mL), micropipette, centrifuge tube (50mL), centrifuge Tube rack, spectrophotometer cuvettes, pipette tips (1000µL), digital thermometer, electronic balance, centrifuge microtube, gloves, volumetric flask (100mL), HPLC autosample vial, parafilm, funnel, filter paper, syringe, syringe filter, mask, reagent bottle (250mL), sieve (500 µm), microwave, blander machine, centrifuge, Ultraviolet-visible (UV-Vis) spectrophotometers, High-Performance Liquid and Chromatography (HPLC)

3.4 Method

3.4.1 Preparation of plant sample

Around 5 kg of *Senna alata* leaves were collected from Kampung Lating, Kelantan. After that, the leaves were washed thoroughly using tap water to remove excess dirt and unwanted particles, and dried the leaves for 3 to 4 days under sunlight until dry and reach a weight of 3 to 4

kilograms. The leaves drying process was performed to remove the water content of the leaves to obtain the exact amount of sample before the next process.

After that, the dried leaves were taken to the laboratory to be ground into powder form. The grinding process is done using a blender machine. Then, the powder sample undergo sieving process using sieve tools to filter the desired particle size and ensure that the particle size obtained is around 425-500 μm. Next, the powder sample was weighed, and a total of 1.5 kg was stored in zip-lock bags before further use.

3.4.2 Extraction process

The extraction method used in this study is the microwave power (MAE) method. The three different powers that was used are 90W, 180W, and 250W (Kaur et al., 2012). Dry sample powder and solvent, which is ethanol, was prepared at a sample to solvent ratio of 1:60 (w/v). The heating process was used during extraction with a fixed temperature control of 40°C and a fixed time of 30 minutes. After extraction, the extract sample was put into a centrifuge tube. Extraction was performed in triplicate for each parameter. Then, the extract was centrifuged at 5800 rpm for 15 minutes at room temperature. After centrifugation, the ethanol extract solution was filtered using filter paper (10 mm). The extract was then stored in a refrigerator for further use. This study, the stored sample was used for the analysis of phenolic compounds found in the plant.

Table 3.1: Range of parameter used for this study

Parameter **The Microwave power :**	Constant Variable
• 90 W, 180W, 250W (To identify the most effective concentration of solvent for the extraction of phenolic from S.alata plant.)	• The ratio of powder dried sample to solvent: 1:60 (w/v). • (5 g powder sample in 150 mL selected solvent) • Temperature control during extraction: 40°C • Fixed time of 30 minutes during extraction

3.4.3 Phytochemical screening (Test of phenol)

Phytochemical tests are a series of procedures used to identify and characterize the different types of chemical compounds present in plants. Phytochemical test is the qualitative analysis in the research study. Qualitative analysis is a subjective analysis that is more concerned with nonstatistical data that cannot be compared and the type of data consists of descriptive statements.

2mL of extract was transferred to a test tube and add 1 mL of water. Next, a few drops of Iron (III) chloride ($FeCl_3$) were added to the extract (2 mL). A blue, green, red or purple color is a positive test. Phenol reacted with $FeCl_3$ to form a purple or bluish-black complex (Shaikh & Patil, 2020). This test was based on the ability of phenol to form a complex with iron ions. Ferric chloride is another name for iron (III) chloride. Iron (III) ions combine with phenol to create complexes that are brightly coloured. From compound to compound, the hue of the complexes

varies. As a phenol test, the reaction with $FeCl_3$ solution can be utilised. $FeCl_3$ solution transforms into an intense greenery-black solution when phenol crystal is added.

3.4.4 UV-Vis Spectroscopy analysis

Quantitative measurement analysis of phenolic compounds in *Senna alata* L. extract was performed using UV-Vis Spectroscopy. The purpose of quantitative analysis is to measure data and identify the types of data that can be measured or expressed in numerical form. The sample were named and labeled with sample ID as shown in Table 3.5 for data collection purposes.

Table 3.2: Sample ID and its naming used for this research

Sample ID	Naming
	Plant part: Leaves
A90/S1	Solvent : Ethanol,60%
A90/S2	Extraction time : 30 minutes
A90/S3	Power Extraction : 90W
	Plant part: Leaves
B180/S1	Solvent : Ethanol,60%
B180/S2	Extraction time : 30 minutes
B180/S3	Power Extraction : 180W
	Plant part: Leaves
C250/S1	Solvent : Ethanol,60%
C250/S2	Extraction time : 30 minutes
C250/S3	Power Extraction : 250W

3.4.4.1 Determination of total phenolic content using UV-Vis Spectroscopy analysis

According to the opinion of Saravia et al, 2018 hydrochloric acid solution can be used to evaluate the total amount of phenolic compounds through slight modifications. 0.25 mL sample extract for each concentration was added to a volumetric flask and diluted up to 25 mL with 0.1 M hydrochloric acid. The absorbance of the standard and tannic acid sample was measured at λ = 276 nm using a Thermo Scientific GENESYS UV-Vis Spectrophotometer. The blank was performed using distilled water. The concentration of total phenolic compounds was calculated based on the tannic acid standard curve.

A calibration curve was obtained by using tannic acid as standard. For the preparation of the tannic acid stock solution, tannic acid (100 mg), from Sigma-Aldrich Co., was dissolved in 0.1 M hydrochloric acid and volume was made up to 100 ml with 0.1 M hydrochloric acid in volumetric flask. Then, 2 mL of stock solution was diluted with 0.1 M hydrochloric acid up to 100 mL in volumetric flask. Standard tannic acid curve was constructed by preparing the dilution of 0.5, 1.5, 2.5, 3.5, and 4.5 mg/mL by standard solution tannic acid and diluted up to 20 mL by using 0.1 M hydrochloric acid. Triplicate measurements were carried out in this analysis.

3.4 HPLC Setting for Detection of phenolic compounds

The standard solution of gallic acid was used in this study. The gallic acid was the standard solution for the estimation of the phenolic content. Approximately 50 mg of gallic acid was mixed into 50 ml of Dimethyl sulfoxide (DMSO) and was well shaken. Through the dilution method, various concentrations of solutions were made (500 mg/L, 250 mg/L, 150 mg/L, 100 mg/L, and 50 mg/L). The calibration curve was plotted, and the total phenolic content was

calculated as gallic acid equivalent, GAE/g of extract and the R^2 = 0.997 (Alothman, Bhat, & Karim, 2009)

The concentration of phenolics in the plant powder was determined using the HPLC method. For sample preparation, the sample was extracted and diluted to 10 mL with 30 HPLC water. According to the manufacturer's instructions filter the extract using a 0.45µm syringe filter or any suitable filtration method to remove any insoluble particles or impurities that may interfere with the HPLC analysis before injection into the HPLC system then set up and prepare the HPLC instrument.

3.5 Research Flow Chart

The research flow chart is divided into three stages. Stages 1 is preparation of *Senna alata* L. sample. Stages 2 is the extraction process and Stages 3 are analysis, phytochemical screening, and characterisation the obtained experimental data as shown in Figure 3.1

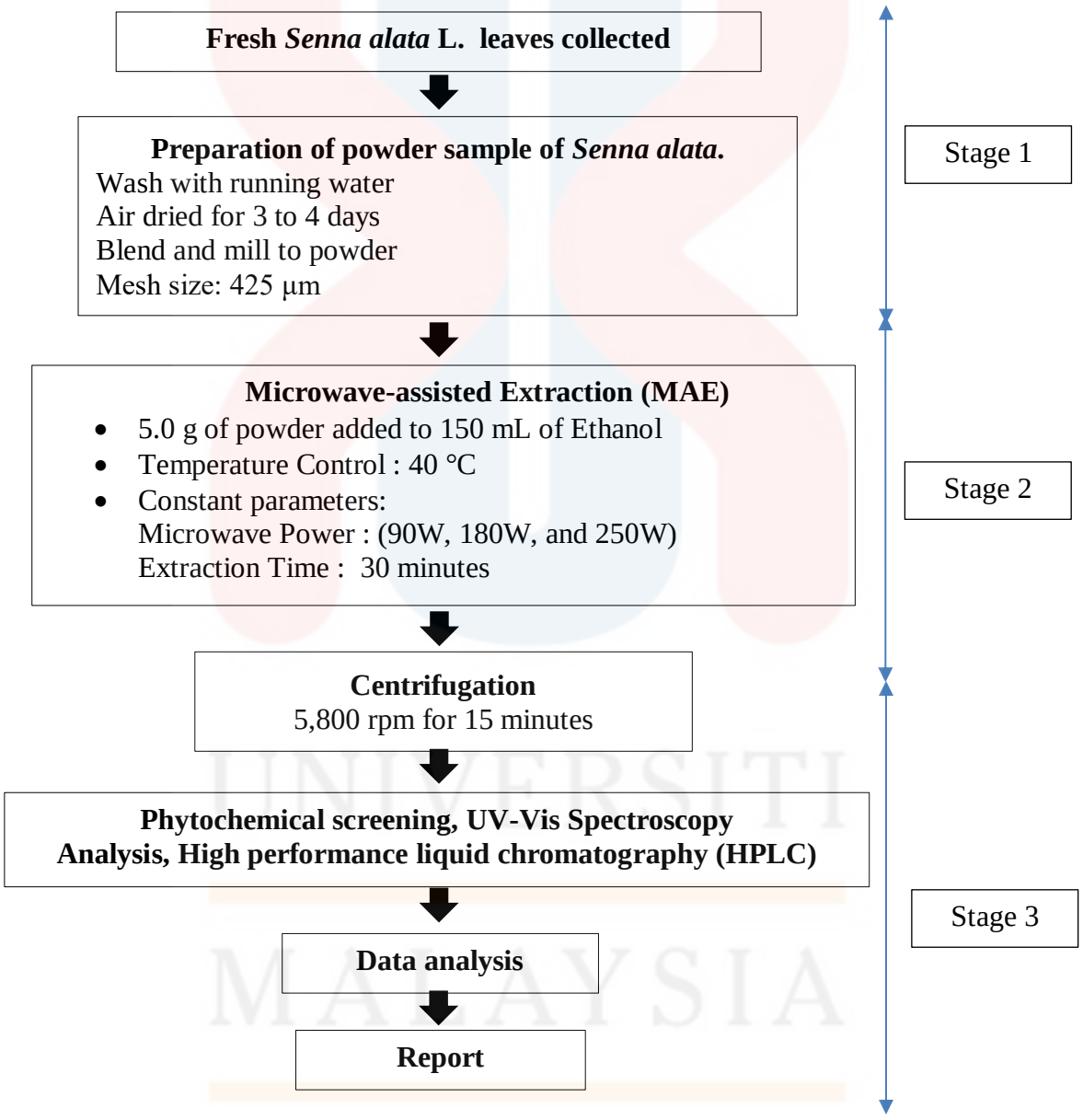


Figure 3.2: Research flow chart for extraction of phenolic compaun from *Senna alata*. plant.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter discuss the results of the phytochemical screening, total phenolic compound using UV-Vis spectroscopy analysis and HPLC analysis for the extraction of phenolic components from leaves of *Senna alata (L.)*

4.1 Phytochemical Screening

Senna alata samples were extracted using different microwave powers, namely ethanol 90W, 180W, and 250W. Microwave power was selected in this study to obtain optimal results and quality. Higher power levels can lead to faster heating and increased agitation, potentially increasing extraction yields for some compounds while at lower power levels, heating is slower and gentler, which can be beneficial for preserving heat-sensitive compounds and maintaining quality extract. In the context of *Senna alata* extraction using different microwave power and ethanol as solvent, the choice of microwave power plays an important role in optimizing the extraction process. The appropriate power level depends on the solvent used. Some solvents have higher boiling points and require more energy to heat, requiring higher power levels (Kremsner et al., 2006). Ethanol has a relatively low boiling point (around 78.37°C). Therefore, it may not necessarily require very high microwave power for effective heating and extraction. However, the choice of a higher power level can still be justified to speed up the process and increase the overall extraction efficiency. The use of lower microwave power, such as 90W, is mentioned as a

strategy for preserving heat-sensitive compounds. This is important because some bioactive compounds in medicinal plants, including Senna alata, may degrade or lose their efficacy at higher temperatures. Therefore, a balance must be struck to maximize extraction efficiency while minimizing the potential degradation of sensitive compounds.

Table 4.1: Results of phytochemical screening

Power	90 watts	180 watts	250 watts
Sample 1	+	+	++
Sample 2	+	+	++
Sample 3	+	+	++

Note: (+): Present

(++): Highly present

Based on Table 4.1, phytochemical screening of *Senna alata* L. showed positive results for phenolic compounds. Phenol turns the solution greenish black colour and a black precipitate forms. The results obtained revealed the presence of phenolic compounds in the leaves of *Senna alata L.* This was proven in a study published by the Brazilian Journal of Biological Sciences using mass spectrometry and phytochemical screening to identify the phenolic compounds found in *Senna alata* leaf extracts. Studies have found that the leaves contain a variety of phenolic compounds, including flavonoids, tannins, phenolic acids, and anthraquinones. The same results were obtained which revealed the presence of flavonoids, phenols, saponins, tannins, alkaloids and anthraquinones in the leaves of *Senna alata L.* by Abubakar et al., (2015) also revealed the metabolites present were determined quantitatively with alkaloid content of 14.09±0.50 and

15.89± 0.72, saponins 40.57±0.57 and 33.02±0.07, flavonoids 42.28±0.90 and 36.52±0.90 and 36.52±0.90 and 36.52±0.90 and 36.52±0.9. , and phenol 7.84±0.49 and 9.91±0.68 mg/100 g for leaf bark and root, respectively.

Referring to Table 4.1, phenolics are abundant in the leaves of *Senna alata L.* Color changes are clearly recorded in the phytochemical examination (see Table 4.2). Through the phytochemical test of phenolic compounds, it can be seen that the higher the microwave power, the darker the color of the senna alata extract sample. In general, higher extraction temperatures can increase the yield of phenolic compounds from plants. This is because heat can help break down cell walls and membranes, making it easier to reduce the content released into the solvent. Santos-Buelga et al. (2012) state that at a given frequency, quicker heating and a higher degree of microwave absorption correspond with a larger dielectric constant value. In contrast to traditional conductive heating techniques, microwaves efficiently and uniformly heat the entire sample at once. The use of ethanol in senna alata extract also has an effect on the production of phenolic compounds. According to Jha et al., (2022), ethanol is a polar solvent that can effectively dissolve various phenolic compounds, often leading to higher yields than non-polar solvents such as hexane. This is because ethanol can interact with both hydroxyl groups and aromatic rings found in many phenolic structures, facilitating their extraction from plant material.

Phenolics act as scavengers, neutralizing harmful free radicals that damage skin cells and contribute to premature aging (Michalak et al., 2022). Referring to the ferric test used in this study, this test is not only to test the presence of phenolic only, but also can test the presence of tannic. This electron transfer reaction forms a complex between phenolic and iron ions. Falcão et al., (2011) reported that the resulting complex usually exhibits a dark blue-black or green-black color, making it visually detectable. This color change acts as a visual indicator for the presence

of phenolics in the sample. Based on the observation of the color formed in the Senna alata extract sample after the phytochemical test, it was found that a high concentration of phenolic compounds is found in the leaves of the plant.

Phytochemical screening as seen in Table 4.1 shows that there is a slight presence of phenolic compounds in the leaves of the ethanol extract of Senna alata L. at a low power level of 90 watts. This happens because the green color of the solution does not change completely but only darkens slightly. Microwaves interact with molecules through a process called dielectric heating. Different molecules absorb microwave energy at different rates, which can create localized hot spots in the extract. When materials are processed, they often undergo physical and structural changes that affect the dielectric properties. These hot spots may concentrate or degrade certain pigments, leading to uneven color changes (Thostenson et al., 1999).

The relationship between microwave power and temperature is direct and proportional, meaning that higher power leads to faster and higher temperature increases. Therefore, the temperature used for extraction is the main factor related to extraction efficiency. High temperatures can result in increased co-extraction of matrix components as well as degradation of active components. Furthermore, because the particle size may have an effect on the extraction yield and phytochemical content (Prasedya et al., 2021). Therefore it needs to be carefully controlled. The leaf sample of Senna alata L. was ground into a powder form because the small particle size is generally favorable for high extraction yield.

Based on the results in this study, this finding supports the findings of a previous study conducted by Ling et al., (2019), that the higher the microwave power, the higher the phenolic compound content. Rapid and targeted extraction offers advantages to produce high-quality bioactive extracts for various applications. However, optimizing parameters such as power level,

extraction time and solvent selection is essential to ensure efficient and selective extraction while minimizing the degradation of sensitive compounds. *Senna alata* is indeed a treasure of valuable secondary metabolites with various bioactivities such as antioxidant and anti-inflammatory. Phenolic compounds of senna alata effectively eliminate free radicals, protect cells from oxidative damage and contribute to its anti-inflammatory effects (Alshehri et al., 2022).

Table 4.2: Phytochemical test observations in *Senna alata L.* extract at 90 watt microwave power level

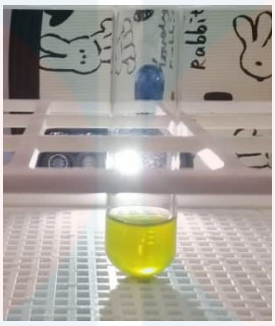
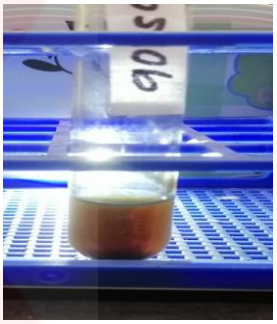
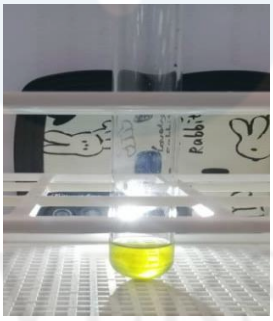
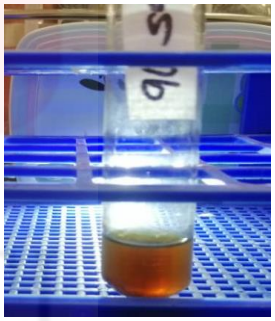
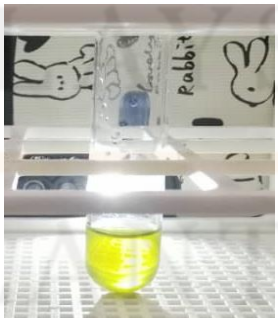
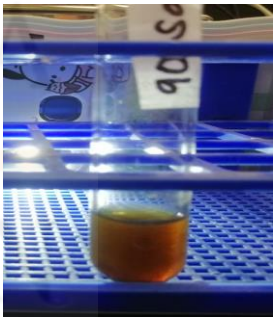
Microwave power (watt,W)	***Senna alata* L. Leaves Sample**		
	Type of sample	**Before**	**After**
90 W	Sample 1		
	Sample 2		
	Sample 3		

Table 4.3: Phytochemical test observations in *Senna alata L.* extract at 180 watt microwave power level

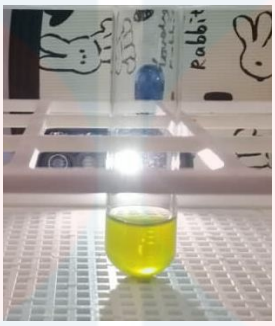
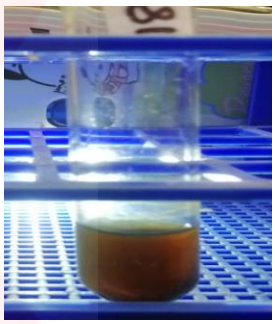
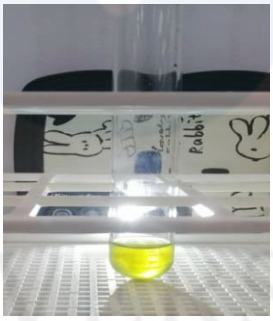
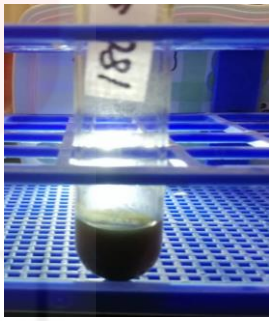
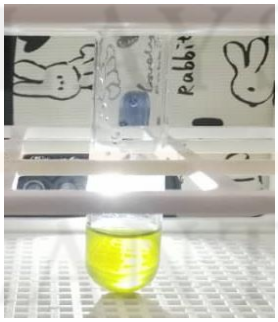
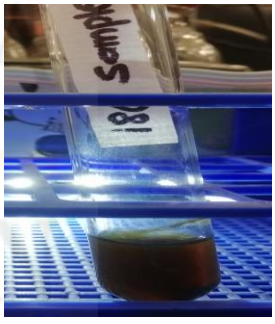
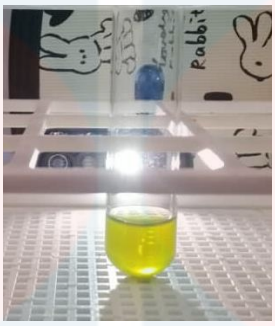
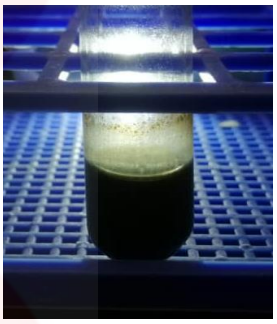
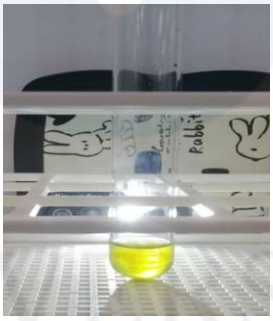
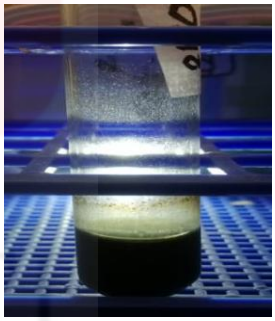
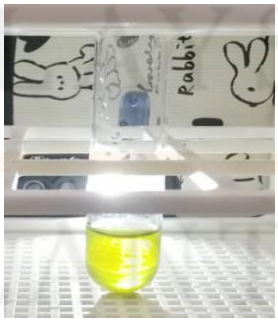
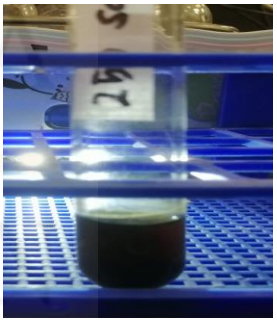
Microwave power (watt,W)	***Senna alata* L. Leaves Sample**		
	Type of sample	**Before**	**After**
180 W	Sample 1		
	Sample 2		
	Sample 3		

Table 4.4: Phytochemical test observations in *Senna alata L.* extract at 250 watt microwave power level

Microwave power (watt,W)	***Senna alata* L. Leaves Sample**		
	Type of sample	**Before**	**After**
250 W	Sample 1		
	Sample 2		
	Sample 3		

4.2 Total phenolic compound using UV-Vis spectroscopy analysis

Table 4.5 shows the readings of tannic acid standard with the concentration at 0.5, 1.5, 2.5, 3.5 and 4.5 mg/mL and measured by using UV-Vis spectrophotometer. The calibration curve of tannic acid standard is plotted as shown in Figure 4.1. R^2 of the calibration curve is 0.993, means the calibration curve for the tannic acid standard was significant. The equivalent equation obtained from the graph is y = 0.0856x + 0.2622 and it is used to calculate the concentration of total phenolic content be extracted from Senna alata.

Table 4.5: Reading of tannic acid standard.

Concentration (mg/mL)	1st reading	2nd reading	3rd reading	average	Std. Deviation	Relative Std. Deviation (%)
0.5	0.298	0.281	0.297	0.292	0.010	3.267
1.5	0.418	0.389	0.412	0.406	0.015	3.770
2.5	0.498	0.488	0.464	0.483	0.014	2.954
3.5	0.584	0.533	0.545	0.554	0.027	4.813
4.5	0.605	0.684	0.649	0.646	0.040	6.128

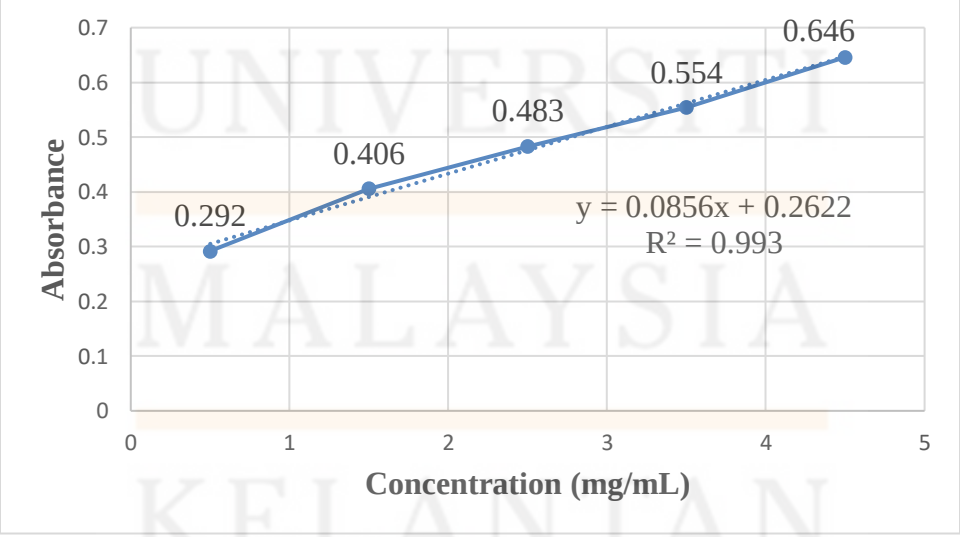


Figure 4.1: Standard calibration curve of tannic acid.

4.2.1 Effect of Microwave Power

Table 4.6 shows the results of UV-Vis analysis of the total phenolic compounds of Senna leaf extract at different microwave power levels. From the results, the highest concentration of total phenolic compounds in Senna alata leaf extract was found to be 5.6581 mg/mL. While the lowest concentration of total phenolic compounds was found to be 2.6597 mg/mL.

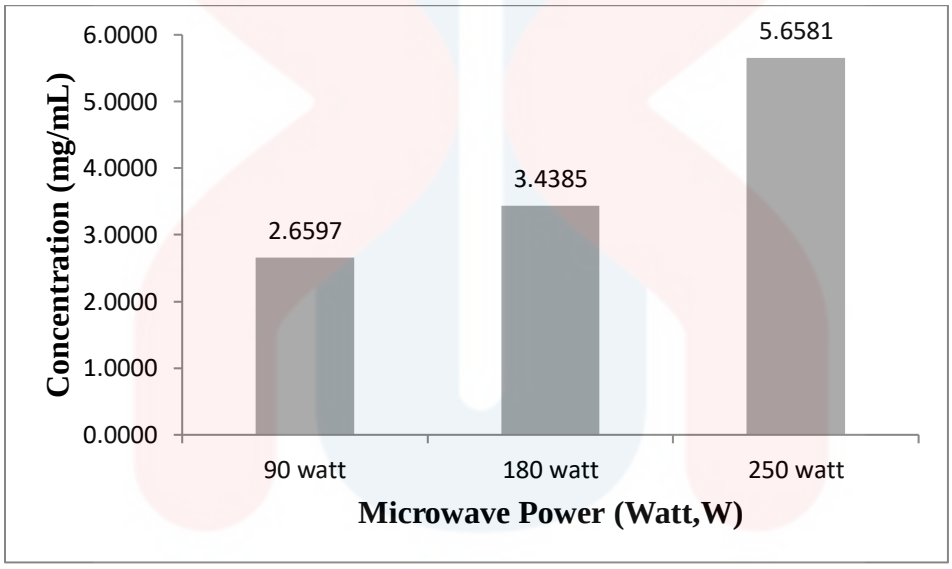


Figure 4.2: The effect of microwave power on the extraction of total phenolic compounds

Figure 4.2 shows the effect of microwave power on the extraction of total phenolic compounds from Senna alata leaves. It has been shown that the amount of phenolic compounds using a micro power of 250 watts is higher than 180 watts and followed by 90 watts. The total concentration of phenolic compounds using 250 watts is 5.6581 mg/mL. The relative standard deviation for all concentrations is small, which indicates that the method has good accuracy and precision.

The solubility of total phenolic compounds can be affected by the extraction temperature. The microwave power is proportional to the extraction temperature. The greater the microwave power, the higher the extraction temperature. The solubility of total phenolic compounds is indeed affected by extraction temperature, and the relationship can be complex depending on the specific plant material, the solvent used, and other extraction parameters. Higher temperatures generally increase the diffusion coefficient of phenolic compounds from the plant matrix into the solvent. This means compounds move more easily, leading to higher extraction yields. Based on the results of the phenolic content of this study, these results support the findings of a previous study conducted by Alara et al., (2021), which is to extract polyphenols from natural sources, it is necessary to ensure that the extraction efficiency is a function of several critical parameters, one of which is temperature.

Additionally, a higher temperature resulted at 250 watts, increasing the kinetic energy of the molecules, leading to a faster diffusion of phenolic compounds from the plant matrix into the solvent. Hotter solvents have a lower viscosity, allowing them to penetrate plant tissue more effectively and extract more phenolic compounds. For some phenolics bound to cell wall components such as cellulose and lignin, high temperatures can weaken these bonds, releasing the bound phenolics and making them available for extraction. However, phenolic compounds are generally heat sensitive. This is proven by Alara et al., (2021). Excessive temperature can cause its degradation, reduction in the yield of extracted compounds and potentially change its chemical properties and bioactivity. This is proven by ElGamal et al., (2023). Therefore, based on the research that has been done, in order to avoid degradation, the extraction temperature has been set not to exceed 40°C.

The temperature decreased gradually during the extraction time of 30 minutes. This can be explained that the low stability of some degraded phenolic amounts is due to the effect of the heat used in the extraction. This extraction process involves a heating process using a microwave power and setting the temperature control which is around 35 - 40 ℃. Therefore, extraction for 30 minutes caused the heat produced to be low and caused the amount of phenolic compounds in the sample not to be degraded.

Based on a previous study conducted by Osorio et al., (2020), the total concentration of phenolic compounds was 227.63 mg GAE/g. Through a comparison of the efficiency of the extraction results from the solvent extraction method (with the one that has been used in this study) and the results from the maceration extraction method (without heat) that has been carried out. The concentration of total phenolic compounds using solvent extraction (with microwave power) in this study is higher than value obtained from the extraction extractor.

4.3 Total phenolic compound using High Performance Liquid Chromatography (HPLC)

Table 4.7 shows the standard reading of gallic acid with concentrations at 20,40,60, 80 and 100 ug/uL and measured using high performance liquid chromatography (HPLC). The calibration curve of the gallic acid standard is plotted as shown in Figure 4.3. The R^2 of the calibration curve is 0.920, meaning that the calibration curve for tshhe gallic acid standard is significant. The equivalent equation obtained from the graph is y = 3062.2x - 42762 and it is used to calculate the concentration of total phenolic content extracted from *Senna alata.*

Table 4.6: HPLC analysis of standard Gallic acid

Sample ID	Peak	Ret. Time	Area	Height	Area %	Height %
20 ug A	1	2.425	3955	446	9.233	11.732
	2	4.063	38878	3358	90.767	88.268
20 ug B	1	2.443	3724	416	8.545	10.990
	2	4.101	39854	3372	91.455	89.010
40 ug A	1	2.415	4568	488	5.529	6.790
	2	4.048	78054	6701	94.471	93.210
40 ug B	1	2.419	4692	508	5.610	6.982
	2	4.039	78952	6761	94.390	93.018
60 ug A	1	2.105	2101	196	1.706	1.834
	2	2.413	4546	485	3.691	4.538
	3	4.033	116512	10012	94.603	93.628
60 ug B	1	2.096	1851	170	1.512	1.599
	2	2.412	4170	475	3.407	4.468
	3	4.032	116369	9990	95.081	93.933
80 ug A	1	2.413	4553	509	2.603	3.363
	2	4.030	170370	14628	97.397	96.637
80 ug B	1	2.099	1988	161	1.108	1.029
	2	2.409	5073	539	2.827	3.444
	3	4.019	172413	14941	96.066	95.527
100 ug A	1	2.081	2866	316	0.935	1.169
	2	2.386	4091	482	1.335	1.780
	3	3.994	299623	26259	97.731	97.051
100 ug B	1	2.079	2740	313	0.897	1.155
	2	2.382	4107	487	1.344	1.800
	3	3.980	298654	26253	97.759	97.045

Table 4.7 : Reading of gallic acid standard.

Concentration (ug/uL)	Peak Area		Average Reading	Std.Deviation	Relative Std. Deviation (%)
	1st reading	2nd reading			
20	39854	38878	39366	690.1362	1.7531
40	78054	78952	78503	634.9819	0.8089
60	116512	116369	116440.5	101.1163	0.0868
80	170370	172413	171391.5	1444.6192	0.8429
100	299623	298654	299138.5	685.1865	0.2291

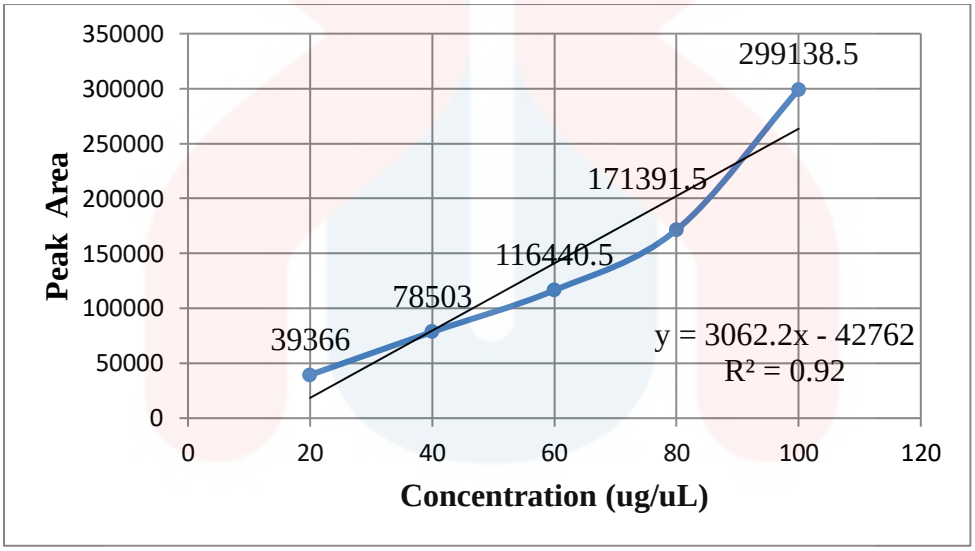
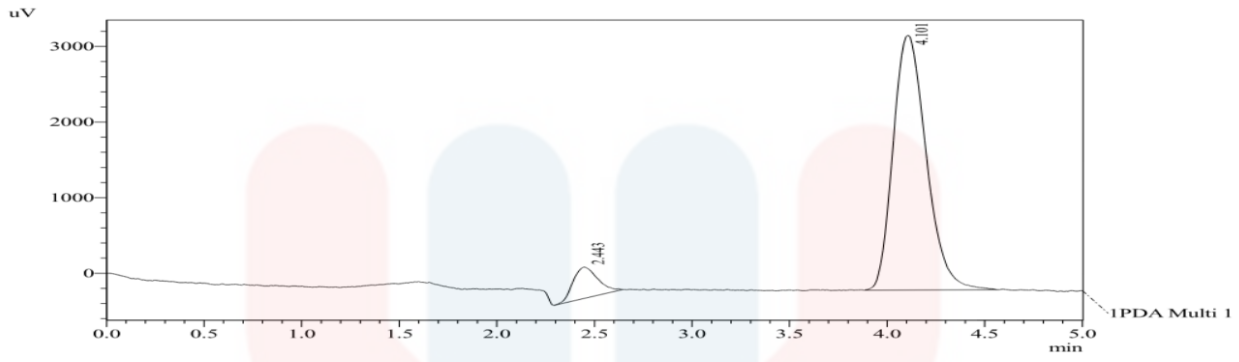
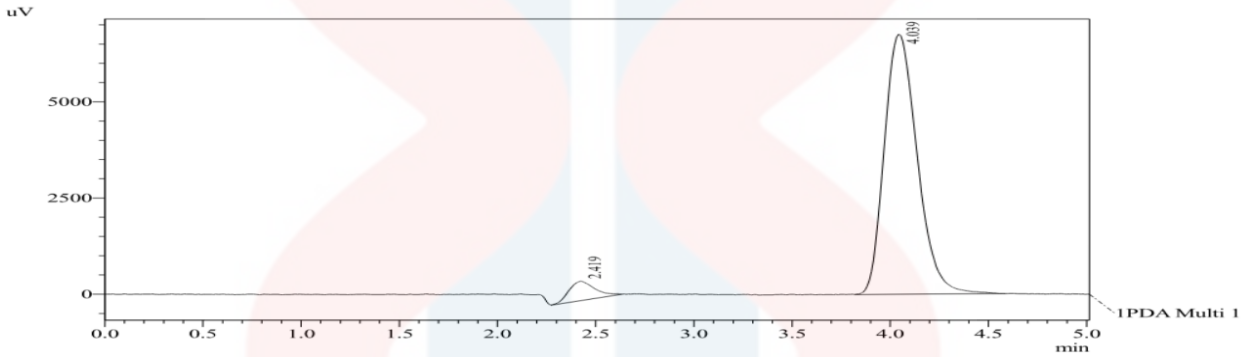


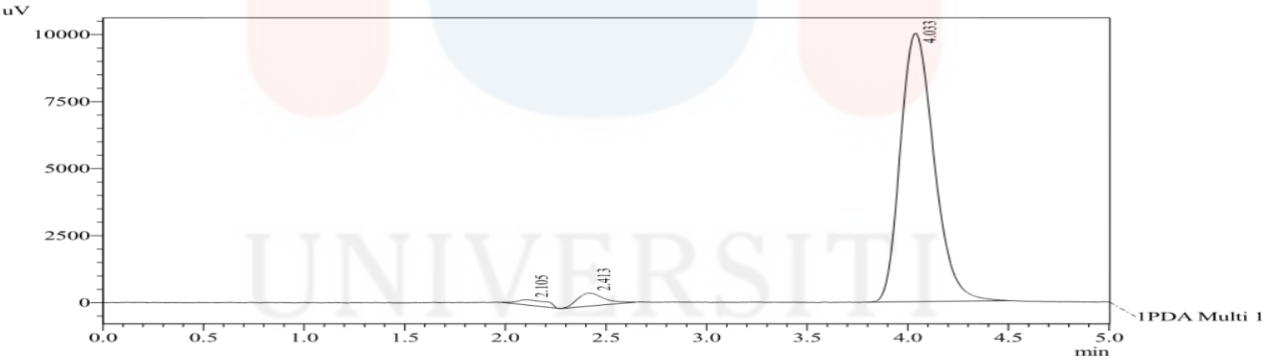
Figure 4.3: Calibration curve of Gallic acid. Concentrations used are: 20, 40, 60, 80 and 100 µg/mL and the absorbance measured at 272 nm.



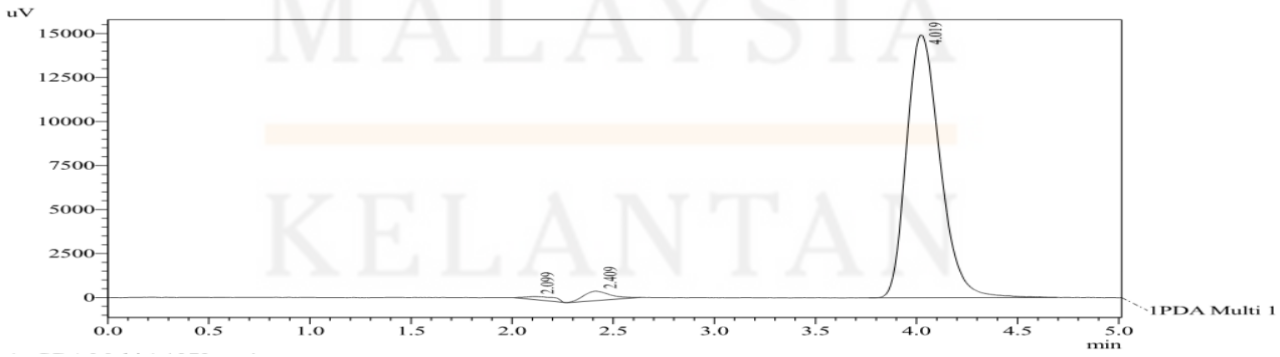
(20 ug B)



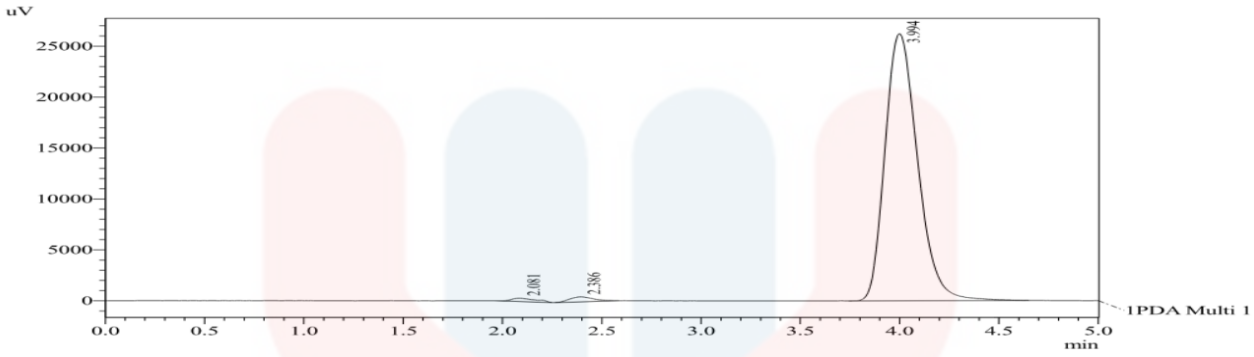
(40 ug B)



(60 ug A)



(80 ug B)



(100 ug A)

Figure 4.4: HPLC chromatograms of standard gallic acid

A calibration curve is a graphical representation of the relationship between the concentration of an analyte and the response of the analytical instrument. In the case of HPLC, the concentration of gallic acid is plotted against the corresponding peak area obtained from the chromatogram. The curve serves as a tool for quantification by allowing the conversion of instrument response to concentration.

To construct the calibration curve for gallic acid, a series of standard solutions with 20, 40, 60, 80 and 100 µg/uL concentrations are prepared. These solutions are then injected into the HPLC system, and the resulting chromatograms are analyzed. The peak areas corresponding to each concentration are recorded, and a linear regression analysis is performed to generate the calibration curve. Several factors can influence the construction and accuracy of the calibration curve. These include the choice of mobile phase, column type, flow rate, and detector wavelength (Hefnawy et al., 2006). Optimization of these parameters is crucial to achieving a stable and reproducible chromatographic separation.

The calibration method for gallic acid can be applied to analyze real samples, extract of Senna alata. The calibration curve of gallic acid using HPLC is a vital tool in analytical chemistry, providing a quantitative method for the accurate determination of this important phenolic compound. The optimization of HPLC parameters, validation of the method, and careful attention to accuracy and precision contribute to the reliability of the calibration curve, making it a valuable asset in both research and industrial settings.

4.3.1 Effect of Microwave Power

HPLC analysis results were obtained in less than 5 minutes for gallic acid calibration curve and identification of phenolic compounds in *Senna alata* leaf extract. HPLC is known for its rapid analysis, providing fast results without compromising data quality. This efficiency is invaluable in high-capacity laboratories, where the analysis of many samples is a routine part of the workflow.

The extracted samples from Section 3.4.2 were analyzed using HPLC with the method described previously (Marília, et al., 2022); the quantification of phenolic compounds (gallic acid) in *S.alata* samples was carried out in the present study with some modifications. A 10 µL sample was injected into the HPLC system, and the PDA was set at 272 nm. The phenolic compounds in the S.alata samples were detected by comparing the peak retention time and peak area (Figure 4.5).

The retention time for gallic acid in HPLC chromatograms may differ from tannic acid in uv-vis spectroscopy analysis due to their structural dissimilarity. Retention time is a critical parameter for identifying and quantifying compounds. This technique offers a higher level of

resolution compared to UV-Vis. HPLC generally offers higher sensitivity and lower detection limits compared to UV-Vis spectrophotometry. This is crucial, especially when dealing with samples with lower analyte concentrations. While UV-Vis spectrophotometry is quick and straightforward, HPLC provides a more detailed and accurate analysis, especially in complex samples. The separation capability of HPLC reduces interferences and enhances precision.

Table 4.8: Result of HPLC analysis on phenolic compounds in *Senna alata L*. extract at 90 watt microwave power level

Sample ID	Peak	Ret. Time	Area	Height	Area %	Height %
AS1 A	1	1.447	338337	37793	7.990	32.041
	2	2.026	3877515	78533	91.568	66.581
	3	3.498	5388	452	0.127	0.383
	4	3.805	13315	1175	0.314	0.996
AS1 B	1	1.646	896204	49564	20.978	34.759
	2	2.011	3357868	91408	78.599	64.105
	3	3.481	4768	421	0.112	0.295
	4	3.801	13286	1198	0.311	0.840
AS2 A	1	1.483	274508	33084	8.221	31.497
	2	2.124	3050985	70420	91.367	67.042
	3	3.380	12378	1394	0.371	1.327
	4	3.962	1387	141	0.042	0.134
AS2 B	1	1.468	238213	27848	6.942	18.626
	2	1.828	960400	55050	27.989	36.820
	3	2.030	2215979	65194	64.581	43.604
	4	3.473	8744	616	0.255	0.412
	5	3.785	7998	806	0.233	0.539
AS3 A	1	1.854	2030120	64405	61.181	42.751
	2	2.181	531735	47373	16.025	31.445
	3	2.372	744062	37735	22.424	25.048
	4	3.473	3725	343	0.112	0.228
	5	3.772	8572	795	0.258	0.528
AS3 B	1	1.439	220975	25875	6.762	18.560
	2	1.899	1131704	55690	34.631	39.946
	3	2.005	1900848	56510	58.167	40.534
	4	3.493	4985	437	0.153	0.313
	5	3.761	9399	902	0.288	0.647

Table 4.9: Result of HPLC analysis on phenolic compounds in *Senna alata L.* extract at 180 watt microwave power level

Sample ID	Peak	Ret. Time	Area	Height	Area %	Height %
BS1 A	1	1.440	442182	47534	8.813	19.927
	2	1.9983	2157896	92113	43.007	38.615
	3	2.133	2402439	97337	47.881	40.805
	4	3.534	6704	582	0.134	0.244
	5	3.734	8269	973	0.165	0.408
BS1 B	1	0.211	3531	237	0.070	0.092
	2	1.648	1088273	66702	21.481	25.730
	3	1.979	1488652	93776	29.384	36.173
	4	2.136	2410563	96218	47.581	37.115
	5	3.345	14477	1647	0.286	0.635
	6	4.053	60697	662	1.198	0.255
BS2 A	1	0.182	2168	217	0.050	0.260
	2	2.112	4288610	82545	99.705	98.606
	3	3.493	5899	438	0.137	0.524
	4	3.785	4637	511	0.108	0.611
BS2 B	1	0.185	1674	110	0.039	0.057
	2	1.433	363368	41464	8.499	21.375
	3	1.994	1960975	77519	45.868	39.962
	4	2.093	1940209	74037	45.383	38.167
	5	3.487	3857	331	0.090	0.171
	6	3.788	5140	520	0.120	0.268
BS3 A	1	1.605	937815	58498	19.719	24.955
	2	1.939	1719364	85615	36.152	36.523
	3	2.132	2045011	85334	43.000	36.403
	4	3.520	12813	1168	0.269	0.498
	5	3.815	40878	3796	0.860	1.619
BS3 B	1	0.232	3507	447	0.074	0.205
	2	1.026	1203	125	0.025	0.057
	3	1.446	335532	38560	7.079	17.695
	4	1.948	2347967	90995	49.535	41.757
	5	2.133	1998396	82763	42.160	37.979
	6	3.488	15480	1228	0.327	0.564
	7	3.754	37958	3798	0.801	1.743

Table 4.10: Result of HPLC analysis on phenolic compounds in *Senna alata L*. extract at 250 watt microwave power level

Sample ID	Peak	Ret. Time	Area	Height	Area %	Height %
	1	0.242	3270	442	0.017	0.071
	2	1.033	1122	116	0.006	0.019
	3	1.436	2276500	256759	12.078	41.030
CS1 A	4	1.954	16139624	340497	85.632	54.411
	5	3.511	94682	5138	0.502	0.821
	6	3.979	193680	18479	1.028	2.9853
	7	4.414	138790	4349	0.736	0.695
	1	0.099	13939	1725	0.073	0.206
	2	0.653	4820	471	0.025	0.056
	3	1.449	2215118	218784	11.557	26.124
CS1 B	4	1.707	2570014	242885	13.407	29.002
	5	1.977	13978699	345803	72.925	41.291
	6	3.483	80444	3684	0.420	0.440
	7	4.066	180905	20043	0.944	2.393
	8	4.381	124565	4089	0.650	0.488
	1	0.123	12619	1669	0.163	0.741
	2	0.683	4505	416	0.058	0.185
	3	1.451	742767	76622	9.619	34.041
CS2 A	4	2.064	6745863	136376	87.363	60.589
	5	3.008	16116	1070	0.209	0.475
	6	3.871	97679	6804	1.265	3.023
	7	4.241	102086	2128	1.322	0.946
	1	0.178	4464	552	0.064	0.209
	2	0.948	2193	232	0.031	0.088
CS2 B	3	1.734	1581493	97351	22.641	36.786
	4	2.063	5313972	155793	76.077	58.870
	5	3.275	82872	10712	1.186	4.048
	1	0.154	2317	299	0.035	0.097
	2	0.909	2369	253	0.036	0.082
	3	1.463	537891	59956	8.191	19.396
CS3 A	4	2.002	537891	124343	49.977	40.225
	5	2.161	3281910	118997	40.344	38.496
	6	3.504	2649360	1930	0.348	0.624
	7	3.779	21158	2253	0.322	0.729
	8	4.370	48964	1084	0.746	0.351

	1	0.210	2355	224	0.033	0.118
	2	1.450	509006	57033	7.194	30.002
CS3 B	3	2.032	637446	124798	90.097	65.650
	4	3.494	28959	2199	0.409	1.157
	5	3.792	41600	3149	0.588	1.656
	6	4.359	1188704	2693	1.678	1.417

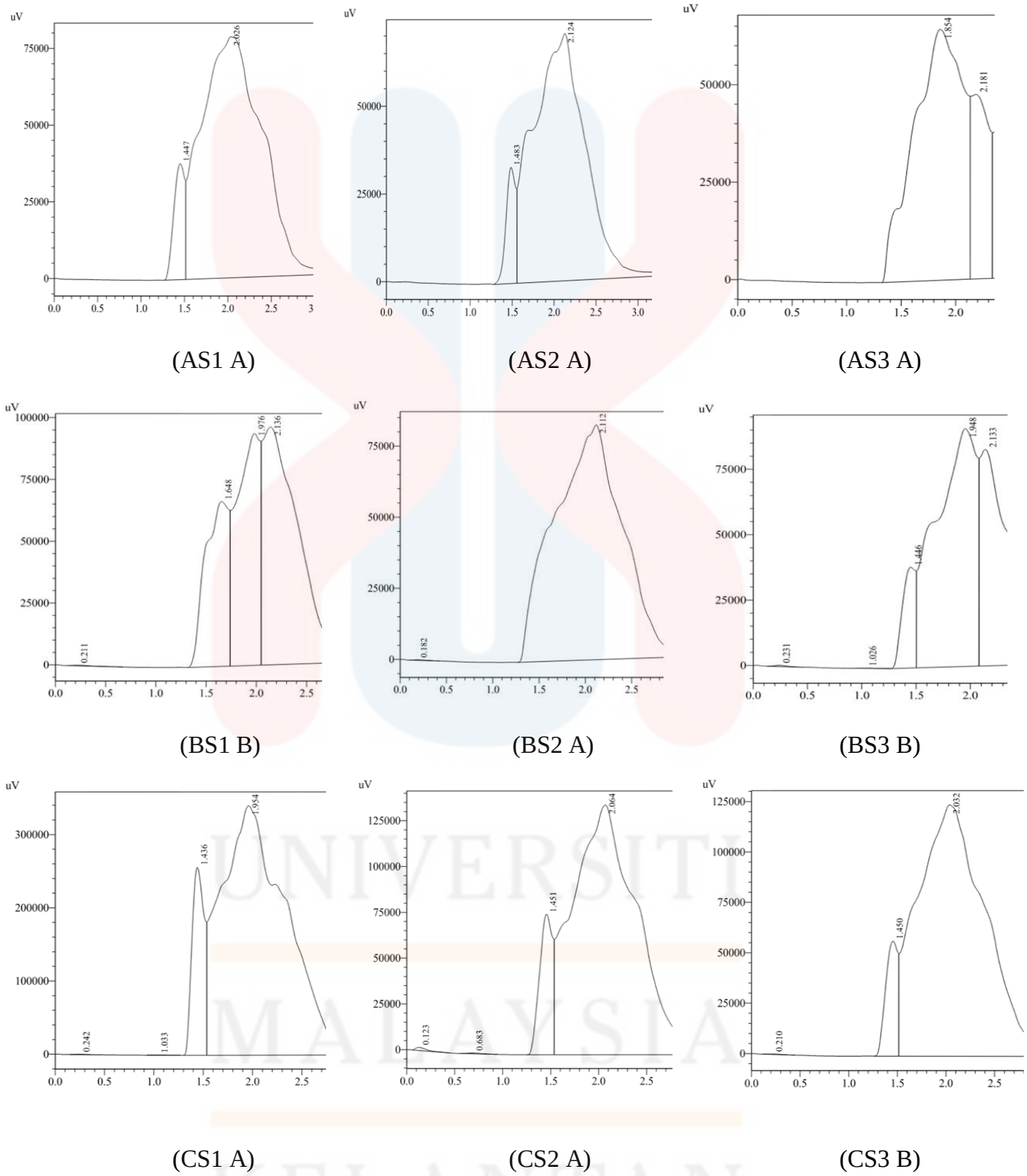


Figure 4.5: Chromatogram of the nine sample extract *Senna alata* at 272nm 4nm

Figure 4.6 shows the results of HPLC analysis of the total phenolic compounds of Senna leaf extract at different microwave power levels. From the results shown in Table 4.9, the highest concentration of total phenolic compounds in Senna alata leaf extract was found to be 47679.7590 ug/uL. While the lowest concentration of total phenolic compounds was found to be 43782.4404 ug/uL.

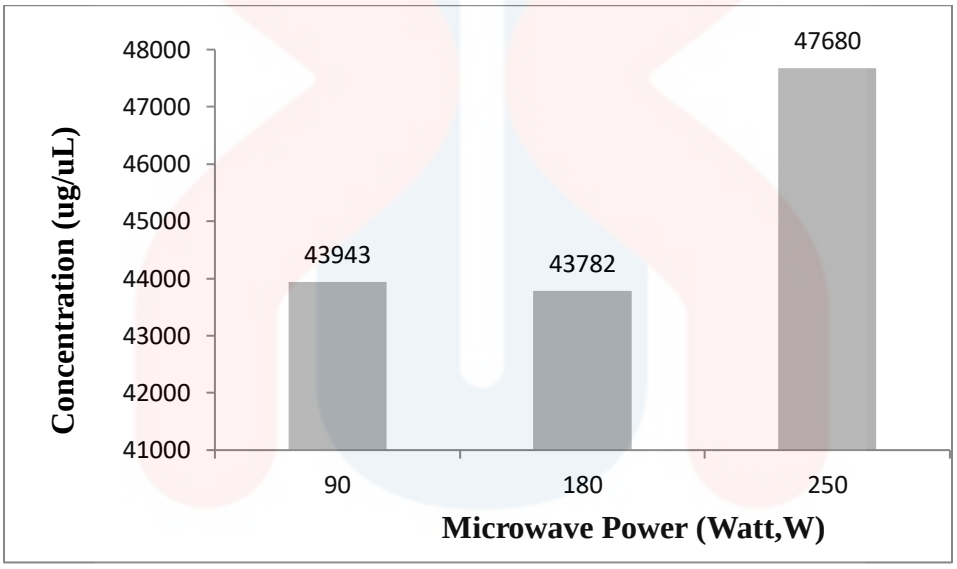


Figure 4.6: The effect of microwave power on the extraction of phenolic compounds in the analysis of HPLC results

The effect of microwave power on the phenolic compound of *Senna alata* is shown in Figure 4.6. In general, samples extracted using the highest microwave power have the greatest concentration of phenolic compounds. It was observed that microwave power at the level of 250W showed a total phenolic content of 47679.7590 ug/uL, followed by microwave power 90W

(43943.4027 ug/uL) and the lowest was 180W (43782.4404 ug/uL) by using the same time rate that is for 30 minutes.

The content of phenolic compounds in Senna alata leaf extract with acid properties was determined through the linear Gallic acid standard curve ($y = 3062.2x - 42762$; $R^2 = 0.92$). The HPLC-PDA results revealed the presence of a major peak in the sample extract, with time retention between 2 to 4 minutes (Figure 4.5)

In this study, UV-Vis and High Performance Liquid Chromatography (HPLC) were used for the quantitative analysis of chemical compounds. They allow researchers to determine the concentration of a specific analyte in a sample. UV-Vis spectrophotometry is selective based on the absorption characteristics of compounds, while HPLC separates individual components for detection. While both methods are employed to quantify the concentration of a specific analyte in a sample, it is not uncommon to observe variations in the results obtained from these techniques when applied to identical samples.

Based on the results of UV-vis and HPLC have discrepancies. UV-Vis spectrophotometry relies on the absorption of light by the analyte at a specific wavelength. This method assumes that absorption is solely due to the presence of the target compound. However, HPLC separates individual components in a mixture prior to detection, providing a higher degree of specificity. However, based on the results HPLC that were obtained, it shows that the sample may experience interference that causes it to also absorb at the selected wavelength.

The calibration curve is a fundamental tool in analytical chemistry that relates the instrumental response (e.g., peak area) to the concentration of a known standard. In the scenario where High-Performance Liquid Chromatography (HPLC) uses gallic acid for calibration, while

UV-Vis spectrophotometry uses tannic acid, it's important to consider the implications and potential challenges associated with using different calibration standards for each technique.

UV-Vis spectrophotometry is usually faster, making it suitable for routine analysis. HPLC, while providing higher specificity, has a longer analysis time. The results obtained from HPLC and UV-Vis analysis reveal the strengths and limitations of each technique. UV-Vis offers rapid analysis but may lack specificity, especially in complex matrices. HPLC, on the other hand, provides greater specificity and accuracy, making it suitable for more complex analyses. The choice between these techniques depends on the specific needs of the analysis, the nature of the sample, and the level of accuracy.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

In conclusion, the active component was successfully extracted from senna alata by using the Microwave-Assisted Extraction (MAE) method in this study. MAE has emerged as a revolutionary technique to harness the abundant phenolic richness of *Senna alata*. Compared to conventional extraction methods, MAE proved to be excellent in terms of efficiency, reduced solvent consumption, and increased phenolic yield and bioactivity. The objective of this study was successfully achieved.

First, the investigation of various microwave power levels resulted in the determination of optimal extraction process parameters to extract phenolic compounds from *Senna alata (L.),* in line with the first objective. After conducting experiments with varying microwave power levels, the optimal extraction process parameters to obtain phenolic compounds from *Senna alata (L.)* were identified. The results have shown the influence of microwave power on the extraction efficiency. Adjusting the microwave power is a critical factor in optimizing the extraction process for phenolic compounds. Next, phytochemical tests confirmed the presence of phenolic compounds in *Senna alata (L.)* extracts, providing confirmation for the second

objective. A little plant extract and adding a few drops of ferric chloride solution has resulted in the development of a color change, such as the greenish-black color of the solution and the formation of a black precipitate indicating the presence of phenolic compounds. Through the phytochemical test of phenolic compounds, it can be seen that the higher the microwave power, the darker the color of the senna alata extract sample. This has shown the second objective has been successful. The final objective, to characterize the extract using phytochemical screening, Ultraviolet-Vis (UV-Vis) spectrophotometer, and high-performance liquid chromatography (HPLC). The final results were obtained through with extracts from *Senna alata (L.)* characterized thoroughly through phytochemical examination, UV-Vis spectrophotometer analysis, and HPLC. The combined results provided a detailed profile of the phenolic compounds present in the extract, confirming the success of the characterization process. This alignment illustrates how each objective has been addressed and confirmed by the final results obtained in the study has been successful.

Selective heating of target compounds by microwaves concentrates and amplifies their bioactivity. This is especially important for *Senna alata*, where phenolic acids have strong antioxidant, anti-inflammatory and antimicrobial properties. The preservation of this bioactivity through MAE paves the way for the development of nutraceuticals and nutraceuticals derived from this versatile plant. Despite the immense potential of MAE, certain limitations warrant consideration. Optimizing process parameters like microwave power, extraction time, and solvent selection is crucial for maximizing yield and bioactivity while minimizing energy consumption. Additionally, the potential leaching of intracellular components and the formation of undesirable degradation products necessitate further investigation and fine-tuning of the MAE process for *Senna alata.*

In addition, the potential of the MAE method has been successfully explored and reported in this research. This refers to the third objective of this research which is *Senna alata L.* leaf extract characterized by using phytochemical screening, Ultraviolet-visible spectrophotometer (UV-Vis), and High performance liquid chromatography (HPLC). Based on the determination of the presence of compounds using phytochemical screening, *Senna alata L.* leaf extract gave positive results for the presence of tannins. By using UV-Vis spectroscopy to analyze the concentration of total phenolic compounds, the highest concentration of total phenolic compounds using MAE extraction was found to be 5.6581 mg/mL at the extraction microwave power parameter.

5.2 Recommendations

After completing this study, some improvements can be taken action in order to make it more reliable for future research. The first recommendation is by gives observations on extraction parameters (time and temperature). Optimization of extraction time and temperature is important to maximize the yield of phenolic compounds. A balance between time and temperature should be achieved to avoid thermal degradation of sensitive compounds.

Next, among the important factors affecting the success of MAE, microwave power and frequency play an important role. Optimization of microwave power and frequency can improve selectivity and extraction efficiency. However, safety precautions should be taken to avoid overheating and degradation of thermolabile compounds. Therefore, optimizing microwave power and frequency is essential to achieve efficient extraction while maintaining the integrity of target bioactive compounds.

In addition, the moisture content of *Senna alata* plant material is an important parameter that can significantly affect various aspects of its use and processing. Proper drying of *Senna alata* leaves before extraction is important to prevent mold growth and ensure the stability of the extracted phenolic compounds. In the MAE extraction process, the moisture content of the plant material can affect the extraction efficiency. Properly dried leaves often yield higher concentrations of bioactive compounds due to better mass transfer during the extraction process. Therefore, the sample storage method must be away from humid temperatures.

To characterize the chemical composition of materials including phenolic compounds, Fourier Transform Infrared Spectroscopy (FTIR) is a recommended analytical technique. FTIR can be used for quantitative analysis of phenolic compounds in a mixture. By comparing the

intensity of specific peaks in the infrared spectrum to calibration curves, the concentration of phenolic compounds can be determined. In addition, FTIR provides structural information about phenolic compounds. For example, the position and intensity of peaks in the spectrum can give insights into the substitution pattern on the phenolic ring and the presence of other functional groups.

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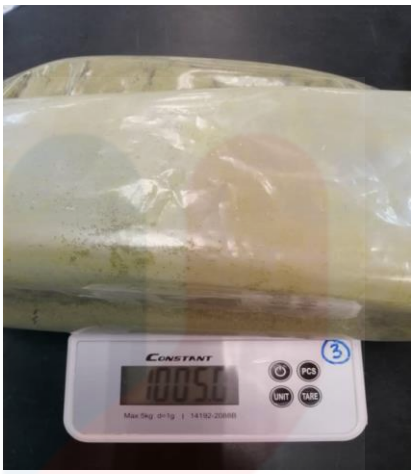
APPENDIX A

Process of preparation of plant sample

Place of temporary sample storage

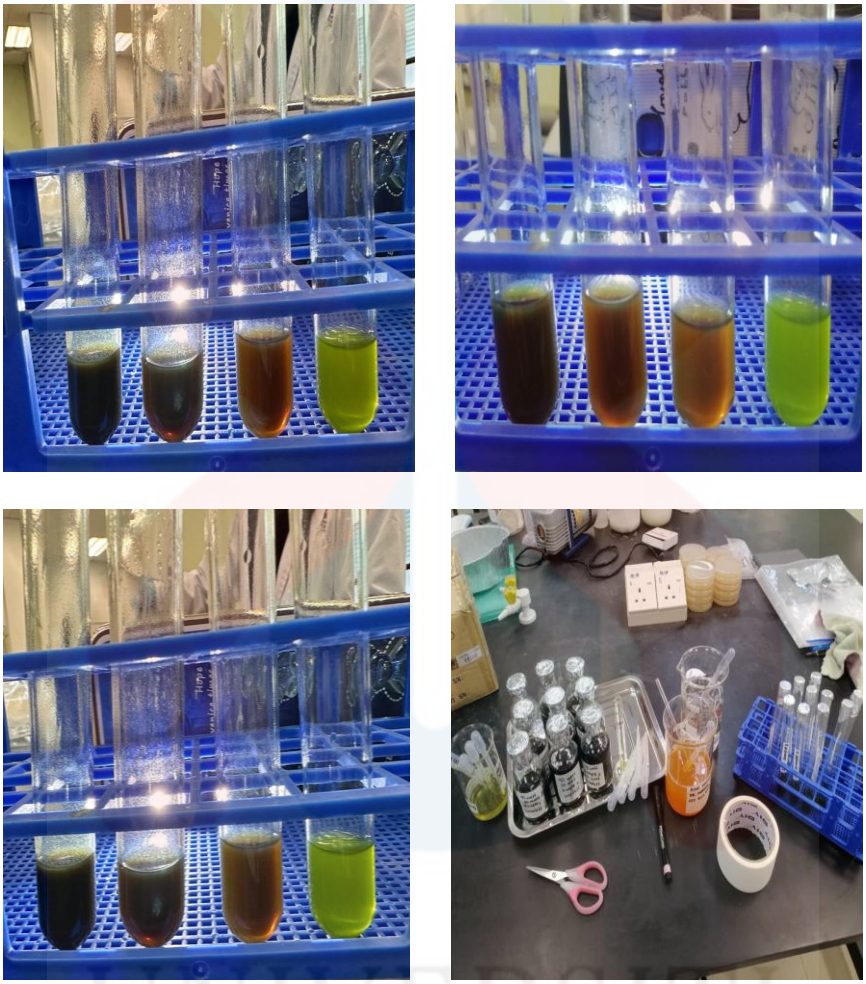


The powder sample undergo sieving process using sieve tools

APPENDIX B

Extraction process using MAE method for extraction of Senna alata

APPENDIX C



Phytochemical test process

APPENDIX D

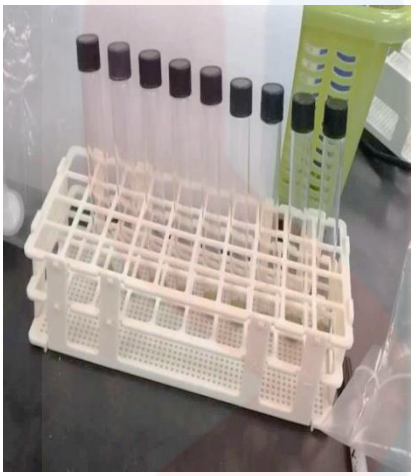
Table D.1: Result of tannic acid standard for UV-Vis spectrophotometers

#	Sample ID	User Name	Date and Time	Triplicates 276nm	Avg Abs276nm
1	Sample1	user	12/12/2023 12:55 PM	0.298 0.281 0.297	0.292
2	Sample2	user	12/12/2023 1:00 PM	0.418 0.389 0.412	0.406
3	Sample3	user	12/12/2023 1:05 PM	0.498 0.488 0.464	0.483
4	Sample4	user	12/12/2023 1:06 PM	0.584 0.533 0.545	0.554
5	Sample5	user	12/12/2023 1:11 PM	0.605 0.684 0.649	0.646

Table D.2: Results of UV-Vis analysis of Senna alata leaf extract of phenolic compounds

#	Sample ID	User Name	Date and Time	Triplicates 276nm	Avg Abs276nm
1	Sample1	user	18/12/2023 2:44 PM	0.239 0.226 0.218	0.228
2	Sample2	user	18/12/2023 2:46 PM	0.170 0.281 0.183	0.211
3	Sample3	user	18/12/2023 2:48 PM	0.248 0.224 0.201	0.225
5	Sample4	user	18/12/2023 2:56 PM	0.285 0.302 0.296	0.294
6	Sample5	user	18/12/2023 2:58 PM	0.258 0.235 0.262	0.252
7	Sample6	user	18/12/2023 2:59 PM	0.250 0.242 0.228	0.240
8	Sample7	user	18/12/2023 3:09 PM	0.493 0.476 0.484	0.484
9	Sample8	user	18/12/2023 3:11 PM	0.386 0.364 0.370	0.373
10	Sample9	user	18/12/2023 3:12 PM	0.367 0.381 0.349	0.366

APPENDIX E



HPLC process