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EXTRACTION OF QUERCETIN FROM *senna alata (L.)* LEAVES USING ULTRASONIC-ASSISTED EXTRACTION (UAE)

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DECLARATION

I declare that this thesis entitled "**Extraction of Quercetin from *Senna Alata (L.)* Leaves Using Ultrasonic-Assisted Extraction (UAE)**" is the results of my own research except as cited in the references.

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Pengekstrakan Kuersetin daripada Daun *Senna Alata (L.)* Menggunakan Pengekstrakan Berbantukan Ultrasonik (UAE)

ABSTRAK

Kajian ini membincangkan pengekstrakan flavonoid daripada *Senna alata (L.)* menggunakan kaedah pengekstrakan berbantu ultrasonik (UAE). *Senna alata (L.)* telah dilaporkan mempunyai potensi anti-alergi, anti-radang, antioksidan, antikanser, antidiabetik dan sifat antikulat. Kuersetin adalah ahli sebatian flavonoid dengan anti-radang dan antimikrob yang tinggi yang biasanya digunakan dalam produk perubatan untuk merawat gatal-gatal, kudis, dan kurap. Kuersetin, memberi kesan biologi dan farmakologinya dengan meningkatkan prestasi fizikal, mengurangkan risiko jangkitan, dan merawat keadaan kulit radang dengan berkesan. Kajian ini bertujuan (1) untuk menyiasat kehadiran kuersetin dalam *Senna alata (L.)* melalui ujian fitokimia, (2) untuk menentukan parameter proses pengekstrakan optimum menggunakan HPLC, dan (3) untuk mencirikan ekstrak flavonoid menggunakan FTIR, Kromatografi cecair berprestasi tinggi (HPLC) dengan pengesanan spektrofotometri ialah kaedah yang dipilih untuk kuantifikasi, membenarkan pengasingan dan penentuan flavonoid dalam ekstrak tumbuhan berdasarkan kawasan atau ketinggian puncak. Dua parameter telah dikaji iaitu, masa sonikasi (10, 20, 30 minit) dan kitaran tugas (40, 50, 60%). Saringan fitokimia mengesahkan kehadiran flavonoid dan fenolik dalam ekstrak dengan melihat perubahan warna daripada putih dan hijau gelap. Parameter optimum yang diperoleh daripada pengekstrakan ialah 20 minit sonikasi menghasilkan 9.842 mg/mL kepekatan kuersetin dan 60% daripada kitaran tugas mencatatkan kepekatan kuersetin sebanyak 8.787 mg/mL. Analisis FTIR berjaya mencirikan ikatan kimia dan kumpulan berfungsi: alkohol alifatik primer dan hidrokarbon alifatik. Ujian resapan cakera menunjukkan aktiviti antibakteria terhadap *E. coli* dan *S. aureus*. UAE terbukti sebagai teknik pengekstrakan hijau yang berkesan untuk kuersetin daripada *S. alata (L.)* berbanding kaedah konvensional, menawarkan kelebihan masa pengekstrakan yang lebih singkat, hasil yang lebih tinggi, penggunaan pelarut yang berkurangan dan mesra alam.

Kata kunci: *Senna alata (L.)*, UAE, Flavonoid, Kuersetin, FTIR

Extraction of Quercetin from *Senna Alata (L.)* Leaves Using Ultrasonic-Assisted Extraction (UAE)

ABSTRACT

This study discusses the extraction of querectin from *Senna alata (L.)* using the ultrasonic-assisted extraction (UAE) method. *Senna alata (L.)* has been reported to have potential anti-allergic, anti-inflammatory, antioxidant, anticancer, antidiabetic, and antifungal properties. Quercetin is a member of flavonoids compound with high anti-inflammatory and antimicrobial that usually used in medicinal product to treat itching, scabies, and ringworm. Quercetin, with its biological and pharmacological effects, improves physical performance, reduces infection risk, and effectively treats inflammatory skin conditions. The study aims (1) to investigate the presence of quercetin in *Senna alata (L.)* through phytochemical tests, (2) to determine the optimum extraction process parameters using HPLC, and (3) to characterize the flavonoid extract using FTIR. High-performance liquid chromatography (HPLC) with spectrophotometric detection is the chosen method for quantification, allowing the separation and determination of flavonoids in plant extracts based on peak areas or heights. Two parameters studied namely, sonication time (10, 20, 30 mins) and duty cycle (40, 50, 60%). Phytochemical screening confirmed the presence of flavonoids and phenolics in the extracts by seeing its colour changing of white precipitate and dark green. Optimum parameters obtained from the extraction are 20 minutes of sonication resulted 9.842 mg/mL quercetin concentration and 60% of duty cycle recorded 8.787 mg/mL quercetin concentration. FTIR analysis successfully characterized the chemical bonds and functional groups: primary aliphatic alcohols and aliphatic hydrocarbon. Disc diffusion tests demonstrated antibacterial activity against *E. coli* and *S. aureus*. UAE proved to be an effective green extraction technique for quercetin from *S. alata (L.)* compared to conventional methods, offering advantages of shorter extraction time, higher yields, reduced solvent usage, and eco-friendliness.

Keywords: *Senna alata (L.)*, UAE, Flavonoid, Quercetin, FTIR

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%	percent	12
°C	degree Celsius	18
ATR-FT-IR	Attenuated Total Reflectance Fourier Transform	22
cm	centimetre	15
cm^{-1}	centimetre	22
COX-1	Cyclooxygenase 1	43
COX-2	Cyclooxygenase 2	43
e-coli	*Escherichia coli*	10
$FeCl_3$	iron chloride	20
FT-IR	Fourier Transform Infrared Spectra	3
g	gram	18
Hcl	Hydrochloric acid	20
HPLC	High Performance Liquid Chromatography	20
Infrared Spectroscopy		
Kg	kilogram	15

kHz	kilohertz	18
MAE	Microwave-Assisted Extraction	10
Mins	minutes	21
ML	millilitre	18
mL/min	milliliter/minute	21
NaOH	Sodium hydroxide	20
PLE	Pressurised Liquid Extraction	10
ROS	Reactive Oxygen Species	10
rpm	revolutions per minute	18
S. aureus	*Staphylococcus aureus*	23
S.alata (L.)	*Senna alata (L.)*	10
UAE	Ultrasonic-Assisted Extraction	2
UMK	University Malaysia Kelantan	15
v/v	volume/volume	21
µm	micrometre	21

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Herbal medicine has played a significant role in the evolution of fundamental health care since prehistoric times, and for good reason: it is the oldest and safest known therapeutic method. According to the World Health Organisation, 80% of the population in developing nations primarily uses herbal medication. Traditional medicine based on the properties of plant extracts has been known as herbalism. The primary sources of therapeutic chemicals that may result in the development of novel therapeutic compounds have been considered to be medicinal plants with extensive biological applications. *Senna alata (L.)* is one of the medicinal plants being explored for its unique and diverse pharmacophore. The Fabaceae family contains the medicinal plant *Senna alata (L.)*. It was found in tropical and wet areas. The plant, which goes by various names, was frequently found in Asia and Africa. According to Fatmawati (2020), this plant was locally called Ketepeng Cina in Indonesia and Gelenggang in Malaysia. *Senna alata (L.)* also known as *Cassia alata (L.)*. *Senna alata (L.)* is a shrub with a flower resembling a yellow candlestick that often grows in the tropics such as Malaysia, India and Indonesia.

It contains a variety of chemical compounds that are bioactive and can be used as traditional medicines to treat a wide range of illnesses, particularly skin-related issues. Additionally, *Senna alata (L.)* has been reported to have potential anti-allergic, anti-inflammatory, antioxidant, anticancer, antidiabetic, and antifungal properties. Flavones, flavonols, flavonoids, glycosides, alatinon, alanonal, and sitosterol-D-glucoside are among the metabolite substances that have been identified from *Senna alata (L.)* (Oladeji, 2020). The leaves are where the chemicals are primarily isolated. In folk medicine, several plant parts were described as medicinal agents for treating a variety of illnesses and infections. The root, for instance, can be used as a laxative and to cure rheumatism.

Because of their medicinal and antifungal properties, seeds and leaves are used in India to treat eczema. In the meantime, *Senna alata (L.)* formulations are used in traditional herbal medicine to treat skin disorders in a variety of countries. Both the extracts and the isolated compounds have distinct pharmacological effects.

Previous studies by Rahmawati & Prihantini et al. (2022) showed that the extraction of *Senna alata's (L.)* leaves contained flavonoids, quinones, tannins, terpenes, sterols, saponins, and alkaloids. In this study, the main considerable chemical compound that wanted to be extracted was quercetin. Quercetin is a pigment that belongs to the flavonoid family. Flavonoids can be found in the leaves, petals, stem, and roots of *Senna alata (L),* but the leaves contain the most metabolites of the active compound.

Based on research done by Chemat and Abert-Vian (2019), eco-friendly plant extraction is a green process that has the least impact on the environment. Green extraction of organic substances is based on the design of extraction procedures that decrease or remove the use of energy and petroleum solvents while ensuring the safety and quality of the extract. It is a theory for meeting today's challenges of the twenty-first century by protecting our surroundings and the public while also increasing academic and industrial competitiveness to be more ecological, economic, and innovative. Examples of green extraction methods were ultrasonic-assisted extraction (UAE), pressurised liquid extraction (PLE), and microwave-assisted extraction (MAE). This advanced extraction method is extensively used for the extraction of bioactive compounds.

Ultrasonic-assisted extraction (UAE) technique was used in this research in order to extract flavonoid from *Senna alata (L).* Due to its high efficacy, UAE can be analysed in an eco-friendlier manner, requiring less solvent and energy (Chaves., 2020). Increased solvent-sample contact enhances mass transfer, resulting in increased yields in less time (Um et al., 2018). UAE allows for quicker extractions with high repeatability, a higher return on investment, simpler manipulation and processing, and a purer final product than the conventional extraction method, which employs hazardous solvents and is not eco-friendly. There are two parameters used in this study which are the sonication time and duty cycle of ultrasonic.

Additionally, duty cycle has an impact on the extract's antioxidant activity and the output of total flavonoids. The intensity and period of the ultrasonic treatment both had an impact on the duty cycle's influence on the extraction yield. Sonification time can affect the yield of flavonoid. Characterisation of flavonoids compounds of *Senna alata (L.)* was performed after ultrasonic-assisted extraction (UAE). Phytochemical screening was used in this study as a qualitative method to determine the existence of flavonoids content. FTIR used as a qualitative method for the extract to identify sample functional groups, while HPLC was used for the determination of quantify compounds.

1.2 Problem Statement

Conventional method, which is solvent-based extraction, required solvent and high temperature to possess a quality and yield of *Senna alata (L.)*. This extraction method faces problems, which result in low yields of extraction, a longer extraction time, and a low-quality end product due to traces of organic solvents. Furthermore, solvent used in conventional methods was methanol which would produce toxic and not even environmentally friendly. Therefore, ultrasonic-assisted extraction (UAE) was crucial for the green option as to replace conventional methods in order to use low consumption of solvent. A parameter study, which was sonication time and duty cycle, was investigated to determine the optimal conditions of the extraction process using UAE. UAE expected to produce high quercetin content, short time, be more ecologically friendly, and more automated to produce high quality products and also reduce greenhouse gas emissions to extraction of bioactive substances (Avinash., 2021).

1.3 Objectives

The main aim of this study was to extract flavonoids from *Senna alata (L.)* by using the ultrasonic-assisted extraction (UAE) method. For further illustrate and guide the study, the following objective was set:

I. To investigate the presence of quercetin from *Senna alata (L.)* by using phytochemical test.

II. To determine the optimum extraction process parameters (sonication time and duty cycle percentage) for the extraction of quercetin from *Senna alata (L.)* by using HPLC.

III. To characterise the flavonoid of *Senna alata (L.)* extract by using FTIR.

IV. To figure out zone inhibition of antibacterial effectiveness of *Senna alata (L.)* extract by using disc diffusion method.

1.4 Scope of Study

In this study, ultrasonic-assisted extraction (UAE) method was used to extract quercetin from *Senna alata (L.)*. There are two parameters, which are sonication time and duty cycle. The extract sample after UAE extraction was undergoes phytochemical screening to identify the presence of flavonoid. HPLC was used to determine the concentration of quercetin as from crude extract compound sample to give quantitative results. FTIR method was used to characterise the chemical bond of quercetin that obtained in the extraction of *S.alata (L.).* Antibacterial test used to evaluate antibacterial properties of the ethanolic extracts of *S. alata*.

1.5 Significant of Study

In this research, ultrasonic-assisted extraction (UAE) was expected to show a good promising method to extract flavonoid in *Senna alata (L.)*. The result expected is increasing extraction yield by improving the solvent ability to penetrate the cells, shorter extraction time with rapid return on investment, higher recovery and reduced solvent used in extraction process compared to conventional extraction methods (Chemat., 2017). Traditional flavonoid extraction methods are being phased out in favour of more effective, selective, solvent- and energy-saving alternatives in order to meet rising consumer demand and environmental regulations. Microwaves, ultrasonic, pressurised liquids, supercritical fluids, and electric fields are among the current options. These advanced extraction methods are often quicker, more eco-friendly, and more automated than traditional ones.

CHAPTER 2

LITERATURE REVIEW

2.1 *Senna alata (L.)* species

Figure 2.1: *Senna alata (L.)* leaves

(Sources: Armah, 2021)

Senna alata (L.) is a plant that can grow wild, plentiful, and prolific in the tropics, particularly in Malaysia, which has a tropical environment with a dry season and a wet season. *Senna alata (L.)* plants typically flourish in valley to mountainous regions at elevations of 1400 metres (Prihantini., 2022). This plant has erect stems that are between 10 and 15 feet tall, skin that is thin and not spiky, and leaves that are slightly wider and yellowish green in colour. The flowers grow in a race and have a vivid yellow colour. When ripe, the brown seeds and hard fruit resemble a brown pod. The dicotyledonous *Senna alata (L.)* plant has a taproot system and is classified as such. The root develops into a prime root and produces smaller branches to increase nutrient absorption and reinforce the stem's upright growth.

Figure 2.1 shows the stem is spherical, reaches heights of 3 to 4 metres, and branches out in a sympodial pattern; the leaves are yellowish green and slightly wide, growing to 50 to 80 centimetres in length; the flowers are brilliant yellow; and the fruit has a spicy, warm flavour. The high protein and carbohydrate content of the seeds makes them a popular alternative to nuts. *Senna alata (L.)* is a plant that typically grows along roadsides, riverbanks, rivers, woodlands, shrubs, meadows, lakesides, and ponds because it prefers open, humid environments. Full sun and moisture are ideal for *Senna alata (L.)* growth. It grows best on soils that are rich to sandy, acidic to slightly alkaline, and properly drained, with a pH range of 5.6 to 7.8. According to study, this species could flourish in areas with typical annual temperatures of 15-30℃ and yearly precipitation of 600-4,300 millimetres. Tannins, alkaloids, flavonoids, terpenes, anthraquinones, saponins, phenolics, and cannabinoid alkaloids are among the metabolites found in *Senna alata (L.).*

Multiple studies demonstrated that the *Senna alata (L.*) showed antibacterial activity against Gram-positive and harmful bacteria namely *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Escherichia coli* and many more.

2.2 Bioactive Compound of *Senna alata (L.)*

Regardless of the desired biological tests, bioactive chemicals derived from medicinal plants are employed for a variety of objectives, but the method utilised to produce them is typically the same. Tannins, alkaloids, flavonoids, terpenes, anthraquinones, saponins, phenolics, and cannabinoid alkaloids are among the metabolites found in *Senna alata (L.). S. alata (L.)* leaves contain metabolites more frequently than other plant parts (Rahmawati, 2022). *S. alata (L.)* possesses antifungal properties, particularly against fungi like *A. niger* that cause skin diseases in people. Due to its secondary metabolites, *senna alata (L.)* extract exhibited antibacterial activity comparable to that of penicillin, chloramphenicol, fluconazole, and ciprofloxacin. Chinese Ketepeng extract has been demonstrated to have anti-inflammatory properties in prior investigations (Christina., 2022).

2.2.1 Flavonoids

Flavonoids are the main polyphenols in human nutrition. All flavonoids are based on the fifteen-carbon flavone C6 (A ring)-C3 (C ring)-C6 (B ring), which is made up of two benzene rings (A and B) joined by an oxygen-containing heterocyclic pyrene ring (C). Flavonols, isoflavones, flavones, chalcones, and anthocyanidins are the six subgroups of flavonoids based on the carbon of the C-ring in which the B-ring is attached and the degree of saturation and oxidation of the C-ring (Nour., 2021). The plant's leaves include antifungal and antibacterial properties, which are traditionally used to treat skin disorders caused by fungi. *Senna alata (L.)* may be used as a supplemental treatment for diabetes and cancer since it has anti-inflammatory and antioxidant qualities. Asthma, urticaria, and itching are all allergic disorders that may be treat alternatively thanks to the plant's active ingredients, which have shown anti-allergic activity.

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

(Source: Oladeji, 2020)

	Parts used	Country	Ethnomedicinal use	Solvent used	Pharmacological activity	Model used	Phytochemicals	References
(1)	Leaves	Nigeria	Treatment of diarrhoea, upper respiratory tract infection, and to hasten labour	Aqueous	Abortifacient	Pregnant rats	Saponins, flavonoids, cardiac glycosides, cardenolides, dienolides, phenolics and alkaloids	[55]
(2)	Leaves	India	To manage diabetes	Ethanolic	Hepato-renal protective effects	Male albino Wistar rats		[56]
(3)	Leaves	India	Treatment of allergy/asthma	Hydromethanolic	Antiallergic	Lipoxygenase (LOX) enzyme	Rhein and kaempferol	[7]
(4)	Leaves	Thailand	To manage diabetes and weight	Aqueous	Antilipogenic	High-fat diet-induced obese mice		[57]
(5)	Leaves	India	Treatment of bacterial infections	Methanol	Antibacterial	Pathogenic bacterial strains		[58]
(6)	Leaves	Cameroon	Treatment of gonorrhoea, gastrointestinal and skin diseases	Methanol	Antibacterial	Multidrug-resistant (MDR) *Vibrio cholerae* and *Shigella flexneri*	Kaempferol, luteolin and aloe-emodin	[59]
(7)	Leaves	India	To manage diabetes	Methanol	Antidiabetic	α-Glucosidase enzyme	Kaempferol 3-O-gentiobioside	[31]
(8)	Leaves	Thailand	Treatment of skin infections	Anthraquinone	Antifungal	STZ-induced diabetic rats	Anthraquinone	[60]
(9)	Leaves	Nigeria	To wash the uterus	Hexane	Anti-implantation, antigonadotropic, antiprogesteronic	Ovariectomized female rats	Alkaloids	[61]
(10)	Flower	Nigeria	Treatment of urinary tract infections and gonorrhoea	Methanol	Antibacterial	Bacterial strains	Steroids, anthraquinone glycosides, volatile oils and tannin	[62]
(11)	Flower	India	To treat scabies and ringworm	Aqueous	Antifungal	Aflatoxin producing and human pathogenic fungi		[63]
(12)	Leaves and barks	Malaysia	To treat superficial fungal infections	Ethanol and water	Antimicrobial	Bacterial and fungal strains		[64]
(13)	Leaves	Cameroon		Ethanol	Antioxidant and anti-inflammatory	White blood cells		[30]
(14)	Leaves	India	Used as purgative expectorant, astringent, vermicide	Ethanol	Anticancer	Male Wistar rats	Alkaloid, flavonoids, saponins, tannins glycosides	[65]
(15)	Leaves	Indonesia	Treatment of malaria; antioxidant and antibacterial	Ethanol	Antiviral	DENV-2 and Huh 7it cells	Flavonoid	[66]
(16)	Petals	India	As an immune stimulant	Petroleum ether	Immunomodulatory	*Garra rufa* fish	Cardiac glycosides, phenols, anthraquinone, alkaloids	[67]

	Parts used	Country	Ethnomedicinal use	Solvent used	Pharmacological activity	Model used	Phytochemicals	References
(17)	Root	Nigeria	As an abortifacient in women, for the termination of early pregnancy	Ethanol	Uterine smooth muscle	Male and female Albino mice	Alkaloids	[68]
(18)	Leaves	Burkina Faso	Treatment of asthma-induced bronchospasm	Aqueous and ethanolic	Bronchorelaxant, genotoxic, and antigenotoxic	Male and female Wistar rats		[69]
(19)	Leaves	Egypt	Treatment of skin tumour	Methanol	Antitumor	Human cancer cell lines (HepG2, MDA-MB-231, and Caco2)	Palmitic, linolenic, linoleic, stearic acid	[70]
(20)	Leaves	Thailand	Laxative	Methanol	Anti-inflammatory	Tert-butyl hydroperoxide-induced oxidative stress in HaCaT cells	Rhein	[71]
(21)	Leaves, flower and fruit	Nigeria	Laxative and treatment of microbial infections	Methanol and ethanol	Antifungal and antibacterial	Clinical bacterial and fungal isolates	Flavonoids	[72]
(22)	Leaves	Thailand	In regulating glucose level in the blood	Aqueous	Antilipogenic	Male ICR mice		[73]
(23)	Leaves	Cameroon	To cure fever	Aqueous and methanolic	Antiplasmodial	RPMI 1640 and albumax		[74]

2.2.2 Quercetin

Quercetin is classified as a flavonol, which is one of the 6 subclasses of flavonoid compound (Kelly., 2011). Quercetin has biological properties that can improve the metal or physical performance and reduce the risk of infection. Quercetin, which possesses pharmacological effects, is a promising discovery in nutritional and medicinal research. Inflammatory skin conditions respond well to the long-lasting anti-inflammatory drug quercetin. It keeps the skin and joints healthy by promoting the production of collagen and fibronectin. Because of this, quercetin benefits those who have inflammation as well as those who seek to treat or prevent wrinkles (Aarti Sharma & Himanshu Gupta., 2010).

Quercetin also inhibits the immune system's response to dendritic cells (Huang et al., 2010). Previous research suggests quercetin has the capacity to fight cancer. Getting rid of free radicals, blocking enzymes that activate carcinogens, altering the pathway of signal transduction, and interacting with receptors and other proteins were said to cause this impact (Nathiya., 2022).

2.2.2.1 Mechanism of Flavonoids as Antioxidant

Plants contain large amounts of flavonoids, a group of chemical compounds. They are appreciated for their antioxidant characterisation of due to their capability to go through free radicals and other reactive oxygen species (ROS) that can injure cells and tissues. Flavonoids have antioxidant capabilities because of its molecular makeup, which includes one or more hydroxyl groups that can donate electrons to neutralise free radicals (Rahmawati., 2022). Numerous studies have shown that flavonoids can protect tissues and cells from oxidative damage, which has been associated with a range of chronic disorders. Research on the antioxidant properties of a few flavonoids, including rutin, quercetin, kaempferol, and myricetin, is extensive.

2.2.2.2 Mechanism of Flavonoid as Antibacterial

Numerous biological actions of flavonoids, including antimicrobial capabilities, have been demonstrated. By interfering with their enzyme systems, disrupting their cell membranes, or preventing DNA replication, certain flavonoids have been discovered to stop the growth of various bacteria. Flavonoids have been demonstrated in multiple trials to be effective against a variety of bacterial strains, including ones that are resistant to antibiotics. For instance, it has been shown that the flavonoid quercetin, which is present in medicinal plants, is efficient against a number of bacteria, such as *E. coli, Salmonella,* and *Staphylococcus aureus.*

2.3 Extraction Method

There are various ways to extract quercetin from *S. alata (L.).* The method used were divided into conventional and non-conventional extraction. Conventional extraction method of flavonoid compound is maceration, decoction, separation, infusion, digestion, serial exhaustive extraction, and soxhlet extraction. For unconventional method, the method involved were ultrasonic-assisted extraction (UAE), microwave-asissted extraction (MAE), and pressurized liquid extraction (PLE). The traditional extraction procedure is more affordable and simpler to utilise. Additionally, investigations have shown that conventional procedures are inefficient and have a negative impact on the environment because it requires a large number of organic solvents. The traditional extraction process frequently includes a recovery step

which is pursued by extract concentration via an evaporation process that is a timewasting consuming process. Based on literature evidence, non-conventional method shows that it has been select as the best technique compared to the conventional method as it propositions plentiful benefits which incorporate fewer solvent volume, higher yields, reduced toxic residue, well process reproducibility, and fewer extraction time (Chaves., 2020).

The effectiveness of ultrasonic-assisted extraction is influenced by a number of factors, including ultrasound frequency and intensity, process length and temperature, liquid-to-solid ratio, solvent type, concentration, pH, and sonicator duty cycle (continuous or pulse).

2.3.1 Advanced Extraction Techniques

2.3.1.1 Ultrasound-Assisted Extraction (UAE)

To extract bioactive components from natural products, industries such as the food and pharmaceutical use ultrasound as an intensification technology (Akbari., 2019). UAE is applicable to a variety of matrices, including those for fruit, seeds, vegetables, and flowers. The most popular solvents for the extraction of flavonoids include ethanol, combinations with water at various ratios, and naturally occurring deep eutectic solvents because of their capability to solubilize moderately polar flavonoids at a relatively less cost and with low ecological damage. The ultrasound-assisted extraction method uses high-intensity sound waves to speed up the extraction of bioactive chemicals from plant-based raw materials. Sonication facilitates the portion fragmentation of plant tissue through acoustic cavitation and accelerates the mass transfer of recovered components into the solvent (Debora., 2017).

2.4 Effect on Sonication Time to Yield of Quercetin

The yield of quercetin by different sonication time are set up to determine the effect on the yield of quercetin. Heat and energy are released during sonication. More than 80 minutes of additional extraction time may cause temperature increases and damage the bioactive component. The cell wall structure is altered during the sonification process, which also speeds up membrane diffusion. Solute degradation

and decreased extraction effectiveness happen when ultrasonic extraction times are very long. Depending on the kind of raw material used, the ideal extraction period for flavonoids compounds has been calculated to be between 10 and 90 minutes (Srivastav., 2021).

2.5 Effect on Duty Cycle to Quercetin Yield

Based on previous studies, the yield of quercetin by different duty cycle percentage during sonification method from 90% to 45% was shown in Table 2.2. Duty cycle was uncovered to have a substantial impact on the antioxidant activity of total flavonoids extract yield. It was demonstrated that the intensity and duration of the UAE treatment determine the duty cycle effect on extraction yield. The ultrasonic processor's duty cycle had a positive effect on flavonoid content.

Table 2.2: Yield of Quercetin Based on Effect of Duty Cycle Parameter

(Source: Kamal, 2021)

No. of sample	Duty cycle (%)	Yield (mg/mL)	Standard deviation
1	90	3.764	0.083
2	75	2.985	0.014
3	60	1.905	0.087
4	45	1.038	0.081

2.6 Characterisation of Quercetin

Characterisation had classified into qualitative and quantitative methods. There are four characterising methods used in this study, which were phytochemical screening, FTIR, antibacterial test and HPLC. Phytochemical screening used to uncover the existence of flavonoids. Qualitative methods of this study were using FTIR to identify functional group in flavonoids and antibacterial test used to study antibacterial features and effectiveness on *S. alata (L.)* leaves. Lastly, the quantitative method was used HPLC to analysis the compound concentration of quercetin obtains from sample extract.

2.6.1 Fourier Transform Infrared Spectra (FTIR)

FTIR analyses the compound by exposing it to infrared radiation. A certain wavelength of energy is selectively absorbed by molecules, changing the sample's dipole moment. The side group and number replacement, when combined with variations in the stretching and bending modes of vibration, cause changes or overlaps in the peaks or several functional groups within the same IT spectrum. Based on the values of the peaks in the region of infrared radiation, the FTIR spectrum is used to identify the presence of functional groups that active chemicals represent during extraction. In order to identify the functional group of each molecule, FTIR analysis was performed on the standard quercetin and the supernatant following extraction in this study.

2.6.2 Antibacterial Tests

Antibacterial properties can be assessed from the ethanolic extract of *S. alata* by examining the zone of inhibition by disc diffusion method. The test microorganism was inoculated onto an agar plate using a standardized inoculum. An antimicrobial agent that diffuses into the agar is usually used to prevent the germination and growth of the test microorganism. The widths of the inhibitory growth zones are then measured.

2.6.3 High Performance Liquid Chromatography (HPLC)

HPLC is a mode of column chromatography that is commonly used in analytical chemistry to identify, separate and measure chemicals. HPLC employed a column containing 54 chromatographic packing materials (the stationary phase), a pump that drove the mobile phase within the column, and a detector that displayed the molecule retaining durations. The retention period is affected by the interactions amongst the stationary phases, the molecules being studied, and the solvents utilised.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Senna alata (L.) leaves were collected at Jeli district around UMK, Lata Keding, and a few villages around the UMK Jeli campus. Around 5 kg of plant leaves were collected for this study. Matured and young *Senna alata (L.)* with yellowish-green and green leaves with a slightly wide shape and a range length of 10-50 cm was used in this study. The leaves then were identified and authenticated by botanist from Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

3.2 Chemicals

Chemicals used in this study are ethanol, Iron III chloride ($FeCl_3$), distilled water, sodium hydroxide, hydrochloric acid, chloramphenicol, *e-coli*, *Staphylococcus aureus*, Natrium broth agar, acetic acid grade in solvent A, acetonitrile (HPLC grade) in solvent B, methanol HPLC grade, mobile phase HPLC water and quercetin standard HPLC analysis.

3.3 Apparatus and Equipment

All apparatus and equipment that were used in this study is stated on Table 3.1.

Table 3.1: List of apparatus and equipment used for this study.

No.	Apparatus	Equipment
1.	250 ml beaker	HPLC machine
2.	Sample bottle	FTIR Spectroscopy model Nicolet ™ iS ™ 10 FTIR Spectrometer (Thermo Scientific, USA)
3.	Dropper	Sieve
4.	Measuring cylinder	Ultrasonic-assisted extraction machine
5.	Falcon tube	Chiller
6.	Beaker	Blender
7.	Syringe filter	Laminar flow hood
8.	HPLC water,	Hot plate
9.	Aluminium foil	Analytical balance
10.	Spatula	Incubator shaker
11.	Zip log bag	Autoclave
12.	50 mL centrifuge bottle	Spectrophotometer
13.	Filter paper	HPLC wire
14.	250 mL centrifuge bottle	Centrifuge machine
15.	Test tube	Water bath machine
16.	Shake flask	
17.	1 L borosilicate glass bottle	
18.	Bunsen burner	
19.	Cotton swab	
20.	Inoculation loop	
21.	Petri dish	
22.	Micro pipette	

23.	Tips	
24.	L-shaped glass	
25.	Parafilm	
26.	2 ml HPLC autosampler short thread vial	

3.4 Preparation Of *Senna alata (L.)* Sample

The leaves of *Senna alata (L.)* were rinsed with running water. The leaves were then enduring two weeks of air drying (Oktaviyanti, 2021). The dried leaves then were blended with heavy duty blender into powder form before extraction process. The powder was undergoing sieve by using durable stainless steel chrome plated frame sieve to constant the mesh size (500 μm). The sample then was kept in zip-lock containers at room temperature and away from direct sunlight.

3.5 Extraction Process

3.5.1 UAE Procedure

2.5 g of powdered sample were soaked in 150 mL of ethanol due to the solid liquid ratio was 1:60. Then, it was stirred using a spatula until the mixture fully mixed. The operating condition was set at 26°C working temperature, 20 kHz frequency and 900 W ultrasonic power. The extraction was performed at different duty cycle 40,50, 60% and the sonication time was set to be fixed at 30 minutes. Next, further perform at different sonication times of 10, 20, 30 minutes and the duty cycle were fixed at 60%. All parameters were repeated three times to get accurate results. After all the parameters are done with sonication process, it was then be transferred into a 50 mL centrifuge bottle to be centrifuge with temperature of 21°C for 15 minutes with 5800 rpm. The supernatant was filtered, collect, and stored at 4°C chiller. All the samples then were tested on phytochemical screening test. All these setting for extraction parameter was stated on Table 3.2, 3.3 and Table 3.4.

Table 3.2: Constant parameter Setting on UAE.

Constant parameter	Ethanol (15 mL) Amplitude 60% 20 kHz frequency 26°C operating temperature 900 W ultrasonic power

Table 3.3: Manipulated and Constant for Parameter 1 (Duty Cycle)

Duty cycle	Sonication time
40%	60 mins
50%	
60%	

From parameter 1, the optimum parameter was selected at 60%. For the effect of sonication time, the selected optimum parameter that was obtained from effect of parameter 1 would be used in the effect of sonication time.

Table 3.4: Manipulated and Constant for Parameter 2 (Sonication Time)

Sonication time	Duty cycle
10 mins	Optimum percentage (60%)
20 mins	
30 mins	

3.5.2 Separation Of Solid and Liquid

To obtain the supernatant, the sample was centrifuged for 15 minutes at 5800 rpm. Filter paper was used to filter each supernatant. Then the substance was being analysed for phytochemicals. Finally, FTIR and HPLC were used to

characterise the supernatant. FTIR was used to identify functional group in extract while HPLC was used to determine the concentration of quercetin.

3.6 Phytochemical Screening

3.6.1 Test For Flavonoid

The test was be used in this part is called alkaline reagent test. Next, 1 mL of the aqueous leaf extract was mixed with two to three drops of diluted sodium hydroxide. the intense yellow colour that first appears and then becomes colourless when a few drops of diluted HCL are added, indicating the presence of flavonoids (Kancherla., 2019).

3.6.2 Test for Phenols

1 mL of water was added to a little amount of ethanolic extract in a test tube along with one or two drops of iron chloride ($FeCl_3$). A positive test result is indicated by the appearance of blue, green, red, or purple (Rao et al., 2016).

3.7 Characterisation Of Crude Extract

3.7.1 Calibration Curve for Quercetin Standard

The range of concentration quercetin was prepared. To obtain the calibration curves, serial dilution was prepared from the stock respectively concentration 0.2. 0.4, 0.6, 0.8, 1.0 mg/ml and was diluted in methanol HPLC grade. The concentration on the x-axis and absorbance on the y-axis was plotted accordingly for quercetin. Since quercetin concentrations in samples may be extremely low, a standard calibration curve can aid to increase measurement accuracy. Next, the calibration curve was used to evaluate the precision of the absorbance measurement device (Wahlang., 2021).

3.7.2 HPLC Setting for Detection of Flavonoids

For the pre-treatment, the standard sample were filtered through 0.45µm syringe filter before being injected into HPLC system. The concentration of flavonoids in plant powder was identified by using HPLC techniques. Then, the extracted sample was diluted to 10 mL with 30 HPLC water. The sample was analysed by MetaChem C18-A (250 × 4.6 mm, 5 µm) column. The mobile phase is HPLC water with acetic acid (99:0.1 v/v) in solvent A and acetonitrile (HPLC grade) in solvent B. The flow rate was fixed at 1 mL/min gradient program that used was 30, 60 and 90 % of solvent B at 1, 3 and 5 minutes respectively. All the setup is presented on **Table 3.5**.

Table 3.5: Conditions of HPLC Required for The Analysis of Quercetin.

HPLC Parameter	**Setting**
Column	MetaChem C18-A (250 × 4.6 mm,5 µ)
Flow Rate	1 mL/min
Injection Volume	20 µL
Running time	5 minutes
Mobile Phase: Solvent A Solvent B	• HPLC Water/ acetic acid (99:0.1 v/v) • Acetonitrile, ACN (HPLC grade)
Gradient Program	30, 60 and 90 % of solvent B at 1, 3 and 5 minutes respectively

3.7.3 Fourier Transform Infrared Spectroscopy (FT-IR)

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) was used to characterise flavonoids. The FTIR machine model used was a Nicolet iS50. Spectrum 400 FT-IR from Perkin Elmer was used for the sample testing. The wavenumber range set for FTIR was 4000-650 cm^{-1}, which was in the mid-infrared region. The liquid samples would be put on the plate for measurement during the FTIR using the ATR. Next, the software programme for FTIR would employ its built-in algorithm. Pre-processing of the spectrum would involve baseline correction to create a horizontal baseline shift and smoothing to remove noise. The pre-processed data that retrieved in Excel form and shows the relationship between absorbance and wavenumber (cm^{-1}) then was noted for statistical data analysis (Che Hafizah et al., 2017).

3.7.4 In-Vitro Antibacterial Assay

3.7.4.1 Agar Medium Preparation and Dilution

28 g of nutritional agar has been suspended in 1 L of distilled water. For the preparation of the medium, distilled water was used. The composition remains in this form until all components have dissolved. The medium then was sterilized in an autoclave for 15 minutes at 121°C. After the petri dish has been sanitized, the nutrient agar was placed on a level surface and allowed it to settle for half an hour so, the medium can solidify then was kept in a chiller.

3.7.4.2 Preparation suspension culture of bacteria

Two kinds of different gram-staining bacteria will be used in this study, *Escherichia coli (e-coli)* as gram-negative bacteria and *Staphylococcus aureus* as gram-positive bacterium. Approximately one colony of bacteria was dissolved in nutrient broth (13 g of nutrient broth dissolved in 1 L of distilled water, sterile by autoclaving at 121°C for 15 minutes before being used). A single colony bacterium was transferred by using a wire loop that has been sterilized with 90% ethanol and heat for at least 30 seconds to avoid cross-contamination. The bacteria culture was incubated overnight in an orbital shaker at 37°C with 150 rpm.

3.7.4.3 Disc Diffusion Method on Quercetin

Antimicrobial test was determined by using a well-diffusion method with *Escherichia coli (e-coli)* and *Staphylococcus aureus.* Two sample was prepared for this test which are sample before and after extraction. 50 µL of the samples was dropped on the divided section on petri dish. The positive control was chloramphenicol while for negative control was distilled water. The diameter of the clear zone of inhibition around the sample after 24 hours of incubation was measured by using a standard ruler.

3.8 Research Flow Chart

The research flow chart is divided into three stages. As illustrated in Figure 3.1, stage 1 is for sample preparation of *Senna alata (L.)* stage 2 is for extraction, and stage 3 is for analysis, assessment, characterisation and comparison of the obtained experimental data.

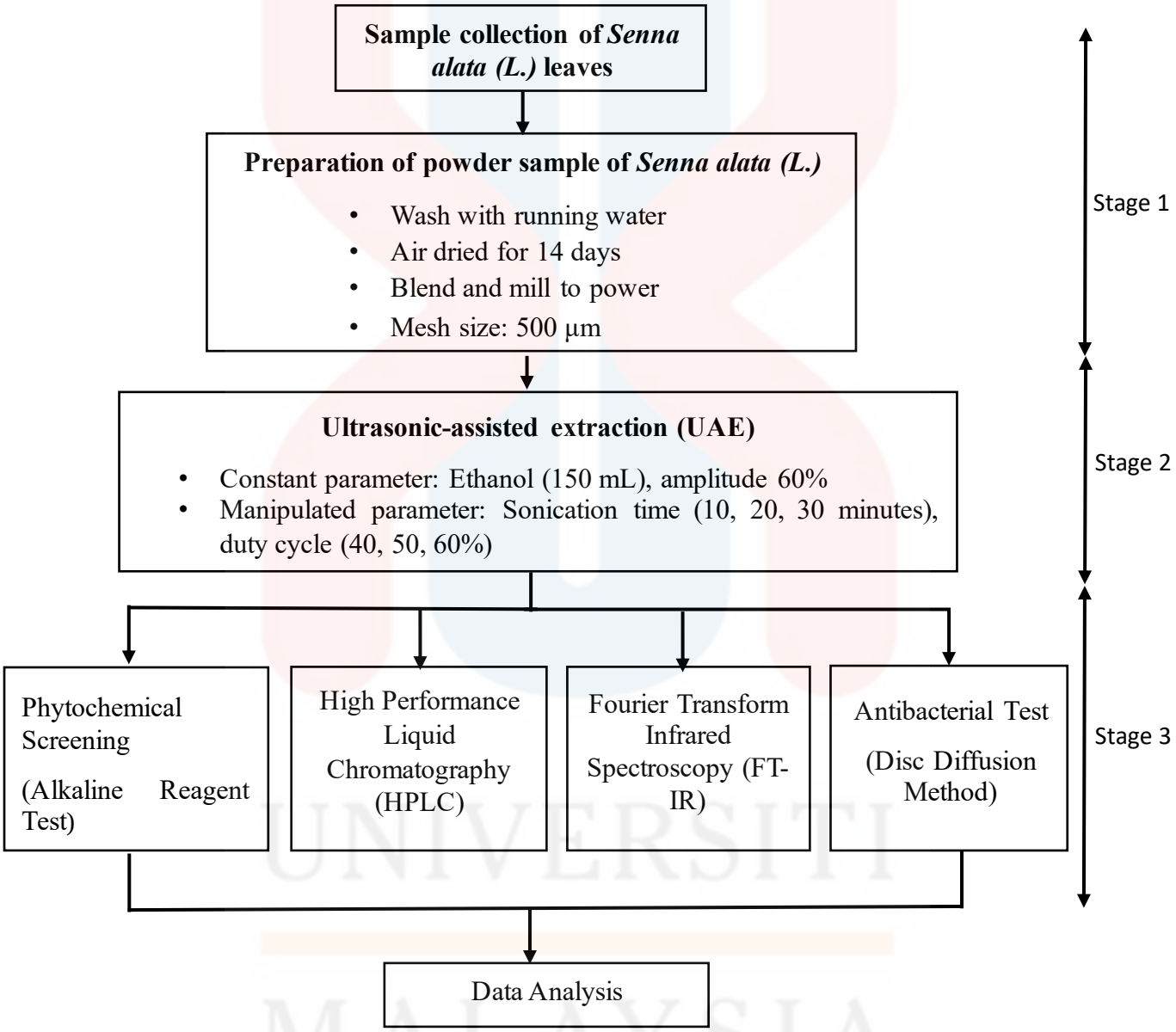

Figure 3.1: Research Flow for *Senna Alata (L).* Leaves Extraction

CHAPTER 4

RESULTS AND DISCUSSION

4.1

In order to extract the active components from *Senna alata (L.)* leaves, this chapter discusses the outcomes of the phytochemical screening, total phenolic and flavonoid compound using HPLC analysis, and FTIR-ATR analysis.

4.2 Phytochemical Screening

The *Senna alata (L.)* sample were extracted by using ethanol. Ethanol was chosen in this study because of ethanol was a universal solvent which can dissolve both polar and nonpolar substances due to its hydroxyl and ethyl groups. Ethanol solution can attract all types of chemical compounds, less toxicity, good absorbance, not easily grown by the microbes and suitable for use in consumer products such as anti-inflammatory products and drug preparation. The observation of *Senna alata (L.)* ethanol extract with different sonication time (10, 20, 30 mins) and duty cycle (40%, 50%, 60 %) were tabulated in Table 4.1.

Table 4.1: Result of phytochemical screening

Phytochemical	Duty cycle (%)			Sonication time (mins)		
	40	50	60	10	20	30
Flavonoids	+	+	+	+	+	+
Phenolics	++	++	++	++	++	++

Note: (+): Present

(++): Highly present

Based on the Table 4.1, the phytochemical screening of the *Senna alata (L.)* showed the positive results on flavonoid and phenolic compound. Flavonoid turned the solution colour from green to intensive yellow colour after added with sodium hydroxide then slightly colourless upon addition of hydrochloric acid. Phenolic turned the colour of the solution from green to dark green. A similar result was obtained that revealed the presence of terpenoid, flavonoids, tannin, saponin, and phenols in *S.alata (L.)* leaves by Prabha Lahare et al., (2020). This also has been proven by Onyegeme-Okerenta Bm et al., (2017) that alkaloid, flavonoid, saponin, tannin, and terpenoids are present in the *S.alata (L.)* leaves. Previously, Halim et al., (2019) reported that *S.alata (L.)* leaf contained several components of flavonoids glycoside. They found that the sun-dried *S.alata (L.)* leaf extract contained a strong inhibitory effect on concanavalin A-induced histamine release.

Referring to the Table 4.3, phenolics was highly present in the leaves of *S.alata (L.)* ethanolic extract. The dark green colour was present on phytochemical screening (see Table 4.2). Phytochemical screening as seen in Table 4.2 proved there was slight presence of flavonoids in the leaves of *S.alata (L.)* ethanolic extract. This happens because the yellow colour solution does not completely change to colourless but only become slightly colourless at the bottom. Flavonoids derived from natural sources are relatively low in toxicity and have bioactive properties in antioxidant effect (Rodríguez De Luna et al., 2020).

In contrast to the methanolic extract of room temperature dried leaf, which is decreased to about half of the value, the maximum total phenolic and flavonoid content was extracted using 95% (v/v) ethanol and dried at room temperature, according to a prior study by Halim et al. (2019). This finding indicates that the kinds of solvents employed in the extraction process have a significant impact on yields, as well as possibly the stability and activity, of the phytochemical components in the plant extract. The solubility of antioxidant chemicals in plant matrices may be influenced by the polarity and chemical properties of the solvents (Fazilah et al., 2019).

Besides type of solvents, the effect of solid-to-liquid ratio may also play an important role in the yields of total phenolic and flavonoid contents. The greater solvent-to-solid ratio used, the higher extraction yield resulted, but too high solvent ratio-to-solid will affect excessive extraction solvent and need a long time (Le et al., 2022).

Temperature used for extraction is a key factor relating to the efficiency of extraction. High temperature can cause the degradation of active components or excessive co-extraction of matrix components. Besides, the particle size must be accurately measured as it can affect the extraction yield and the phytochemical structure (Kobus et al., 2022). The *S. alata (L.)* leaves sample have been grinded into ground powder form as the small particle sizes are commonly favourable to high extraction yield.

Based on the results in this study, this finding supported previous research finding conducted by (Atanu et al., 2022), that leaves of *S. alata (L.)* has high phenolic content compared to flavonoid content.

Table 4.2: The observation of Flavonoid compound in *S. alata (L.)* ethanolic extract.

Sample	*Senna alata (L.)* ethanolic leaf extract sample		
	Before	After NaOH	After Hcl
1A3 (20 mins)			
2A1 (30 mins)			
3A1 (10 mins)			

Table 4.3: The observation of Phenolic compound in *S. alata (L.)* ethanolic extract.

Sample	*Senna alata (L.)* leaves sample	
	Before	After $FeCl_3$
4A1 (40%)		
5A3 (50%)		
6A3 (60%)		

4.3 Quantitative Method (HPLC Analysis)

In this study, the quantitative methods that used was high-performance liquid chromatography (HPLC). HPLC with spectrophotometric detection was the methods of choice for the separation of complex mixtures containing non-volatile compound such as flavonoids in plant extract. Quantification uses peak areas or heights to determine the concentration of a compound in the sample.

4.3.1 Calibration Curve

The quantity and response data for a particular analyse (compound) acquired from one or more calibration samples were represented graphically by a calibration curve. An aliquot of the standard solution at a different concentration was injected, and the peak area that resulted was measured to generate the curve (Al-Rifai et al., 2015). The peak area of the standard quercetin using HPLC analysis is illustrated in Figure 4.1. The retention time for the standard quercetin was 1.830 minutes and the area for 0.2 mg/mL concentration of quercetin was 151429 AU.

Figure 4.1: The HPLC peak of 0.20 mg/mL standard quercetin.

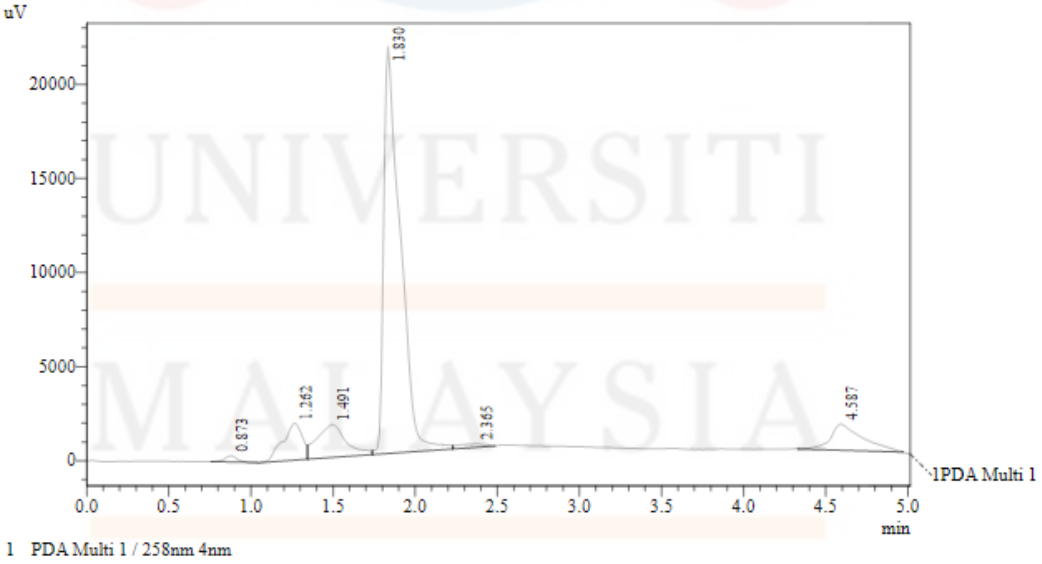

Table 4.4: The HPLC peak of 0.20 mg/mL standard quercetin.

Peak	Ret. Time	Area	Height	Area (%)	Height (%)
1	0.873	1680	325	0.791	1.192
2	1.262	16646	1966	7.832	7.213
3	1.491	21575	1754	10.151	6.435
4	1.830	151429	21634	71.246	79.378
5	2.365	2250	185	1.058	0.680
6	4.587	18963	1390	8.922	5.101

Table 4.5 shows the peak area reading from HPLC analysis for different quercetin concentration. The peak area increases according to increasing concentration of quercetin. The stock solution was diluted to 0.2, 0.4, 0.6, 0.8, 1.0 mg/mL.

Table 4.5: The peak area reading from HPLC analysis for different quercetin concentration.

Concentration of quercetin (mg/mL)	Peak area (AU)
0.2	151,429
0.4	268,307
0.6	317,200
0.8	504,725
1.0	552,667

A linear calibration curve was established for quercetin had been obtained as shown in Figure 4.2. Linearity was tested by analysing the peak area of standard quercetin of different concentration. The equivalent equation y=502669x + 36500 was obtained and the correlation coefficient (R^2) of the calibration curve is 0.9903, means the calibration curve for the quercetin standard was significant. A perfect line has the correlation coefficient value of one, and most R^2 value for calibration curves are greater than 0.95. The equivalent equation attained from the standard curve is used to calculate the concentration of total quercetin content from *S. alata (L.)* leaves.

Table 4.6: Concentration of quercetin standard obtained from HPLC analysis.

Concentration (mg/mL)	1st Reading (AU)	2nd Reading (AU)	3rd Reading (AU)	Average (AU)	Std. Dev (AU)
0.2	148426.000	151429.000	143845.000	147900.000	3819.263
0.4	222060.000	227353.000	268307.000	239240.000	25311.496
0.6	317200.000	307085.000	307328.000	310537.667	5771.029
0.8	409079.000	504725.000	422052.000	445285.333	51883.332
1	552667.000	546006.000	543967.000	547546.667	4550.026

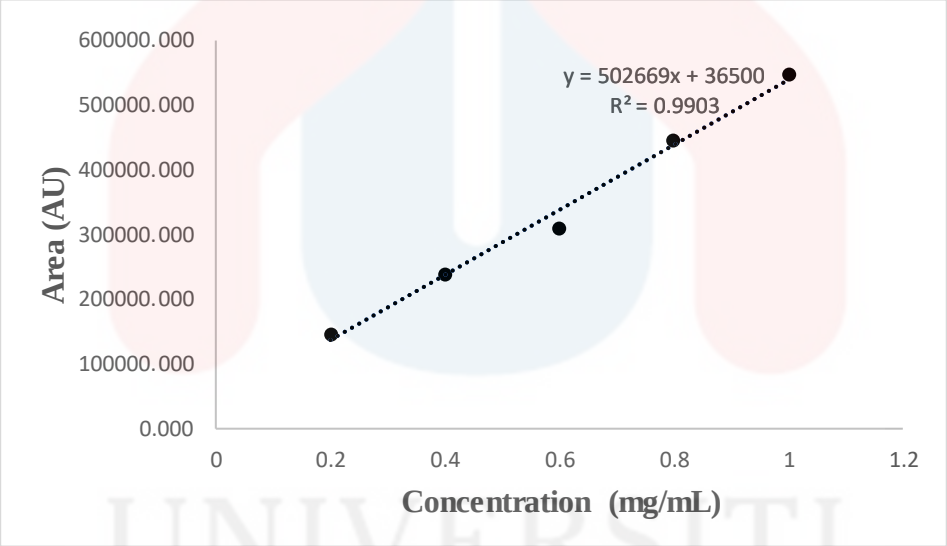

Figure 4.2: Standard calibration curve of quercetin.

Table 4.7: Concentration of quercetin extract from *Senna alata (L.)* leaves obtained from HPLC analysis.

Sonication Time (mins)	Highest Quercetin analysis on HPLC (AU)	Concentration (mg/mL)
10	3866927	7.620
20	4983870	9.842
30	3723834	7.336

Table 4.8: Analysis for HPLC on *Senna alata (L.)* leaves extract on total quercetin compound at different extraction sonication time.

Sonication Time (mins)	1st Reading (AU)	2nd Reading (AU)	3rd Reading (AU)	Average (AU)	Std. Dev (AU)
10	3866927.000	2914213.000	3031440.000	3270860.000	519526.172
20	2601293.000	3380303.000	4983870.000	3655155.333	1214835.851
30	3723834.000	3074958.000	3210411.000	3336401.000	342293.917

Table 4.9: Concentration of quercetin extract from *Senna alata (L.)* leaves obtained from HPLC analysis.

Duty cycle (%)	Highest Quercetin analysis on HPLC (AU)	Concentration (mg/mL)
40	3764280	7.416
50	3827840	7.542
60	4453337	8.787

Table 5.1: Analysis for HPLC on *Senna alata (L.)* leaves extract on total quercetin compound at different duty cycle.

Duty Cycle (%)	1st Reading (AU)	2nd Reading (AU)	3rd Reading (AU)	Average (AU)	Std. Dev (AU)
40	3764280.000	3143974.000	3190396.000	3366216.667	345513.478
50	4177574.000	3524460.000	3827840.000	3843291.333	326831.045
60	4375037.000	3719072.000	4453337.000	4182482.000	403229.889

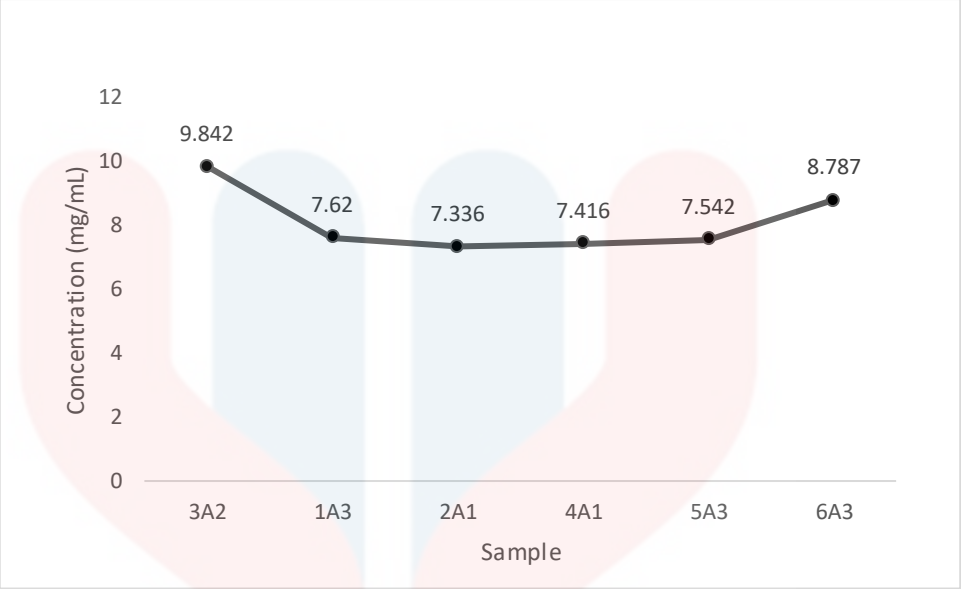

Figure 4.3: Total of quercetin compound concentration in *Senna alata (L.)* leaves ethanolic extract.

4.3.2 Effect of Sonication Time

Figure 4.4 showed the analysis result of HPLC on total flavonoid compound of *S. alata (L.)* leaf extract at different sonication time. In this study, three different sonication times were selected which are 10, 20, and 30 minutes. The solvent extraction method was analysed for its efficiency on extraction of total flavonoid compound as shown in Figure 4.3.2. From the result presented in, the highest concentration of total flavonoid compound was found to be 9.842 mg/mL at 20 minutes of extraction time.

As can be seen in Figure 4.4, the concentration of quercetin was doubled after 20 minutes of sonication period, whereas 30 minutes yielded the lowest results. The earlier study by Ha et al. produced the similar result (2022). The extraction that undergoes ultrasonic process by assisted of homogenizer process causes the plant cell undergoes two time of cell was lysis, so more quercetin compound release from plant cell (Kamal et al., 2021).

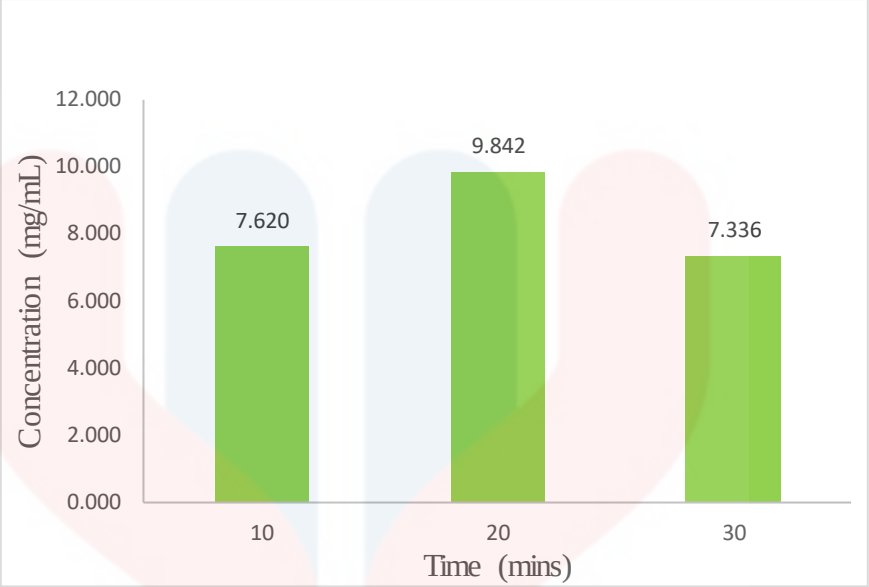

Figure 4.4: Effect of sonication time on quercetin concentration (duty cycle: 60%)

The effect of sonication time on extraction concentration of quercetin was shown in Figure 4.4. This phenomenon can be explained that the longer time taken for extraction of the sample, the more concentration of active compounds will be damaged (Le at el., 2022.

The process of sonication releases heat and energy. Extraction times more than 20 minutes may cause temperature increases that could impact the bioactive ingredient. At higher temperatures, some beneficial compounds, such flavonoids, may mobilise and disintegrate (Kamal et al., 2021). There is no effect when the extraction time is extended after the solute has achieved equilibrium both inside and outside the solid material. The flavonoid content increased from 7.620 mg/mL to 9.842 mg/mL, and subsequently declined with a longer extraction time.

According to a prior study by Mohamad et al. (2021), lengthening the sonication period enhanced the production of polysaccharides from lyceum. This phenomenon can be explained by the fact that the longer sonication probes are in contact with the substance, the more cells are broken down and quercetin gets produced as a result. Certain mechanical effects from ultrasound-assisted extraction can boost extraction

efficiency; these effects can be described by sonic cavitation (Mohamad et al., 2021). The duty cycle did not alter the idea of acoustic cavitation or the mechanical effect on the sample's cell wall. Declared in different ways, the acoustic cavitation process and specific mechanical effects happen more frequently at higher sonication periods than at lower sonication times during the process of sonication to breach the cell wall. This result supports the previous findings by Li et al. (2019) and Aspergillus et al. (2020) that the sonication period was a key influence in the UAE of flavonoids.

4.3.3 Effect of duty cycle

Figure 4.5 displays the concentration of quercetin by duty cycle percentage during the sonication process, ranging from 40% to 60%. The quercetin sample's retention period was found to be 1.803 minutes. According to the results, quercetin concentration peaked at a duty cycle of 60% and started to decline at 50%. This may have occurred due to the duty cycle being reduced, which also reduced the solvent's and the targeted compound's contact surface area (Lim et al., 2021). 60% duty cycle refers to five on and five off times during a one-minute sonication cycle. This indicates that a high percentage of duty cycle during the sonication time process increased the sonicator probe's contact with the sample, leading to a high quercetin concentration (Lim et al., 2021). The highest percentage of duty cycle, at 8.787 mg/mL, 60% duty cycle, corresponds to the maximum quercetin content, as Figure 4.5 illustrates.

Acoustic cavitation and certain mechanical impacts from the ultrasound can improve the extraction efficiency in the UAE process. According to Lim et al. (2021) and Mohamad et al. (2021), acoustic cavitation disrupts the cell wall, making it easier for the solvent to enter the plant material and release the intracellular product.

One mechanical impact of ultrasound is agitation of the extraction solvent. The impact of increased solvent penetration into the sample matrix can lead to an increase in the contact surface area between the solvent and the targeted chemicals (Ajit et al., 2021). Consequently, it can be concluded that, at the maximum percentage of duty cycle during the process of sonication to shatter the cell wall, the process of acoustic cavitation and certain mechanical effect occur more frequently than at other duty cycle percentages. The UAE method of flavonoid extraction from a previous study by Kamal

et al., (2021) also revealed a similar pattern, with 60% duty cycle proven to perform better in terms of flavonoid yield among the tested variations of duty cycle. In 150 mL mobile extractor, by using 60% duty cycle and 30 sonication time resulted 8.787 mg/mL extraction yield.

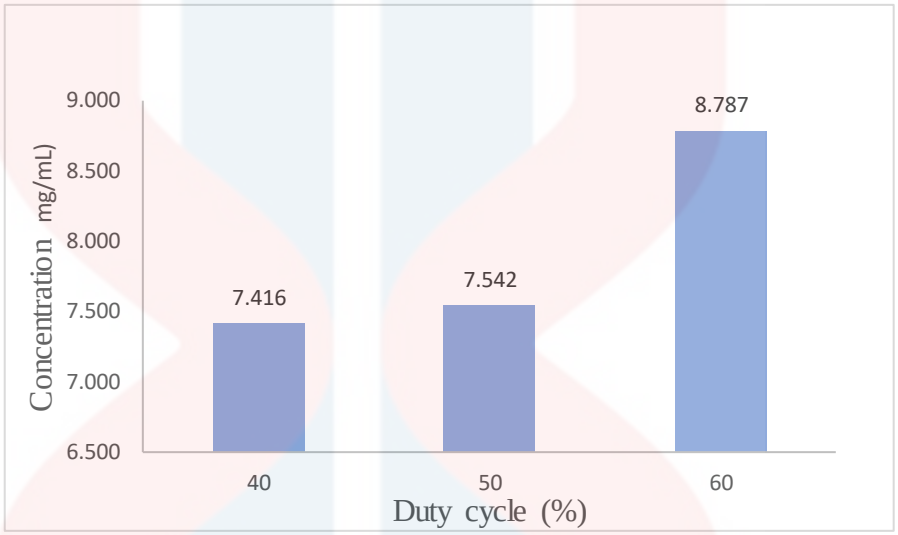

Figure 4.5: Effect of duty cycle on quercetin concentration (sonication time: 30 minutes)

4.4 Physical Characterisation

4.4.1 FTIR

Using the Fourier transform infrared (FTIR) spectrum, the presence of organic compounds in the sample was predicted by measuring the wavelengths at which ultraviolet light was absorbed. By examining the peak that is between the frequencies, the functional group that is present can be determined. The standard quercetin and the supernatant after the *S. alata (L.)* plant extract were examined using FTIR in this study in order to identify the functional group of each component. The FTIR of the plant extract *S. alata (L.)* is displayed in Figure 4.6.

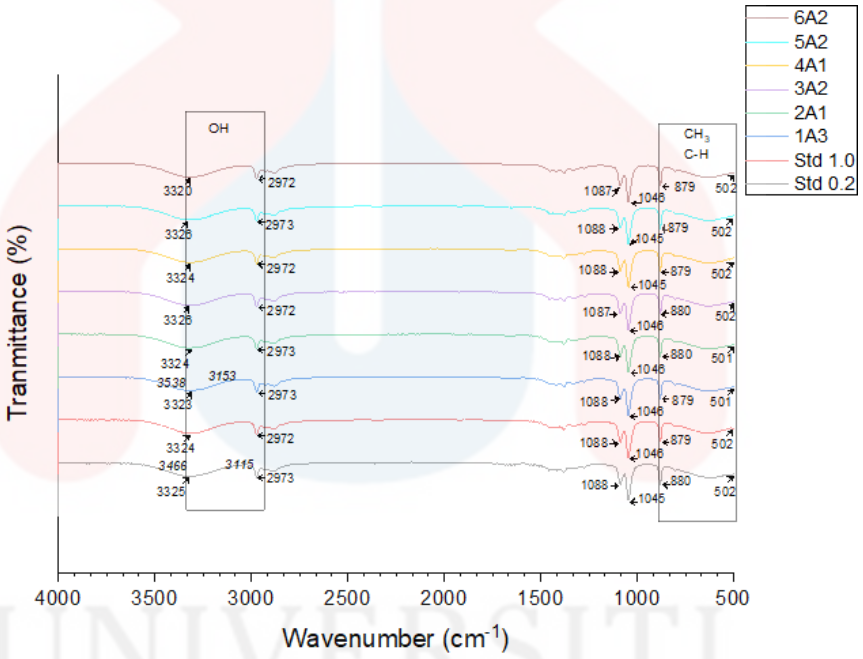

Figure 4.6: FTIR of *S. alata (L.)* plant extract with different sonication time, duty cycle and pure quercetin standard for 0.2 and 1 mg/mL concentration.

The FTIR spectrum for pure standard quercetin and sample is shown in Figure 4.6, where its characteristics bands were detected. Strong appearance of absorption band observed at 3325-3353 cm^{-1}. OH groups stretching were detectable at 3566-3116 cm^{-1} for standard quercetin, whereas OH bending of the quercetin from sample were detect from 3538-3153 cm^{-1}. The strong band at 1088 cm^{-1} with shoulder at 1045 cm^{-1}, and the peak at 879 cm^{-1} and 502 cm^{-1}, were ascribed to asymmetric and symmetric CH_3 stretching motions and the bending C-H modes, respectively. The FTIR band extract

was almost similar to the previous study conducted by Catauro et al., (2015) extract of quercetin has stretch vibration at 3448 cm^{-1}. This was due to the plant extract had -OH and CH stretching vibration.

Two types of functional group are detected on this characterize which is primary aliphatic alcohols and aliphatic hydrocarbon. Compounds with the hydroxyl (-OH) group are known as alcohols. These substances are divided into three groups based on how many additional carbon atoms are joined to the oxygen-bound carbon: primary, secondary, and tertiary. The highly-polar -OH group is present in alcohols. In the condensed phase, it enables hydrogen bonding between molecules. Alcohols have substantially higher boiling temperatures than the equivalent alkane with the same amount of carbon atoms because of this hydrogen bonding (Papale et al., 2020).

Several compounds with linear, long-chain aliphatic hydrocarbons and deuterated alkanes have aliphatic groups. The C-H stretching vibration modes are around 3000 cm^{-1}, while the -CH deformation modes are approximately 1460 cm^{-1} and 1380 cm^{-1}. The atoms that are immediately bonded to the aliphatic group may cause the standard frequencies to vary significantly. Specifically, the band positions will be shifted to higher frequencies by nearby atoms with significant electro negative (Piccolella et al., 2020). At 2973-2950 cm^{-1}, the CH_3 asymmetric stretching vibration takes place, and at roughly 2930 cm^{-1}, the CH_2 absorption takes place. While CH_2 absorption takes place at roughly 2870-2973 cm^{-1}, symmetric CH_3 vibration takes occur at 2885-2865 cm^{-1}.

4.4.2 Antibacterial Test

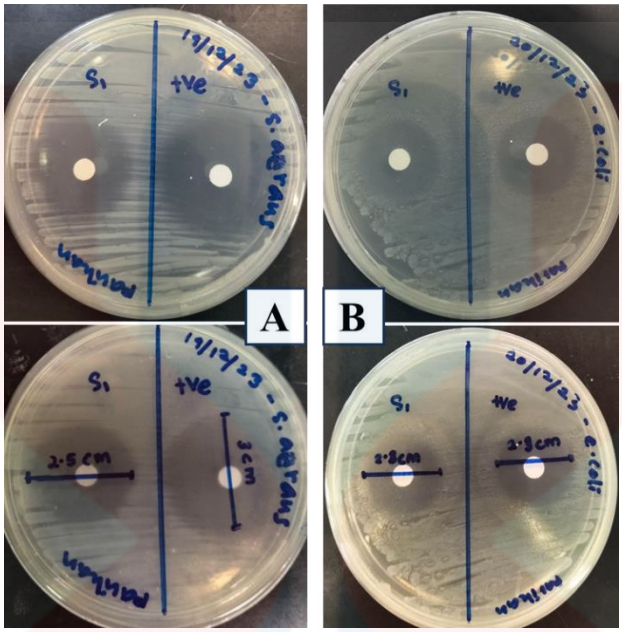

Figure 4.7: Inhibition zones produces by *S. alata (L.)* leaves ethanolic extraction on *Escherichia coli (e-coli)* and *Staphylococcus aureus.*

The formation of zone inhibition by *S. alata (L.)* leaf extract is shown in Figure 4.7. The antibacterial potential of the antibacterial extract of the *S. alata (L.)* leaves was determined from the clear zone around the paper disc containing the extract. The antimicrobial activity exerted by *S. alata (L.)* leaves extracts was found to be approximately that *Staphylococcus aureus* has a bigger clear zone, which is 2.5 cm, compared to *E. coli*, which is 2.3 cm.

Both *Escherichia coli (E. coli)* and *Staphylococcus aureus* bacteria gave positive results on *S. alata (L.)* leaves ethanolic extracts, which showed zones of inhibition on the agar disc diffusion method. The results of this investigation suggest that the components responsible for the antibacterial activity's yielding and potential stability are influenced by temperature, sonication duration, and duty cycle. The antibacterial activity that the plant presents is associated with the presence of phytochemical constituents such as flavonoids, steroids, saponins, and tannins (Nordin et al., 2019).

The result corresponded to the earlier research by Doughari et al. (2021), which reported that *S. alata (L.)* contains saponins, alkaloids, tannins, phenols, and

flavonoids based on early phytochemical tests. Reportedly, plants use these bioactive substances, which have antimicrobial activity, to defend against bacterial, fungal, and pesticidal diseases. Tests of the extracts' antibacterial susceptibility revealed that they were all highly active against both gram-positive and gram-negative bacteria.

Numerous biological and medicinal qualities are said to be present in these secondary metabolites (Arif et al., 2019). Sun-dried *S. alata (L.)* leaves extract was found to have a potent inhibitory impact on concanavalin A-induced histamine release, COX-1 and COX-2, and 5-lipoxygenase activities in a previous study reported by Uwangbaoje et al., (2015).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this study, active compound was successfully extracted from leaves of *S. alata (L.)* by using solvent extraction method in this study. The objective of this study was achieved successfully, which the quercetin was successful extracted from *S. alata (L.)* by using UAE. Firstly, the presence of quercetin from *Senna alata (L.)* was detected by using phytochemical test which can be observed through the colour changes after added with NaOH and Hcl from yellow to slightly colourless at the bottom. Besides, the second objective, optimum extraction process parameters (sonication time and duty cycle percentage) for the extraction of quercetin from *Senna alata (L.)* by using HPLC was recognised. Based on the result gained, longer sonication time use on UAE can affect the quercetin content, while higher percentage used on duty cycle increased the quercetin concentration. Optimum concentration of quercetin (9.842 mg/mL) was detected at 20 minutes sonication time and (8.787mg/mL) was recorded at 60% duty cycle.

The functional group of solvent extraction was successfully explored and reported in this research. This referring to the third objectives of this research which is to characterise the flavonoid of *Senna alata (L.)* extract by using FTIR. Lastly, the fourth objective was to figure out zone inhibition of antibacterial effectiveness of *Senna alata (L.)* extract by using disc diffusion method on gram positive and gram-negative bacteria, which both showed positive results on zone inhibition. This finding indicates that the kinds of solvents employed in the extraction process have a significant impact on yields, as well as possibly the stability and activity, of the phytochemical components in the plant extract. The solubility of antioxidant chemicals present in plant matrices may be influenced by the polarity and chemical properties of the solvents (Turkmen et al., 2006).

In addition to solvent types, the amount of water in the aqueous solvent may have a significant impact on the yield of total flavonoid content.

In conclusion, under mild circumstances, the UAE's ultrasonic cavitation might generate powerful forces that could mechanically shatter cell walls, allowing for a large extraction yield. As a result, it was determined that the UAE approach, which may require less time, energy, and temperature during extraction, is an alternate method for phenolic and flavonoid component extraction from *Senna alata (L.)* leaves.

5.2 Recommendations

Further study by using another parameter, such as the mesh size of the plant powder, is needed to see the effect of surface area on increasing the yield of bioactive components in plants. Phytochemical screening to observe the flavonoid content in this study can be improved by using other chemical like ammonia to get a better result. Based on an antibacterial test on *Senna alata (L.)*, this plant extract was suggested to be used as an ingredient in medicinal products, such as soap or cream, to treat itching, scabies, and ringworm. UAE can be used to extract other bioactive compound since its produce high concentration and short time required.

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APPENDIX A

Calculation A: Stock quercetin.

$$\frac{50 \text{ mg quercetin}}{25 \text{ mL HPLC water}} = 2\text{mg/mL}$$

Calculation B: Serial dilution of standard quercetin

Formula: $M_1V_1=M_2V_2$

0.2 mg/mL quercetin: (0.2 mg/mL)(10 mL) = 2 mg/mL (V_2)

= 1 mL (*Used: 1 mL stock quercetin + 9 mL HPLC methanol)*

0.4 mg/mL quercetin: (0.4 mg/mL)(10 mL) = 2 mg/mL (V_2)

= 2 mL (*Used: 2 mL stock quercetin + 8 mL HPLC methanol)*

0.6 mg/mL quercetin: (0.6 mg/mL)(10 mL) = 2 mg/mL (V_2)

= 3 mL (*Used: 3 mL stock quercetin + 7 mL HPLC methanolr)*

0.8 mg/mL quercetin: (0.8 mg/mL)(10 mL) = 2 mg/mL (V_2)

= 4 mL (*Used: 4 mL stock quercetin + 6 mL HPLC methanol)*

1 mg/mL quercetin: (1 mg/mL)(10 mL) = 2 mg/mL (V_2)

= 5 mL (*Used: 5 mL stock quercetin + 5 mL HPLC methanol)*

Calculation C: Concentration of quercetin

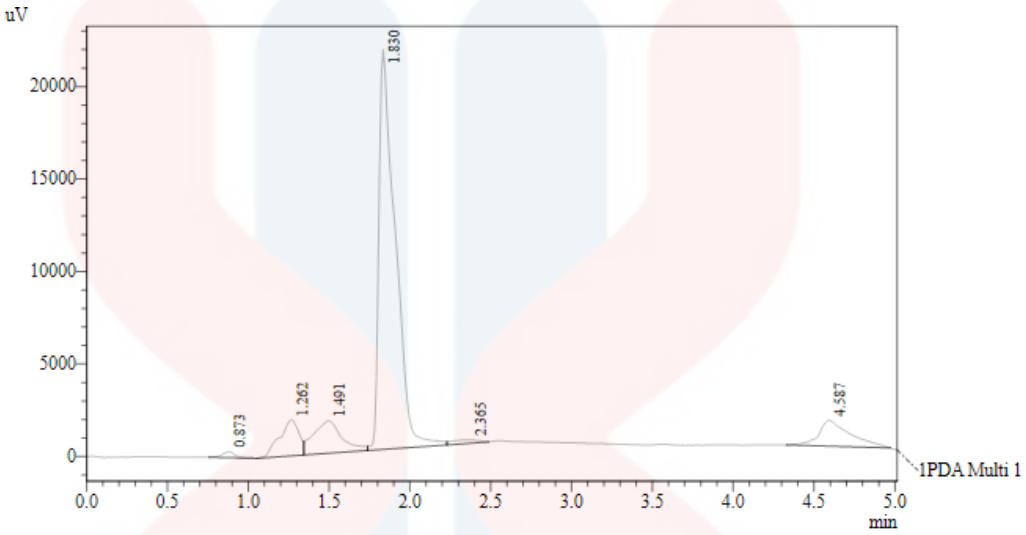
Y = mx + c, where y=area and x=concentration of quercetin

4983870 = 502669 x + 36500

$$x = \frac{(4983870 - 36500)}{502669}$$

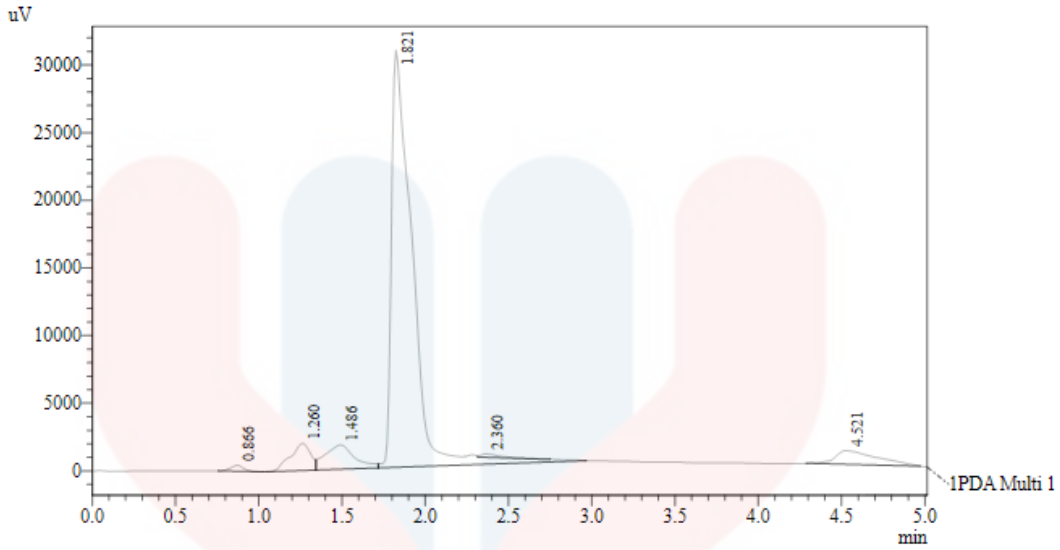
x = 9.842

APPENDIX B



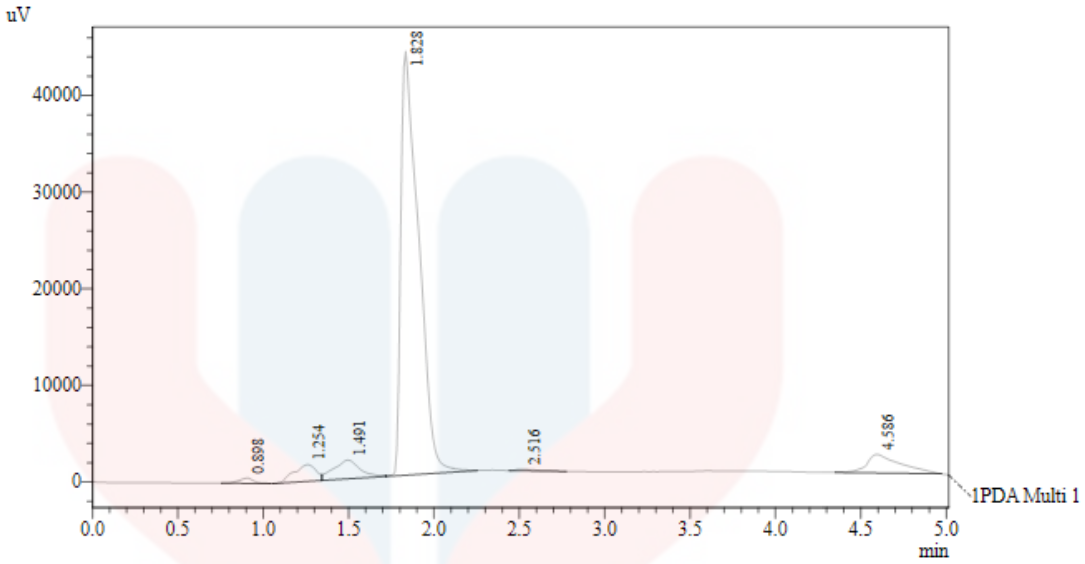
Peak	Ret. Time	Area	Height	Area (%)	Height (%)
1	0.873	1680	325	0.791	1.192
2	1.262	16646	1966	7.832	7.213
3	1.491	21575	1754	10.151	6.435
4	1.830	151429	21634	71.246	79.378
5	2.365	2250	185	1.058	0.680
6	4.587	18963	1390	8.922	5.101

Figure C.1: Peak of 0.20 mg/mL standard quercetin.



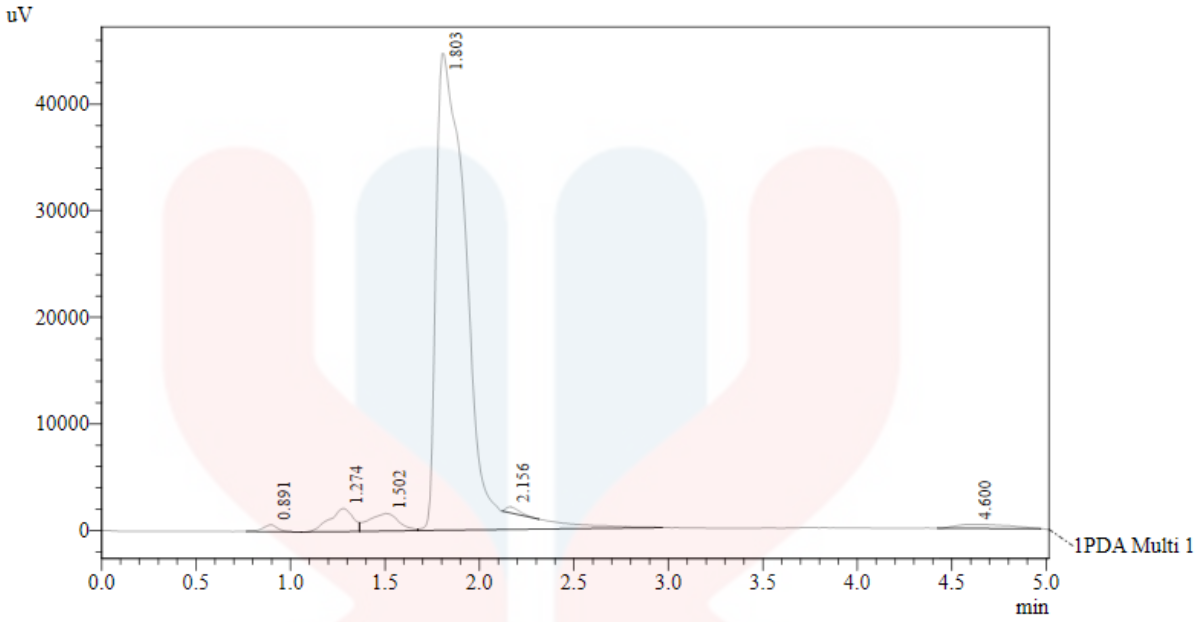
Peak	Ret. Time	Area	Height	Area (%)	Height (%)
1	0.866	2438	441	0.711	1.213
2	1.260	17242	2006	5.224	5.521
3	1.486	22068	1793	6.687	4.934
4	1.821	268307	30829	81.296	84.850
5	2.360	2201	255	0.667	0.701
6	4.521	17870	1011	5.414	2.781

Figure C.2: Peak of 0.40 mg/mL standard quercetin.



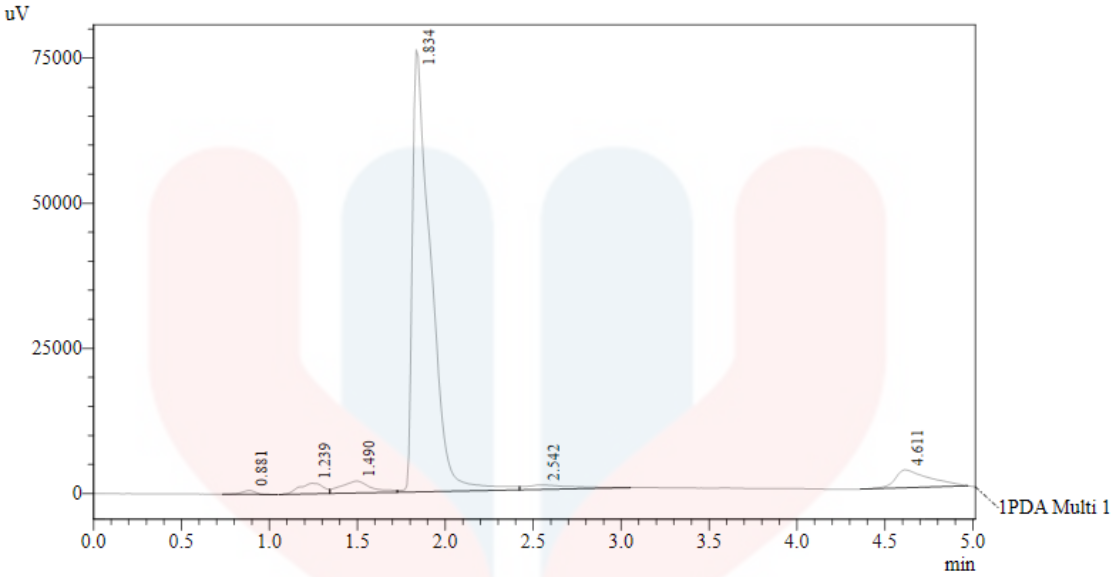
Peak	Ret. Time	Area	Height	Area (%)	Height (%)
1	0.898	3242	549	0.842	1.095
2	1.254	15797	1747	4.103	3.481
3	1.491	20859	1939	5.417	3.863
4	1.828	317200	43862	82.375	87.398
5	2.516	1279	156	0.3372	0.311
6	4.586	26691	1933	6.932	3.852

Figure C.3: Peak of 0.60 mg/mL standard quercetin.



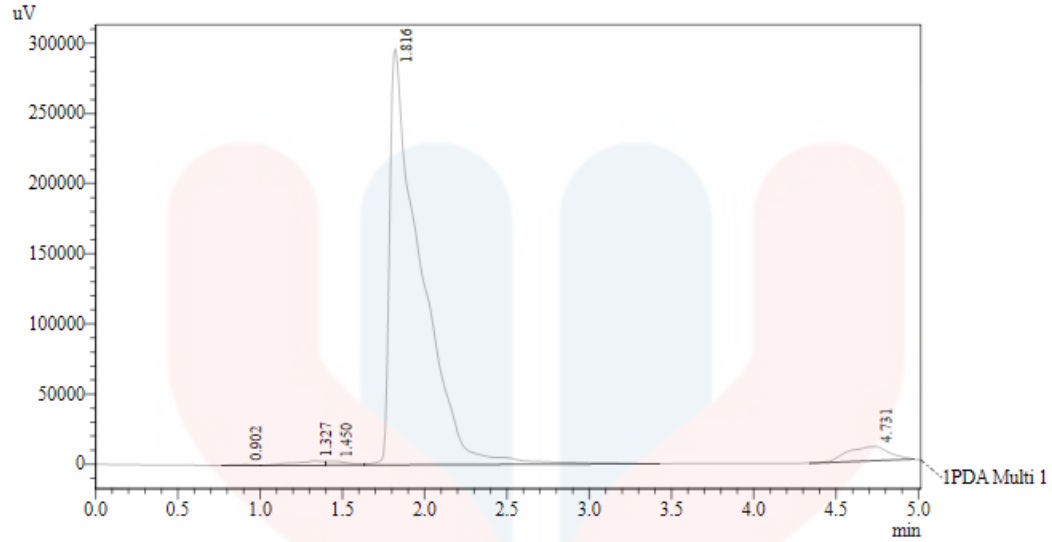
Peak	Ret. Time	Area	Height	Area (%)	Height (%)
1	0.891	3326	644	0.600	1.285
2	1.274	18827	2151	3.394	4.295
3	1.502	17768	1624	3.203	3.243
4	1.803	504725	44728	90.991	89.321
5	2.156	2639	542	0.476	1.082
6	4.600	7415	387	1.337	0.773

Figure C.4: Peak of 0.80 mg/mL standard quercetin.



Peak	Ret. Time	Area	Height	Area (%)	Height (%)
1	0.881	3544	660	0.542	0.779
2	1.239	17659	1839	2.702	2.174
3	1.490	24470	2063	3.744	2.438
4	1.834	552667	76180	84.571	90.027
5	2.542	13949	782	2.135	0.924
6	4.611	41205	3095	6.305	3.658

Figure C.5: Peak of 1 mg/mL standard quercetin.

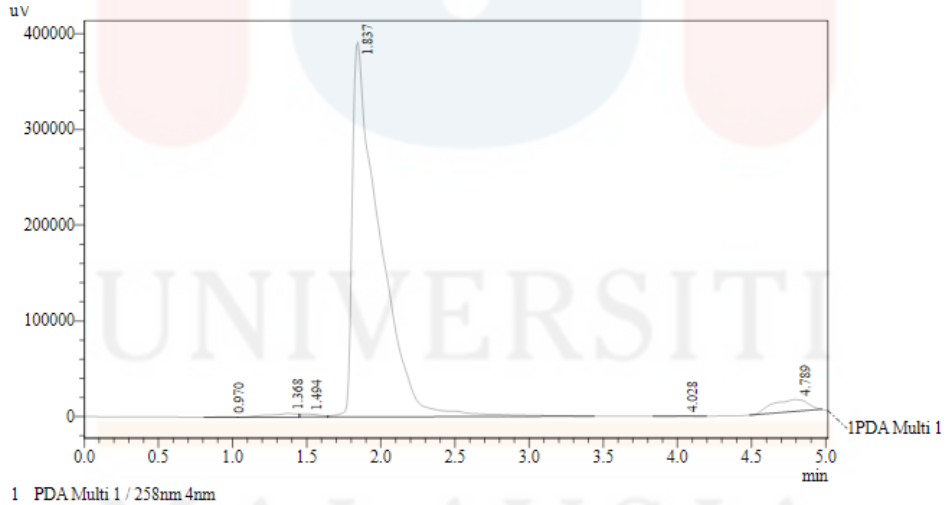


1 PDA Multi 1 / 258nm 4nm

PDA Ch1 258nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %	Name
1	0.902	3211	504	0.078	0.161	
2	1.327	39586	3203	0.963	1.023	
3	1.450	23956	2543	0.583	0.812	
4	1.816	3866927	296949	94.044	94.865	
5	4.731	178129	9823	4.332	3.138	

Figure C.6: Peak of 10 minutes sonication time.

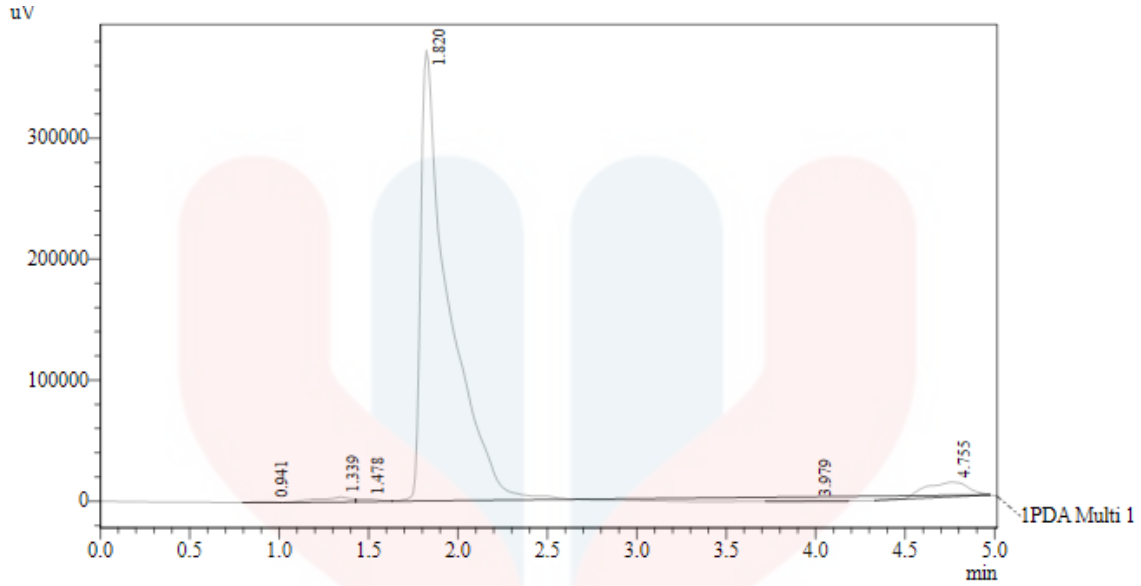


1 PDA Multi 1 / 258nm 4nm

PDA Ch1 258nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %	Name
1	0.970	1580	223	0.030	0.054	
2	1.368	51611	3831	0.978	0.931	
3	1.494	27148	3096	0.514	0.752	
4	1.837	4983870	391881	94.415	95.250	
5	4.028	1343	132	0.025	0.032	
6	4.789	213148	12262	4.038	2.980	

Figure C.7: Peak of 20 minutes sonication time.

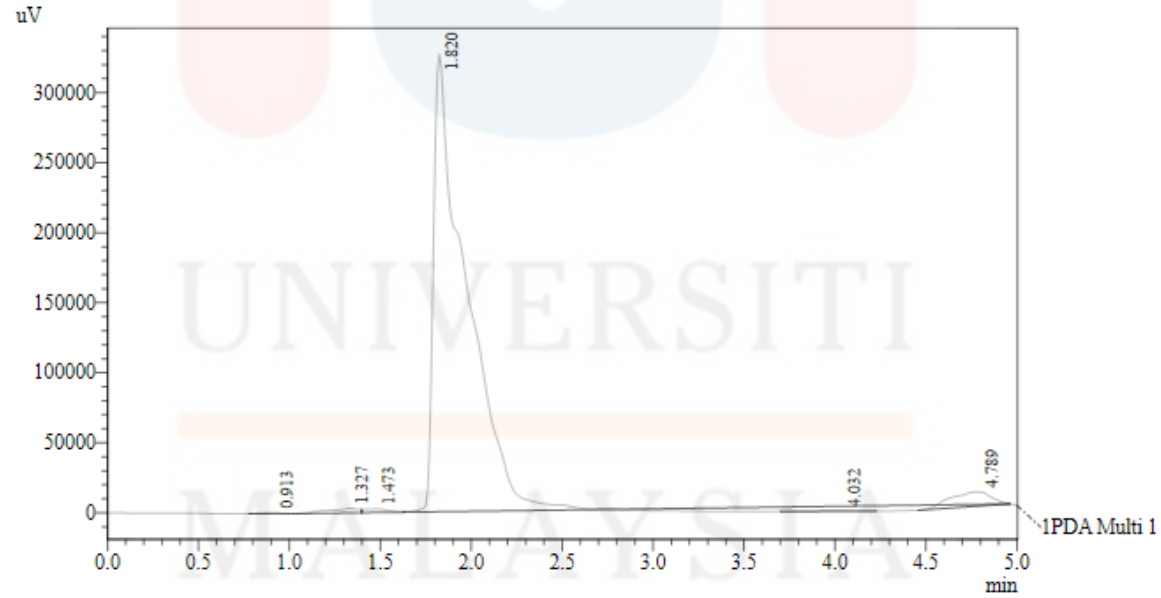


1 PDA Multi 1 / 258nm 4nm

PDA Ch1 258nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %	Name
1	0.941	2578	339	0.064	0.086	
2	1.339	53997	3988	1.347	1.018	
3	1.478	17335	2269	0.433	0.579	
4	1.820	3723834	372938	92.910	95.235	
5	3.979	2358	134	0.059	0.034	
6	4.755	207879	11933	5.187	3.047	

Figure C.8: Peak of 30 minutes sonication time.

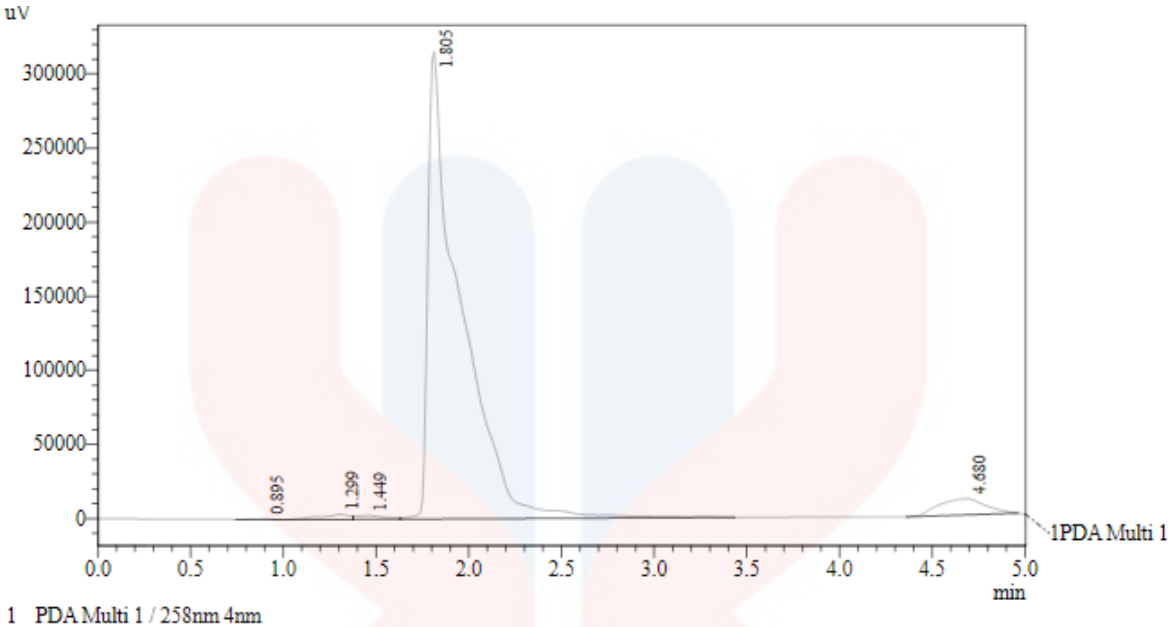


1 PDA Multi 1 / 258nm 4nm

PDA Ch1 258nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %	Name
1	0.913	4012	596	0.100	0.174	
2	1.327	41055	3270	1.027	0.952	
3	1.473	23810	2506	0.595	0.730	
4	1.820	3764280	326793	94.143	95.182	
5	4.032	2902	149	0.073	0.043	
6	4.789	162402	10022	4.062	2.919	

Figure C.9: Peak of 40% duty cycle.

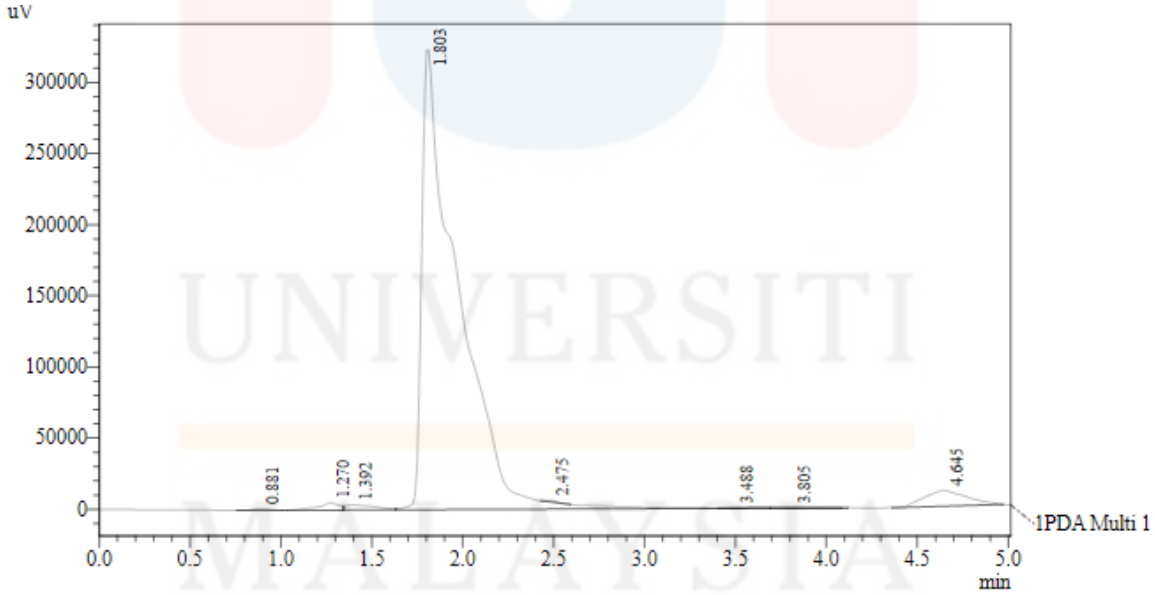


1 PDA Multi 1 / 258nm 4nm

PDA Ch1 258nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %	Name
1	0.895	3320	538	0.081	0.162	
2	1.299	38422	3414	0.940	1.026	
3	1.449	26331	2395	0.644	0.720	
4	1.805	3827840	315510	93.620	94.830	
5	4.680	192797	10855	4.715	3.262	

Figure C.10: Peak of 50% duty cycle.



1 PDA Multi 1 / 258nm 4nm

PDA Ch1 258nm 4nm

Peak#	Ret. Time	Area	Height	Area %	Height %	Name
1	0.881	6622	1058	0.140	0.306	
2	1.270	43304	5298	0.915	1.533	
3	1.392	40453	3730	0.855	1.079	
4	1.803	4453337	323376	94.103	93.560	
5	2.475	3068	862	0.065	0.250	
6	3.488	1239	94	0.026	0.027	
7	3.805	2781	186	0.059	0.054	
8	4.645	181601	11032	3.837	3.192	

Figure C.11: Peak of 60% duty cycle.

APPENDIX C

Figure C.12: *Senna alata (L.)* after air dried for 14 days.

Figure C.13: *Senna alata (L.)* leaves after blended and milled.

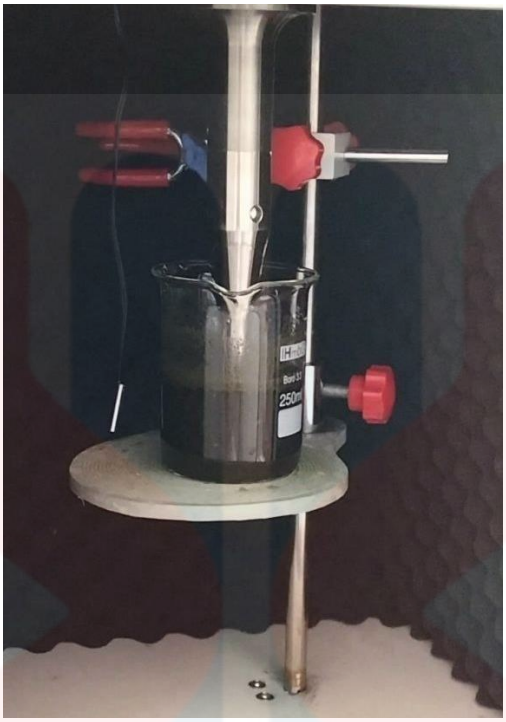


Figure C.14: *Senna alata (L.)* on UAE process.

Figure C.15: 0.8 mg/mL standard quercetin.