CHARACTERIZATIONS OF SAPONIN AND PHENOLIC BIOACTIVE COMPOUNDS EXTRACTED FROM FENUGREEK SEED AND ALOE VERA LEAVES VIA MICROWAVE-ASSISTED EXTRACTION METHOD

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ABSTRAK

Dalam kajian ini, potensi benih fenugreek dan Aloe vera sebagai sumber saponin, fenolik dan antioksidan telah dikaji. Pengekstrakan dibantu oleh ketuhar gelombang mikro (MAE) dan pengekstrakan Soxhlet digunakan untuk mendapatkan ekstrak. Kesan faktor eksperimen MAE seperti masa pengekstrakan (2-12 min), kuasa gelombang mikro (300-700 W), kepekatan etanol (20-100%), nisbah pelarut (F:S) (1:8-1:16 dan 1:18-1:22 g/mL) dan suhu pengekstrakan (40-80°C) dinilai menggunakan (OFAT). Faktor pengekstrakan Soxhlet termasuk masa pengekstrakan (1-5 h), kepekatan etanol (20-100%) dan F: S (1:14-1:24 g/mL) juga dikaji. Keputusan menunjukkan hasil pengekstrakan yang lebih tinggi, jumlah kandungan saponin (TSC) dan jumlah kandungan fenolik (TPC) diperoleh melalui MAE berbanding pengekstrakan Soxhlet dalam penjimatan masa. Hasil maksimum dalam pengekstrakan Soxhlet diperoleh pada 3 jam masa pengekstrakan, kepekatan etanol 60% dan nisbah 1:20 g/mL F:S yang 19.35 ± 0.75%, 125.04 ± 1.55 mg DE/g d.w. dan 60.13 ± 2.04 mg GAE/g d.w. untuk benih fenugreek dan 22.45 ± 0.76%, 44.78 ± 1.01 mg OAE/g d.w. dan 49.99 ± 0.56 mg GAE/g d.w. untuk Aloe vera. Penyaringan terhadap "factors via two-level factorial design" dijalankan untuk parameter MAE. Keputusan menunjukkan bahawa kepekatan etanol dan suhu pengekstrakan adalah faktor yang paling penting dan paling tidak penting dalam mencapai hasil maksimum. Pemilihan tahap faktor untuk proses pengoptimuman diperoleh berdasarkan faktor pra-penilaian (OFAT). Faktor optimum yang terbaik adalah masa pengekstrakan (2-4 min), kuasa gelombang mikro (500-700 W dan 400-600 W), kepekatan etanol (40-80% dan 20-60%), F:S (1:8-1:12 dan 1:18-1:22 g/mL) dan suhu ialah 70 °C. Kondisi optimum untuk benih fenugreek dan Aloe vera menghasilkan hasil pengekstrakan, TSC dan TPC berada pada 2.84 min, 572.50 W, 63.68%, dan 1:9 g/mL. Berdasarkan keadaan optimum, hasil pengekstrakan, TSC dan TPC benih fenugreek adalah 26.04 ± 0.88%, 195.89 ± 1.07 mg DE/g dw, 81.85 ± 0.61 mg GAE/g dw, dan ekstrak daun Aloe vera di MAE adalah 2.79 min, 478.95 W, 43.38% etanol, dan 1:19 g/mL. Berdasarkan kepada kondisi tersebut, hasil ekstrak daun TSC dan TPC dari Aloe vera adalah 36.17 ± 1.13%, 65.89 ± 0.77 mg OAE/g d.w dan 73. 05 ± 1.05 mg GAE/g d.w. Ekstrak yang diperoleh melalui MAE dan Soxhlet juga diuji untuk kapasiti antioxidant melalui DPPH dan ABTS, struktur dan ikatan menggunakan FTIR dan kajian morfologi menggunakan SEM. Keputusan menunjukkan bahawa ekstrak yang diperoleh melalui MAE menunjukkan kapasiti antioksida yang lebih tinggi dengan nilai IC_{50} yang rendah (195.27 ± 0.56 µg/mL; DPPH), (157.92 ± 1.11 µg/mL; ABTS), 12 puncak yang dikenal pasti dalam FTIR untuk benih fenugreek dan (275 ± 1.45 µg/mL; DPPH) (215.58 ± 0.57 µg/mL; ABTS), 11 puncak di FTIR dan masing-masing tekstur kemas dan tekstur terbuka melalui SEM. Walau bagaimanapun, ekstrak Soxhlet (224.47 ± 0.77 µg/mL; DPPH), (199.67 ± 0.96 µg/mL; ABTS) untuk benih fenugreek dan (305.79 ± 0.66 µg/mL; DPPH), (263.29 ± 1.21 µg/mL, ABTS), dengan 6 puncak yang dikenal pasti melalui FTIR dan liang yang tidak tertutup dan liang tertutup ditunjukkan melalui SEM pada kedua-dua tanaman. kajian kinetik dan sifat dielektrik untuk MAE juga dilakukan. Hasil LC-QTOF-MS dari optimum ekstrak mengesahkan kehadiran 58 saponin dan 27 fenolik dalam benih fenugreek dan 29 saponin dengan 32 fenolik dalam ekstrak Aloe. Optimum ekstrak juga menunjukkan sifat surfaktan seperti pembasahan, pengurangan ketegangan permukaan air, sifat berbuih dan emulsifikasi. Oleh itu, ekstrak ini boleh menjadi sumber saponin, fenolik, antioksidan dan pengemulsi semula jadi untuk makanan, kosmetik dan produk farmaseutikal.

ABSTRACT

In this study, the potential of fenugreek seed and Aloe vera leaves as a source of saponins, phenolics and antioxidants were investigated. Microwave-assisted extraction (MAE) and Soxhlet extraction (SE) were used to obtain the extracts. The effects of experimental factors in MAE such as extraction time (2-12 min), microwave power (300-700 W), ethanol concentration (20-100%), feed-to-solvent ratio (1:8-1:16 and 1:18-1:22 g/mL) and extraction temperature (40-80 °C) were evaluated using one-factor-at-a-time (OFAT), respectively. The SE factors including extraction time (1-5 h), ethanol concentration (20-100%) and feed-to-solvent ratio (1:14-1:24 g/mL) were also investigated. Results indicated the higher extraction yield, total saponin content (TSC) and Total phenolic content (TPC) were obtained via MAE compared to SE in a time saving process. The maximum yields in SE were obtained at 3 h of extraction time, 60 % ethanol concentration and 1:20 g/mL F:S ratio which were 19.35±0.75%, 125.04±1.55 mg DE/g d.w. and 60.13±2.04 mg GAE/g d.w. for fenugreek seed and 22.45±0.76%, 44.78±1.01 mg OAE/g d.w. and 49.99±0.56 mg GAE/g d.w. for Aloe vera leaves, respectively. Further screening of the factors via two-level factorial design was carried out for MAE parameters. Results indicated that ethanol concentration and extraction temperature were the most and least significant factors in achieving maximum recoveries of the yields, respectively. The selection of factor levels for optimization process was obtained based on the pre-evaluation of factors (OFAT). The best points for optimizing the factors were extraction time (2-4 min), microwave power (500-700 W and 400-600 W), ethanol concentration (40-80% and 20-60%), feed-to-solvent ratio (1:8-1:12 and 1:18-1:22 g/mL) and constant temperature of 70 °C, respectively. The optimal MAE conditions for fenugreek seed and Aloe vera leaves extraction yield, TSC and TPC were at 2.84 min, 572.50 W, 63.68%, and 1:9 g/mL. Based on the optimum condition, the responses of extraction yield, TSC and TPC of fenugreek seed were 26.04 ± 0.88%, 195.89 ± 1.07 mg DE/g d.w., 81.85 ± 0.61 mg GAE/g d.w, and for Aloe vera leaves extracts in MAE were 2.79 min, 478.95 W, 43.38% ethanol, and 1:19 g/mL. Where, based on these conditions, the extraction yield, TSC and TPC of Aloe vera leaves extract were 36.17 ± 1.13%, 65.89 ± 0.77 mg OAE/g d.w and 73. 05 ± 1.05 mg GAE/g d.w, respectively. The extracts obtained via MAE and SE were also tested for its antioxidant capacity via 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,20-azino- bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), structure and bonding using Fourier transform infrared (FTIR) and morphological studies using SEM. Results indicated that extracts obtained via MAE showed higher antioxidant capacity with low IC_{50} values of (195.27 ± 0.56 µg/mL; DPPH), (157.92 ± 1.11 µg/mL; ABTS), 12 identified peaks in FTIR for fenugreek seed and (275 ± 1.45 µg/mL; DPPH) (215.58 ± 0.57 µg/mL; ABTS), 11 peaks in FTIR and more wrapped and opened texture via scanning electron microscope (SEM), respectively. However, in SE it was (224.47 ± 0.77 µg/mL; DPPH), (199.67 ± 0.96 µg/mL; ABTS) for fenugreek seed and (305.79 ± 0.66 µg/mL; DPPH), (263.29 ± 1.21 µg/mL; ABTS), with 6 identified peaks via FTIR and closed pores showed via SEM in both plants, respectively. kinetic studies and dielectric properties for MAE were also carried out. The LC-QTOF-MS result of optimized extracts also confirmed the presence of 58 saponins and 27 phenolic compounds in fenugreek seed and 29 saponin with 32 phenolic compounds in Aloe extract. The optimized extracts also indicated surfactant properties such as wetting, reduction of water surface tension, foaming and emulsification properties. Thus, these extracts can be a promising source of saponins, phenolics, antioxidants and natural co-emulsifier for food, cosmetics and pharmaceutical products.

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R2	R- square
Adj. R2	Adjacent coefficient of determination
C	Carbon
A	Alpha
Θ	Theta
Y	Response
β_0	Constant term
β_i	Coefficient of linear factor
β_{ii}	Coefficient of quadratic parameter
β_{ij}	Coefficient of interaction parameters
ε'	Dielectric constant
ε''	Dielectric loss
τ	Relaxation time
ω	Angular frequency in radians per second
ε_∞	Complex permittivity
ε_s	Static permittivity
λ	Wavelength
λ_0	Wavelength in free space

LIST OF ABBREVIATIONS

MAE	Microwave-assisted extraction
UAE	Ultrasonic-assisted extraction
DCM	dichloromethane
TSC	Total saponin content
TPC	Total phenolic content
DPPH	2,2-diphenyl-1-picrylhydrazyl (DPPH),
ABTS	2,20-azino- bis (3-ethylbenzothiazoline-6-sulfonic acid
RSM	Response surface methodology
ANOVA	Analysis of variance
CV	Coefficient of variation
FCCCD	Face-centred central composite design
FTIR	Fourier transform infrared (FTIR) spectroscopy
SEM	Scanning electron microscopy
LC-QTOF-MS	Liquid chromatography-mass spectrometry quadrupole time-of-flight
OFAT	One-factor-at-a-time
DNA	Deoxyribonucleic acid
WHO	World health organization
DOE	Design expert
D_P	Penetration depth

CHAPTER 1

INTRODUCTION

1.1 Introduction

Currently, plants are in a considerable interest due to their numerous benefits and applications. Plants with high nutritional values have received more attentions as a dietary supplement to prevent chronic immune disorders. Researchers have claimed that chronic ailments are treatable or could be averted by using the sources of bioactive-compounds extracted from plants and fruits. Many secondary metabolites with different functionality and properties exists in plants such as phenols, steroids, tannins, alkaloids, saponins, flavonoids and triterpenoids (Alara et al., 2017). Secondary metabolites specially polyphenols and flavonoids have been used as natural antioxidants to control the oxidative stress or cell damage (Ashraf et al., 2013). Saponins are classified as steroid and triterpenoid glycosides. The bioactive compounds of saponins and phenolics have demonstrated potential benefits for the treatments of many diseases in humans (Chan et al., 2014).

Saponins are commonly found secondary metabolites in plants. Saponins are also found in foodstuff such as onion, spinach, peanuts, garlic, asparagus and many other plants. Essentially, it protects plants from the attacks of pathogens and herbivores. In addition, food and medicine industries are interested to use them as an active constituent in their products (Augustin et al., 2011). The division of saponins as triterpenoids and steroidal glycosides helps to distinguish the types of saponins by the sugar chains attached at different positions (Pagureva et al., 2016; Poojary et al., 2015). In fact, many plants have indicated a great phytochemical and medicinal properties which are useful in treating some diseases such as tumor, cancer, diabetes, heart problems and infections (Riasat et al., 2018). In addition, the bioactive compounds of plants also act as natural antioxidants by donating hydrogen atom to eliminate the unpaired electron. Since

phenolic compounds contain the hydroxyl (-OH) group, this free OH is in charge to eliminate the oxidative damage and scavenge the radicals (Altemimi et al., 2017; Paj et al., 2019). Researchers have revealed that plants containing phenolics and saponin compounds are able to combat with many pathogens and infections and are useful in preventing some ailments (Bahmani et al., 2016; Iqbal et al., 2017). Medicinal plants have a long history of application in Malaysia which goes back to more than a thousand year ago. Studies have shown that only in Peninsular Malaysia more than 1300 species of medicinal plants have been recorded. In 1999, the value of aromatic and medical plants of Malaysia were estimated to be around 4.6 billion with a growth rate of 15-20% per year in herbal product market (Sultana et al., 2014).

Fenugreek or Helba (*Trigonella foenum-graecum L.*) is a known medicinal plant with a long history of usage as a prevalent and natural medicine in Asia, Africa, Egypt, Middle East and European countries. Fenugreek is an annual plant of the Leguminosae or Fabaceae family, the genus *Trigonella* and species is *foenum-graecum*. The Legume or bean family is the third largest family of flowering plants with more than 18000 species and 650 genera (Doyle, 2001). Different parts of this plant such as seed, leaves and steam have indicated high pharmacological and nutritional value, hence, this plant attracted many researchers to take it under consideration. The seeds and leaves of this plant have indicated anti-diabetic, anti-microbial, lactation aid, anti-inflammation, anticancer, antifungal, and antioxidant activity (Arivalagan et al., 2013).

Aloe vera with more than 400 species of the genus Aloe belonging to the family of Liliaceae. It is commonly known as *Aloe barbadensis* Miller. According to the international botanical rule, it is named Aloe vera. Originally, it is native to Sudan but is also cultivated in South Africa, Asia, America and Venezuela. This plant can survive in temperature up to 45°C and minimum temperature 6°C (OM et al., 2015). Aloe vera have been used since ancient time due to its medicinal and nutritional properties. It has achieved a considerable view for the treatment of skin problems such as burns, eczema and cuts. The popularity of this plant is due to its high biological benefit and nutritional value, which help in treating inflammation, diabetes, wound healing, lowering blood cholesterol, and preventing cancer.

The recovery of biologically active "compounds from plants are potentially influenced by" different solvent formulations and extraction methods. The extractions of bioactive compounds have usually been done through conventional and unconventional extraction methods such as Soxhlet, maceration, hydro-distillation, and ultrasound assisted extraction (UAE) (Rababah et al., 2004; Wani et al., 2016). However, these methods have some disadvantages such as longer processing time, high energy and solvent consumption, low yield and efficiency, and possible thermal degradation of bioactive compounds. Over the years, microwave-assisted extraction (MAE) has replaced some traditional extraction methods in industries and laboratories due to its high extraction efficiency and low solvent, time and energy consumption (Veggi et al., 2013). The extraction of bioactive compounds can also be affected by the extraction solvent, a proper selection of solvent can enhance the recovery yield and bioactivity of the extract. The common solvents used in obtaining bioactive compounds of plants are ethanol, water, dichloromethane (DCM), chloroform, methanol, acetone, ethyl acetate and combination of one or more of these solvents. On the other side, ethanol and water are the most preferred extraction solvents which are considered safe and clean to the environment (Middlesworth & Cannell, 1998). The lack of information in providing a depth understanding on extraction of bioactive compounds in an optimized condition of MAE from fenugreek seed and Aloe vera leaves made this study a target for investigation. Therefore, this study was carried out to investigate the extraction and characterization of bioactive compounds of total saponin content (TSC) and total phenolic content (TPC) from fenugreek seed and Aloe vera leaves in the optimized conditions of MAE process using face centred-central composite design (FCCCD) subjected under response surface methodology (RSM). The extraction kinetic was also evaluated using first and second-order kinetic model.

1.2 Problem Statement

The use of synthetic medicines or drugs involves adverse or side effects on humans. Reports revealed that yearly around 100,000 people die due to the side effects or sensitivity to certain medicines (Karimi et al., 2015). According to the World Health Organization (WHO), since the early civilization more than 80% of people all around the

world consume herbs as traditional medicines to cure and treat various ailments (Baruah et al., 2016). The side effects and negative impact of herbal remedies are very rare and even hard to find. Therefore, in developed countries, such as United Status (US) and Europe, people turn to herbal medicine as they believe that medicines produced from herbal remedies are free of risk and other side effects to the health (Karimi et al., 2015). The use of commercial synthetic pharmaceutical drugs for curing diseases such as cancer, diabetes, and heart related ailments are suspected to have harmful effects (Karimi et al., 2015).

Tavakoly et al. (2018) conducted a research on improving inflammation and oxidative stress in 48 type II diabetes patients by feeding them 15g of fenugreek seed powder per day. The result of their study suggested a promising effect on biomarkers of inflammation and oxidative stress in type II diabetes patients, but they also suggested further investigation on the effect of fenugreek seed powder related to these ailments. The research conducted by Belguith-Hadriche et al. (2010) and the result showed that fenugreek seed extract have a significant hypocholesterolemic effects and antioxidant activity when fed to Wistar rats. Another study on total phenolic and flavonoid content of fenugreek seed extracted by maceration using ethyl acetate as extraction solvent was carried out by Belguith-Hadriche et al. (2010), the result of their study showed that after 24 hours of extraction duration the content of total phenolic and flavonoids were 75.60 (mg GAE/g d.w) and 19.01 (mg. CE/g. d.w), respectively. The research conducted by Haritha et al. (2014) and Devaraj and Karpagam, (2011) on the antidiabetic and antioxidant activities of Aloe vera leaves reported that the leaves of Aloe vera have demonstrated a protective role as antidiabetic and antioxidant in female and Wistar albino male rats. Devaraj and Karpagam (2011) also reported the aqueous extract of Aloe vera leaves has potential anti-inflammatory activity. Raphael (2012) carried out and investigation on the screening of phytochemical compounds such as saponins, alkaloids, tannins, steroids, flavonoids, glycosides terpenoids and carbohydrates from the leaves of Aloe vera extracted via Soxhlet extraction method using chloroform. However, his research was not able to detect the compounds such as steroids, saponins and glycosides in the extract of Aloe vera and they obtained an extraction yield of 8.6%. Another study conducted by Arunkumar and Muthuselvam (2009) on the extraction and screening of

tannin, saponin, flavonoids, terpenoids and steroids from the leaves of Aloe vera extracted using Soxhlet extraction method and different solvents. Among the targeted compounds, the result was negative for steroids. Table 1.1 indicates bioactive compounds of plants extracted via different extraction method from different plant sources.

Table 1.1 Different plants and extraction methods used for obtaining biologically active compounds.

Plant material	**Compou-nds**	**Extraction method/Parameter**	**Solvent**	**Outcome**	**Reference**
Fenugreek seed	TPC and TFC	Maceration	ethyl acetate	75.60 mg GAE/g d.w and 19.01 mg CE/g d.w	(Belguith-Hadriche et al., 2010)
Nettle	TPC	MAE, 10 min, 1:30 g /mL, 50% (407 W)	Water	24.64 mg GAE/g d.w	(Ince et al., 2012)
Nettle	TPC	UAE, 30 min, 1:30 g/mL, 50 % power	Water	23.86 mg GAE/g d.w	(Ince et al., 2012)
Allium ampeloprasum var. porrum	New steroid Saponin	Maceration, 72 h,	Methanol	Steroid Saponin	(Adão et al., 2011)
licorice root	TPC	Soxhlet, 6 h	80% ethanol	41.7 mg GAE/g d.w	(Karami et al., 2015)
licorice root	TPC	MAE, 6 min	80% ethanol	47.47 mg GAE/g d.w	(Karami et al., 2015)
Aloe vera	Aloe emodin	MAE, 3 min, 425 W	Ethanol	2.62%	(Wang et al., 2011)
Aloe vera	Aloe emodin	Soxhlet, 6 h	Ethanol	2.53%	(Wang et al., 2011)
Aloe vera	TPC	cold percolation	Methanol	58.4 mg GAE/g d.w	(Kumar et al., 2017)
Fenugreek seed	TSC	UAE, 15 min,1:10 g/mL, 75 °C	Ethanol	129 mg DE/g d.w	(Navarro et al., 2018)

It is clearly seen in the table that extraction of plants and herbal medicines have been carried out through different methods such as maceration (Li et al., 2007), Soxhlet, (Wang et al., 2010), ultrasound-assisted extraction (Wang et al., 2011) and microwave-assisted extraction (Karami et al., 2015; Wang et al., 2011; Wang et al., 2010). However, studies revealed that MAE is the best method in terms of high extraction yield, low solvent and time consumption, and extraction quality compared to other extraction methods. From the previous finding it has been found that fenugreek seed and Aloe vera leaves contain saponin, phenolic and antioxidant properties. However, the methods used previously, provided low extraction yield and bioactive compounds. Therefore, this study is aimed to extract total saponin content (TSC) and total phenolic content (TPC) from fenugreek seed and Aloe vera leaves via MAE in their optimized conditions which have not been previously explored.

1.3 Objectives of the Study

The objectives of this research are described as follows:

i. To evaluate the potentials of microwave-assisted extraction in obtaining the bioactive compounds of saponin and phenolics from fenugreek seed and Aloe vera leaves.
ii. To optimize the extraction process parameters using response surface methodology (RSM) in microwave-assisted extraction method.
iii. To characterize the extracts of fenugreek seed and Aloe vera leaves in terms of physicochemical properties.
iv. To compare the performance of MAE with the Soxhlet extraction in terms of extraction yield and efficiency.

1.4 Scopes of the Study

To achieve the objective of this study the following activities were applied:

i. Extraction of fenugreek seed and Aloe vera leaves via MAE using parameters namely; extraction times (2, 3, 4, 6 and 12 min) microwave powers (300, 400, 500, 600 and 700 W), ethanol concentrations (20, 40, 60, 80 and 100 %), feed-to-

solvent ratios (fenugreek seed; 1:8, 1:10, 1:12, 1:14 and 1:16 g/mL and Aloe vera leaves 1:8, 1:14, 1:18, 1:20, 1:22 g/mL), and extraction temperatures (40, 50, 60, 70 and 80 °C) using one factor at time (OFAT) experimental method prior to optimization.

ii. Determination and quantification of saponins and phenolic bioactive compounds in the extracts of fenugreek seed and Aloe vera leaves using UV-Vis spectrophotometry.

iii. Screening of MAE parameters such as extraction time, microwave power, ethanol concentration, feed-to-solvent ratio, and temperature via two-level factorial design to find the most significant parameters in achieving high recovery.

iv. Extraction of fenugreek seed and Aloe vera leaves using various Soxhlet extraction parameters such as extraction times (1, 2, 3, 4, and 5 hours), ethanol concentrations (20, 40, 60, 80, and 100 %), feed to solvent ratios (1:14,1:18, 1:20, 1:22, 1:24 g/mL) using OFAT experimental method.

v. Optimization of MAE parameters for optimal recoveries of total saponin content (TSC) and total phenolic content (TPC) using face-centered central composite design (FCCCD) of RSM under design of expert (DOE) software.

vi. Evaluation of dielectric constant, dielectric loss factors and extraction kinetic in MAE.

vii. Comparing the performance of MAE with the Soxhlet extraction in terms of high recovery yield of TSC and TPC.

viii. Identification of saponins and phenolic compounds via liquid chromatography-mass spectrometry quadrupole time-of-flight (LC-MS-QTOF) in the optimal extracts of MAE, evaluation of 2,2- diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) ($ABTS^{+\bullet}$) antioxidant capacities and identification of functional groups and morphological studies of the extracts using Fourier transform infrared (FTIR) and scanning electron microscopy (SEM) analysis, respectively.

ix. Evaluation of the emulsification properties of the extracted compounds of fenugreek seed and Aloe vera leaves in optimal MAE.

x. Evaluation of surface activity and wettability properties of the optimal MAE extracts.

1.5 Significance of the Study

Along with the growing of environmental problems, the awareness of peoples is rising worldwide. Due to the pollution of environment, heart related diseases, formation of free radicals in the body, cell damage and death have become a global concern. The production of free radicals inside our body is the result of normal metabolism system, exposure to environmental pollutants and some other factors. The functions of these radicals are very important in human bodies. However, due to their high reactivity as an oxidant and inhibitor of enzymes, they lead to the biomolecules oxidation such as lipids, protein, DNA and amino acids which finally result into cell damage and consequently cell death and cancer. Previous findings have revealed that chronic illnesses can be treated by consuming dietary antioxidants. Besides, they have achieved a potential interest in food and pharmaceutical sciences (Semmar et al., 2017). Since application of synthetic antioxidants such as BHA and BHT are suspected to have harmful effects to human health. Phenolic compounds are one of the secondary metabolites which have strong antioxidant activity. On the other hand, saponin is also a secondary metabolite which acts as chemical barrier in protecting plants from bacteria and insect attacks. Saponins derived from plants have pharmaceutical properties of anti-inflammation, anticancer, anti-bacterial and antitumor. Studies indicated that saponin is very effective in treating vascular diseases (Cheok et al., 2014). Studies also reported that saponins extracted from plants are able to prevent or treat cancer (Amin et al., 2005). In addition, previous findings revealed that fenugreek seed and Aloe vera leaves are a good source of phenolics and flavonoids which are known as natural antioxidants (Belguith-Hadriche et al., 2010; Haritha et al., 2014).

The quality and quantity of bioactive compounds extracted from plants are related to the extraction methods and solvent formulation. Bioactive compounds are sensitive to temperature. Conventional extraction techniques such as Soxhlet, maceration and boiling are commonly involved high extraction temperature and continuously in contact with the extraction solvent for period of long time, hence it results to the biodegradation of thermolabile compounds. In addition, the consumption of extraction solvent is very high in conventional methods. However, MAE is a technique that provides high extraction

yield and less solvent consumption. It maintains the bioactivity of the compounds due to fast microwave irradiation. Therefore, the potential of MAE and Soxhlet extraction of fenugreek seed and Aloe vera leaves were evaluated in this study.

CHAPTER 2

LITERATURE REVIEW

2.1 Background of the Study

Fenugreek seed and Aloe vera leaves are among the medicinal plants which are used since long time before the production of modern medicines. These plants contains variety of phytochemical compounds including alkaloids, phenols, tannins, flavonoids, saponins, and many other secondary metabolites, which have potential health benefit for humans due to their antioxidant, anticancer, antitumor, antiinflammation, and antidiabetic properties (Maan et al., 2018; Yadav & Baquer, 2014).

This chapter discusses a review of the past findings relevant to this work. This chapter has 15 sections, where each section explains the main points relevant to the present study. Section 2.2 gives an overview of the medicinal plants. Sections 2.3 and 2.4 describes about saponins and phenolic compounds of the plants, respectively. Sections 2.5 and 2.6 discuss about fenugreek seed and Aloe vera leaves which are the selected plants for investigation. The plant extracts and overview of the extraction methods are discussed in sections 2.7 and 2.8, respectively. Sections 2.9 and 2.10 demonstrates about microwave-assisted extraction and factors affecting microwave-assisting extraction including extraction time, microwave power, ethanol concentration, feed-to-solvent ratio and extraction temperature, respectively. Soxhlet extraction is described in section 2.11. One-factor-at-a time and response surface methodology are discussed in sections 2.12 and 2.13, respectively. The methods of characterization and analysis are briefly explained in section 2.14. Finally, a brief summary of the chapter is discussed in section 2.15.

2.2 Medicinal Plants

Plants species that grow on the earth are estimated to be around 250,000-500,000 (Kregiel et al., 2017). Many of these plants have been utilized as traditional herbal medicine since the ancient time for the treatment of humans and animals. According to the World Health Organization (WHO) more than 75% of people in the world are still using the traditional medicines for primary health care. In recent years, natural products derived from plants and herbs are achieving more interest due to low or free risk and side effects. The direct use of natural products in pharmacies as food supplements and complementary medicine is rapidly growing in North America and Europe (Middlesworth & Cannell, 1998).

In addition, most of the medicinal plants also possess antioxidant activity, which is very effective in reducing the toxic and harmful effects of synthetic drugs and environmental pollutions, which are suspected to be the reason in formation of free radicals and reactive oxygen species (ROS) and hence lead to cancer and cell damages. For example, synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which are used in food and medicines are known to have potential risk and toxicity to health (Dudonné et al., 2009). One of the main reasons of type II diabetes is obesity (Farhan et al., 2017). On the other hand, most of the pregnancy related ailments are treated by herbal medicines in most of the countries. It is also reported that risk of diabetes stricken is very high during pregnancy. Studies indicated that consumption of herbal medicines during pregnancy was very popular and safe (Sooi & Keng, 2013). Heart related disease are also one of the human concerns in recent years. Increasing the air pollution level and environmental problems are the main reason behind various health problems in human.

In Malaysia, China and India, traditional medicines have been used since a long time for well-being and health improvements among people. A study conducted by Matthews et al. (2010) indicated that in many countries herbal medicines were applied for curing of illness in pregnant women. Previous researches indicated that although the consumption of herbal medicines in treatment of pregnant women was very popular, but

in some cases, they use it without the lack of awareness about its benefit and safety (Sooi & Keng, 2013). A large number of secondary metabolites are biosynthesized by plants such as saponins, terpenoids, steroids, tannins, flavonoids, phenols, glycosides, alkaloids and etc. These biomolecules have been reported to have anticancer, antiinflammation, antibacterial, antidiabetics, properties (Khan et al., 2018). The secondary metabolites obtained from plants are very useful additive in foods, drinks, medicines, as fragrance and pigment.

2.3 Saponins

Saponins are one of the secondary metabolites with higher molecular weight. These metabolites can be found in plants and marine organisms. Saponins are categorized in two groups of triterpenoid and steroid glycosides (Figure 2.1). It has been reported that, saponins possess a significant anti-tumorigenic and anticancer activities (Tiwari et al., 2017). These natural compounds are consisting of a sugar part (glycon) and non-sugar part (aglycon) connected by a glycoside compound Figure 2.2. The aglycone part is called sapogenin which can be either a triterpenoid or steroid. The chemical structure of each saponins describes it's biological activities such as anti-inflammatory, antibacterial, antifungal, antidiabetic, and anticancer properties (Khan et al., 2018).

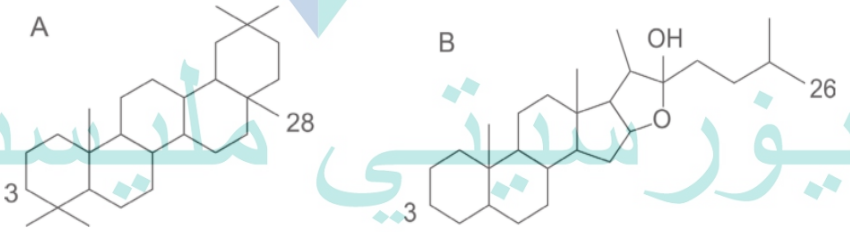


Figure 2.1 Structure of triterpenoid saponin (A) and steroidal saponin (B)

Source: Kregiel et al. (2017)

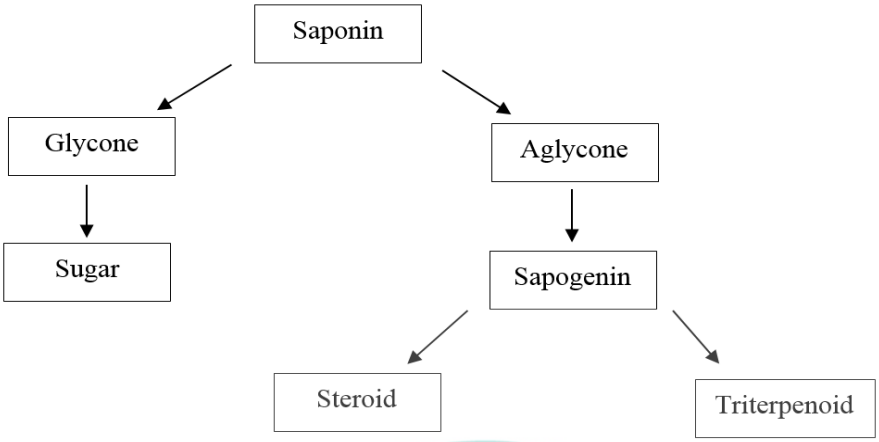


Figure 2.2 Saponin properties and classification

Source: Kaur et al. (2015)

Despite of having the above properties, saponin also possess emulsifying and foaming characteristic which makes it a useful additive for food, pharmaceutical and cosmetic products. Saponins are also known as biosurfactant or surface-active materials with bioactivity. Currently, the production of biosurfactants from natural sources are becoming very attractive for industries due to its low risk and eco-friendly properties (Marchant & Banat, 2012). The surfactants obtained from natural sources have similar properties to the commercially available surfactants by containing hydrophobic (water-heating or oil-loving) and hydrophilic (water-loving) compartments (Fracchia et al., 2012) as seen in Figure 2.3. Natural surfactants (saponins) can be acquired from plants and some sea animals (Boruah & Gogoi, 2013; Cheeke, 2010; Cheok et al., 2014) and it is available in different parts of plants, including leaves, roots, seeds, barks, flowers, and fruits (Goel & Panchkula, 2010; Oleszek & Hamed, 2010b; Samal et al., 2017). The word saponin means foaming agent and it implies a Latin word. Saponins are dissolvable in water with higher molecular weights in the ranges of 600 to 2000 Daltons (Goel and Panchkula, 2010; Peter et al., 2004).

One of the important characteristics of saponins is the foaming properties which makes them able to act as surface-active agents. Surface active agents are widely used chemicals in different industries which include paper products, detergent, paint,

petroleum, pharmaceuticals, textile, cosmetics, food, water and soil treatment (Elazzazy et al., 2015; Guclu-Ustundag & Mazza, 2007; Gupta et al., 2013) .

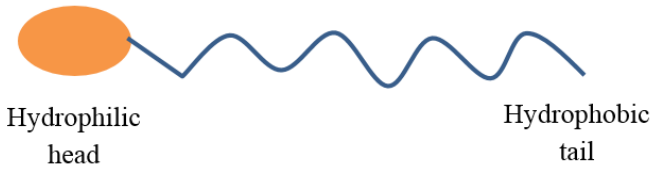


Figure 2.3 Surfactant monomer showing head and tail.

Source: Ghosh et al. (2014)

Biosurfactants are amphiphilic molecules with head and tail containing hydrophobic and hydrophilic properties. In aqueous media, the terms hydrophilic groups are used as lyophilic and hydrophobic groups as lyophobic (Fracchia et al., 2012). Surfactants are found in soaps and detergents, they have the ability to reduce the interfacial tension and surface tension at the water-oil and air-water interfaces (De Almeida et al., 2016; Rufino et al., 2014). Biosurfactants have several advantages over the synthetic surfactants and they can be obtained from different sources. The advantages of biosurfactants over the chemical derived surfactants are the bioavailability, biodegradability, environment-friendly, high foaming and low-cost. Hence, their application in food, cosmetics and pharmaceutical products are considered safe and less harmful (Bhadoriya & Madoriya, 2013; Fracchia et al., 2015; Nitschke & Costa, 2007). The wide applications of biosurfactants have made it a multifunctional agent from food to petroleum industries. The bulk application of chemically derived surface-active agents in industrial processes may result to contamination of soils, rivers, and even oceans by the industrial discharges. The characteristic and usefulness of biosurfactants as presented in Figure 2.4 include solubility enhancement, reduction of surface tension and low critical micelle concentrations (CMC) (Jha et al., 2016; Mulligan, 2009). When a biobased surfactant is able to reduce water surface tension from 72 to around 40 mN/m and interfacial tension between a polar and non-polar liquid and for water against n-hexadecane from 40 to 1 mN/m (Mulligan, 2005) is considered effective. In surface and colloid chemistry, CMC is an important characteristic of surfactants defined as the maximum concentration of surfactant monomers in water which can be influenced by

temperature, pH and ionic strength of the solution (Mondal et al., 2015; Pacheco et al., 2010; Shah et al., 2016).

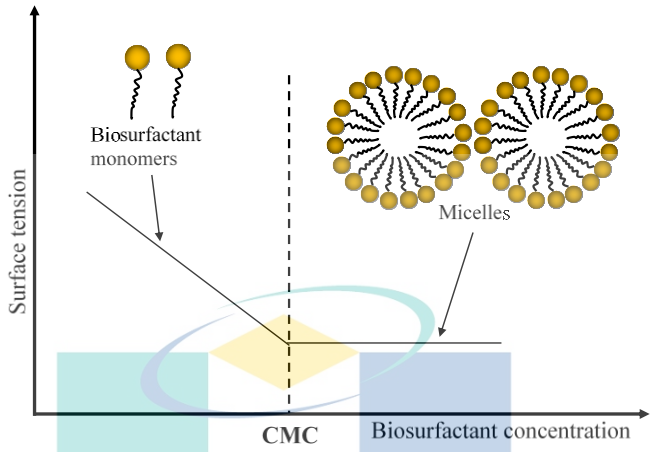


Figure 2.4 Biosurfactant monomer and micelle formation.

2.4 Phenolic Compounds

There are many bioactive compounds exist in different parts of plants. Phenolic compounds are reported to be one of the most valuable compounds with beneficial effects on human health such as antimicrobial, antioxidant, anticancer and antidiabetic properties. Phenolic compounds such as phenolic acid, flavonoids and tannins are known to have antioxidant activities (Floegel et al., 2011). This property is due to their ability in scavenging and neutralizing free radicals as well as metal chelating especially iron and copper cations. Plants that are rich in antioxidant activity are very attractive to food industries, since they are looking for such compounds to promote the use of their product and make them a valuable alternative to the synthetic food additives. Studies also shown that vegetables and fruits are a very good source of phenolic compounds and higher consumption of these food sources prevents some dangerous diseases. The presence of phenolic compounds is not uniformly distributed in plants. The diversity is possible within the same plant species due to environment condition, genetic factors and growth stages (Iness et al., 2010). Phenolic compounds such as phenolic acids and flavonoids have great health benefits as they can reduce the risk of type 2 diabetes. Many vegetables and fruits such as tomatoes, berries and grapes have phenolic compounds and they are

also a good source of antioxidants. The production of free radicals inside the body is mainly due to the air pollution, weak immune system, inflammation and UV light which can cause cancer and some other diseases. Hence, to prevent these risks, consumption of foods that contain phenolic compounds are important to protect our body against oxidative damage (Lin et al., 2016). The main classes of phenolic compounds are shown in Table 2.1.

Table 2.1 Main classes of phenolic compounds with respect to carbon chain

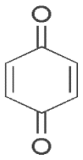
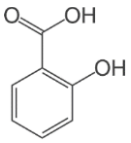
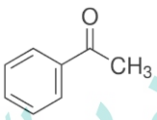
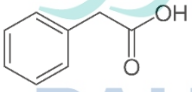
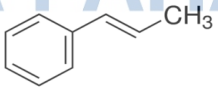
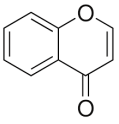
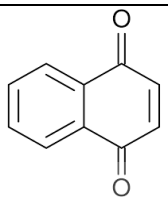
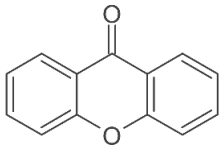
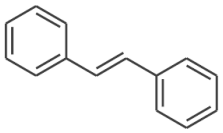
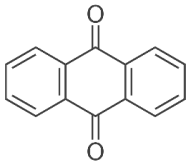
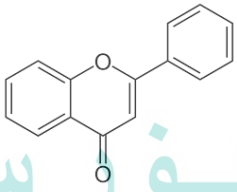
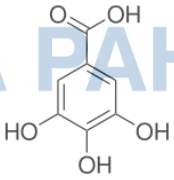
Class	Basic Skeleton	Basic Structure
Simple phenol	C_6	
Benzoquinones	C_6	
Phenolic acids	$C_6 - C_1$	
Acetophenones	$C_6 - C_2$	
Phenylacetic acids	$C_6 - C_2$	
Phenylpropenes	$C_6 - C_3$	
Chromones	$C_6 - C_3$	

Table 2.1 Continued

Class	Basic Skeleton	Basic Structure
Naphthoquinones	$C_6 – C_4$	
Xanthones	$C_6 – C_1 – C_6$	
Stilbenes	$C_6 – C_2 – C_6$	
Anthraquinones	$C_6 – C_2 – C_6$	
Flavonoids	$C_6 – C_3 – C_6$	
Tannins	$(C_6\text{-}C_3\text{-}C_6)_n$	

Source: Giada (2013)

2.5 Fenugreek

Fenugreek (*Trigonella foenum-graecum*) is a multi-regional plant commonly found in the Middle East, Asia and some European countries. Many countries in Asia, Africa, and Europe use this plant especially the seed and leaves as a food source. Fenugreek belongs to the Leguminosae or Fabaceae family, the genus *Trigonella* and species is *foenum-graecum*. The Legume or bean family is the third largest family of flowering plants with more than 18000 species and 650 genera (Doyle, 2001). The use of fenugreek documented in ancient Egypt. Egyptians were used this herb to embalm mummies and in incense. The Greeks and Romans utilized it for animal feed. *Foenum-graecum* represents the Latin name of fenugreek means Greek-hay (Al-Asadi, 2004). Morton (1990), reported that the height, leaves and seedpods of this plant are around 60 cm, 2-2.5 cm and 14 cm, respectively. Each pod has 10-20 brownish-yellow seeds with a dimension of 3×4 mm. Different parts of this plant such as seed, leaves and steam have indicated high pharmacological and nutritional value, hence, this plant attracted many researchers to take it under consideration. The seeds and leaves of this plant have shown anti-diabetic, anti-microbial, lactation aid, anti-inflammation, anticancer, antifungal, and antioxidant activity (Arivalagan et al., 2013; Yadav & Baquer, 2014). A study carried out by Kaviarasan et al. (2006), on experimental rats and suggested that fenugreek seed can protect liver from ethanol-induced oxidative stress. Cancer is one the most dangerous diseases in worldwide. Where in some cases it remains untreatable. Comparing to conventional therapeutic treatments, the natural sources, fruits and vegetables containing anticancer activity are mostly recommended for prevention of cancer. Another study conducted by Amin et al. (2005) on rats fed fenugreek seed extract, showed that the extract has potential activity against breast cancer in rat. Prabhu and Krishnamoorthy (2010), reported that the ethanolic extract of fenugreek seed also provided anticancer activity when tested in Swiss albino mice.

Recently, the intake of dietary supplements produced from plants and herbs have achieved more attention due to high nutritional values for treating and preventing chronic immune disorders. Some research has claimed that bioactive compounds extracted from plants and fruits are very useful to treat or avert chronic ailments. This is due to the

existence of pharmacological compounds such as flavonoids, tannins, saponins, alkaloids, phenols, steroids and triterpenoids (Alara et al., 2017). Bioactive compounds of polyphenols and flavonoids are known as a good source of natural antioxidants for controlling oxidative stress (Ashraf et al., 2013). The bioactive compounds of saponins are also known for its health benefits. The saponin and phenolic compounds of plants possess potential medicinal values in treating various ailments such as heart problems, cancer, tumor, diabetes and infections (Chan et al., 2014). In fact, the extraction method and different solvent formulation may also affect the recoveries of bioactive compounds. In addition, previous studies have also reported the presence of alkaloids, tannins, flavonoids, saponins, phenolics and steroids in seeds of fenugreek. However, determinations of these bioactive compounds in fenugreek seed are commonly performed via conventional extraction techniques such as maceration, Soxhlet and ultrasound assisted extraction (Rababah et al., 2004; Wani et al., 2016). In addition, different types of solvents have been used to extract biologically active compounds from fenugreek seed in previous studies. Fenugreek plant, seed and leaves are indicated in Figure 2.5.

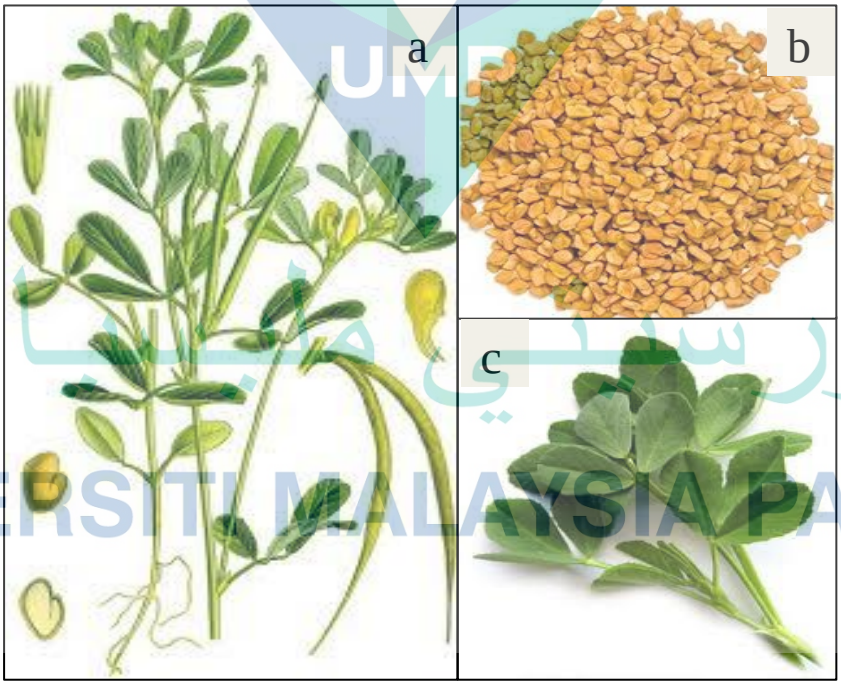


Figure 2.5 Fenugreek plant (a); fenugreek seed (b); fenugreek leaves (c)

Source: Saikat (2019)

2.6 Aloe Vera

Aloe vera (*Aloe barbadensis Miller*) a known medicinal plant belonging to the family of Liliaceae is originally cultivated in South Africa. This plant has more than 400 species and can be found in countries with warm and arid climate. The height of Aloe vera leaves can go from 10 cm to almost 100 cm (Figure 2.6). The leaves have tick epidermis with viscose gel where 95-99% of the leaves are water (Ray et al., 2015). Aloe vera is one of the most popular medicinal plant due to its potential biological activity and availability. Aloe vera leaves contain various biological compounds such as glycosides, proteins, chromones, amino acids, carbohydrates, lipids, vitamins, sugars, and minerals. Studies also revealed that Aloe vera leaves have many pharmaceutical properties including anticancer, antioxidant, antimicrobial, anti-inflammation and antidiabetic (Nandal & Bhardwaj, 2012). This plant has also been used in the dietary supplements and some food products. Due to existence of amino acids in Aloe vera, it has been reported to be very effective for the wound healing. Besides, the presence of inorganic electrolytes like potassium, iron, chromium, magnesium, sodium, copper, zinc and calcium which are vital portion of wound healing process (Maan et al., 2018). A study that was conducted on the application of Aloe vera in wound healing showed that the treatment process was 8.79 days shorter than non-treated patients (Maenthaisong et al., 2007).

In addition, the anti-inflammatory effects of the Aloe vera is also useful for the treatment of joint pain. The complex mechanism in human body results to the painful inflammation which involves the production of inflammatory mediator (bradykinin). Studies revealed that Aloe vera possess a bradykinase enzyme which has anti-bradykinin activity. This enzyme, leads to the breakdown of the bradykinin and hence reduces the inflammation (Maan et al., 2018). The existence of glycoproteins and polysaccharides in Aloe vera make it a potent anti-cancer agent. also shows its anticancer property. Moreover, the presence of some bioactive compounds and vitamins in Aloe vera possess strong antioxidant activity which is useful for the treatment of various diseases including cancer (Maan et al., 2018; Mukherjee et al., 2014).

Figure 2.6 Aloe vera plant.

Source: Khaniabadi et al. (2016)

2.7 Justification of Plants Selection

The selection of fenugreek seed and Aloe vera leaves in this study were based on their geographical and economical aspects in Malaysia. Fenugreek is an annual plant widely found in Malaysia and other Asian countries. In addition, Aloe vera is also easily found in Malaysia, since this plant is a tropical plant and can live long in warm countries with high humidity. This plant can tolerate at least 6°C. The best temperature is between 13 to 30°C. Fenugreek seed and Aloe has indicated many health benefits for humans. Previous studies have revealed many applications of these two plants in medical studies. The existence of saponin and phenolic compounds has been confirmed in most of plants, vegetables, and fruits. However, the application of fruits and vegetables for medical purposes are not very encouraged to be used for medical research or production of medicines in large scale. Since, most of them are considered very important for daily intake as a food source in human life (Pem & Jeewon, 2015). Therefore, there are also some other types of plants that are not considered as the main source of food in human and animal life. Hence, their application in medical research with good outcome are not limited. Fenugreek seed and Aloe vera leaves are mostly used in medical and health care products. This study also focuses on the application of these two plants as a good source of TSC and TPC for food, medical and pharmaceutical industries.

2.8 Plant Extracts

Plant extracts have been used for different purposes for many years due to their safety and potential medicinal advantage. Traditionally, humans have used the extracts of different parts of plants to cure many infectious and chronic diseases. In fact, medicinal plants have received much attention as a source of bioactive compounds which are relatively safe for humans and rarely reported to have any side effect. In addition, they can be consumed as a source of standardized plant extract or pure compounds. Concisely, different parts of plants such as bark, fruit, leaves, peels, steam, and seeds have been reported to have pharmacological and phytochemical properties (Rakholiya et al., 2013). The secondary metabolites produced from plants are able to inhibit fungi, bacteria, viruses, and pests. According to the chemical structure, secondary metabolites are classified to phenolics, alkaloids, saponins, lipids, terpenes, and carbohydrates. Table 2.2 shows some secondary metabolites retracted from of plants.

Table 2.2 Secondary metabolites extracted from different plants.

Plant name	Plant photo	Secondary metabolite	References
Origanum		Phenolics	(Jose et al., 2010)
Uncaria sinensis		Alkaloid	(Tan et al., 2011)
Allium macrostemon Bunge		Saponin	(Chen et al., 2009)

Table 2.2 Continued

Plant name	Plant photo	Secondary metabolite	References
Xanthoceras sorbifolium		Lipid	(Venegas-Calerón et al., 2017)
Fraser Fir		Terpene	(Bowman et al., 1997)
Opuntia cochenillifera (L.)		Carbohydrate	(Monrroy et a., 2017)

2.9 Overview of the Extraction Methods

The selection of a proper extraction method is very important in the process of plant extraction. The advantages and disadvantages of extraction method would mainly depend on the factors such extraction yield, time of extraction, extraction cost, safety, and quality of the extract. The quality and quantity of bioactive compound extraction depends on extraction techniques and types of extraction solvent. Different techniques of extraction including, Soxhlet, ultrasonic-assisted extraction (UAE), maceration, reflux extraction, microwave-assisted extraction (MAE), and accelerated-solvent extraction (ASE) have been applied in previous studies (Gholamreza et al., 2011; Ince et al., 2012; Navarro et al., 2018; Sporring et al., 2005). However, many studies reported that MAE is the best technique to obtain bioactive compounds of plants, since this method is

performed with low energy and solvent consumption, has short time and high extraction recovery (Alara et al., 2018; Cheok et al., 2014).

2.10 Microwave-Assisted Extraction

In fact, MAE is a non-conventional technique of extraction with more advantages than conventional methods of extraction. In MAE, the conduction and convection process are very fast within seconds and they are usually neglected and assumed zero. In microwave heating process, the heat transfers to the material trough radiation process (Akbari et al., 2016). This technique has been considered as green extraction method where the extraction process is based on the direct impact of microwave irradiation on polar compounds. Microwaves generate electromagnetic radiation with frequencies in the ranges of 0.3 GHz to 300 GHz and a wavelength from 1m to 1mm. The mechanism of internal heating in a microwave is based on the dielectric polarization caused by microwave irradiation and hence the process involves three sequential steps. i) increased temperature and pressure of the system which results to the separation of the solutes from the active sites of the plant matrix. ii) solvent diffusion across the plant matrix and iii) transfer of the solutes from the plant matrix to the solvent (Barba et al., 2016). In fact, the mechanism of microwave heating is not the same as conventional heating. In conventional heating, the thermal energy from the heating source is transferred to the material through convection, conduction, and radiation processes. However, in microwave heating two mechanisms are involved to transfer the energy which are called ionic conduction and dipole rotation (Figure 2.7). Ionic conduction refers to the electrophoretic migration of ions when an electromagnetic field is applied, and hence the friction between ions and then generation of heat. Dipole rotation is defined as the reorientation of the dipoles under microwave radiation. Polar solvents such as water, ethanol and methanol have high dielectric properties and hence better heating. Non-polar solvents such as petroleum ether and n-hexane provide poor heating in a microwave process as they have low dielectric constant (Desai et al., 2010; Zhang et al., 2011).

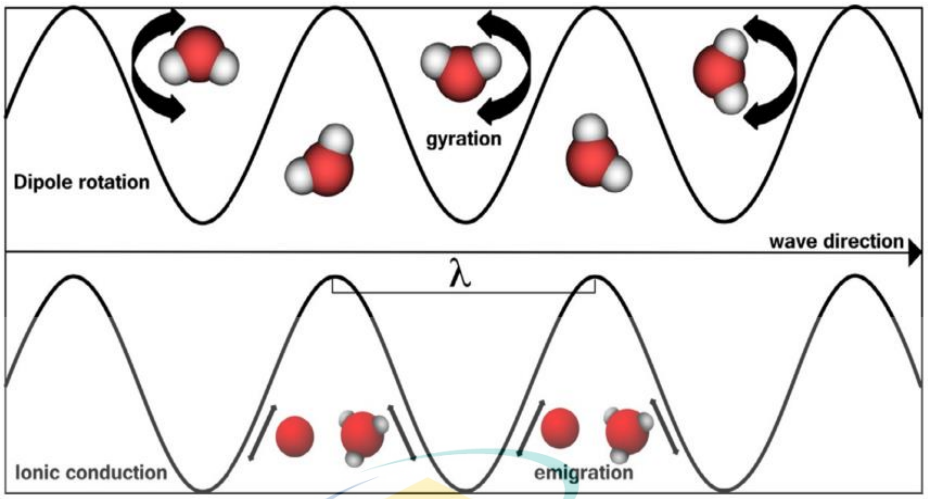


Figure 2.7 Mechanism of microwave heating

Source: Aguilar-Reynosa et al. (2017)

The application of polar solvents in microwave extraction has been very effective in the extraction of bioactive compounds from plants (Azwanida, 2015). Water is highly polar and the polarity of ethanol is lower than water. Hence, combination of water and ethanol may provide better heating and efficiency in plant extraction. Table 2.3 indicates some of the bioactive compounds of plants extracted via MAE. Considering the economic and environmental aspects lower extraction time and diluted ethanol is a good reference in achieving saponin and phenolic compounds. MAE has received much attention in extraction of bioactive compounds from plants. The reduced extraction time and solvent using MAE has attracted industries to use this technique in industrial scales. Liazid et al. (2011) studied that extraction of anthocyanins from grape skins and carried out that in MAE the extraction time reduced from 5h to 5min when compared with the conventional solid-liquid extraction method. Figure 2.8 represents the heating distribution in conventional and microwave heating.

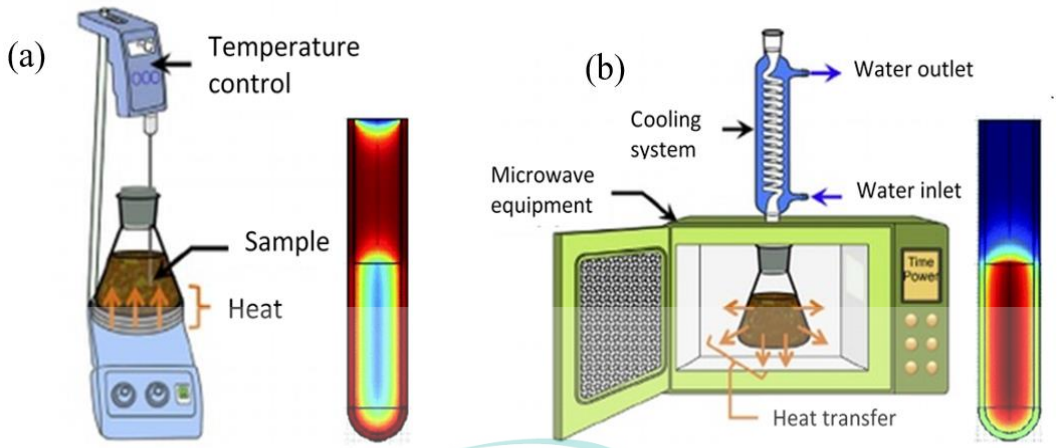


Figure 2.8 (a) conventional heating method, (b) microwave-assisted extraction method

Source: Barba et al. (2016)

Table 2.3 Microwave-assisted extraction of medicinal plants.

Plant material	**Compounds**	**Solvent**	**Optimal condition**	**References**
Green tea leaves	polyphenols	Water	3 min, 600 W, 1:20 g/mL	(Li & Jiang, 2010)
Eclipta prostrata	Phenolics	Ethanol-water	2 min, 400 W, 70 °C, 1:30 g/mL, 50 % ethanol	(Fang et al., 2015)
Xanthoceras sorbifolia Bunge	Saponins	Ethanol-water	7 min, 900 W, 1:32 g/mL, 51 °C, 42 % ethanol	(Li et al., 2010)
lotus plumule	Alkaloid	Methanol	4.3 min, 200 W, 65 % methanol,	(Xiong et al., 2016)

Table 2.3 Continued

Plant material	Compounds	Solvent	Optimal condition	References
Myrtus communis L. leaves	TPC	Ethanol - water	62 s, 500 W, 1:32 g/mL, 42 % ethanol	(Dahmoune et al., 2015)
Vernonia amygdalina leaf	TPC, TFC, DPPH, ABTS	Ethanol-water	4 min, 558 W, 1:10 g/mL, 76 % ethanol	(Oluwaseun et al., 2018)
Phyllanthus amarus	Total extraction yield	Methanol	3 min, 600 W, 1:10 g/mL, 65 % methanol	(Garg et al., 2016)
Yuanhu Zhitong prescription	Tetrahydropalmatine, imperatorin and isoimperatorin	Ethanol-water	27 min, 500 W, 70 % ethanol	(Liao et al., 2008)
Chaenomeles sinensis	oleanolic acid and ursolic acid	Methanol	7 min, 600 W, 52 °C, 1:32 g/mL,	(Fang et al., 2010)
Coriolus versicolor mushroom	TPC, DPPH, ABTS	Ethanol-water	3.8 min, 125 W, 40 % ethanol,	(Maeng et al., 2017)
Sophora japonica L.	Extraction yield	Methanol	1.3 min, 287 W, 1:50 g/mL,	(Liu et al., 2016)

2.11 Factors Affecting Microwave Extraction

Microwave-assisted extraction can be influenced by many factors such as extraction time, microwave power, solvent concentration, feed-to-solvent ratio, and extraction temperature. The effects of these factors are described in the following subsections.

2.11.1 Effect of Extraction Time

Extraction time is an important factor influencing the extraction of bioactive compounds of the plant. Generally, this factor has a positive effect on the yield of bioactive compounds. In fact. Time of extraction in MAE is very short when compared to conventional extraction methods. Commonly, it varies from few seconds to less than an hour (Barbosa-Cánovas, 2013). Increasing the extraction time in MAE results to an increase in extraction yield. However, longer irradiation time in MAE will cause to the degradation of thermolabile compounds and hence reduce the quality of the extract (Chen et al., 2007). In a MAE, the overheating occurs when a solvent with high dielectric properties such as water, methanol and ethanol is used. Since water and methanol have higher dielectric properties compared to ethanol. So, a combination of water and ethanol in a proper ratio will provide better heating during microwave irradiation. Since ethanol is considered safe to the environment and also provides high yield during MAE (Karami et al., 2015).

2.11.2 Effect of Microwave Power and Temperature

Microwave power is strongly interrelated to the extraction temperature and time. The extraction temperature in MAE increases with the microwave power, hence shorter extraction time is required to perform the extraction. Increasing the microwave power results to the increase in the yield of extraction. However, further power enhancement will cause to the overheating of the plant matrix and hence degradation of bioactive compounds (Wang et al., 2010). At high microwave power, the temperature will increase, and the rupture of plant cells occurs rapidly.

2.11.3 Effect of Solvent Concentration and Solvent-to-Feed Ratio

Solvent selection is the most important factor in a MAE process. In microwave extraction, the selection of extraction solvent is usually based on solvent penetration, solubility of the components, interaction of solvent with plant matrix and dielectric constant of the solvent. Generally, solvents with high dielectric constant such as water, methanol and ethanol provide better absorption of microwave energy and hence effective

extraction. None-polar solvents such as hexane and petroleum ether are not a good choice to be used in MAE for the extraction of bioactive compounds. The addition of water to the extraction solvent, also facilitate better extraction process and heating (Barbosa-Cánovas, 2013; Veggi et al., 2013).

Solvent-to-feed ratio is also one of the important parameters in MAE. During the extraction process, the solvent volume must be sufficient to immerse the sample during the extraction process. If the ratio of feed-to-solvent is not selected properly, the extraction of sample will not be very effective, since the contact of solvent and sample is very essential (Atirah et al., 2017).

2.12 Calculation of Microwave Parameters

Microwaves are a form of electromagnetic energy, the most significant characteristic of microwave heating is its volumetric heating, which makes it quite different from conventional heating. Volumetric heating means that the microwave energy can be absorbed directly and internally by materials and then converts into heat. In conventional heating, the energy is transferred to the material via convection, conduction, and radiation phenomena through the external surface of the material. However, in microwave, energy is delivered to the material directly through the molecular interactions with the electromagnetic field via conversions of electromagnetic energy into thermal energy or with the electric field of the incident radiation (Venkatesh and Raghavan, 2004). Since, the microwave has the volumetric heating property; hence the volume rate of heat generation in a microwave can be calculated as Eq. (2.1).

$$Q_{mw} = \underbrace{\frac{hA}{V}(T_m - T_a)}_{\text{Convection}} + \underbrace{\frac{\varepsilon A \sigma}{V}[(T_m + 273.15)^4 - (T_a + 273.15)^4]}_{\text{Conduction}} + \underbrace{\rho C_p \left(\frac{dT}{dt}\right)}_{\text{Radiation}} \quad (2.1)$$

The contribution of heat transfer for convection and conduction was not significant, thus they can be ignored. In Eq. (2.2), the rate of heat transfer for convection and conduction is assumed zero, due to the rapid transformation of heat from emulsified

water droplets to the continuous phase (oil). Accordingly, the temperature of water and oil is almost at the same rate (Fang and Lai, 1995; Anisa, 2011). Therefore, the volume rate of heat generation of mixture can be simplified as below:

$$Q_{mix} = \rho_{mix} C_{p,mix} \left(\frac{dT}{dt}\right) \qquad 2.2$$

Where, Q_{mix} (cal/s.cm^3) is the volumetric rate of heat generation of the mixture, ρ_{mix} and $C_{p,mix}$ are the density and heat capacity of materials in (g/cm^3) and (Cal/g.^{0}C) respectively. *dT/dt* is the rate of temperature increase (^{0}C/sec).

For calculation of volume rate of heat generation in Eq. (2.2), the density (ρ) and (Cp) of the solvents calculated from mixing rules as described in the following Eqs. (2.3 and 2.4):

$$\rho_{mix} = \rho_w \emptyset + \rho_o (1 - \emptyset) \qquad 2.3$$

$$C_{p,mix} = C_{P,w} \emptyset + C_{p,o} (1 - \emptyset) \qquad 2.4$$

In microwave system, energy transfers directly to the materials through molecular interactions with the electromagnetic field via conversions of electromagnetic energy into thermal energy as a result of dielectric loss that caused by rotation of polarized molecules (Venkatesh and Raghavan, 2004; Routray and Orsat, 2012). The dielectric properties of materials related to the complex relative permittivity (ε) as described by Eq. (2.5), the imaginary unit (j) is shown in Eq. (2.6).

$$\varepsilon = \acute{\varepsilon} - j\varepsilon'' \qquad 2.5$$

$$j = \sqrt{(-1)} \qquad 2.6$$

Where ε', is the dielectric constant that reflects the ability of the material to store electric energy when in an electromagnetic field; ε'', is the dielectric loss factor that

influences the conversion of the electromagnetic energy into thermal energy (heat). The ratio of dielectric constant and dielectric loss factor represent the tangent loss factor, tan δ, also called the dissipation factor or the dielectric loss tangent as expressed by Eq. (2.8).

$$tan\,\delta = \frac{\varepsilon''}{\varepsilon'} \qquad 2.8$$

The dielectric constant is a measure of a material's ability to store the microwave energy as it passes through, while the dielectric loss factor measures the material's ability to dissipate that energy.

The dielectric constant and dielectric loss of water were given by (Wolf, 1986):

$$\varepsilon'_w = 85.2 - \ 0.3358T \qquad 2.9$$

$$\varepsilon''_w = 320.66T^{-1.03} \qquad 2.10$$

The Debye equation for obtaining dielectric constant (ε') and dielectric loss (ε'') of polar dielectric materials at various frequencies is described in Eqs. (2.11- 2.13). The temperature enters the discussion by way of the parameter known as the relaxation time, τ. In this work, the value of relaxation time is obtained using the relationship between $\omega = 2\pi f$ the angular frequency in radians per second and $\tau = 1/\omega$.

Equations (2.11) and (2.13) were used to compute the values of the dielectric constant, loss factor, and relaxation time, respectively. The values of the complex permittivity ε_∞ and static permittivity ε_s used in this work were adapted from the previous finding computed by (Barthel & Buchner, 1991).

$$\varepsilon'_e = \varepsilon_\infty + \frac{(\varepsilon_s - \varepsilon_\infty)}{1 + \omega^2\tau^2} \qquad 2.11$$

Where $\tau = 1/\omega$

$$\varepsilon'_e = \frac{(\varepsilon_{s+}\varepsilon_{\infty})}{2} \qquad 2.12$$

$$\varepsilon''_e = \frac{(\varepsilon_{s-}\varepsilon_{\infty})}{2} \qquad 2.13$$

Since the frequency for most types of the microwave apparatus is set at 2.24 GHz, the wavelength of the electromagnetic fields in the liquids as a function of temperature expressed by Eq. (2.14).

$$\lambda = \frac{\lambda_0}{\sqrt{\acute{\varepsilon}}} \qquad 2.14$$

Where, λ is the wavelength, (λ_0 = 12.2 cm) is the wavelength in free space (Patil, 2012).

The penetration depth (D_P) is also one of the important parameters in microwave heating. The D_P within a sample for a radiation is related to dielectric constant and dielectric loss as expressed in Eq. (2.15).

$$D_P = \frac{C}{2\pi f \sqrt{2\acute{\varepsilon}}\ [\sqrt{1 + tan^2\delta - 1}]^{-1/2}} \qquad 2.15$$

Where, C is the speed of light (cm/sec), and f is the applied frequency (Hz). However, when tan δ<1, the D_P is as Eq. (2.16), which is usually the case (Veggi et al., 2013).

$$D_p = \frac{\lambda_0\sqrt{\acute{\varepsilon}}}{2\pi\varepsilon''} \qquad 2.16$$

2.13 Kinetic Modelling

The kinetic studies in an extraction would allow a better understanding of the extraction mechanism. Kinetic studies have been carried our using different models. However, the commonly used models are Fick's law, Patricelli empirical model, First and Second-order Rate Laws. However, first order and second-order models have been widely used to describe the rate of adsorption in liquid-solid interactions. In a MAE the modelling is essential for predication of extraction behaviour and scaling up purposes (Chan, 2013). A study carried out by Kusuma & Mahfud (2017) on kinetic modelling of oil extraction from sandalwood by microwave-assisted hydro distillation using first and second-order kinetic models. The study found that the second-order model was suited better for the experiment by providing highest coefficients of correlation (R^2= 0.9961). Another kinetic study performed by Alara & Abdurahman (2019) on effects of extraction techniques on bioactive compounds from *Vernonia cinerea* leaf. Their findings also indicated that the second-order model better fitted the study with higher coefficients of correlation.

2.13.1 First Order Kinetic Model

The equation in a first order model was first described by Lagergren (1898) and modified by Kusuma and Mahfud (2017) the equation of first-order extraction kinetic model can be written as shown in Eq. (2.17). It is assumed that the first-order rate equation is correlated with the idea of a linear driving force (Liu and Shen 2008).

$$\frac{dC_t}{dt} = K_1(C_s - C_t) \qquad 2.17$$

where C_s the concentration of water-soluble compounds of plants extract at saturation. C_t is the extraction capacity at different extraction time t, and k_1 indicates the first-order extraction rate coefficient in per minute (min^{-1}). Eq. (2.17) was further integrated at the boundary conditions of $C_t = 0$ and $C_t = C_t$ to obtain the Eq. (2.18).

$$\ln\left(\frac{C_s}{C_s - C_t}\right) = k_1 t \qquad 2.18$$

The Eq. (2.18) was expressed in a linear form as shown in Eq. (2.19) whereby $\log_{10}$ (C_s - C_t) was plotted against t to obtain a slope and intercept that are used in determining the first-order extraction rate and extraction capacity.

$$log_{10}(C_s - C_t) = \log(C_s) - \frac{k_1}{2.303}t \qquad 2.19$$

2.13.2 Second-Order Kinetic Model

According to Kusuma and Mahfud (2017) a second-order kineitc model for determining the extraction rate of fenugreek seed and Aloe vera leaves extracts can be expressed in differential form. In a second-order kinetic model mechanism, the extraction occurs in two simultaneuos process. The quantity of extract improves with time in the beginning and decline gradually with time until the extraction process ends (Man et al. 2012). Thus, the rate of dissolution of extract present in the plant sample to a solvent can be described as Eq. (2.20).

$$\frac{dC_t}{dt} = k_2(C_s - C_t)^2 \qquad 2.20$$

Where k_2 is the coefficinet of second-order extraction rate in litre per gram per minute (L/g.m). Eq. (2.20) was further integrated using the boundary conditions $C_t = 0$ at $t = 0$ and $C_t = C_t$ at $t = t$ to obtain final Eq. (2.23) through Eqs. (2.21–2.22).

$$\frac{1}{(C_s - C_t)} - \frac{1}{C_s} = k_2 t \qquad 2.21$$

By rearranging the Eq. 2.21, Eq. 2.22 and 2.23 can be obtained as follows, respectively.

$$C_t = C_s - \frac{C_s}{1 + C_s k_2 t} \qquad 2.22$$

$$C_t = \frac{C_s^2 k_2 t}{1 + C_s k_2 t} \qquad 2.23$$

Equation (2.23) was then rearranged in linearize form through Eqs. (2.24 and 2.25) to obtain Eq. (2.26) as follows:

$$\frac{1}{C_t} = \frac{1}{k_2 C_s^2} + \frac{t}{C_s} \qquad 2.24$$

$$\frac{t}{C_t} = \frac{1}{k_2 C_s^2} + \frac{t}{C_s} \qquad 2.25$$

By inversing the Eq. 2.25, Eq. 2.26 will be achieved as sollows:

$$\frac{C_t}{t} = \frac{1}{(\frac{1}{k_2 C_s^2} + \frac{t}{C_s})} \qquad 2.26$$

Thus, extraction rate (C_t/t) can be evaluated from Eq. (2.26). If the initial extraction rate (m) with C_t = t as t approaches zero. Then,

$$m = k_2 C_s^2 \qquad 2.27$$

By substituting Eq. (2.27) in Eq. (2.25), the Eq. 2.28 can be obtained as follows:

$$\frac{t}{C_t} = \frac{1}{m} + \frac{t}{C_s} \qquad 2.28$$

Hence, the second-order extraction rate coefficient (k_2) the initial extraction rate coefficient (m), and extraction capacity can be calculated through experimental procedure from the slope and intercept of a plot between t/Ct and t.

2.14 Soxhlet Extraction

Soxhlet extraction has been a widely used extraction technique since more than a century. It has been considered a standard method for obtaining bioactive compounds of different plants. In fact, the extraction techniques are divided in two categories of conventional and non-conventional extraction methods. Soxhlet is referred as a conventional technique of extraction. Since it has been used for over a century. Initially, in 1879 this technique was first proposed by a German agricultural chemist, Franz Ritter

von Soxhlet and named it as Soxhlet extractor (Luque et al., 2010). The procedure of extraction in this technique starts with placing the sample inside a thimble (sample holder) and then transferring them to the Soxhlet column. Then the distillation flask which is connected to the Soxhlet column and heat source will heat up the solvent to the boiling point. When the solvent reaches to the flow level, a siphon aspirates the solute from the sample holder and unloads it back into the distillation flask. This operation will repeat many cycles continuously until the extraction time is completed (Figure 2.9). Once the extraction completed, the solvent is removed mostly using a rotary evaporation system. The extracted compound can be collected in a sample container and the solid residue will be discarded (Luque et al., 2010). The advantages of this method are the simplicity of the technique and after extraction process, no filtration is required. The major drawbacks and disadvantages of Soxhlet extraction method are the long processing time and high solvent consumption (Karami et al., 2015; Luque et al., 2010). However, when comparing this method with other conventional extraction techniques like maceration, higher extraction yield can be obtained through Soxhlet extraction (Dhobi et al., 2009). Many researchers have used this technique for the extraction of bioactive compounds from different plant sources. Table 2.4 shows the researchers used this method for the extraction of bioactive compounds of the plants.

Table 2.4 Soxhlet extraction of plants bioactive compounds

Plant material	**Compounds**	**Solvent**	**Optimal condition**	**References**
licorice root	Phenolic compounds	Ethanol	6 h, 80% ethanol	(Karami et al., 2015)
Piper nigrum	Piperine	Methanol	22 h, pure methanol	(Subramanian et al., 2016)
Silybum marianum	Flavonolignan	Ethanol	12 h, 80% ethanol	(Dhobi et al., 2009)
Perilla Frutescens leaves	Flavonoids	Water	4 h, water	(Shao et al., 2012)
licorice root	Glycyrrhizic acid	Ethanol	10h, ethanol 60%	(Pan et al., 2000)

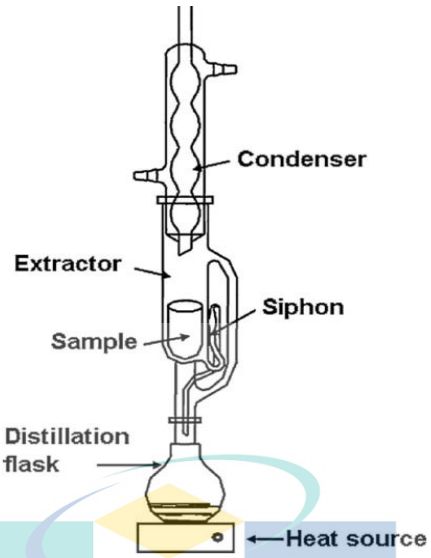


Figure 2.9 Soxhlet extraction apparatus.

Source: Luque et al. (2010)

2.15 One-Factor-at-a-Time Experiment

One-factor-at-a-time (OFAT) experiment was a commonly used method for optimization process long time ago. In this method only one factor is variable at a time while other factors are kept constant. This approach is usually used to determine the advantages of one factor during the process over another factor. However, this technique is time consuming and expensive, besides it has not been very useful for designing an experiment due to the lack of sufficient data and estimation of interactions between factors. Therefore, some studies have reported that OFAT has advantages in improving the system rather than in designing and modelling of the system (Fang et al., 2010; Frey & Jugulum, 2006).

2.16 Response Surface Methodology

Response surface methodology (RSM) is a statistical tool widely used for designing the experiment through statistical and mathematical techniques. RSM explores the influence of several variables on one or more responses. The independent variables are known as the input and the dependent variable is called response variable (Bezerra et

al., 2008; Demirel and Kayan, 2012) . The utilizations of RSM for optimizing the process parameters are very common, it reduces the time and cost of experimental designs. Usually, RSM is consists of either first-order or second-order model. If the response can be defined by a linear function of independent variables, then the approximating function is a first-order model. A two-level factorial (2^k) is the most common first-order design which can be expressed as Eq. 2.29.

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k + \varepsilon \qquad 2.29$$

In an experimental design, the second-order design is used when there is a curvature in the response surface. Typically, if the lack of fit is significant in a first-order model, then a second-order model can be used in order to locate the optimum or best condition. The second-order model is presented in Eq. (2.30).

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon \qquad 2.30$$

Where, y, β_0, k, β_i, x_i, β_{ii}, β_{ij} and ε are the response, the constant term, number of variables, the coefficient of the linear factor, variables, the coefficients of the quadratic parameter, the coefficients of the interaction parameters, and the residual associated to the experiments, respectively. In second order model the widely used design are central composite, 3^k factorial and Box–Behnken (Khuri and Mukhopadhyay, 2010). However, central composite design (CCD) is the most popular design among all second-order designs. Because, this design not only provides a rapid screening for a wide range of conditions, but it also indicates the role of each variable in the process. In addition, the face centred central composite design (FCCCD), under the CCD design is very attractive design since no need to repeat the central points and in this case $\alpha = 1$. Besides, this design does not require extra levels except (-1,0,1) (Anderson, 1997; Rai & Bai, 2014). Therefore, this design is popular in optimizing the analytical process parameters. This design is illustrated in Figure 2.10.

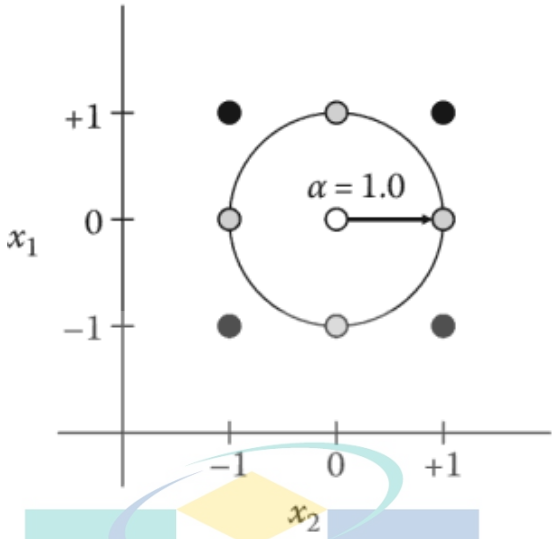


Figure 2.10 Face centred central composite design

Source: Rai and Bai (2014)

2.17 Methods of Characterization and Analysis

The characterization and analysis of bioactive compounds extracted from fenugreek seed and Aloe vera leaves in this study were performed using Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), liquid chromatography-mass spectrometry quadrupole time-of-flight (LCMS-QTOF) and UV-Vis spectrophotometry. In addition, further properties of the extracts were evaluated using the measurements of wettability, surface tension, foaming properties and emulsification. Each of these characterizations is discussed in the following subsections.

2.17.1 Fourier Transform Infrared (FTIR) Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) analysis is a form of vibrational spectroscopy that is useful in determining the bonding mechanisms in samples. Basically, FTIR operates on the basis of functional groups of the sample and gives the information in the form of peaks. FTIR spectroscopy facilitates to understand the molecular vibration of molecules and also to determine that how a molecule is bonding on the surface of a solid compound based on its infrared spectrum (Peak, 2005). FTIR spectrometer can collect the spectral data of the sample in a wide spectral range at the same time (Sharpet

al., 2018). Traditionally, this technique has been used for analysing materials in chemical industries. However, recently, it has been introduced as an easy and useful method for biological and medical analyses.

2.17.2 Scanning Electron Microscopy

Scanning electron microscopy (SEM) is a wieldy used technique for determination of microstructure, chemistry and morphology of materials. The primary components of a SEM system include electron column, electron detectors, electromagnetic lenses, chamber, and computer control system as shown in Figure 2.11**.** The electrons placed at the top of the column moves downwards, and to produce a fine beam of electros they pass through a combination of apertures and lenses. The surface of the sample come in contact with electron beam under vacuum. When the electron-beam coils are moving, it scans the surface of the sample. The interaction between the sample and electron beams produces a number of signals and then the microstructure of the sample can be detected by detectors.

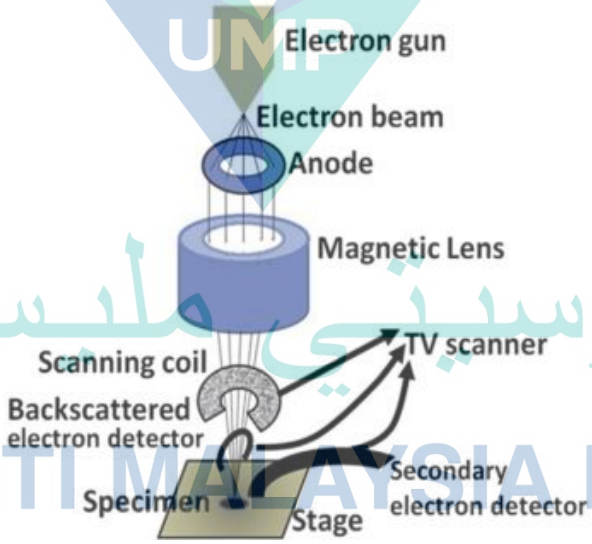


Figure 2.11 Components of scanning electron microscopy

Source: Singh (2016)

2.17.3 Liquid Chromatography-Mass Spectrometry Quadrupole Time-of-Flight

Liquid chromatography-mass spectrometry quadrupole time-of-flight (LC-QTOF-MS) is an analytical chemistry technique which is very sensitive in determining the components of a sample. LC-QTOF-MS is an excellent way to determine bioactive compounds and to confirm the existence of some compounds with unknown retention time, since it provides accurate mass spectra (m/z) value to each component. The QTOF systems provides superior detection limits of a sample in full spectrum mode as well as it gives invaluable information through high mass accuracy and resolution. Recently, this technique has become a widely used method for the identification of bioactive compounds in plant extracts (Chen et al., 2011).

2.17.4 UV-Vis Spectrophotometry

UV-Vis spectrophotometry is an extensively used technique in analytical chemistry for the identification, characterization and quantitative analysis of the samples. This technique is based on the passed or reflected light through the surface of the sample cuvette and then the absorption measurements can be at a specific wavelength or wide range of spectral. UV-Vis spectrophotometry works based on the electronic transitions between orbitals or bonds of atoms, molecules and ions in liquid, solid and gaseous states (Hameed et al., 2018). This method is also as an excellent analytical tool identify the unique optical properties of individual samples. UV-Vis spectrophotometry functions based on the absorption of electromagnetic radiations in the wavelengths of 180-780 nm (Worsfold & Zagatto, 2017).

2.17.5 Wettability, Surface Tension and Foaming Properties

Saponins are group of natural surfactants (biosurfactants) that can be obtained from plants or marine animals. Saponins are one of the secondary metabolites that can be found as steroid or triterpenoid glycosides. Saponins are also called as multifunctional agents due to their structural diversity and functional properties that make them an attractive and useful compound with wide range of application in cosmetic, food, pharmaceutical, water treatment, soil treatment, and agricultural industries (Rekiel et al.,

2020). Saponins are surface-active agents containing both hydrophobic and hydrophilic moieties. The important parameters of surfactants are the foaming, wetting and emulsification properties which reduce the surface tension of the water or an emulsion (Varjani & Upasani, 2017). Surface tension is the measurement of a liquid-gas or air.

The wettability of a sample is related to the contact angle measurement which indicate the degree of wetting when a liquid and solid interact. Duan et al. (2018) reported that the smaller the contact angle the better the wettability of the surfactant. The purpose of surfactant application is to enhance the wettability of aqueous solutions on a hydrophobic surface. The wettability of a surfactant is usually evaluated by the degree of the contact angle (θ) created on a solid substrate. When the θ is above 90° the surfactant is considered to have poor or non-wetting. In the case if the θ is less than 90° then the surface can get easily wetted and provides a good wetting property as shown in Figure 2.12. The foaming property of the saponins are due to the combination of aglycone backbone and sugar chain in their structure. However, in rare cases, some saponins without foaming property have also been observed (Kregiel et al., 2017). Basically, the foam testing is usually performed according to Ross-Mile method (Ross & Miles, 1941) in which after the mechanical agitation of the surfactant solution the height of the produced foam is measured. Initially, the first foam observed after the agitation is called the foamability while the foam by the time is the measure of foam stability.

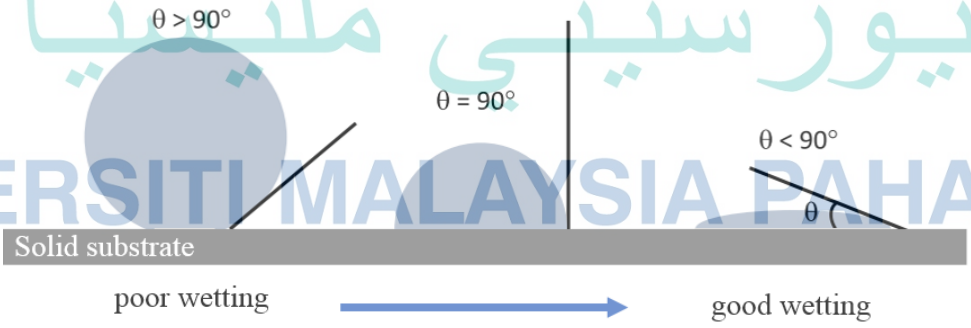


Figure 2.12 Wetting property of a surfactant solution

Source: Chang et al. (2017)

2.17.6 Emulsification

Emulsification is the process of emulsion formation. It is a dynamic process and to form a stable emulsion a mechanical energy is required to break the dispersed phase into very small droplets and disperse it in the continuous phase. Commonly, in emulsification process, two different phases with different densities are required. Emulsifications are carried out using many methods such as mixing with rotor-stator systems, simple shaking, liquid injection through porous membranes, ultrasound generators and high-pressure homogenizers. To produce a stable emulsion, only homogenization is not sufficient. In this case, surfactants or stabilizers are required to make stable emulsion system. Emulsification plays an important role in many industries such as food, cosmetic, pharmaceutics and crude oil industries (Mnif & Ghribi, 2016).

2.18 Summary

In this chapter, the related information about the current research were gathered and discussed. The previous studies about fenugreek seed and Aloe vera leaves properties and bioactivities were outlined and used to support the outcome of this research. The existence of bioactive compounds of saponin and phenolics were reported in the previous studies. However, microwave-assisted extraction and optimization of extraction parameters and recovery yields with formulation of solvent were investigated for the first time in this research. The selection of extraction method and solvent formulation plays an important role in the recovery of phytochemicals from plants. Based on the review of the literature, it is revealed that most of the literature reports MAE as an unconventional and green extraction technology for increasing the recovery yields and decreasing the extraction cost, time and solvent consumption during the process. Studies also reported that comparing to other chemical solvents such as methanol, petroleum ether, dichloromethane, acetone, ethyl acetate and hexane, ethanol is the best choice to use as an extraction solvent for obtaining bioactive compounds of the plants. Since, it has less toxicity and harmful effect to human health and environment, and also it can be used in any combination with water for the purpose of extraction. Hence, in this study, extraction of saponin and phenolic bioactive compounds via MAE and Soxhlet extraction were carried out.

CHAPTER 3

METHODOLOGY

3.1 Introduction

In this chapter, the extraction of saponin and phenolic compounds from fenugreek seed and Aloe vera leaves are discussed. Fenugreek seed and Aloe vera leaves were extracted using MAE and Soxhlet extraction at different parameters and the extracts were evaluated for the contents of total saponin and total phenolic compounds. The extracts were also tested using LC-QTOF-MS, SEM, FTIR, wettability, contact angle, surface tension and emulsification properties. Besides, the antioxidant activities of the extracts were also evaluated using DPPH and ABTS essays.

This chapter includes 14 sections. Section 3.2 explains the experimental plant, chemicals and reagents used in this study. Section 3.3 describes the general procedure and methodology of the study including MAE and Soxhlet extraction methods. Section 3.4 discusses about the experimental procedures and optimization processes. Section 3.5 explains about the model validation and statistical analysis. The dielectric properties of extraction solvents and kinetic studies are discussed in section 3.6. The determination of total saponin content, total phenolic content, DPPH and ABTS antioxidant activities are described in sections 3.7-3.9, respectively. The characterization of the extracts such as SEM, FTIR, wettability, surface tension, foaming and emulsification properties are discussed in sections 3.10-3.14, respectively. Finally, a brief summary of the chapter is explained in section 3.14.

3.2 Experimental Plant, Chemicals and Reagents

Seeds of fenugreek were collected from local retail market located in Kuantan, Pahang, Malaysia. Before, drying the seeds at 50 °C in an air-oven for 24 h, the seeds were manually cleaned to separate all the unwanted species and foreign matters. The

seeds had moisture content of 5.51 ± 0.14 % (d.w basis). Further, the dried seeds were ground using an ultra-centrifugal grinder (Retsch ZM-200, Germany) equipped with a ring sieve at size of 0.5 mm trapezoid holes. The crushed seeds were kept away from the light at 4 °C before extraction. Aloe vera (*Aloe barbadensis Mill.*) was collected from a botanical garden in Kuantan, Pahang, Malaysia. The whole leaves were freshly taken from the plant, washed, cut in two layers from the centre and each cut was scratched to ease and fasten the drying process, then the cuts putted in a tray. The thin layer pieces were pre-dried for 1 day under shade drying and then dried under 50 °C for 26 ± 2 h in air-dried oven. The leaves of Aloe vera had 97.18 ±0.5 % water. The dried leaves were then crashed in a mortar and passed through 1 mm sieve. The powdered leaves were kept in a dark airtight plastic container and stored at 4 °C before extraction.

Ethanol (99.5%), methanol (99.9), vanillin, diosgenin, gallic acid (GA), Folin-Ciocateu reagent, 2,20-azino- bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium carbonate, sulphuric acid (H_2So_4), oleanolic acid, ascorbic acid and potassium persulfate ($K_2S_2O_8$) were obtained from Sigma Aldrich, Malaysia. In this research, high purity chemicals and reagents with well stored condition were used.

3.3 Procedures of Plant Extraction

In this study, two different types of extraction methods (MAE and Soxhlet) were applied to obtain the extracts of fenugreek seed and Aloe vera leaves. Each extraction technique is described in the following sections. Figure 3.1 shows the methodology of this research. The experiment started with the preparation of fenugreek seed and Aloe vera leaves such as cleaning, drying, grinding and sieving. The prepared seed and leave powder were stored at 4 °C in a refrigerator. After that, two extraction process namely, MAE and Soxhlet extraction was applied to extract the plant. The factor levels in each method were evaluated using OFAT experiment. The TSC and TPC of the extracts was determined via Uv-Vis spectrophotometer. After OFAT, screening of the factors was carried out using two level factorial analysis to determine the most significant factors affecting the response. An optimization was also performed for MAE to obtain TSC and

TPC in the optimal condition. Afterwards, the ABTS and DPPH bioassays with IC_{50} values were also carried out to evaluate the antioxidant capacities of the extract. The optimal extracts were characterised via FTIR, SEM, LC-QTOF-MS, wettability testing, surface tension, foaming and emulsification.

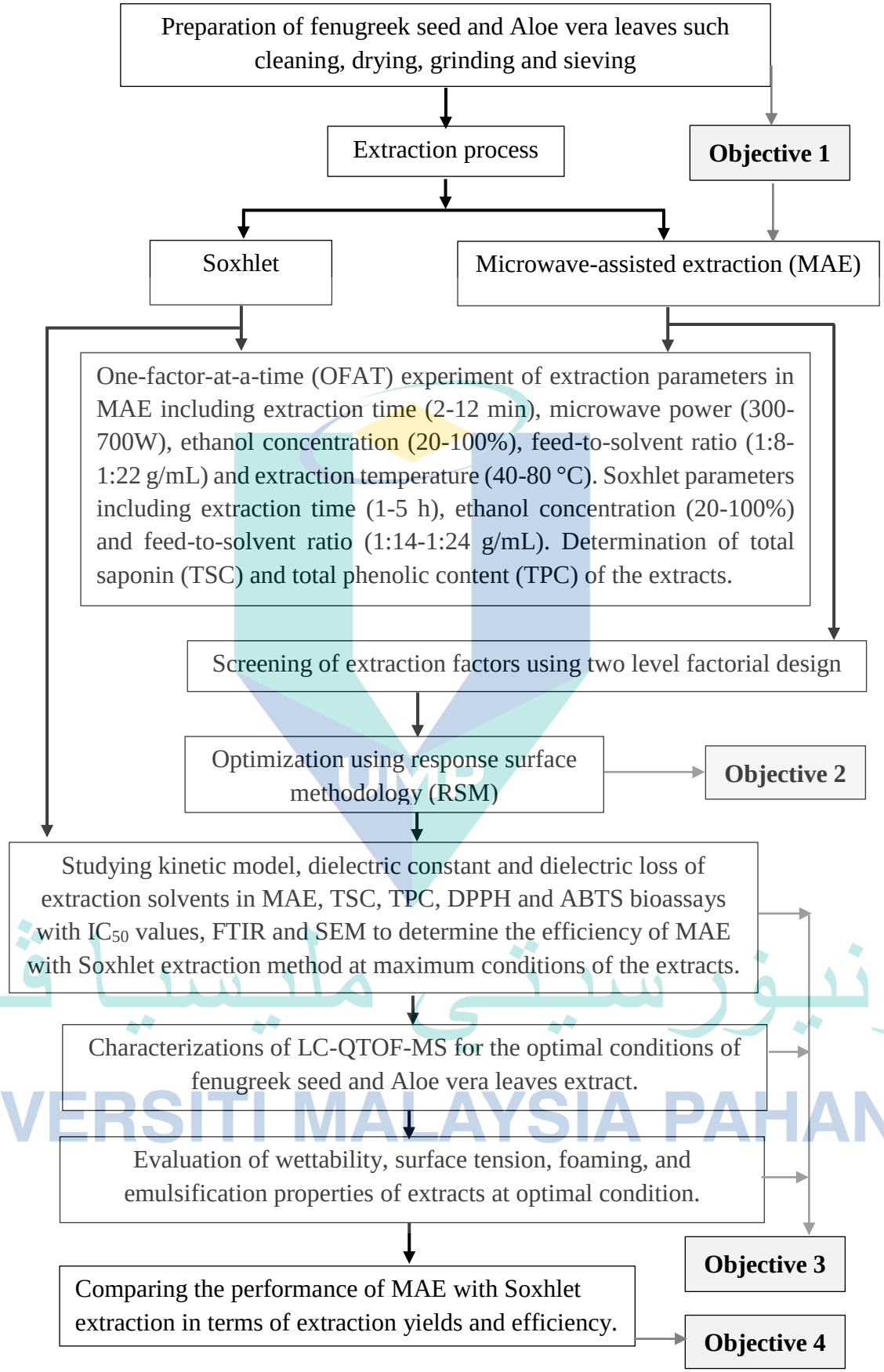


Figure 3.1 Flow of the experiment.

3.3.1 Microwave-Assisted Extraction

The process of microwave-assisted extraction was performed using a laboratory microwave oven (ethos 1000 W, Milestone, Italy) with frequency of 2450 as shown in Figure 3.2. As it can be seen, the whole microwave extractor system is including of a microwave oven, a condenser connected to the extraction flask and a water-cooling system that connected to the inlet and outlet of the condenser. The ethos microwave system was connected to a parameter control device which was used for setting up the experimental parameters. This device was applied to set up the extraction time at three different levels, preheating for 2 min, the desired extraction time and 2 min for cooling the extract inside the system. The purposes of pre- and post-treatment were to efficiently heat the sample before applying further heat; however, post-treatment was applied to cool up the samples at room temperature. The MAE was operated at various extraction parameters at different levels. Fenugreek seed (10g) and Aloe vera leaves (3g) was extracted using one-factor-at-a-time (OFAT) experiment at parameters of extraction time, microwave power, ethanol concentration, feed-to-solvent ratio and temperature. Ethanol was used as extraction solvent due to the environmental concern and high efficiency.

After removing the sample from the oven, a qualitative filter paper No.1 (Advantec®) was used to separate the particles from the mixture and a vacuum filtration system was used to accelerate the process. Afterwards, the solvents (ethanol and water) were removed at 50 °C from the extract via a rotary evaporation system (Büchi, R-200, Germany). Then, based on Eq. (3.1) the yield of extraction was calculated. The extracts were stored at 4°C to avoid the biodegradation of compounds. Then, a UV-vis spectrophotometer (Hitachi, U-1800; Japan) was used for the measurement of TPC and TSC in the extract. The experiments repeated three times.

$$Extraction\ yield\ (\%) = \frac{Weight\ of\ extracted\ yield\ (w)}{Weight\ of\ dry\ sample\ used\ (w)} x\ 100 \qquad 3.1$$

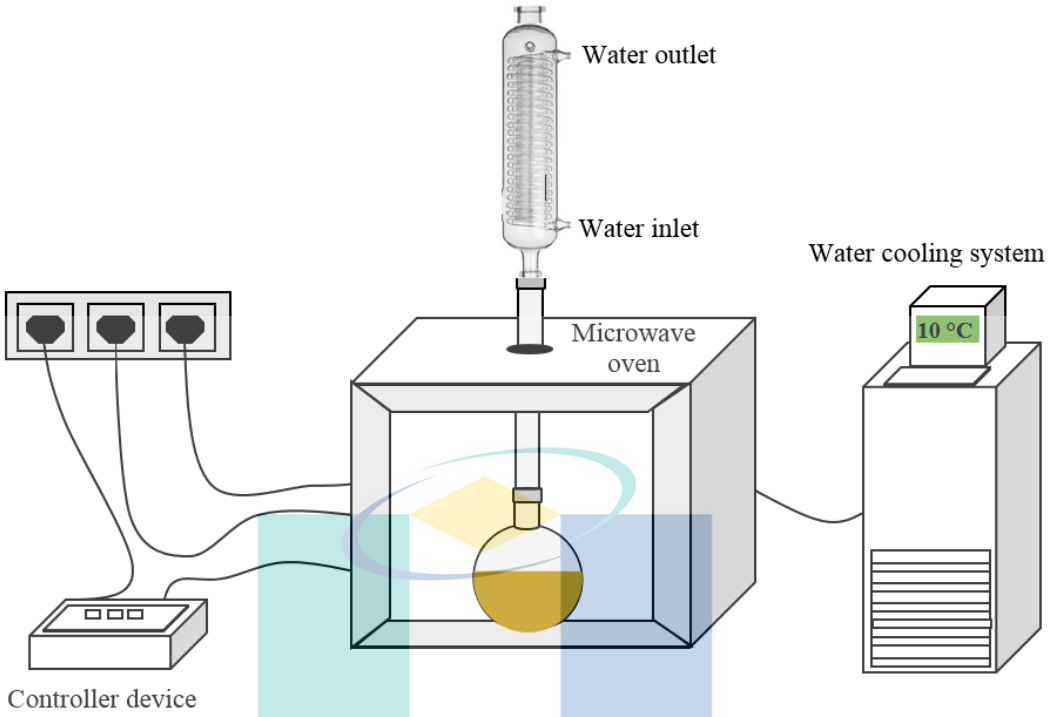


Figure 3.2 Schematic diagram of microwave-assisted extraction method.

3.3.2 Soxhlet Extraction

The fenugreek seed and Aloe vera leaves were pre-treated before the extraction at the same conditions carried out for MAE method. Then the dried fenugreek seed (20 g) and Aloe vera leaves (6 g) were placed inside a thimble. Afterwards, the thimble was transferred inside the Soxhlet extractor column which was connected to the condenser from the top and a round flask filled with aqueous ethanol from the down. The solvent flask was placed inside a heating mantle as illustrated in Figure 3.3. As the solvent started heating to the boiling point, it began evaporating and passing through the Soxhlet extractor to the condenser. The cooled vapors dropped back to the Soxhlet extractor chamber which contains the sample inside the thimble. Once the solvent level reached to the siphon, the solvents containing the extract reflexed back to the round flask. The procedure continued until the extraction time completed. Then, to cool down the surface of the heating mantle at room temperature it was switched off for few minutes. The cooled solvent containing the extract was filtered using Advantec® qualitative No. 1 filter paper and rotary evaporated using a rotary evaporator (Büchi, R-200, Germany) at 50 °C. The

extraction yields were calculated using Eq. (3.1) and the dried extract was kept at 4 °C in a fridge for further analysis. Then, the extraction yield, total saponin content (TSC) and total phenolic content (TPC) of both materials was evaluated. Afterwards, the target compounds of TSC and TPC were selected for the optimization process. All the experiments were done three times.

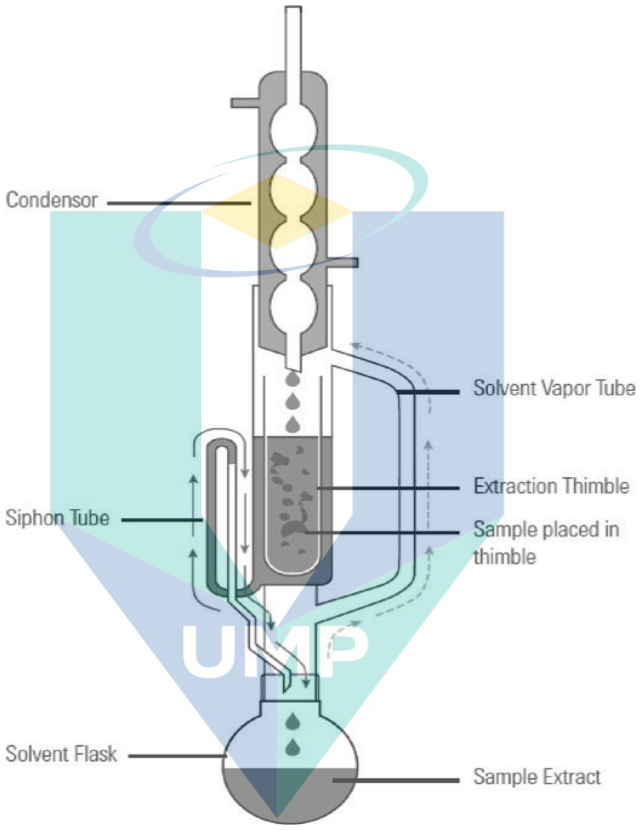


Figure 3.3 Schematic diagram of Soxhlet extraction method.

3.4 Experimental Procedure and Optimization of MAE parameters

3.4.1 One-Factor-at-a-Time Experiment

Fenugreek seed and Aloe vera leaves were extracted using OFAT experiment at parameters of extraction time (A), microwave power (B), ethanol concentration (C), feed-to-solvent ratio (D) and temperature (E) . Ethanol was used as extraction solvent due to the environmental concern and high efficiency. To perform OFAT, one factor was varied at different levels while other factors kept constant. The OFAT experiment for fenugreek

seed and Aloe vera leaves via MAE was carried out at irradiation time 2-12 min, microwave power 300-700 W, ethanol concentration 20-100%, feed-to-solvent ratio 1:8-1:16 g/mL and 1:10-1:22 g/mL and extraction temperature 40-80 °C, respectively. OFAT was used prior to two-level factorial design (TLFD) and optimization process was performed using response surface methodology (RSM) under Design-Expert (DOE) (Version 7.1.6, Stat-Ease, Inc. USA) software.

3.4.2 Screening of MAE Factors

There were five variables analyzed using two-level factorial analysis, namely microwave extraction time (min), microwave power (W), ethanol concentration (%), F:S ratio (mg/mL), and temperature (°C). Each variable was evaluated at two coded levels and randomized in 16 experimental runs. Tables 3.1 and 3.2 show the two-level experimental design (TLED) of fenugreek seed and Aloe vera leaves, respectively. The significance of the model and P-value were evaluated using analysis of variance (ANOVA). DOE software was used to design the experiment.

Table 3.1 Two-level experimental design of fenugreek seed extraction.

Run	A Extraction Time (min)	B Microwave power (W)	C Ethanol concentration. (%)	D F:S (g/mL)	E Temperature (°C)
1	2	700	40	1:12	80
2	4	700	80	1:12	80
3	2	700	80	1:12	60
4	2	700	80	1:8	80
5	4	700	40	1:12	60
6	2	700	40	1:8	60
7	4	500	40	1:12	80
8	4	500	40	1:8	60
9	2	500	40	1:8	80
10	2	500	80	1:8	60
11	4	500	80	1:8	80
12	4	700	40	1:8	80
13	4	500	80	1:12	60
14	4	700	80	1:8	60
15	2	500	40	1:12	60
16	2	500	80	1:12	80

Table 3.2 Two-level experimental design of Aloe vera leaves extraction.

Run	A Extraction Time (min)	B Microwave power (W)	C Ethanol concentration. (%)	D F:S (g/mL)	E Temperature (°C)
1	2	600	20	0.22	80
2	4	600	60	0.22	80
3	2	600	60	0.22	60
4	2	600	60	0.18	80
5	4	600	20	0.22	60
6	2	600	20	0.18	60
7	4	400	20	0.22	80
8	4	400	20	0.18	60
9	2	400	20	0.18	80
10	2	400	60	0.18	60
11	4	400	60	0.18	80
12	4	600	20	0.18	80
13	4	400	60	0.22	60
14	4	600	60	0.18	60
15	2	400	20	0.22	60
16	2	400	60	0.22	80

3.4.3 Optimization of the Process

The optimization process was accomplished via RSM by applying the face centred central composite design (FCCCD). From the experiment, three responses were recorded including yield of extraction (Y_{Ex}), total content of saponin (Y_{TSC}) and phenolic (Y_{TPC}). Basically, a total of 30 runs including 6 centre points with a quadratic polynomial equation were generated as shown in Eq. (3.2).

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} x_i x_j \quad 3.2$$

Where, the response, the coefficients of regression for the intercept, linear, square, and interaction, the independent variables and number of factors were indicated as *Y*, *β0*, *βi*, *βii*, *βij*, *Xi*, Xj and n, respectively.

The model terms, adequacy based on R-squares, lack-of-fit, F-value, interaction of factors and adequate precision were evaluated from the analysis of variance (ANOVA).

Table 3. 3 Independent variables and factor levels used in the experiment.

Independent variables	**Factor levels**			**Factor levels**		
	(fenugreek seed)			**(Aloe vera leave)**		
(input)	-1	0	+1	-1	0	+1
Extraction Time (min)	2	3	4	2	3	4
Microwave Power (W)	500	600	700	400	500	600
Ethanol Concentration (%)	40	60	80	20	40	60
Feed-to-solvent Ratio (g/mL)	1:8	1:10	1:12	1:18	1:20	1:22

The experimental factors of A, B, C, and D were optimized via FCCCD design and responses were collected. Table 3.4 shows the factors based on coded values and experimental responses for fenugreek seed and Aloe vera leaves.

Table 3.4 FCCCD of coded factors and responses of the extracts.

Run	Factors of fenugreek seed extract				Factors of Aloe vera leaves extract				Responses		
	A (min)	B (W)	C (%)	D (g/mL)	A (min)	B (W)	C (%)	D (g/mL)	Y_{EX}	Y_{TSC}	Y_{TPC}
1	3	600	60	1:10	3	500	40	1:20	-	-	-
2	4	500	40	1:8	4	400	20	1:18	-	-	-
3	3	700	60	1:10	3	600	40	1:20	-	-	-
4	2	500	40	1:12	2	400	20	1:22	-	-	-
5	2	700	40	1:8	2	600	20	1:18	-	-	-
6	3	600	60	1:10	3	500	40	1:20	-	-	-
7	3	600	60	1:8	3	500	40	1:18	-	-	-
8	4	700	80	1:12	4	600	60	1:22	-	-	-
9	3	600	60	1:10	3	500	40	1:20	-	-	-
10	2	700	80	1:8	2	600	60	1:18	-	-	-
11	4	600	60	1:10	4	500	40	1:20	-	-	-
12	2	500	80	1:8	2	400	60	1:18	-	-	-
13	4	500	80	1:8	4	400	60	1:18	-	-	-
14	3	600	60	1:10	3	500	40	1:20	-	-	-
15	3	600	60	1:10	3	500	40	1:20	-	-	-
16	3	500	60	1:10	3	400	40	1:20	-	-	-
17	4	700	80	1:8	4	600	60	1:18	-	-	-
18	4	500	40	1:12	4	400	20	1:22	-	-	-
19	4	700	40	1:12	4	600	20	1:22	-	-	-
20	2	700	40	1:12	2	600	20	1:22	-	-	-
21	2	700	80	1:12	2	600	60	1:22	-	-	-
22	2	600	60	1:10	2	500	40	1:20	-	-	-
23	2	500	80	1:12	2	400	60	1:22	-	-	-
24	3	600	40	1:10	3	500	20	1:20	-	-	-
25	3	600	80	1:10	3	500	60	1:20	-	-	-
26	3	600	60	1:12	3	500	40	1:22	-	-	-
27	4	700	40	1:8	4	600	20	1:18	-	-	-
28	3	600	60	1:10	3	500	40	1:20	-	-	-
29	4	500	80	1:12	4	400	60	1:22	-	-	-
30	2	500	40	1:8	2	400	20	1:18	-	-	-

3.5 Model Validation

The validation and adequacy of the model were carried out by conducting verification experiment in the optimum conditions of extraction yield, TPC and TSC. To validate the model, the obtained results compared with the actual and predicted values.

The model accuracy was evaluated by triplicating the experimental in the optimal condition and R-squares were assessed. The results were reported as mean ± SD. Paired samples t-test was performed to evaluate the difference between actual and predicted values.

3.6 Dielectric Properties of Extraction Solvent and Kinetic studies

In a microwave, the heating is also referred as dielectric heating. In microwave range, the interaction of dielectric materials with electromagnetic radiation results in energy absorbance in the material. The dielectric properties of water and ethanol were studied according to the methods applied by Wolf (1986) and Onimisi (2015), respectively. Wolf was suggested the Eqs. of (3.3) and (3.4) for calculation of dielectric constant ε' and dielectric loss ε'' of water. However, the Debye equation for obtaining dielectric constant and dielectric loss of polar dielectric materials at various frequencies were as expressed in Eqs (3.6 and 3.7).

The dielectric constant and dielectric loss of water were given by (Wolf, 1986), where T shows the process temperature:

$$\varepsilon'_{rw} = 85.2 - \ 0.3358T \qquad 3.3$$

$$\varepsilon''_{rw} = 320.66T^{-1.03} \qquad 3.4$$

The Debye equation for obtaining dielectric constant (ε') and dielectric loss (ε'') of polar dielectric materials at various frequencies is described in Eqs. (3.6 and 3.7). The temperature enters the discussion by way of the parameter known as the relaxation time, τ. In this work, the value of relaxation time is obtained using the relationship between $\omega = 2\pi f$ the angular frequency in radians per second and $\tau = 1/\omega$.

$$\varepsilon'_e = \varepsilon_\infty + \frac{(\varepsilon_s - \varepsilon_\infty)}{1 + \omega^2\tau^2} \qquad 3.5$$

Where $\tau = 1/\omega$

$$\varepsilon'_e = \frac{(\varepsilon_{s+}\varepsilon_\infty)}{2} \quad 3.6$$

$$\varepsilon''_e = \frac{(\varepsilon_{s-}\varepsilon_\infty)}{2} \quad 3.7$$

Where ε_∞ is the complex permittivity, ε_s is the static permittivity, and these the values of ε_∞ and ε_s were obtained from National Physical Laboratory Report Mat 23 (Gregory & Clarke, 2012).

The extraction kinetics of fenugreek seed and Aloe vera leaves were studied using first and second-order kinetic models. The equation in a first order model was first described by Lagergren (1898) and modified by Kusuma and Mahfud (2017). In first order, the plot of $\ln(\frac{C_s}{C_s - C_t})$ extract concentration (mg/mL) was plotted against time t (min) to obtain a slope and intercept that are used in determining the first-order extraction rate and extraction capacity as shown in Eq. (3.8).

$$\ln\left(\frac{C_s}{C_s - C_t}\right) = k_1 t \quad 3.8$$

where C_s the concentration of water-soluble compounds of plants extract at saturation. C_t is the extraction capacity at different extraction time t, and k_1 indicates the first-order extraction rate coefficient in per minute (min^{-1}).

According to Kusuma and Mahfud (2017) a second-order kineitc model for determining the extraction rate of fenugreek seed and Aloe vera leaves extracts can be expressed in differential form. In a second-order kinetic model mechanism, the extraction occurs in two simultaneuos process. The secomd-order model shows an inverse plot between extraction time (min) and extract concentration (mg/mL) as of Eq. (3.9).

$$\frac{1}{(C_s - C_t)} - \frac{1}{C_s} = k_2 t \quad 3.9$$

3.7 Determination of Total Saponin Content in the Extracts

The TSC in the extracts of fenugreek seed and Aloe vera leaves was measured according to the methods described by Hu et al. (2012), Moyo et al. (2013) and Venegas-Calerón et al. (2017). Initially, 0.2 mL of the extracted Aloe vera leaves with 0.35 mL of 8% vanillin in absolute ethyl alcohol and 0.80 mL of methanol were mixed in a test tube. Then, from a 72% sulphuric acid about 1.25 mL was added and shaken vigorously. The mixture was then transferred into a water bath to heat for 10 min at 60 °C. Then the sample was cooled in ice crystals bath for 300 sec. The TSC in the extracts of fenugreek seed and Aloe vera leaves were then determined using UV-Vis spectrophotometer device (Hitachi, U-1800, Japan) at a wavelength of 544 nm. The standard curves were prepared using diosgenin and oleanolic acid at concentrations of 100-600 mg/L, respectively. Diosgenin was used as representative standard for steroid saponins (fenugreek seed) and oleanolic acid was used as representative standard for triterpenoid saponins (Aloe vera) (Hierro et al., 2018; Neelesh K. Nema, 2012). The TSC in the extract of fenugreek seed was presented as milligram of diosgenin equivalent (DE) per gram of dry weight (mg DE/g d.w.) and milligram of oleanolic acid equivalent (OAE) per gram of dry weight (mg OAE/g d.w.) for Aloe vera leaves extract (see Appendix A). The absorbance was read against methanol (blank) and the measurements were repeated thrice. Then, the total content of saponin was calculated using Eq. (3.10) showing milligrams of standard equivalent (StE) per gram of dry weight sample.

$$TSC\ (mg\ StE/g\ d.w) = \frac{V \times C}{m} \qquad 3.10$$

In the above equation, V, C and m indicate the volume of solvent (mL), sample concentration from the standard curve (mg/L) and weight of dry sample used for extraction (g).

3.8 Determination of Total Phenolic Content in the Extract

The determination of TPC in the extracts of fenugreek seed and Aloe vera leaves was done by applying the methods used by Sookjitsumran et al. (2016) and Nickel et al.

(2016) with minor changes. First, the equal amount (0.2 mL) of extract and reagent of Folin-Ciocalteu were added together in a test tube and placed in dark for around 5 min at 30°C. Afterwards, 0.6 mL of 20% sodium carbonate (Na_2CO_3) solution was added to the mixture and stored for another 2 h. The wavelength of 765 nm was selected in a UV–vis Spectrophotometer to take the absorbance of the sample using methanol as blank. A standard curve at concentrations of (100-500 mg/L) was prepared from gallic acid (GA) to measure the sample concentration. Result was expressed as milligram of GA equivalent per g of the extract (mg GAE/g d.w.) (see Appendix A). Eq. (3.11) was used to calculate the amount of TPC in the extract.

$$TPC\ (mgGAE/g\ d.w) = \frac{V \times C}{m} \qquad 3.11$$

Where V is the volume of solvent (mL), C is the sample concentration taken from standard curve (mg/L) and m is the weight of sample (g) used for extraction.

3.9 Antioxidant Activities

3.9.1 DPPH Radical Scavenging Activity

The DPPH antioxidant activity of the extract was performed using the methods described by Alara et al. (2018b) with some changes. To prepare a 0.1 mM of DPPH stock solution, 0.004 g of DPPH solid particles dissolved in 100 mL of pure methanol and the mixture was stored at 4°C. Next, 10 mL of DPPH stock solution was mixed with 90 mL of pure methyl alcohol to make a 0.1 mM of fresh DPPH working solution. The dried sample (extract) or ascorbic acid were dissolved in methanol as 10 mg of dried extract in 10 mL (1:1 g/mL) of methyl alcohol to make the stock solution of (1 mg/mL). Then, different concentrations of 100-500 µg/mL were prepared from the stock extraction solution of plant extract of Soxhlet extraction and optimized MAE (see Appendix B). Afterwards, the same amount (0.2 mL) of the extract or the standard ascorbic acid and DPPH working solution mixed together and kept in dark for half an hour. The mixture was then stored at room temperature in the dark for 30 min. The antioxidant properties of extracts were presented as IC_{50} which indicates the concentration of µg/mL of the extract

to inhibit 50% of the DPPH radical. Finally, the absorbance recorded at 517 nm using a UV visible Spectrophotometer and the analysis were done three times. The DPPH inhibition was calculated using Eq. (3.12) against methanol as blank.

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{A_1 - A_0}{A_1} x\, 100 \qquad 3.12$$

where A_1 and A_0 indicate the absorbance of DPPH-methanol mixture, and the mixture of extract-DPPH prepared solution, respectively.

3.9.2 ABTS Radical Scavenging Activity of the Extract

The ABTS assay of the extract in the optimized condition of MAE was determined according to Zielinski et al. (2014) and Cheng et al. (2013) with little modifications. The assay was started by preparing 7mM and 2.45 mM stock solution of ABTS and $K_2S_2O_8$, respectively. To prepare the solutions 7.6 mg of ABTS and 1.32 mg of $K_2S_2O_8$ w was separately dissolved in 2 mL of water (solution a and b), respectively. Then, the solutions of a and b mixed together in a same ratio (1:1 v/v) and incubated at room temperature for 12-16 h in the dark. The dried sample (extract) or ascorbic acid were dissolved in methanol as 10 mg of dried extract in 10 mL (1:1 g/mL) of methanol to prepare the stock solution of (1 mg/mL). Then, different concentrations of 100-500 µg/mL were prepared from the stock extraction solution of plant extract of Soxhlet extraction and optimized MAE (see Appendix B). Next, to get a constant absorbance of 1.1 ± 0.02 at 734 nm, 1 mL of the solution was diluted with 60 mL of methanol. Afterwards, 0.15 mL of the extracts and 2.85 mL of ABTS solution added together. The sample mixture was stored at 26±2 °C for 2 h and the absorbance obtained via UV-Vis spectrophotometer at 734 nm. The antioxidant properties of extract were represented as IC_{50} which indicates the concentration of µg/mL of the extract to inhibit 50% of the ABTS radical. The blank was ethanol and the ABTS scavenging activity calculated as shown in Eq. (3.13).

$$ABTS\ radical\ scavenging\ activity\ (\%) = \left(1 - \frac{A_{sample}}{A_{control}}\right) x\ 100 \qquad 3.13$$

where $A_{control,}$ and $A_{sample,}$ represent the mixture of ABTS and methanol absorbance and the blend of extract and ABTS solution, respectively.

3.10 Analysis of LC-QTOF-Mass Spectrometer

The phenolic and saponin compounds in extract of fenugreek seed and Aloe vera leaves obtained at the optimal condition were identified using LC-QTOF-MS analysis (Waters® Vion IMS, USA). The LC-QTOF-MS was equipped with a PDA detector and symmetry C18 column (100 mm x 2.1 mm, 1.8 µm particle size) was used for the tentative identification. Ionization was performed in positive and negative electrospray (ESI) modes. The extract of fenugreek seed and Aloe vera leave was prepared at 20 ppm in LCMS grade methanol. The identification of saponins and phenolic compounds were performed at negative and positive ion modes and different concentrations of acetonitrile and water were used as mobile phase. The condition of MS was selected between 100-1000 m/z. The temperature of desolvation, column and sample were set to 550 °C, 40 and 15, respectively. The flow rate (0.5mL/min) and injection volume (20 µL) were set to operate the system. The capillary voltage and desolvation gas flow rate were set at 1.50 kV and 800 L/h, respectively. UNIFI software with scientific library was used to identify the bioactive compounds.

The positive ion mode was operated with the mobile phase of A (water +0.1% formic Acid) and B (acetonitrile). The initial conditions were set at 90% phase A and 10% phase B and the time was changed from 0.00 to 8.34 min during the entire operation. Then, the operation conditions were changed based on the operational time of 4.17 min A 45% and B 55%, at 6.25 min the mobile phases were A 10% and B 90%, while at 8.34 min, A 90% and B 10%. The operation was carried out at constant flow rate of 0.5 mL/min. The low collision energy was at 4.00 eV and the high collision energy stated at 10.00 eV and ended at 45.00 eV. The same operation method was applied for the negative ion mode, but no formic acid was used in the mobile phase. UNIFI software with scientific library was used to identify the bioactive compounds.

3.11 Scanning Electron Microscopy

The microphotographs of the dried fenugreek seed and Aloe vera leaves powders before and after the extraction were captured by scanning electron microscopy (SEM) at 500×magnification (HITACHI TM3030Plus, Japan). The samples were dried at 50 °C in an oven (Memmert UN55, Germany) then a small amount of samples were coated by thin layer of gold to prevent charging of the surface and provide a better conduction.

3.12 Fourier Transform Infrared Spectroscopy

The FTIR analysis of fenugreek seed and Aloe vera leave extracts obtained in the optimal condition and performed using Nicolet iS5 FTIR spectrometer equipped with a DTGS detector and OMNIC software. FTIR spectrum of the extract was recorded between 4000- 400 cm^{-1} using KBr pellet methods at room temperature. The dry sample was flatted on plate surface containing a diamond crystal and the lid was closed to measure the bonding structure directly.

3.13 Wettability, Surface Tension and Foaming Properties

The wettability analysis of the sample was performed by contact angle measurement using a goniometer (Beijing united test co. ltd., Beijing, China). The measurement was carried out according to ASTM D 5725 on a hydrophobic substrate. The extract solution was prepared at 1% extract in-deionized water. Initially, 5 µL of extract solution was horizontally dropped on the surface of substrate and images were captured at 25 °C. For each sample, ten images were analysed using JY-82 software and the average was obtained to find out the contact angle.

The measurement of surface tension was performed using a standard testing method of ASTM (American Society for Testing and Materials), ASTM 1331. The testing was carried out using Du Nouy Interfacial Tensiometer at 27 ºC. The instrument was equipped with a 6cm circumference platinum ring which were hanged on the top of the sample holder. The tensiometer was calibrated first by placing the water in the sample boat. Then, the surfactant aqueous solution (1% w/v) was added to measure the surface

tension of the solution. The measurements were taken after reaching to an equilibrium value and repeated thrice.

Foaming properties were measured according to the method applied by Tmáková et al. (2015) and Chen et al. (2010) with some modifications. The aqueous solutions of the fenugreek and Aloe vera leaves extracts prepare at 1% w/v in deionized water were placed inside a plastic beaker and agitated via a homogenizer at 500-1000 rpm for 2 min at room temperature. The sample was then transferred into a cylinder larger than the prepared volume. The height of the foam was measured immediately after agitation and every 5 min till 30 min and finally after 1 h. The R5 parameter was measured for the samples. R5 represents the ratio of the foam height at 5 min to that at the initial stage, generally it shows the foam stability. The commercial and synthetic surfactant Tween 80 was used to compare the results. The foaming capacity and stability were measured according to Eqs. (3.14) and (3.15), respectively.

$$Foming\ capacity\ (\%) = \left(\frac{V_2 - V_1}{V_1}\right) x\ 100 \qquad 3.14$$

$$Foam\ stability\ (R5, \%) = \left(\frac{foam\ height\ at\ 5\ min}{initial\ foam\ height\ at\ 0\ min}\right) \times 100 \qquad 3.15$$

Where in Eq. 3.14, V_1 indicates the volume of solution before mixing and V_2 shows the volume of solution after homogenizing.

3.14 Emulsification Index and Emulsion Stability

The emulsification index and emulsion stability test of fenugreek seed and Aloe vera leaves extracts were performed by forming the emulsion of sunflower oil-in-water (O/W). The emulsions were prepared by using the plant extracts (1% w/v) and Tween 80 as emulsifier standard at concentrations of 1% and 40-60% O/W homogenized for 5 min at room temperature. To prepare the O/W emulsion, the emulsifiers were dissolved into the continuous phase (water) and sheared vigorously for 2 min. Then the dispersed phase (oil) was added slowly to the mixture and mixed for another 3 min using a homogenizer

at 2000 rpm. The prepared emulsions were transferred to a glass cylinder for further evaluations. The stability of the emulsions was carried out through the gravity separation of the phases. The longer the separation time is the better the emulsification properties. The emulsification property of the emulsifiers were measured according to the method applied by Chakraborty et al. (2015). The emulsification index was measured for the first 10 min and then after each hour until to the 5th hour, then the evaluation followed to the next 24 hours. The emulsification index was calculated using Eq. (3.16).

$$Emulsification\ stability\ (\%) = \left(\frac{Total\ height\ of\ emulsion\ layer}{Total\ height\ of\ the\ system}\right) x\ 100 \qquad 3.16$$

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

By increasing the global health risk as a result of environmental problems, researchers are more in the stage of investigating better solution for preventing and declining diseases in human. In this age, the major health concerns are about cancer, heart problems, diabetes, skin problems and infectious diseases. Many studies are focusing about providing plant-based medicines for the treatment of these illnesses. Natural medicines are believed to possess a wide range of bioactive compounds such as phenolics, flavonoids, saponins, alkaloids, antioxidants, steroids, triterpenoids and many others, which are potentially useful for averting and preventing different types of sicknesses. Taking these into consideration, the present study has focused on the extraction and determination of the bioactive compounds of saponins and phenolic compounds of fenugreek seed and Aloe vera leaves extracts.

This chapter explains about results and discussion part of this research. Section 4.2 discusses about the extraction based on OFAT experiment. Section 4.3 explains the two-level factorial design and screening. Section 4.4 describes about optimization of MAE for obtaining maximum yield of TSC and TPC. The analyses of response surface plots and factor effects are described in section 4.5. Section 4.6 explains about dielectric properties of solvents and kinetic models. Section 4.7 discusses about effects of Soxhlet extraction. Characterization and analyses of samples are discussed in section 4.8. Finally, a brief summary of the chapter is discussed in section 4.9.

4.2 Extraction Based on One-Factor-at-a-Time Experiment

The extraction of fenugreek seed and Aloe vera leaves was initially evaluated via OFAT experiment to simplify the evaluation of factor levels and reduces the time and

cost of experiment. Figures 4.1 and 4.2 show the result of experiment carried out by OFAT for fenugreek seed and Aloe vera leaves using MAE method. As it can be seen in Figure 4.1, maximum extraction yields of fenugreek seed were obtained at 3 min extraction time, microwave power 600 W, ethanol concentration 20%, feed-to-solvent ratio (F:S) 1:10 g/mL and extraction temperature 70°C. This finding is similar with those found by Yu et al. (2013). However, results indicated that despite of high yields of extraction at 20%, the bioactivity of the extract improved when the 60% ethanol was applied. This may be due to the high dielectric properties of water molecules that absorbed more energy during the microwave heating and hence resulted to the extraction of more fenugreek viscose gums rather than biologically active compounds of phenolics and saponins. In this regard, as stated by Wani et al. (2016) that 60% ethanol was effective to obtain a steroidal saponin called diosgenin from microwave extraction of fenugreek seed. They also suggested that ethanol concentration less than 60% results in the extraction of fenugreek seed gum which can create problem during filtration . Figure 4.2 indicates the effect of extraction parameters on extraction yield, TSC and TPC of Aloe vera leaves. As seen the maximum extraction yields of Aloe vera were obtained at 3 min irradiation time, microwave power 500 W, ethanol concentration 40%, F:S ratio 1:20 and extraction temperature 70 °C.

Figure 4.1 (a-e) show the effect of extraction time (2-12 min), microwave power (300-700 W), ethanol concentration (20-100 %), F:S ratio (1:8-1:16 g/mL) and extraction temperature (40-80 °C) on extraction recoveries of fenugreek seed. As seen in Figure 4.1a, the extraction yield (%), TSC (mg DE/g d.w) and TPC (mg GAE/g d.w) recoveries increased by increasing of the time from (2-3 min) to 26.12±0.17, 158.976±0.44, and 67.94±0.89, respectively. However, raising the time to 12 min caused a significant reduction of recoveries to 19.33±1.16%, 110.096±0.67 (mg DE/g d.w) and 46.31±0.66 (mg GAE/g d.w), respectively. Figure 4.2 (a-e) indicate the effect of extraction time (2-12 min), microwave power (300-700 W), ethanol concentration (20-100%), F:S ratio (1:10-1:22 g/mL) and extraction temperature (40-80 °C) on extraction recoveries of Aloe vera leave via MAE. The effect of time on extraction yield, TSC and TPC of Aloe vera was same as fenugreek seed. Where the maximum recoveries were 22.16±0.56%, 47.62±0.63 mg OAE/g d.w and 51.25±0.71 mg GAE/g d.w at 3 min.

Longer time beyond 3 min resulted to the reduction of yields. This might be due to the degradation of sample beyond 3 min of extraction time. Yu et al. (2013) reported that longer exposure of time in a MAE above 3 min in the extraction of polyphenols from grape peel also resulted to the overheating of the sample and hence reduced the extraction efficiency and yield recoveries. Another study carried out by Xu et al. (2012) also claimed 3 min of extraction time as optimum condition in a MAE.

Looking to the effect of microwave power (Figure 4.1b), the highest recoveries of fenugreek seed were achieved at 600 W with the maximum yield's extraction, TSC and TPC at 27.21±0.57%, 161.94±0.45 (mg DE/g d.w) and 72.32±0.23 (mg GAE/g d.w), respectively. But the yields were reduced to 25.38±0.65%, 134.416±0.65 (mg DE/g d.w) and 69.23±0.22 (mg GAE/g d.w), when the power reached to 700 W. This result is in a good agreement with those found by Fang et al. (2010), as reported that higher microwave power induces the degradation of compounds since the microwave power and internal temperature in the microwave is interrelated. Thermal degradation of bioactive compounds can also affect the properties, nutritional value and overall quality of the extract (Nakilcioglu-Taş & Otleş, 2018). Figure 4.2b illustrates the effect of microwave power on yields of Aloe vera leave extract. As seen, the maximum yields of 26.42±0.25%, 53.34±0.37 mg OAE/g d.w and 60.25±0.67 mg GAE/g d.w for Aloe vera were obtained at 500 W. Where in the same vein, other researchers also found that the optimum yield of phenols and triterpenoid saponins from plant extracts can also be achieved at 500 W (Bale and Shinde, 2013; Dahmoune et al., 2015; Xu et al., 2012).

Ethanol concentration is also an effective factor on the extraction of bio-compounds. Figure 4.1c illustrates the effect of ethanol concentration on extraction yield, TSC and TPC of fenugreek seed extract. As seen, increasing the ethanol concentration from 20% to 60%, enhanced the recovery yields of TSC and TPC in the extract to 171.376±0.78 (mg DE/g d.w) and 76.09±0.95 (mg GAE/g d.w), respectively. While the extraction yield was the highest at 20% (27.58±0.60%) and the main reason is the extraction of fenugreek gums at higher water content. This may be due to water soluble polysaccharide gums which are less soluble in alcohol (Amid & Mirhosseini, 2012). In addition, the yields were significantly dropped to 14.43±0.80%, 148.896±0.61 (mg DE/g

d.w) and 57.36±0.66 (mg GAE/g d.w), respectively when the ethanol concentration increased to 100%. Wang et al. (2010) also found that 60% ethanol concentration is the optimum point for extraction of bioactive compounds from tea using MAE. Compared to other chemical solvents ethanol is an efficient solvent for extracting phytochemical compounds of the plants. It has low toxicity and high extraction efficiency in a MAE when it is mixed with water at different concentrations. Water has a high dielectric property therefore it is considered a good co-solvent to be used in MAE for obtaining plant phytochemicals. The low polarity of ethanol can be improved by mixing it at different concentration with water for the purpose of extraction in a MAE method. Most of the bioactive compounds such as phenolics, saponins, and flavonoids have high polarity, therefore a good formulation of ethanol and water is required. Figure 4.2c show the effect of ethanol concentration of extraction yield, TSC and TPC of Aloe vera leaves extract. As seen, in Aloe vera, the highest recoveries of yields 29.01±0.18%, 56.66±1.21 mg OAE/g d.w and 62.42±0.62 mg GAE/g d.w were obtained at 40% ethanol concentration, respectively. Beyond 40%, the yields started to decline. The difference between the solvent concentration in fenugreek seed and Aloe vera leaves might be due to the variance in properties and reaction of these two plants in MAE. By the extraction of fenugreek seed with high water concentration, more water-soluble gums will be extracted rather than bioactive compounds of saponins and phenolics. The extracted gums create some difficulty in filtration process after MAE. These findings are in a good agreement with those found by Amid and Mirhosseini (2012). Dahmoune et al. (2015) and (Liu et al., 2016) also reported that the optimum yields of *Myrtus communis L*. leaves and *Flos Sophorae Immaturus* plant were achieved at 40% of ethanol concentration, respectively.

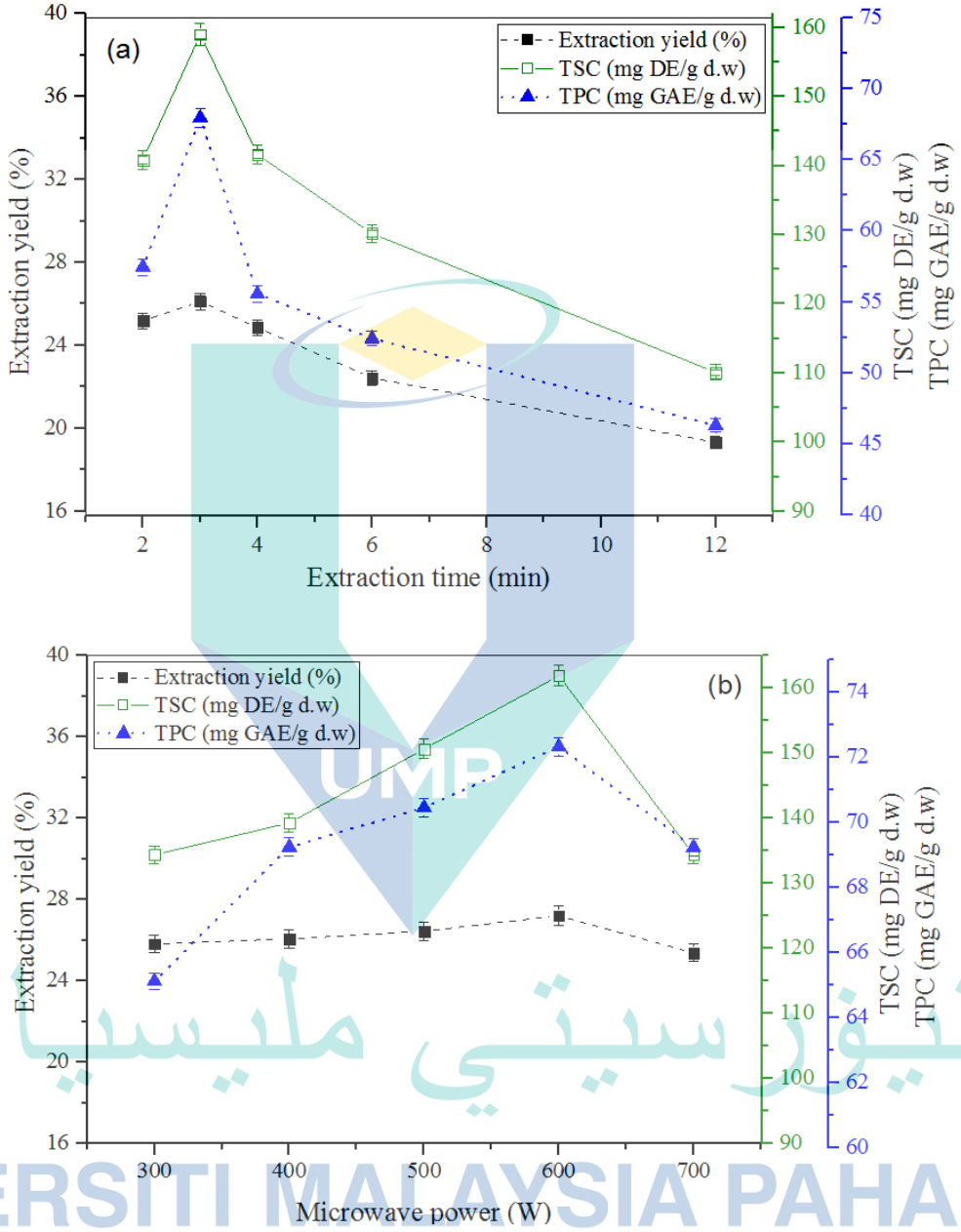
Another important parameter in a MAE is the ratio of feed-to-solvent. The determination of this factor is important to maximize the yields of recovery and minimize the use of solvent during the extraction process. The effect of feed-to-solvent ratio on the extraction yield, TSC and TPC is shown in Figure 4.1d. It can be seen that the yields varied by F:S ratio from 1:8-1:16 g/mL, the maximum recoveries of 26.75±0.74%, 177.12±1.98 mg DE/g d.w and 77.64±0.21 mg GAE/g d.w) were obtained at 1:10 g/mL. However, beyond 1:10 g/mL, resulted to the reduction of yields to 22.11±0.96%,

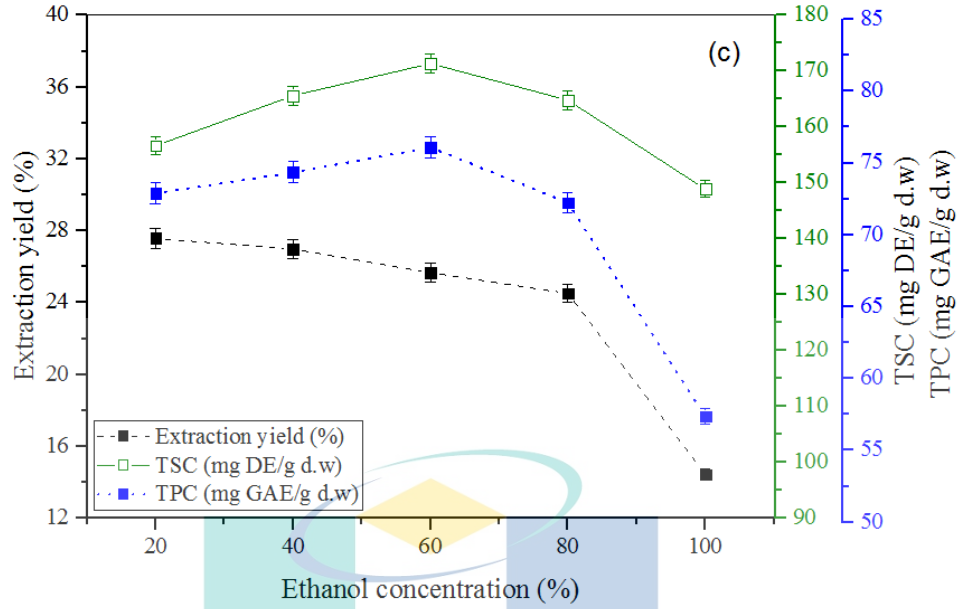
120.032±0.38 mg DE/g d.w and 62.66±0.21 mg GAE/g d.w, respectively. On the other hand, in MAE of Aloe vera leaves (Figure 4.2d), the selection of F:S ratio was varied due to the properties of Aloe vera leaves during the extraction. The F:S ratio was selected at 1:10-1:22 g/mL, for Aloe vera extraction. It is seen that increasing the ratio of F:S from 1:10-1:20 g/mL resulted to an increase in yields production of 34. 21±0.52%, 64.98±0.45 mg OAE/g d.w and 69.74±0.72 mg GAE/g d.w, respectively.

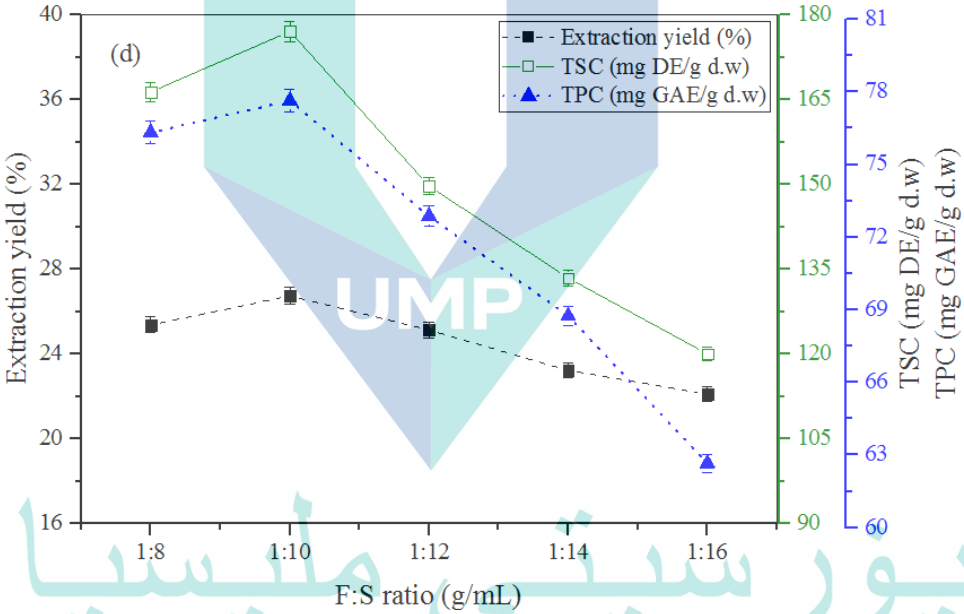
In a study that conducted by Xu et al. (2012) on the MAE of triterpenoid saponins from *Pulsatilla turczaninovii*, the optimum condition of F:S ratio was found at 1:20 g/mL. This study also claimed that the yields of triterpenoid saponins increased by increasing F:S ratio from 1:10-1:20 g/mL, this is due to excessive swelling, wetting, and solvent absorption of the sample. In a microwave-assisted extraction, one of the key factors is extraction temperature. As seen in Figure 4.1e and 4.2e, enhancement of the temperature from 40-70 °C resulted to an increase in extraction yield, TSC and TPC of fenugreek seed to 27.98±0.67%, 193.02±0.22 mg DE/g d.w and 81.78±0.23 mg GAE/g d.w and for Aloe vera leaves extract to 35.65±0.28%, 66.95±1.21 mg OAE/g d.w and 71.21±0.38 mg GAE/g d.w, respectively. However, increasing the temperature beyond 70 °C caused to decline in yields of extraction in both fenugreek seed and Aloe vera leaves. In this regard, Alara et al. (2017) also found 70 °C as the optimum temperature for MAE of Phenolic compounds from *Vernonia amygdalina* leaf. In MAE due to volumetric heating mechanism the solvent temperature increases rapidly, especially if the solvent contain water. Since water has high dielectric property and can be heated in seconds by microwave radiation. In addition (Proestos & Komaitis, 2008). In this case, higher temperature will cause to overheating of the sample and hence evaporation of some light compounds which results to lower yield.

One-factor-at-a-time experiment is a technique that facilitates the effect of different ranges of a factor on the yield of extract. On the other hands, OFAT experiment is not a developed statistical method to contribute in identifying the most significant factors in achieving high yields of recovery. Therefore, two-level factorial design was applied to identify the most significant factors and evaluate the interaction and contribution of each factor in obtaining the response with a statistical data. The high and

low level of each factor for two-level factorial analysis were obtained from OFAT experiment based on the adjacent levels of best points of each factor.







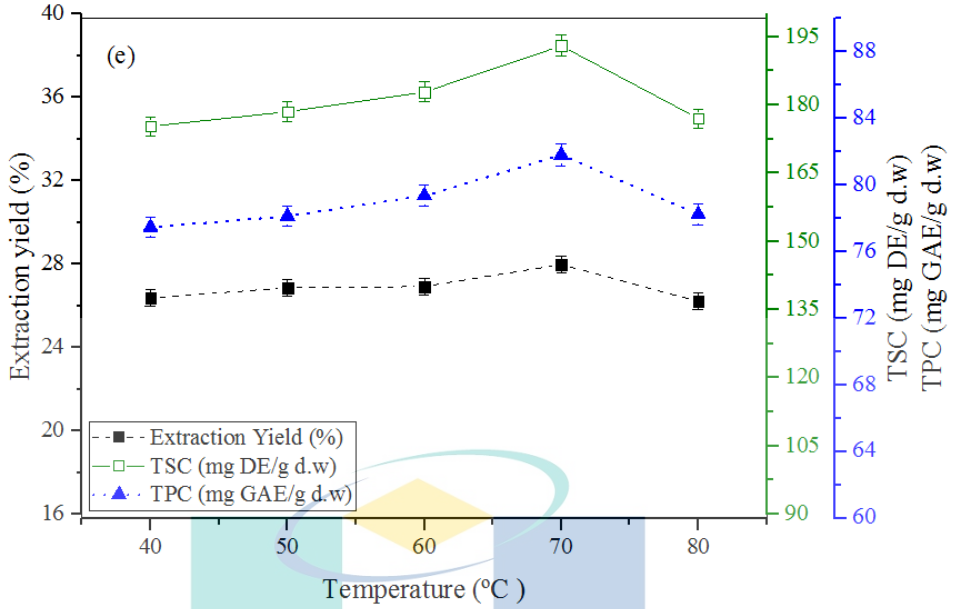
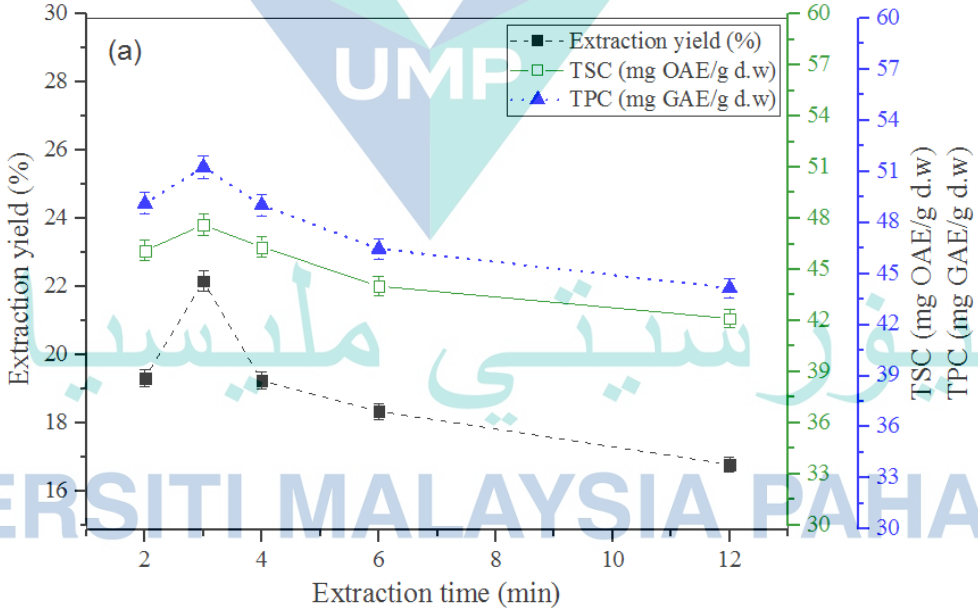
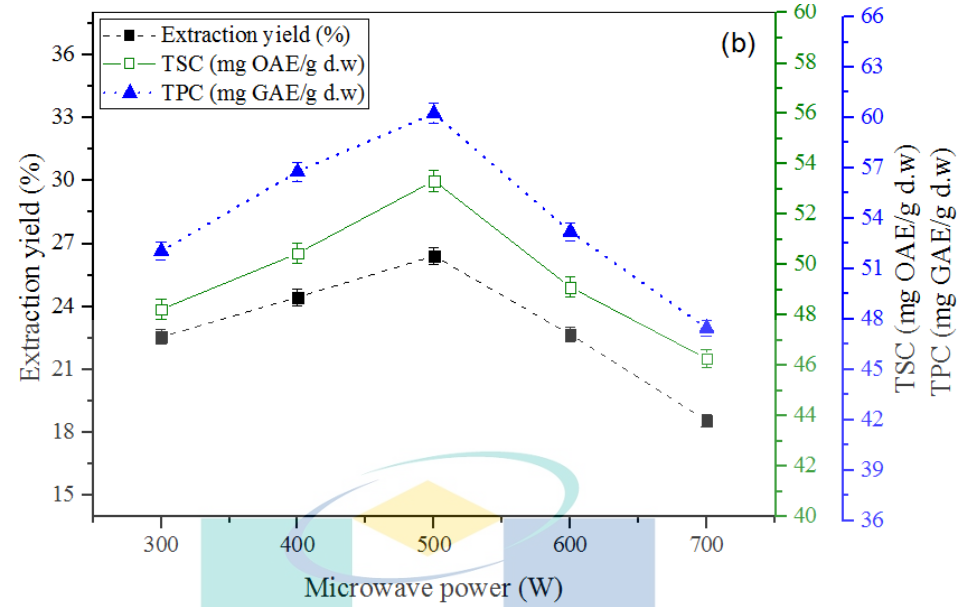
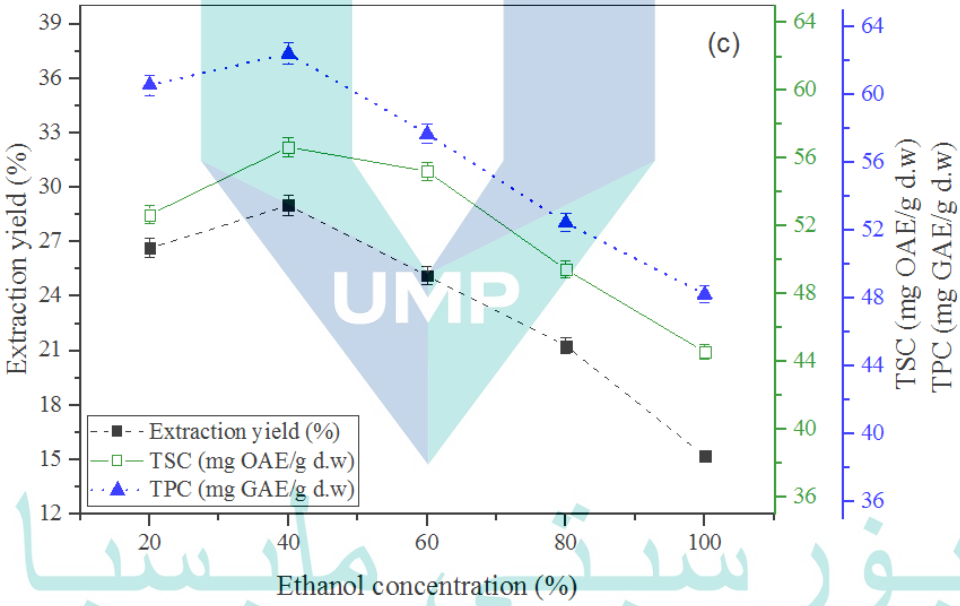


Figure 4.1 Effect of extraction time (a); Microwave power (b); ethanol concentration (c); feed-to-solvent ratio (d) and temperature (e) on extraction yield, TSC and TPC of fenugreek seed extracted via MAE using OFAT experiment.







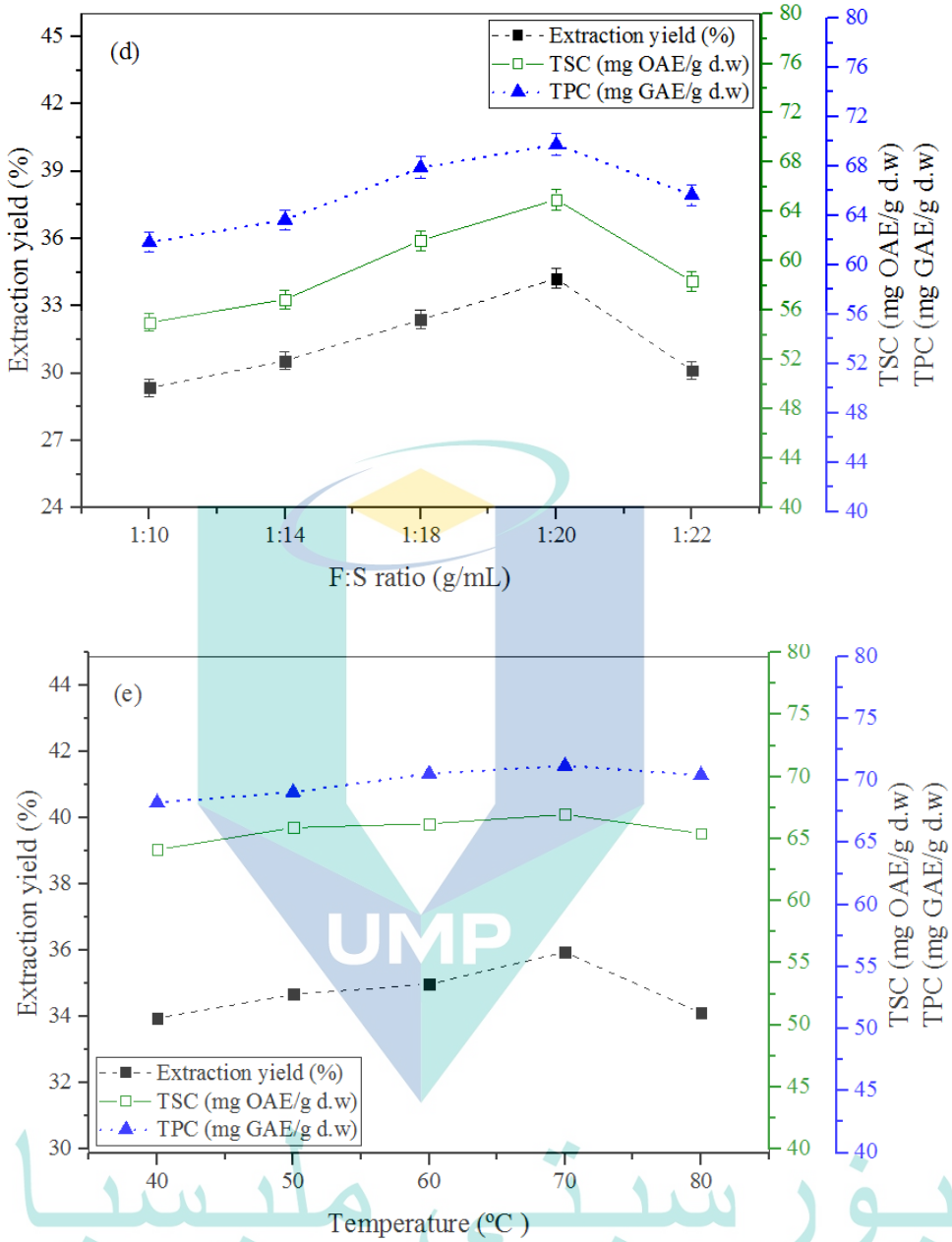


Figure 4.2 Effect of extraction time (a); Microwave power (b); ethanol concentration (c); feed-to-solvent ratio (d) and temperature (e) on extraction yield, TSC and TPC of Aloe vera extracted via MAE using OFAT experiment.

4.3 Two Level Factorial Design, Screening and Analysis

The screening of MAE process parameters namely extraction time (A), microwave power (B), ethanol concentration (C), feed-to-solvent ratio (D) and temperature (E) was carried out using two level factorial design (TLFD) to evaluate the

most contributing factor of the experiment. Factor screening indicated the concentration of ethanol (C) is the most contributing factor effecting all the response namely extraction yield, total saponin content (TSC) and total phenolic content (TPC) of fenugreek seed and Aloe vera leave extracts. While extraction temperature (E) was insignificant to obtain high recovery of yield and bioactive compounds. Other MAE parameters such as time of irradiation (A), microwave power (B) and ratio of feed-to-solvent (D) also displayed a significant contribution based on their effects and *p*-values <0.05. The levels of factors were selected based on OFAT experiment as (-1, 0, +1) as shown in Table 4.1.

Table 4.1 Levels of independent factors for TLFD.

Parameters	**Unit**	**Fenugreek seed**		**Aloe vera leaves**	
		Low level	**High level**	**Low level**	**High Level**
Extraction time (A)	Min	2	4	2	4
Microwave power (B)	W	500	700	400	600
Ethanol concentration (C)	%	40	80	20	60
Feed-to-solvent (D)	g/Ml	1:8	1:12	1:18	1:22
Temperature (E)	°C	60	80	60	80

4.3.1 Effect of MAE Factors on Extraction Yield

The screening of experimental factors is considered important if there are many factors involved in an experiment. Application of many factors during the experimental work will complicate the experiment and increase the cost as well. To reduce the cost, time, and complication of the experiment, the experimental factors have been screened using two-level factorial design. Figures 4.3 and 4.4 indicate the influence of MAE factors on yield of extraction of fenugreek seed and Aloe vera leaves. As it can be seen in Figures 4.3 and 4.4 (b, c, f) increasing in the level of irradiation time (A), microwave power (B) and temperature (E) resulted in increasing the recovery of yield with positive effects. However, raising the level of ethanol concentration (C) and ratio of feed-to-solvent (D)

resulted to a decrease in the yield of extraction of fenugreek seed and Aloe vera leaves as seen in Figures 4.3 and 4.4 (d and e). In this vein, the Pareto charts show that ethanol concentration showed the most significant effect (Figures 4.3a and 4.4a). The sequence of parameters in extraction of fenugreek seed and Aloe vera leaves in terms of their contribution can be generated as C>B>A>D>E and C>A>B>D>E, respectively. On the other hand, the interaction between factors also created based on the contribution of each factor with a sequence of BC>AC>BD>AB>BE>CE and BD>BC>AC>AB>BE>CE, respectively. Previous findings revealed that the ratio of 1:10 g/mL to 1:20 g/mL can be the best selections for optimization of bioactive compounds from different types of plants (Alara et al., 2017; Maeng et al., 2017; Xu et al., 2012). Abayomi et al. (2018) also claimed that microwave power and time have significant influence on recovery of bioactive compounds. The contribution of each factor on recovery yields of fenugreek seed is displayed in Table 4.2. It is seen that power, time, ethanol concentration and ratio of feed to solvent had a significant effect ($p < 0.05$) while the influence of temperature was insignificant to obtain a high recovery yield ($p > 0.05$). The major contribution was indicated by ethanol concentration at 26.03%, followed by microwave power (8.39%), irradiation time (5.52%), feed-to-solvent ratio (2.49%) and microwave temperature (0.34%).

The same trend was found for Aloe vera leaves extract. As seen in Table 4.3, the effect of factors on yield of Aloe extract is represented in the following sequence, ethanol concentration (27.09%)>irradiation time (5.25%)> microwave power (4.57%)> F:S ratio (2.78%). Temperature shows a very insignificant amount (<1%). The contribution trend of these results is in a good correlation with the previous study on *Vernonia amygdalina* leaf extraction using MAE method (Alara et al., 2017). Despite of having the major contribution, the effect of ethanol concentration was negative, since increasing the percentage of ethanol, declined the yields of extraction. This is due to the extraction of polysaccharide gums which are more soluble in water rather than in alcohols (Amid & Mirhosseini, 2012), as well as dielectric properties of water and ethanol during the extraction process (Proestos & Komaitis, 2008). The interaction and effects of factors are also described clearly in pareto chart (Figures 4.3a and 4.4a). Where the blue color displays the negative and orange color refers to the positive effect of factors.

Table 4.2 Extraction variables and their contribution percentage from fenugreek seed extract

Variables	**Y_1 Extraction yield (%)**	**P-value**	**Y_2 TSC (mg DE/g d.w)**	**P-value**	**Y_3 TPC (mg GAE/g d.w)**	**P-value**
Model	**Contribution (%)**	**0.0003 (significant)**	**Contribution (%)**	**0.0002 (significant)**	**Contribution (%)**	**0.0003 (significant)**
A	5.52	0.0017	8.67	0.0011	11.27	0.0002
B	8.39	0.0008	4.22	0.0055	7.53	0.0003
C	26.03	<0.0001	25.58	<0.0001	23.45	< 0.0001
D	2.49	0.0074	1.41	0.0429	2.68	0.0008
E	0.34	0.1360	0.01	0.8248	0.00	0.5206
AB	8.39	0.0008	0.25	-	9.06	0.0002
AC	14.24	0.0003	0.02	-	20.32	0.0001
AD	0.18	-	13.00	0.0004	9.32	0.0002
AE	0.07	-	15.11	0.0003	0.00	0.0013
BC	17.69	0.0002	6.30	0.0023	1.56	0.0006
BD	10.90	0.0005	0.05	-	3.55	-
BE	4.73	0.0023	0.88	0.0863	0.00	-
CD	0.02	-	0.52	-	2.02	0.0010
CE	0.87	0.0415	0.12	-	2.28	0.0009
DE	0.13	-	23.86	0.0001	6.94	0.0003

A = irradiation time (min); B = microwave power (W); C = ethanol concentration (%); D = feed-to-solvent ratio (g/mL); E = temperature (°C)

Table 4.3 Extraction variables and their contribution percentage from Aloe vera leaves extract.

Variables	**Y_1 Extraction yield (%)**	**P-value**	**Y_2 TSC (mg OAE/g d.w)**	**P-value**	**Y_3 TPC (mg GAE/g d.w)**	**P-value**
Model	**Contribution (%)**	**0.0002 (significant)**	**Contribution (%)**	**0.0005 (significant)**	**Contribution (%)**	**0.0004 (significant)**
A	5.25	0.0016	12.53	0.0004	11.74	0.0003
B	4.57	0.0021	10.79	0.0005	10.33	0.0003
C	27.09	< 0.0001	20.48	0.0002	24.69	0.0001
D	2.78	0.0052	1.93	0.0063	1.63	0.0019
E	0.00	0.9524	0.14	0.1584	0.05	0.0579
AB	8.66	0.0006	0.01	-	0.00	-
AC	13.91	0.0002	4.55	0.0018	3.09	0.0010
AD	0.13	-	6.25	0.0011	7.72	0.0004
AE	0.03	-	1.85	0.0067	1.04	0.0029
BC	15.22	0.0002	9.75	0.0006	7.02	0.0004
BD	16.52	0.0002	3.91	0.0023	5.43	0.0006
BE	5.21	0.0016	0.05	-	0.01	-
CD	0.06	-	4.05	0.0021	3.59	0.0009
CE	0.42	0.0969	0.06	-	1.76	0.0017
DE	0.14	-	23.64	0.0002	21.90	0.0001

A = irradiation time (min); B = microwave power (W); C = ethanol concentration (%); D = feed-to-solvent ratio (g/mL); E = temperature (°C

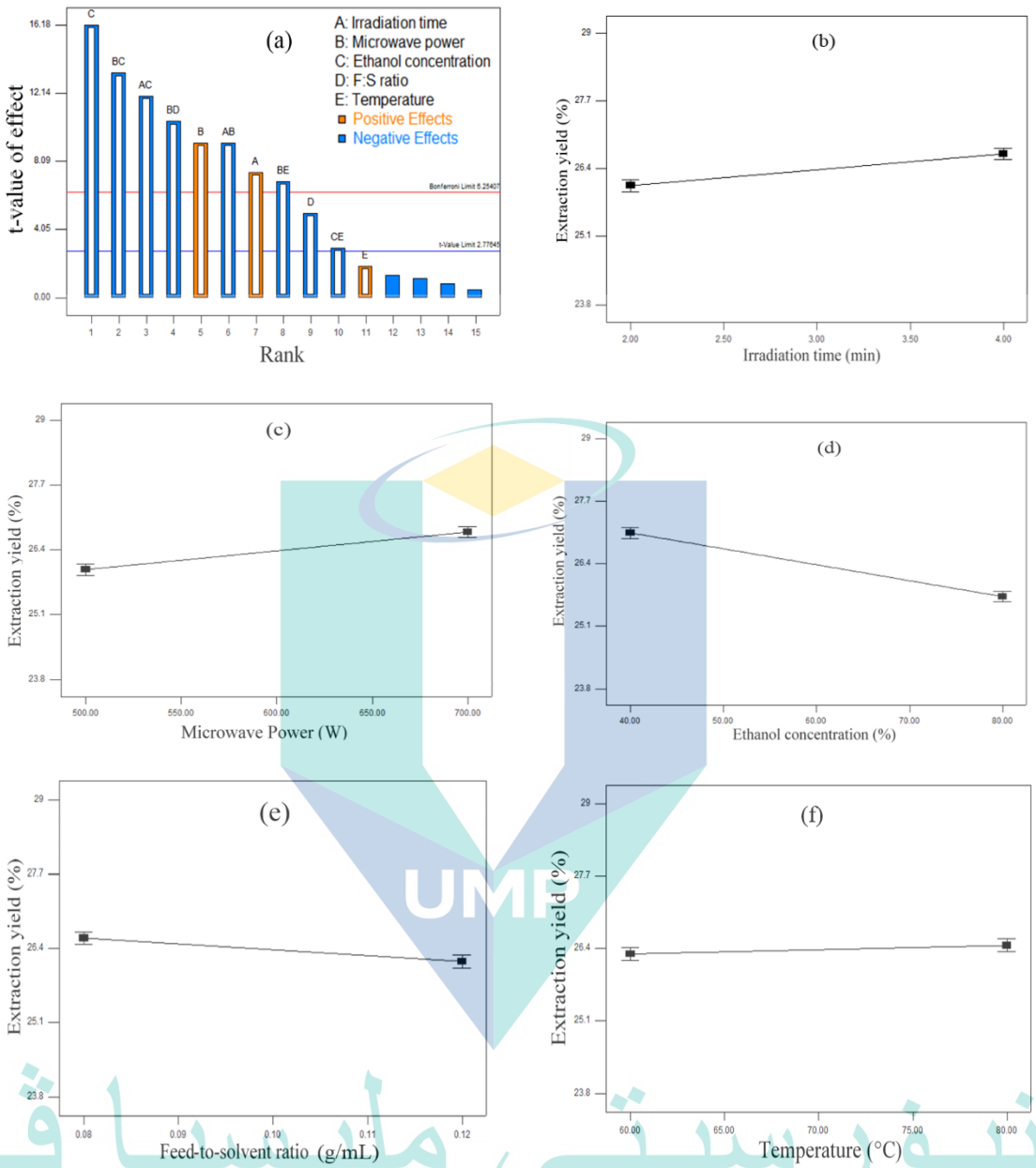


Figure 4.3 (a) Pareto chart, (b) effect of irradiation time, (c) microwave power, (d) concentration of ethanol, (e) feed-to-solvent ratio, and (f) effect of temperature on extraction yield of fenugreek seed.

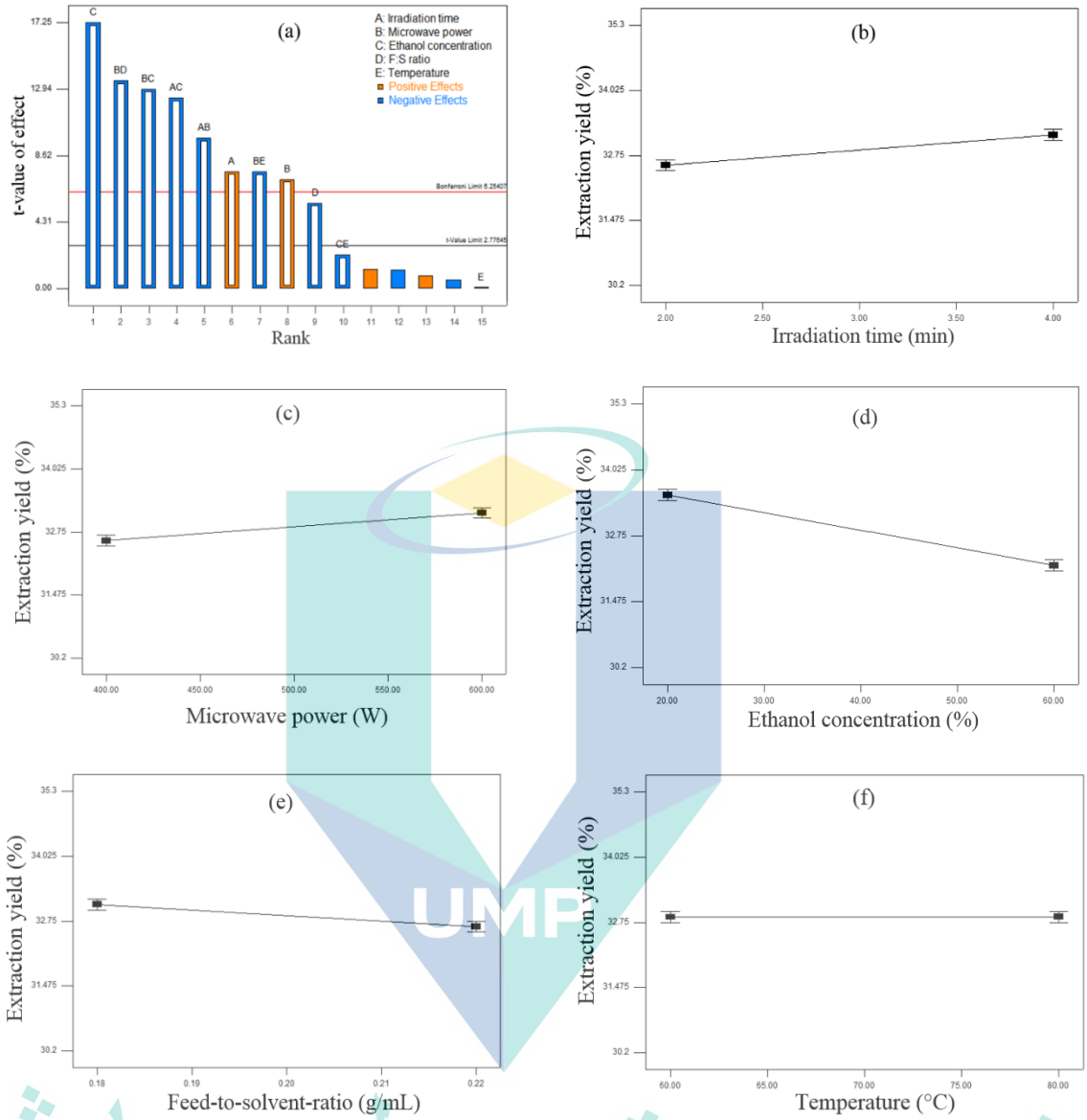


Figure 4.4 (a) Pareto chart, (b) effect of irradiation time, (c) microwave power, (d) concentration of ethanol, (e) feed-to-solvent ratio, and (f) effect of temperature on extraction yield of Aloe vera leaves.

4.3.2 Effect of MAE Factors on Total Saponin and Total Phenolic Contents

The effects of five variables from A to E on the yield of total saponin and total phenolic content of fenugreek seed and Aloe vera leaves are shown in Figures 4.5-4.8, respectively. It can be seen that increasing the time of irradiation (A), microwave power (B), ethanol concentration (C) and temperature (E) resulted in increasing the total saponin and total phenolic content of Fenugreek seed and Aloe vera leaves. However, TSC and

TPC reduced with increasing the levels of feed-to-solvent ratio (D) more than 1:10 g/mL for fenugreek seed and 1:20 g/mL for Aloe vera leaves extract as seen in Figure 4.5 and 4.6 (b-f). In a microwave extraction, extraction factors such as temperature, time and microwave power are interrelated. Therefore, the recovery yields of bioactive compounds can be improved by enhancing the levels of these factors to an adequate level. The reason behind the negative influence of feed-to solvent ratio on recovery yields of TSC and TPC could be the poor transformation of the plant extract to extraction solvent. Different plant materials interact different under extraction process. The heat and solvent adsorption mechanism vary by the properties of plants sources, due to the existence of various phytochemicals with different polarities (Sasidharan et al., 2011). In addition, more extraction solvent than the equilibrium point would result into the low transformation of bioactive compounds during the extraction process (Barbosa-Cánovas, 2013). The influence and contribution of each factor on recovery of TSC and TPC yields of fenugreek seed and Aloe vera leaves are displayed in Table 4.2, 4.3 and pareto charts Figure 4.5a and 4.6a, respectively.

It is seen in Table 4.2 that the most contributing, effective and significant parameter on TSC of fenugreek seed and Aloe vera leaves (fenugreek seed; Aloe vera leaves) was ethanol concentration (25.58%; 20.48%) followed by irradiation time (8.67%; 12.53%), microwave power (4.22%; 10.79%), and feed-to-solvent ratio (1.41%;1.93%) with p-values of <0.05, respectively. However, temperature was the least contributing factor (0.011%; 0.14%) with insignificant effect p>0.05 in both plants. In fact, as it can be seen in pareto charts the extraction temperature falls below the t-value which indicates the insignificance of the factor. The interaction between factors also generated based on the contribution and effect of each factor with a sequence of DE>AE>AD>BC>BE and DE>BC>AC>CD>BD>AE, respectively (Figures 4.5 and 4.6).

The contribution of factors in TPC of fenugreek seed and Aloe vera leaves extracts were similar to the TSC results. As seen in Table 4.3 the contribution of each factors in TPCs of these plants were as ethanol concentration (23.45%; 24.69%), irradiation time (11.27%; 11.74%), microwave power (7.53%; 10.33%) and feed-to-solvent ratio (2.68%;

1.63%) with p-values less than 0.05, respectively. However, temperature indicated not a significant effect (p>0.05). In addition, the factor interaction sequences in TPCs were as AC>AD>AB>DE>BD>CE>CD>BC and DE>AD>BC>BD>CD>AC>CE>AE, respectively (Figures 4.7 and 4.8).

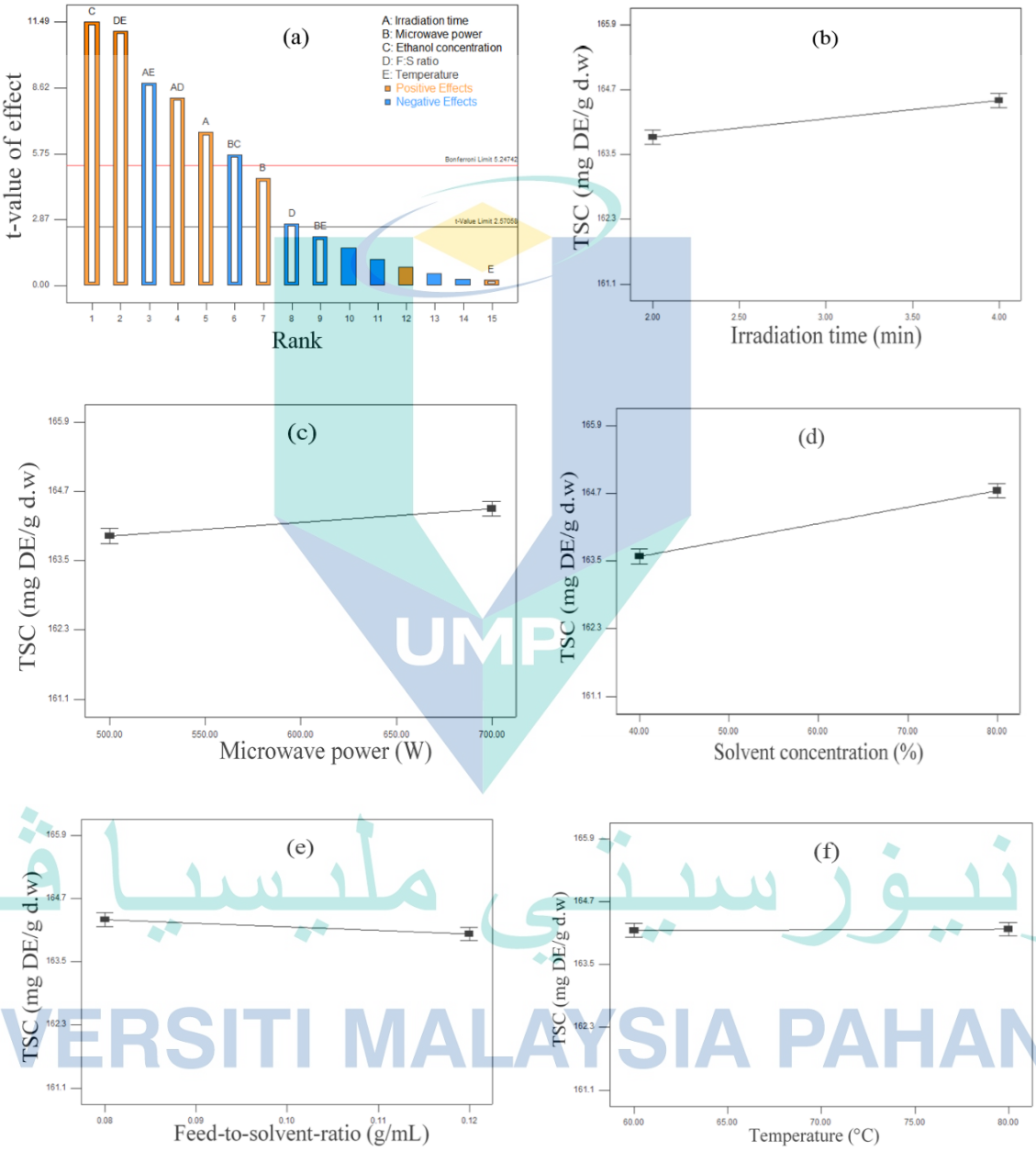


Figure 4.5 (a) Pareto chart, (b) effect of irradiation time, (c) effect of microwave power, (d) effect of ethanol concentration, (e) influence of feed-to-solvent ratio, (f) effect of temperature on total saponin content of fenugreek seed extract.

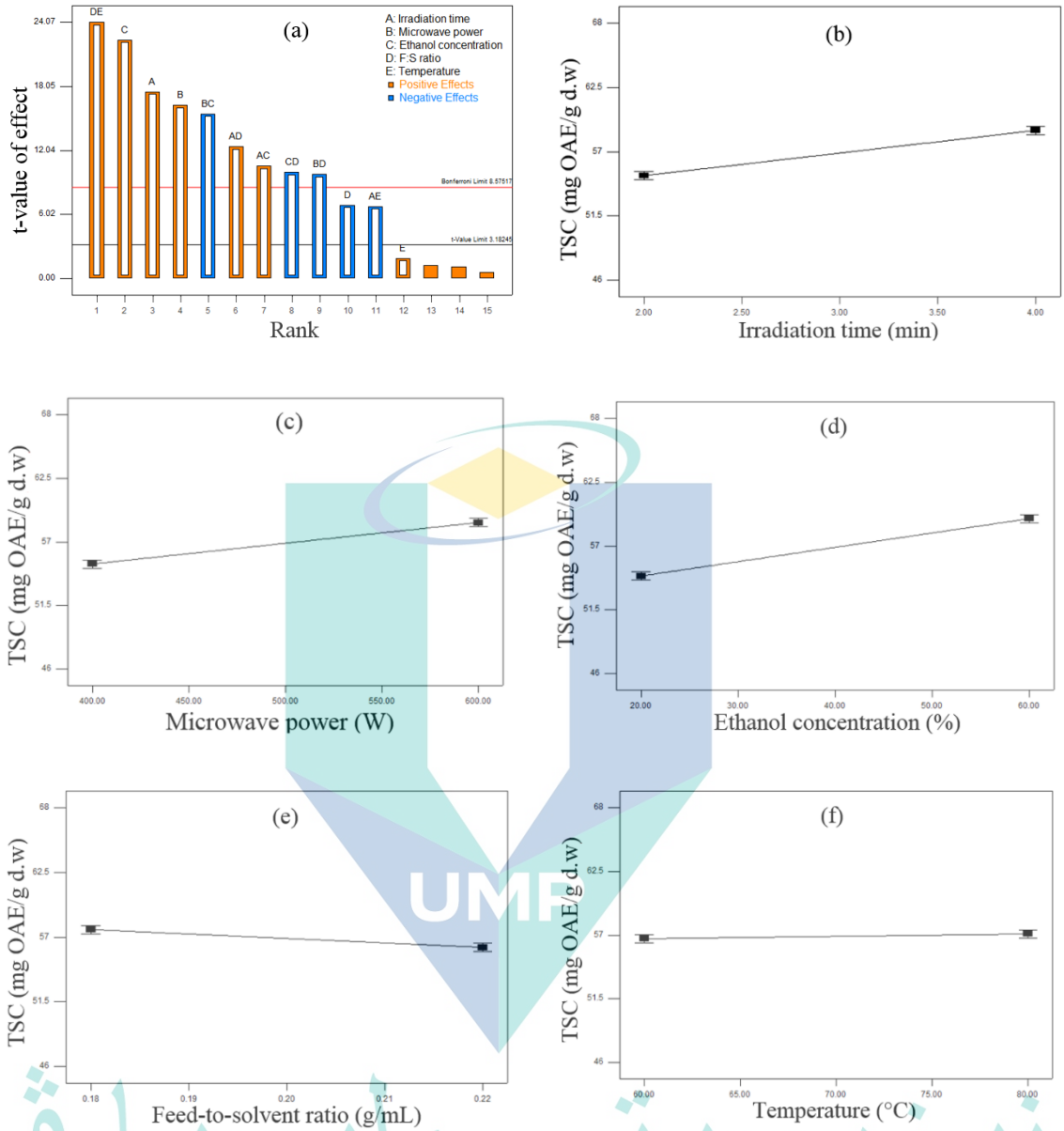


Figure 4.6 (a) Pareto chart, (b) effect of irradiation time, (c) effect of microwave power, (d) effect of ethanol concentration, (e) influence of feed-to-solvent ratio, (f) effect of temperature on total saponin content of Aloe vera leave extract.

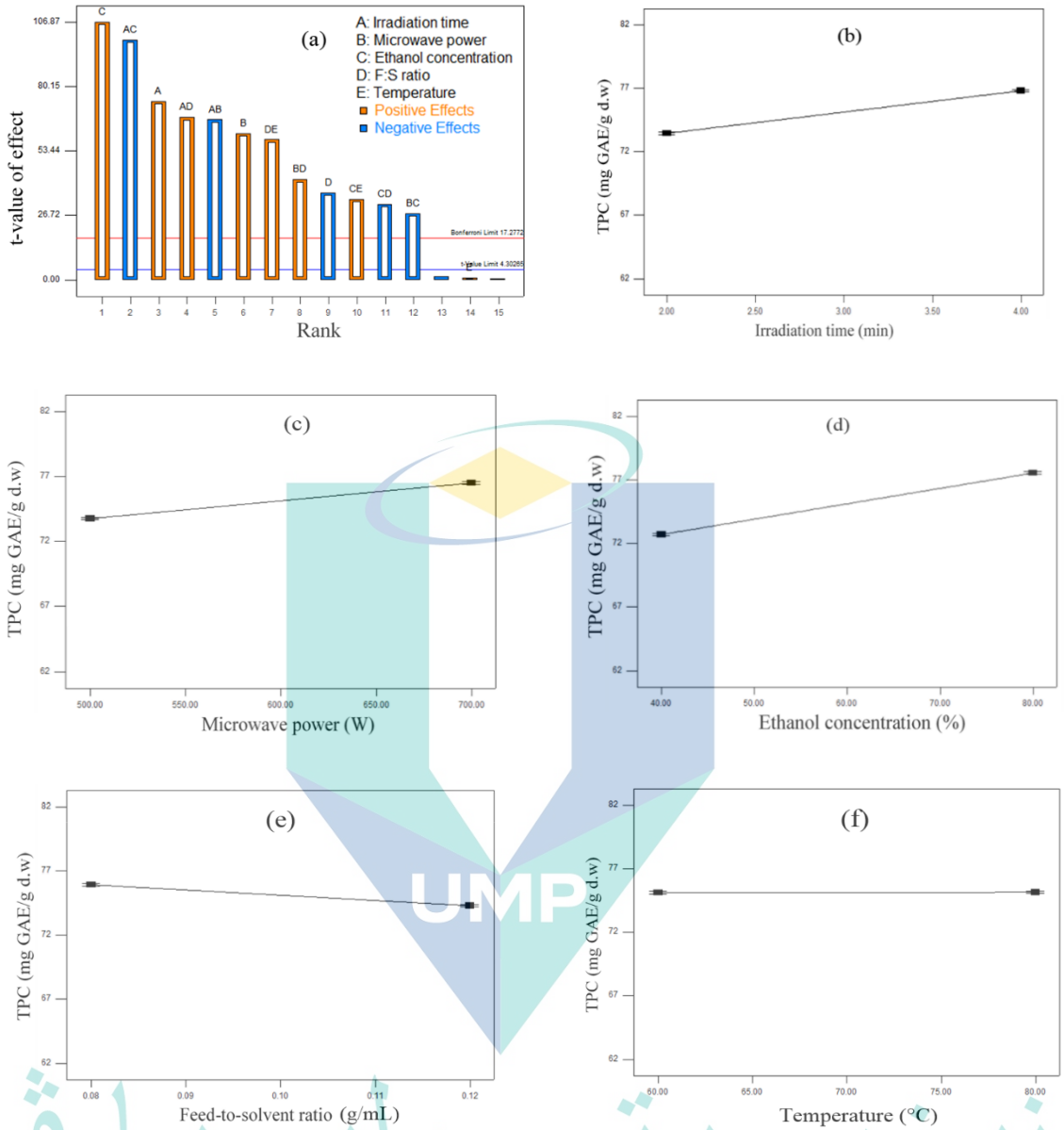


Figure 4.7 (a) Pareto chart, (b) effect of irradiation time, (c) effect of microwave power, (d) effect of ethanol concentration, (e) effect of feed-to-solvent ratio, (f) effect of temperature on total phenolic content of fenugreek seed extract.

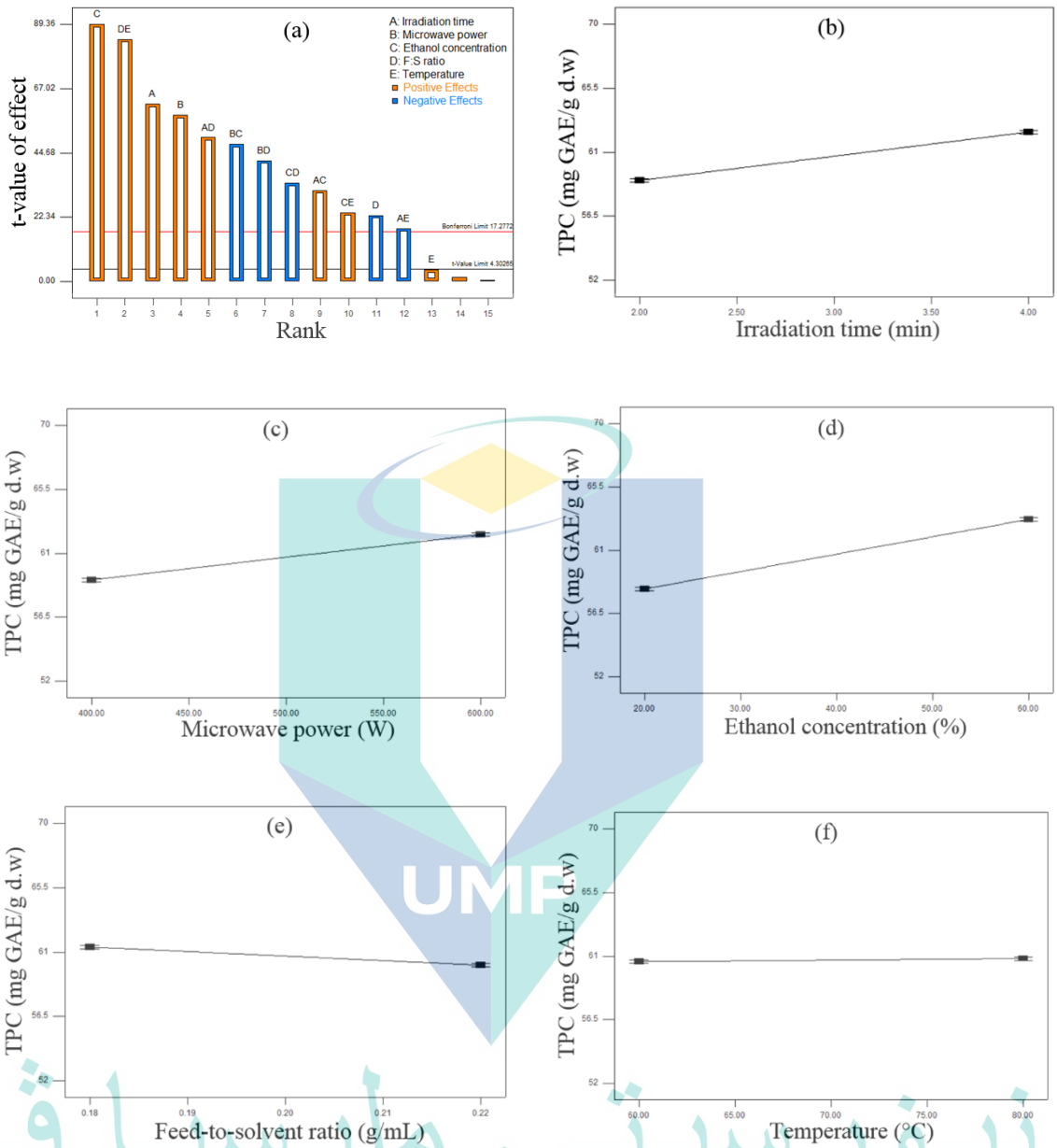


Figure 4.8 (a) Pareto chart, (b) effect of irradiation time, (c) effect of microwave power, (d) effect of ethanol concentration, (e) effect of feed-to-solvent ratio, (f) effect of temperature on total phenolic content of Aloe vera leaves extract.

4.4 Optimization of MAE Factors for Achieving Optimum Yields of Extraction, TSC and TPC

4.4.1 Optimization and Model Fitting using RSM

The ranges of factors for optimization of MAE process parameters were selected based on one-factor-at-one-time experiments. In regard to OFAT results, for fenugreek seed the independent variables of optimization process that affect the extraction yield, TSC and TPC were irradiation time A (2-4 min), microwave oven power B (500-700 W), ethanol concentration C (40-80%) and feed-to-solvent ratio D (1:8-1:12 g/mL). The factor ranges for optimization of Aloe vera extract were also selected based on OFAT results as irradiation time A (2-4 min), microwave power B (400-600 W), ethanol concentration C (20-60%) and F:S ratio D (1:18-1:22 g/mL) as seen in Table 4.4. The model terms, adequacy based on R-squares, lack-of-fit, F-value, interaction of factors and adequate precision were evaluated from the analysis of variance (ANOVA).

Table 4.4 Experimental factors with their levels used in the experiment.

Parameters	Unit	Fenugreek seed			Aloe vera leaves		
		Low	Centre	High	Low	Centre	High
		-1	0	+1	-1	0	+1
Extraction time (A)	Min	2	3	4	2	3	4
Microwave power (B)	W	500	600	700	400	500	600
Ethanol concentration (C)	%	40	60	80	20	40	60
Feed-to-solvent (D)	g/mL	1:8	1:10	1:12	1:18	1:20	1:22

The temperature during the extraction process did not indicate a considerable contribution, therefore, it was fixed at 70 °C based on best points of OFAT. The FCCCD results based on actual and predicted values are shown in Table 4.5. The second-order polynomial equations of optimized conditions for extraction yield, TSC and TPC of fenugreek are shown in Eqs. (4.1- 4.3), respectively. The Eqs. (4.4-4.6) indicate the second-order polynomial equations of optimized conditions for extraction yield, TSC and TPC of Aloe vera leaves extract.

$$Y_{Ext(F.S)} = +27.23 - 0.44A - 0.41B - 1.37C - 0.49D - 0.17AB + 0.10AC - 0.48AD$$
$$+0.36BC - 0.034BD + 0.51CD - 0.63A^2 - 1.48B^2 + 0.58C^2 + 0.070D^2 \quad 4.1$$

$$Y_{TSC(F.S)} = +192.80 - 4.49A - 4.49B + 6.52C - 8.97D - 4.01AB - 3.64AC - 3.89AD$$
$$+1.70BC + 4.67BD - 5.43CD + 1.62A^2 - 11.18B^2 - 14.38C^2 - 14.23D^2 \quad 4.2$$

$$Y_{TPC(F.S)} = +81.01 - 0.95A - 0.95B + 1.04C - 0.90D - 1.26AB + 0.14AC + 0.81AD$$
$$-1.47BC + 0.31BD + 0.25CD - 5.29A^2 - 2.24B^2 - 5.16C^2 - 3.84D^2 \quad 4.3$$

$$Y_{Ext(Aloe)} = +36.56 - 2.19A - 1.15B + 1.23C - 0.98D - 1.35AB - 0.21AC + 1.46AD$$
$$-1.26BC - 1.00BD - 0.39CD - 7.71A^2 - 2.46B^2 - 3.23C^2 - 1.05D^2 \quad 4.4$$

$$Y_{TSC(Aloe)} = +65.91 - 1.76A - 1.31B + 1.42C - 1.61D - 0.66AB + 0.17AC + 1.49AD$$
$$-0.87BC - 0.44BD - 0.26CD - 3.28A^2 - 2.56B^2 - 6.72C^2 - 3.44D^2 \quad 4.5$$

$$Y_{TPC(Aloe)} = +71.35 - 1.72A - 1.33B + 1.31C - 3.07D + 0.41AB - 0.35AC$$
$$+0.28AD - 1.54BC - 1.09BD + 0.86CD - 4.69A^2 - 2.73B^2 - 4.13C^2 - 4.55D^2 \quad 4.6$$

where *Y* is the response, *A*, *B*, *C*, and *D* indicate the variables, respectively. Table 4.5 depicts the results of fenugreek seed extract in experimental and predicted values obtained from FCCCD. As seen, the obtained experimental and predicted values were in good correlation without any significant differences. Table 4.6 shows the ANOVA of quadratic model for optimization of extration yield, TPC and TSC of extract. It is seen that, the models and most of the factor terms for extraction yield, TSC and TPC are significant ($P < 0.0001$) and *F*-values fo 47.394, 71.34 and 201.23, respectively. The lack of fit F-values of of 0.62, 3.22 and 0.76 indicate non-significant Lack of Fit. For a fit model, non-significant lack of fit is desirable. As seen, the Pred R^2 values of .9276, 0.9448 and 0.9849 are not very different from the Adj R^2 values of .9573, 0.9714 and 0.9898. The Adeq Precision values of 25.031, 25.824 and 37.255 indicate adequate signals and desirable values, respectively. An adjacent between R^2 and Adj R^2 also shows a well fitted model, as seen, there is no significant difference between them.

The interaction factors varied between different batches, only *CD* was significant in all responses ($P < 0.05$) except in TPC. In addition, most of the factors and their interactions indicated significant terms. The coefficient of determination (R^2) for

extraction yield, TSC and TPC were 0.9779, 0.9852 and 0.9947, respectively. Where these indicate that there is a desirable correlation between actual and predicted values. The good correlation between *pred* R^2 and *adj* R^2 and non-significant of the model also implies the fitting of the model. Therefore, the design obtained in this experimental study shows a reliable model which can be reused. As indicated in Table 4.5, the values of predicted and actual results are not significantly different, and this supports a fitted model. The findings of this study have conformity with the previous works done by Dahmoune et al.(2015), Alara et al. (2018b) and Xiong et al. (2016).

In the same way, the results of ANOVA for Aloe vera extracts were similar in some conditions with the fenugreek seed. Table 4.7 indicates the experimental results of Aloe vera extract in actual and predicted values obtained from FCCCD. As seen, the obtained experimental values were much closed with the predicted values without any significant differences. Table 4.8 shows the analysis of variance of quadratic model for optimization of extration yield, TSC and TPC of Aloe vera leaves extract. It is seen that, the models and most of the factor terms for extraction yield, TSC and TPC are significant at a *P*-value of < 0.0001 and *F*-values of 113.65, 108.22 and 85.00, respectively. There is only a 0.01% chance that a "Model F-value" this large could occur due to noise. The "Lack of Fit F-value" of 1.05, 3.10 and 3.69 indicate that the Lack of Fit is not significant relative to the pure error. There are 50.95%, 11.16% and 8.10% chance that a "Lack of Fit F-value" this large could occur due to noise, respectively. For a fit model, non-significant lack of fit is good. As seen, the "Pred R-Squared" values of 0.9494, 0.9567 and 0.9122 are in reasonable agreement with the "Adj R-Squared" values of 0.9819, 0.9810 and 0.9759."Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable, therefore the obtained ratios of 29.52, 27.62 and 26.005 indicate adequate signals, respectively. An adjacent between R^2 and Adj R^2 also shows a well fitted model, as seen, there is no significant difference between them. This model can be used to navigate the design space.

The interaction factors varied between different batches, only *BC* was significant in all responses (P < 0.05) except in TPC. In addition, most of the factors and their interactions indicated significant terms. The coefficient of determination (R^2) for

extraction yield, TSC and TPC were 0.9907, 0.9902 and 0.9876, respectively. Where these indicate that there is a desirable correlation between actual and predicted values. The good correlation between *pred* R^2 and *adj* R^2 and non-significant of the model also implies the fitting of the model. Therefore, the design obtained in this experimental study shows a reliable model which can be reused. As indicated in Table 4.7, the values of predicted and actual results are not significantly different, and this supports a fitted model. The findings of this study have conformity with the previous works done by Dahmoune et al.(2015) and Alara et al. (2018b).

4.4.2 Validation of the Model

The model validation was performed based on the suggested optimal condition of RSM which were 2.84 min, 572.50 W, 63.68%, and 1:9 g/mL. Where, based on these conditions, the extraction yield, TSC and TPC of Aloe vera leaves extract were 27.14%, 196.49 mg DE/g d.w and 81.01 mg GAE/g d.w, respectively. To evaluate the validity of the predicted model, experimental runs were performed in suggested optimal conditions and the responses were 26.04 ± 0.88%, 195.89 ± 1.07 mg DE/g d.w., 81.85 ± 0.61 mg GAE/g d.w, respectively. By applying the paired t-tests for each optimization processes it is found that there was no statistically significant difference observed between predicted and actual values ($p > 0.05$). Thus, it is suggested that the obtained model is suitable for the study.

Likewise, the suggested optimum condition for Aloe vera leaves extracts in MAE were 2.79 min, 478.95 W, 43.38% ethanol, and 1:19 g/mL. Where, based on these conditions, the extraction yield, TSC and TPC of Aloe vera leaves extract were 37.18%, 66.58 mg OAE/g d.w, and 72.26 mg GAE/g d.w, respectively. To evaluate the validity of the predicted model, experimental runs were performed in suggested optimum conditions and the responses were 36.17 ± 1.13%, 65.89 ± 0.77 mg OAE/g d.w and 73. 05 ± 1.05 mg GAE/g d.w, respectively. By applying the paired t-tests for each optimization processes it is found that there was no statistically significant difference between actual and predicted values ($p > 0.05$) were observed. Therefore, it can be claimed that the obtained response is a good indication of the adequate model with a suitable design.

Table 4.5 Actual and predicted responses based on face centred central composite design and coded factors for fenugreek seed extract.

Run	Factors				Responses					
	A	B	C	D	Y_{Ext} (Act)	Y_{Ext} (Pred)	Y_{TSC} (Act)	Y_{TSC} (Pred)	Y_{TPC} (Act)	Y_{TPC} (Pred)
1	0	0	0	0	27.25	27.23	191.22	192.80	80.10	81.0
2	1	-1	-1	-1	28.89	28.96	170.46	169.54	63.70	63.7
3	0	1	0	0	25.01	25.33	178.12	177.12	77.40	77.8
4	-1	-1	-1	1	27.82	27.80	146.42	146.80	60.36	60.5
5	-1	1	-1	-1	27.56	27.61	140.22	141.75	68.05	68.0
6	0	0	0	0	26.89	27.23	195.02	192.80	81.40	81.0
7	0	0	0	-1	27.45	27.79	187.38	187.54	77.92	78.1
8	1	1	1	1	23.13	23.33	131.62	132.59	61.28	61.5
9	0	0	0	0	27.46	27.23	196.02	192.80	82.60	81.0
10	-1	1	1	-1	24.37	24.38	175.62	176.31	65.92	66.3
11	1	0	0	0	26.08	26.15	189.02	189.92	74.40	74.8
12	-1	-1	1	-1	24.25	24.08	184.58	183.23	69.57	69.3
13	1	-1	1	-1	24.58	24.70	180.58	182.77	68.24	68.5
14	0	0	0	0	27.98	27.23	197.02	192.80	82.16	81.0
15	0	0	0	0	27.21	27.23	197.82	192.80	81.56	81.0
16	0	-1	0	0	26.11	26.15	180.02	186.10	79.06	79.7
17	1	1	1	-1	24.48	24.33	160.66	159.82	61.06	60.6
18	1	-1	-1	1	26.01	26.07	146.86	145.32	62.76	62.4
19	1	1	-1	1	24.13	24.13	133.38	134.28	61.60	61.6
20	-1	1	-1	1	26.56	26.52	154.82	151.79	64.88	64.7
21	-1	1	1	1	25.55	25.31	164.18	164.65	64.40	64.0
22	-1	0	0	0	26.75	27.04	194.72	198.91	75.96	76.7
23	-1	-1	1	1	24.91	25.15	153.98	152.87	65.80	65.7
24	0	0	-1	0	29.01	29.17	167.72	171.89	74.30	74.8
25	0	0	1	0	26.23	26.44	184.02	184.94	76.33	76.9
26	0	0	0	1	26.78	26.81	164.66	169.59	75.36	76.3
27	1	1	-1	-1	27.31	27.15	139.54	139.81	61.49	61.6
28	0	0	0	0	27.67	27.23	195.02	192.80	81.46	81.0
29	1	-1	1	1	24.06	23.84	138.82	136.84	68.48	68.2
30	-1	-1	-1	-1	28.88	28.76	157.26	155.45	65.17	65.0

A = Irradiation time; B = Microwave power; C = Ethanol concentration; D = F:S ratio; Act = Actual value; Pred = Predicted value.

Table 4.6 Analysis of variance showing optimization of bioactive compounds of fenugreek seed extract.

Factors	Extraction yield			TSC			TPC			
Source	Sum of Squares	F Value	p-value Prob > F	Sum of Squares	F Value	p-value Prob > F	Sum of Squares	F Value	p-value Prob > F	
Model	72.185	47.394	< 0.0001	13174.54	71.34	< 0.0001	1709.26	201.23	< 0.0001	Significant
A	3.538	32.519	< 0.0001	363.24	27.54	< 0.0001	16.25	26.78	0.0001	
B	3.05	28.04	< 0.0001	362.88	27.51	< 0.0001	16.18	26.67	0.0001	
C	33.647	309.285	< 0.0001	765.45	58.03	< 0.0001	19.57	32.25	< 0.0001	
D	4.322	39.726	< 0.0001	1448.80	109.83	< 0.0001	14.65	24.15	0.0002	
AB	0.446	4.096	0.0612	256.96	19.48	0.0005	25.23	41.59	< 0.0001	
AC	0.17	1.564	0.2302	211.70	16.05	0.0011	0.32	0.54	0.4756	
AD	3.715	34.151	< 0.0001	242.42	18.38	0.0006	10.39	17.13	0.0009	
BC	2.081	19.127	0.0005	46.10	3.49	0.0812	34.38	56.66	< 0.0001	
BD	0.019	0.174	0.6827	349.32	26.48	0.0001	1.51	2.49	0.1352	
CD	4.091	37.6	< 0.0001	471.32	35.73	< 0.0001	0.99	1.64	0.2201	
A^2	1.028	9.45	0.0077	6.77	0.51	0.4848	72.58	119.63	< 0.0001	
B^2	5.713	52.512	< 0.0001	324.06	24.57	0.0002	13.03	21.48	0.0003	
C^2	0.857	7.876	0.0133	536.03	40.63	< 0.0001	68.97	113.68	< 0.0001	
D^2	0.013	0.117	0.7371	524.91	39.79	< 0.0001	38.13	62.84	< 0.0001	
Residual	1.632			197.87			9.10			
Lack of Fit	0.901	0.616	0.7596	171.26	3.22	0.1045	5.49	0.76	0.6688	not-significant
Pure Error	0.731			26.61			3.62			
Cor Total	73.817			13372.41			1718.36			
C.V. %	1.2519			2.14			1.10			
PRESS	5.3416			738.82			25.91			
R^2	0.9779			0.9852			0.9947			
Adj R^2	0.9573			0.9714			0.9898			
Pred R^2	0.9276			0.9448			0.9849			
Adeq Precis	25.031			25.8237			37.2552			

Table 4.7 Actual and predicted responses based on face centred central composite design and coded factors for Aloe vera leaves extract.

Run	Factors				Responses					
	A	B	C	D	Y_{Ext} (Act)	Y_{Ext} (Pred)	Y_{TSC} (Act)	Y_{TSC} (Pred)	Y_{TPC} (Act)	Y_{TPC} (Pred)
1	0	0	0	0	35.30	36.56	67.82	65.91	71.33	71.35
2	1	-1	-1	-1	18.71	18.26	47.14	47.06	53.51	54.49
3	0	1	0	0	33.20	32.95	61.38	62.04	66.59	67.29
4	-1	-1	-1	1	20.16	20.35	48.42	47.79	51.99	52.37
5	-1	1	-1	-1	26.45	27.35	53.30	53.91	61.19	60.39
6	0	0	0	0	36.30	36.56	66.14	65.91	71.26	71.35
7	0	0	0	-1	36.52	36.48	63.92	64.08	68.99	69.86
8	1	1	1	1	16.78	16.28	46.00	46.09	47.81	49.02
9	0	0	0	0	36.40	36.56	66.02	65.91	72.79	71.35
10	-1	1	1	-1	29.07	28.50	55.93	55.19	56.99	58.91
11	1	0	0	0	27.10	26.66	59.10	60.87	64.13	64.94
12	-1	-1	1	-1	29.17	28.65	56.50	57.36	64.31	63.31
13	1	-1	1	-1	22.74	23.62	53.12	52.53	57.71	57.80
14	0	0	0	0	37.86	36.56	66.46	65.91	71.53	71.35
15	0	0	0	0	37.36	36.56	66.18	65.91	72.33	71.35
16	0	-1	0	0	34.56	35.25	63.94	64.65	69.99	69.95
17	1	1	1	-1	17.76	18.09	47.25	47.73	56.51	55.05
18	1	-1	-1	1	22.06	22.00	47.67	48.22	50.37	49.37
19	1	1	-1	1	16.50	17.54	46.18	45.17	48.54	48.47
20	-1	1	-1	1	22.78	21.27	46.97	47.37	48.98	49.81
21	-1	1	1	1	19.90	20.86	47.67	47.60	53.82	51.76
22	-1	0	0	0	30.16	31.04	64.78	64.39	68.53	68.37
23	-1	-1	1	1	25.20	24.98	51.28	51.51	59.32	60.50
24	0	0	-1	0	31.70	32.10	56.90	57.76	65.66	65.91
25	0	0	1	0	34.53	34.57	60.10	60.61	68.13	68.53
26	0	0	0	1	34.06	34.53	59.64	60.86	63.94	63.73
27	1	1	-1	-1	18.19	17.78	46.17	45.75	58.19	57.93
28	0	0	0	0	37.46	36.56	66.94	65.91	70.79	71.35
29	1	-1	1	1	26.18	25.79	53.39	52.63	56.39	56.11
30	-1	-1	-1	-1	22.57	22.45	52.87	52.59	58.91	58.61

A = Irradiation time; B = Microwave power; C = Ethanol concentration; D = F:S ratio; Act = Actual value; Pred = Predicted value

Table 4.8 Analysis of variance showing optimization of bioactive compounds of Aloe vera leaves extract.

Factors	Extraction yield			TSC			TPC			
Source	Sum of Squares	F Value	p-value Prob > F	Sum of Squares	F Value	p-value Prob > F	Sum of Squares	F Value	p-value Prob > F	
Model	1502.40	113.65	< 0.0001	1708.63	108.22	< 0.0001	1842.32	85.00	< 0.0001	Significant
A	86.45	91.56	< 0.0001	55.80	49.48	< 0.0001	52.98	34.22	< 0.0001	
B	23.85	25.25	0.0002	30.66	27.19	0.0001	31.68	20.46	0.0004	
C	27.42	29.03	< 0.0001	36.49	32.35	< 0.0001	31.05	20.05	0.0004	
D	17.21	18.22	0.0007	46.57	41.29	< 0.0001	169.27	109.34	< 0.0001	
AB	29.03	30.75	< 0.0001	6.92	6.14	0.0256	2.73	1.77	0.2038	
AC	0.71	0.76	0.3982	0.48	0.43	0.5239	1.94	1.25	0.2804	
AD	34.05	36.06	< 0.0001	35.44	31.42	< 0.0001	1.25	0.81	0.3823	
BC	25.48	26.99	0.0001	12.17	10.79	0.0050	38.19	24.67	0.0002	
BD	15.86	16.79	0.0010	3.03	2.69	0.1219	18.84	12.17	0.0033	
CD	2.45	2.60	0.1280	1.11	0.98	0.3371	11.79	7.61	0.0146	
A^2	154.02	163.11	< 0.0001	27.88	24.72	0.0002	57.08	36.87	< 0.0001	
B^2	15.68	16.61	0.0010	16.98	15.06	0.0015	19.27	12.45	0.0030	
C^2	26.98	28.57	< 0.0001	117.01	103.75	< 0.0001	44.13	28.50	< 0.0001	
D^2	2.88	3.04	0.1014	30.66	27.19	0.0001	53.73	34.70	< 0.0001	
Residual	14.16			16.92			23.22			
Lack of Fit	9.60	1.05	0.5095	14.57	3.10	0.1116	20.45	3.69	0.0810	not-significant
Pure Error	4.56			2.35			2.77			
Cor Total	1516.56			1725.55			1865.54			
C.V. %	3.4842			1.8861			2.0171			
PRESS	76.7930			74.7747			163.7192			
R^2	0.9907			0.9902			0.9876			
Adj R^2	0.9819			0.9810			0.9759			
Pred R^2	0.9494			0.9567			0.9122			
Adeq Precis	29.5216			27.6209			26.0054			

4.5 Analysis of Response Surfaces Plots

To evaluate the interactive effects of independent factors and their interactions on extraction yield, TSC and TPC of fenugreek seed and Aloe vera leaves, three dimensional (3D) plots were created (Figures 4.9-4.14). The effects of each factor such as irradiation time (min), microwave power (W), ethanol concentration (%) and F:S ratio (g/mL) on extraction yields, TSC and TPC of fenugreek seed and Aloe vera leaves are discussed in subsections (4.5.1-4.5.4).

4.5.1 Effect of Microwave Irradiation Time

Irradiation time is one of the important factors in a MAE. It is reported that increasing the irradiation time results to increase the recovery yields in MAE, however higher extraction time more than an appropriate point cause a poor extraction (Xu et al., 2012). To optimize the extraction recoveries with the time of irradiation the time were set at 2-4 min and other factors were at the centre points (600 W, 60% and 1:10 g/mL). The results of fenugreek seed extract indicated that time of irradiation has a significant effect on recoveries of phytochemicals. It can be seen in Figures 4.9-4.11 (a, b and c) that initially, the extraction yield, TSC and TPC of extract were 26.75%, 194.72 mg DE/g.d.w. and 75.96 mg GAE/g d.w., respectively when the time was 2 min. While, there was a fluctuation from the average of centre points at 3 to 4 min of irradiation time from 27.41%, 195.32 mg DE/g.d.w. and 81.55 mg GAE/g d.w. to 26.08%, 189.02 mg DE/g.d.w. and 74.40 mg GAE/g d.w, respectively. Figures 4.12-4.14 (a, b and c) show the influence of irradiation time on extraction yield, TSC and TPC of Aloe vera leaves extracts. As seen, at first the extraction yield, TSC and TPC were 30.16%, 64.78 mg OAE/g d.w. and 68.52 mg GAE/g d.w. While there was a fluctuation from 3 to 4 min of irradiation time from 35.29%, 67.82 mg OAE/g d.w. and 71.32 mg GAE/g d.w. to 27.09%, 59.1 mg OAE/g d.w. and 64.12 mg GAE/g d.w., respectively.

This might be due to bioactive compound degradation when exposed to longer time beyond 3 min of irradiation. Maeng et al. (2017) and Xu et al. (2012) also reported that 3 min of irradiation in a MAE as the optimum condition for extracting saponins and phenolic from *Coriolus* versicolor mushroom and *Pulsatilla turczaninovii*, respectively.

4.5.2 Effect of Microwave Power

The effects of microwave power on the recovery of extraction yield, TSC and TPC from fenugreek seed extract were investigated under different ranges (500-700 W). Table 4.5 and Figures 4.9-4.11 (a, d and e) reflected that at 500 W, the recoveries of extraction yield, TSC and TPC were 26.11%, 180 mg DE/g.d.w. and 79.06 mg GAE/g d.w., respectively. As the microwave power reached 600 W, significant increase of 27.41%, 195.32 mg DE/g.d.w. and 81.55 mg GAE/g d.w., were observed, respectively; these values were obtained from the average of 6 centre points in FCCCD design. However, the recoveries declined significantly to 25.01%, 178.12 mg DE/g.d.w. and 77.40 mg GAE/g d.w., when the level of microwave power was increased to 700 W.

In the same vein, the effects of MAE factors on recovery yield of extraction, TSC and TPC of Aloe vera leaves extract are also represented in Table 4.6 and Figure 4.12-4.14 (a, d and e). It can be seen that similar to fenugreek seed extraction condition the optimum recoveries were optioned at centre points. The maximum recoveries of 36.78%, 66.59 mg OAE/g d.w., and 71.67 mg GAE/g d.w. were obtained at 500 W. The reduction of these bioactive compounds and extraction yield beyond 500 W and 600 W was also reported by Xu et al.(2012), Karabegović et al. (2013), Zhong et al. (2016) and Shao et al. (2012) from MAE of *Pulsatilla turczaninovii, Prunus laurocerasus* leaves, *Radix astragali* and *Perilla Frutescens* leaves, respectively. They claimed that higher microwave power results in the thermal degradation of plant matrix and thus, declined the yields.

4.5.3 Effect of Ethanol Concentration

Ethanol is a common extraction solvent for obtaining bioactive compounds of plants due to its low environmental impact and toxicity. The mixture of ethanol and water can be an excellent formulation in a MAE. The dielectric property of water is higher than ethanol and ethanol is a good solvent for obtaining bioactive compounds. Hence, better energy absorption and extraction efficiency will occur during the process. Figures 4.9-4.11 (b, d and f) illustrate the effect of 40 to 80% ethanol on the recoveries of extraction yield, TSC and TPC of fenugreek seed. As seen, the recoveries of these bioactive

compounds at 40% ethanol were 29.01%, 167.72 mg DE/g.d.w. and 74.30 mg GAE/g d.w., respectively. Further increase in ethanol concentration to 60% enhanced the yields of TSC and TPC to 195.32 mg DE/g d.w., 81.55 mg GAE/g d.w. except the extraction yield which was reduced by increasing the ethanol concentration to 27.41%. This is due to the properties of fenugreek seed, which tend to provide more gums than bioactive compounds of saponin and phenols. However, when ethanol concentration reached 80% in the extraction medium, the yields declined to 184.02 mg DE/g d.w. and 76.33 mg GAE/g d.w., respectively. This indicated that only extraction yield of fenugreek seed cannot be optimized at centre points or optimum factor levels. Since, this study focus more in optimization of TSC and TPC of the extracts. Therefore, the optimized levels were selected based on the maximum recoveries of TSC and TPC in fenugreek seed extract.

Likewise, Figures 4.12-4.14 (b, d and f) indicate the 3D plots of Aloe vera leaves extract affected by ethanol concentration. It is seen that, increasing the ethanol concentration from 20% to 40% increased the recovery yields of extraction, TSC and TPC from 31.69%, 56.9 mg OAE/g d.w. and 65.66 mg GAE/g d.w. to 36.78%, 66.59 mg OAE/g d.w. and 71.67 mg GAE/g d.w., respectively. However, further increment of ethanol concentration in the solvent to 60% reduced the yields to 34.53%, 60.1 mg OAE/g d.w. and 68.13 mg GAE/g d.w., respectively. Water is highly polar, and the polarity of ethanol is lower than water. Hence, combination of water and ethanol may provide better heating and efficiency in plant extraction.

Pure ethanol is not capable to provide efficient heating in a microwave extraction to obtain more bioactive compounds since most of biologically active compounds such as flavonoids, phenolics and saponins have high polarity. Therefore, a polar solvent can increase the rate of extraction. However, more than 60% of water in ethanol can decline the recovery of TSC and TPC in the extract (Maeng et al., 2017). This may be due to the high dielectric properties of water molecules that absorbed more energy during the microwave heating and hence resulted to the extraction of more fenugreek viscose gums rather than biologically active compounds of phenolics and saponins. In this regard, as stated by Wani et al. (2016) that 60% ethanol was effective to obtain a steroidal saponin

called diosgenin from microwave extraction of fenugreek seed. They also suggested that ethanol concentration less than 60% results in the extraction of fenugreek seed gum which can create problem during filtration. The results from this study are similar to the study carried out by Amid and Mirhosseini (2012) where biopolymer was obtained from *Durio zibethinus* seed. It was reported that the protein fractions are more soluble in alcohols while polysaccharide fractions (gums) have better solubility in water rather than alcohols. Therefore, the yields of TSC and TPC increased by increasing the concentration of ethanol to 60% in fenugreek seed extract. As the concentration exceeded 80% of ethanol concentration, significant declination was observed in both TSC and TPC of fenugreek seed due to protein denaturation in the extraction medium (Yang et al., 2010). This result was in good correlation with the findings of Maeng et al. (2017), they studied the optimization of MAE of bioactive compounds from *Coriolus versicolor* mushroom and reported that ethanol concentration is the most effective independent variable in the extraction of bioactive compounds.

4.5.4 Effect of Feed-to-Solvent Ratio

The ratio of feed to solvent is also considered important factor in a MAE. A proper ratio will provide a desirable wetting of the plant matrix and better microwave adsorption. However, ratio of F:S depends on the heating property of plant matrix. Different plants require different amount of solvent formulation (Cheok et al., 2014). In this study, the recoveries of extraction yield, TSC and TPC from fenugreek seed extract increased from 27.45% 187.38 mg DE/g d.w. and 77.92 mg GAE/g d.w. to 27.41%, 195.32 mg DE/g d.w. and 81.55 mg GAE/g d.w., respectively, as the feed-to-solvent ratio increased from 1:8 to 1:10 g/mL, but there was no significant difference between these two ratios in achieving the extraction yield. However, further increment of the ratio to 1:12 g/mL minimized the recoveries of TSC and TPC to 26.78%, 164.66 mg DE/g.d.w. and 75.36 mg GAE/g d.w., respectively (Figures 4.9-4.11 c, e and f). An increase in the ratio from 1:8 to 1:10 g/mL provided a better wettability and microwave adsorption of plant matrix during the microwave heating. On the other hand, further enhancement of the ratio to 1:12 g/mL means more ethanol and water content in the extraction medium, which results to more adsorption of microwave energy due to the dielectric property of water and hence,

reduced the yields. Nevertheless, ratio of feed-to-solvent depends on the heating property of plant matrix. Different plants require different amount of solvent formulation (Cheok et al., 2014).

In the same vein, Figures 4.12-4.14 (c, e and f) show the effect of feed-to-solvent ratio on extraction yield, TSC and TPC of Aloe vera leaves extracted via MAE. It is seen that the recoveries of extraction yield, TSC and TPC increased by increasing the ratio of F:S from 36.51%, 63.91 mg OAE/g d.w. and 68.99 mg GAE/g d.w. to almost 36.8%, 66.59 mg OAE/g d.w. and 71.67 mg GAE/g d.w., respectively when the ratio increased from 1:18 to 1:20 g/mL. However, further enhancement in ratio of F:S to 1:22, declined the yields to 34.05%, 59.64 mg OAE/g d.w. and 63.93 mg GAE/g d.w., respectively. Accordingly, Xu et al. (2012) found 1:20 g/mL of volume ratio as the optimum condition for extraction of triterpenoid saponin from *Pulsatilla turczaninovii* using MAE. In addition, Xiao et al. (2012), also reported the ratio of 1:20 g/mL as the optimized extraction condition for extraction of aloin from Aloe vera via MAE. As mentioned, ratio of feed-to-solvent depends on the heating property of plant matrix. Different plants require different amount of solvent formulation (Cheok et al., 2014). The obtained findings were in correlation with previous results reported by Alara et al. (2018) and Xu et al. (2012) on MAE of phytochemicals from *Vernonia amygdalina* leaf at an F:S ratio of 1:10 g/mL as an optimized condition and determination of triterpenoid saponins in *Pulsatilla turczaninovii* at F:S ratio of 1:20 g/mL.

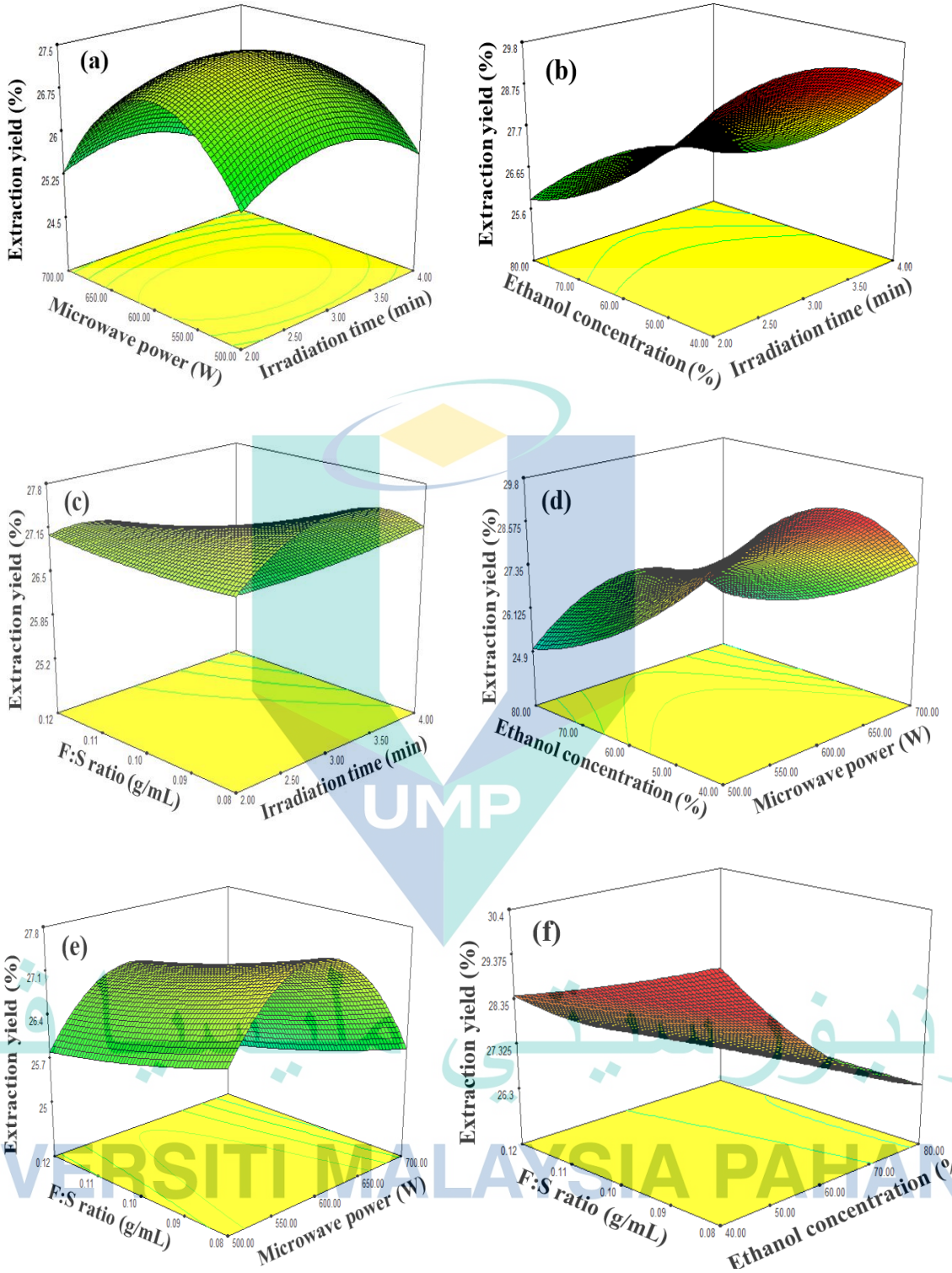


Figure 4.9 Three dimensional plots of fenugreek seed extract showing the effect of MAE factors on extraction yield.

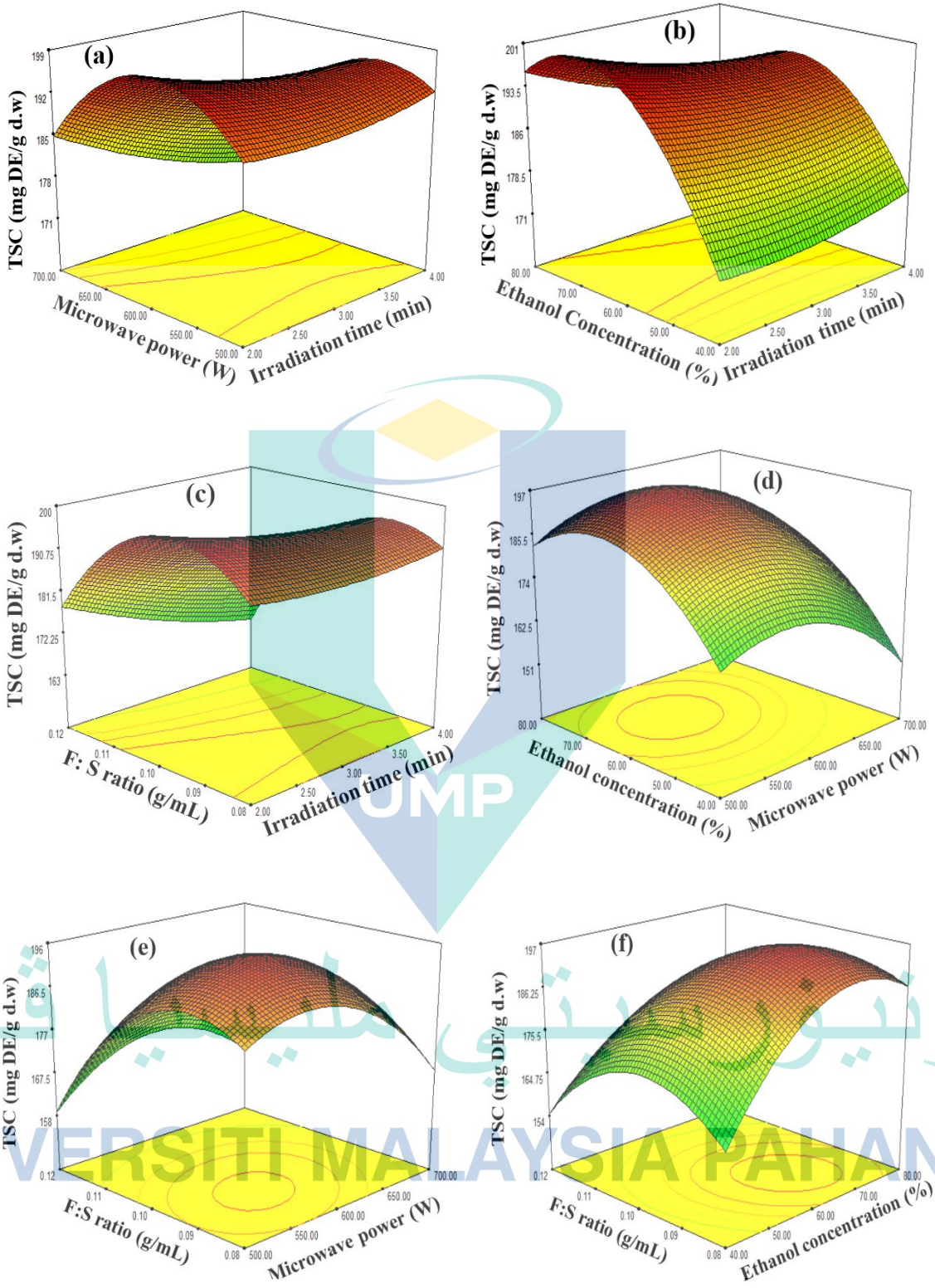


Figure 4.10 Three dimensional plots of fenugreek seed extract showing the effect of MAE factors on TSC of the extract.

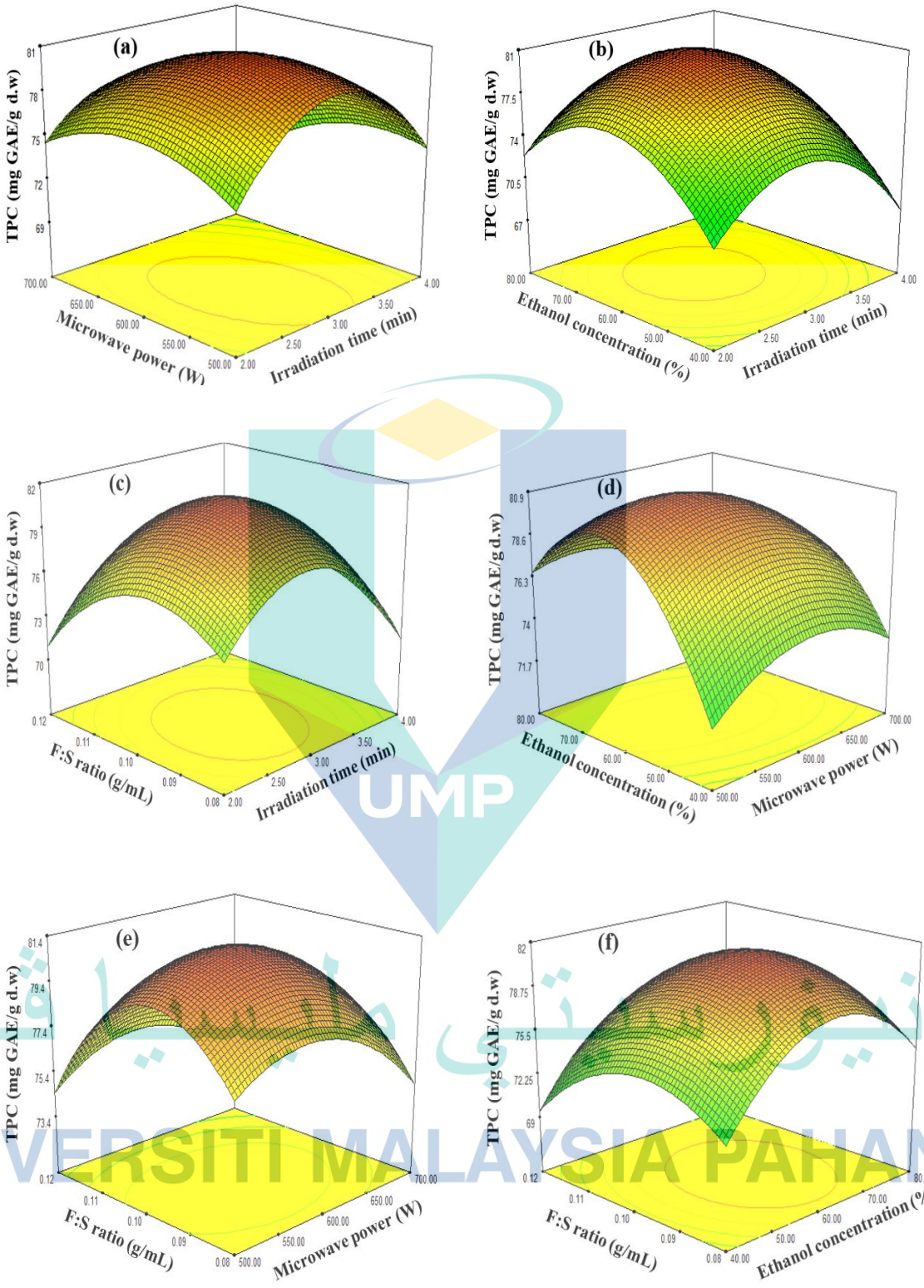


Figure 4.11 Three dimensional plots of fenugreek seed extract showing the effect of MAE factors on TPC of the extract.

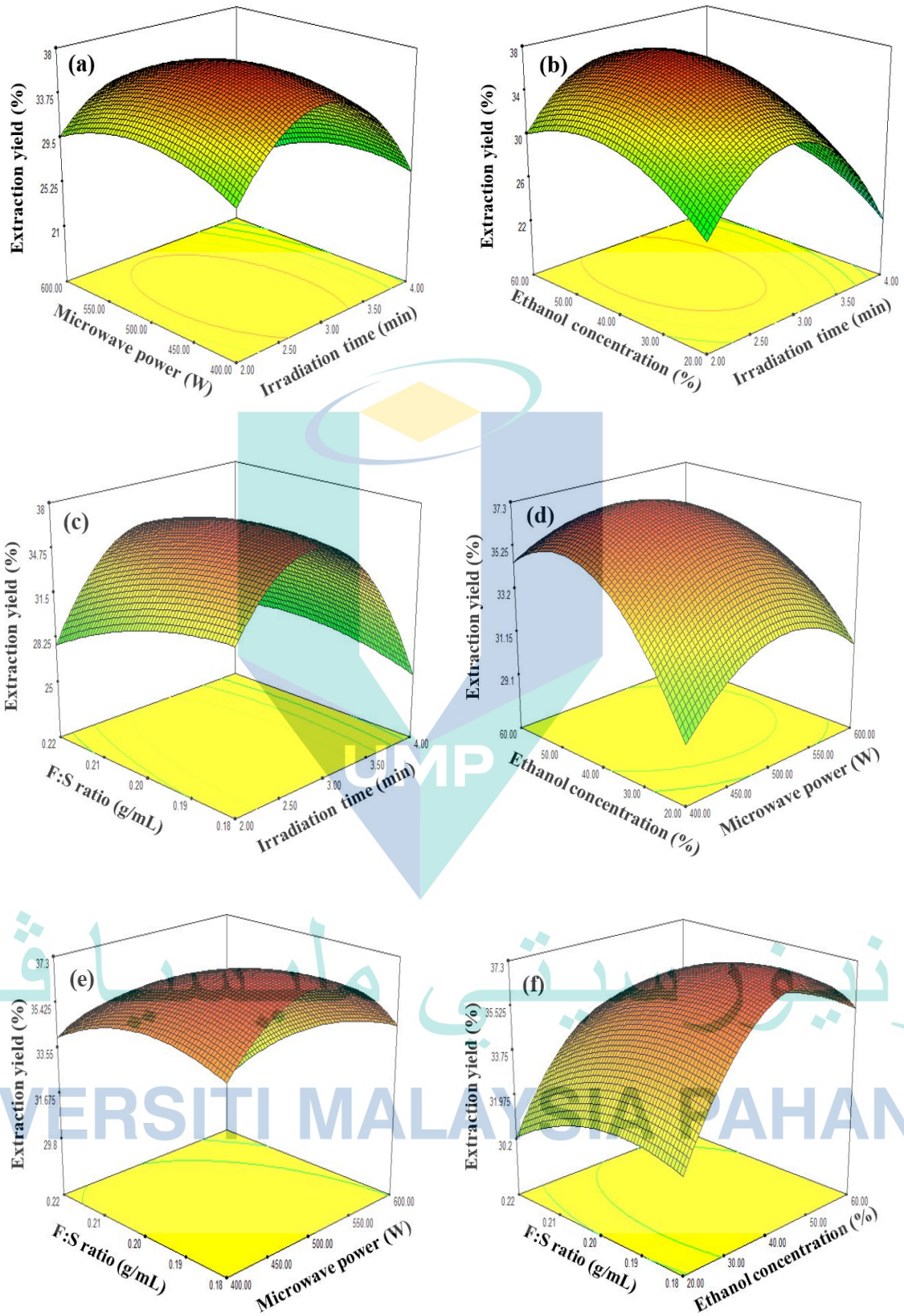


Figure 4.12 Three dimensional plots of Aloe vera leaves extract showing the effect of MAE factors on extraction yield.

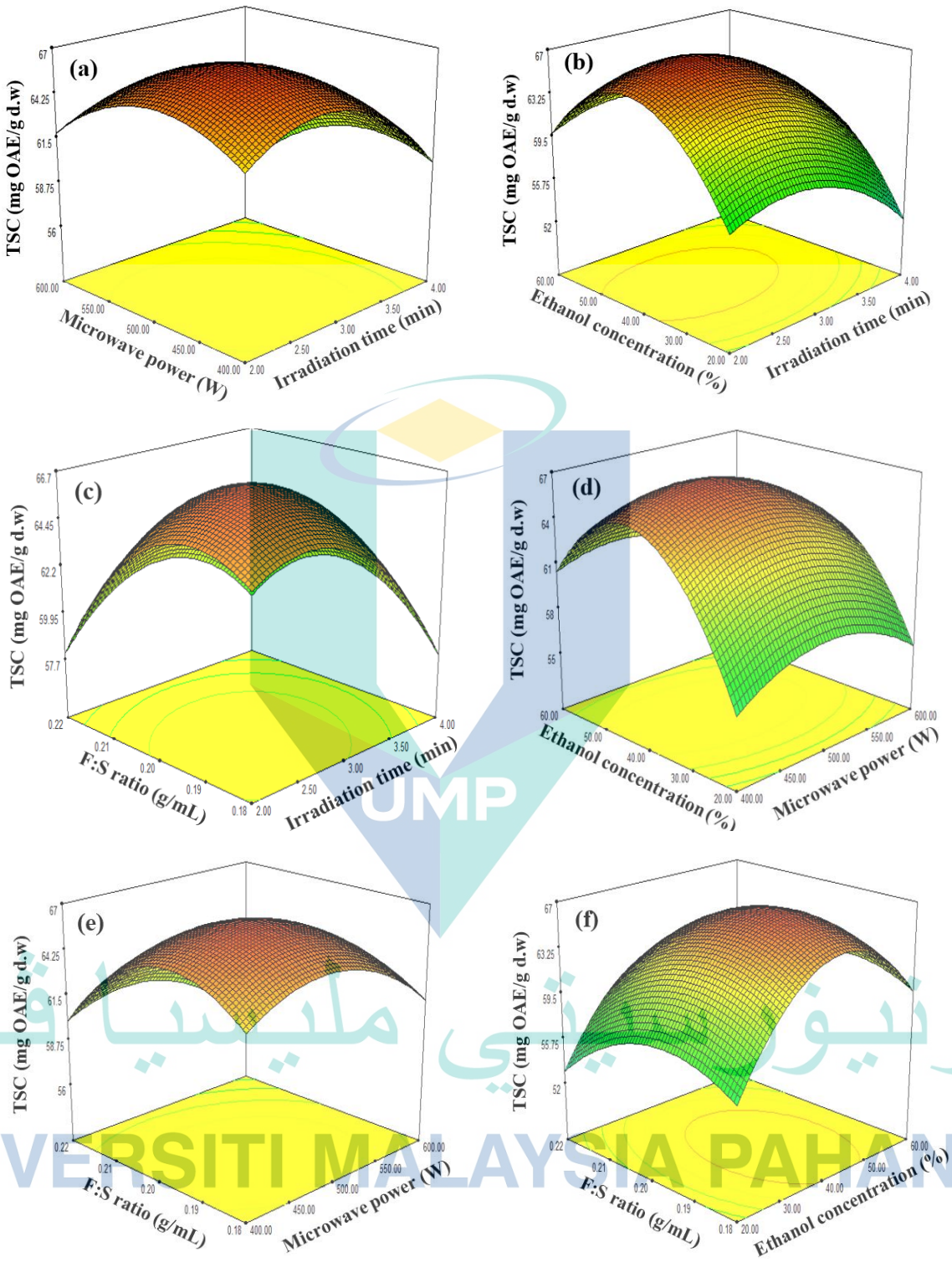


Figure 4.13 Three dimensional plots of Aloe vera leaves extract showing the effect of MAE factors on TSC of the extract.

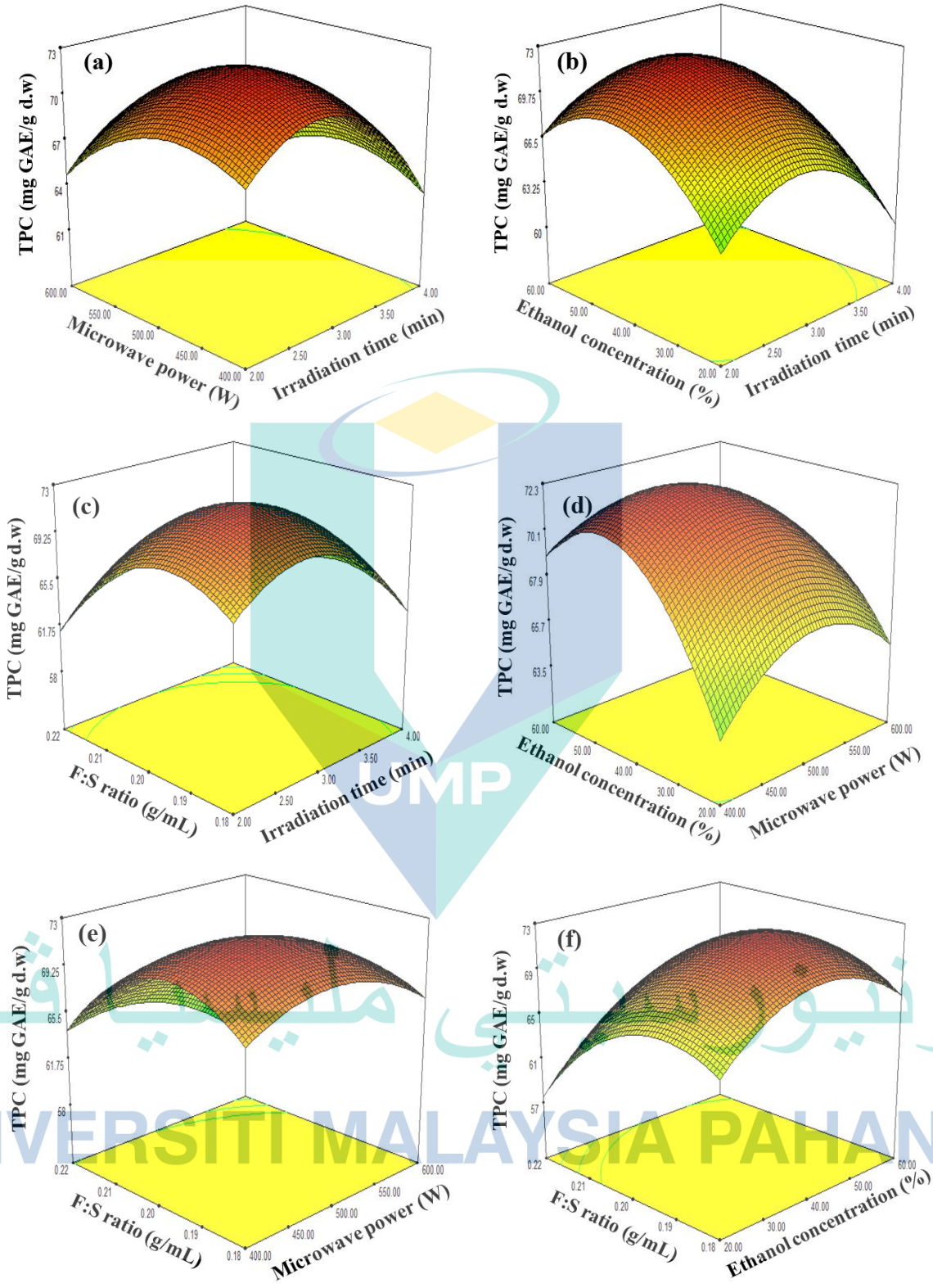


Figure 4.14 Three dimensional plots of Aloe vera leaves extract showing the effect of MAE factors on TPC of the extract.

4.6 Dielectric Properties and Kinetic Modelling

4.6.1 Dielectric Properties

The dielectric properties are also responsible for converting the microwave energy to the heat (Eskilsson and Bjorklund, 2000; Erdogan, 2011). Materials that absorb the microwave radiation are called dielectrics. Therefore, microwave heating is also referred to as dielectric heating. In microwave range, the interaction of dielectric materials with electromagnetic radiation results in energy absorbance in the material. The dielectric constant and dielectric loss of the extraction solvents such as water and ethanol were studied at room temperature (25°C) and final hearting temperature (70°C). Table 4.9 shows the dielectric constant, dielectric loss of water, ethanol and the water-ethanol mixture, loss tan δ, wavelength (WL) and penetration depth (PD) for fenugreek seed and Aloe vera leaves extracts, respectively. It is seen in Table 4.9 that the dielectric constant and loss of water much higher compared to ethanol at 25°C (76.80; 11.64) and (14.84; 10.31) respectively. However, by increasing the temperature to 70°C, the dielectric properties of both solvents reduced to (61.69; 4.03) and (14.36; 10.21), respectively. As seen the dielectric constant and dielectric loss of fenugreek seed and Aloe vera during microwave heating were same, but the wavelength and penetration depth were achieved different. This is due to the different concentration of ethanol and water. Since, for fenugreek seed extraction 60% ethanol was the best concentration, while for Aloe vera 40% ethanol was the best as discussed in previous sections.

Table 4.9 Dielectric properties of extraction solvents.

Fenugreek seed extraction solvents										
T	Water		Ethanol		Mixture		Water	Ethanol	WL	PD
°C	έ	ε″	έ	ε″	Έ	ε″	tan δ	tan δ	(cm)	(cm)
25	76.80	11.64	14.84	10.31	36.15	10.77	0.15	0.69	2.02	1.08
70	61.69	4.03	14.36	10.21	30.64	8.08	0.06	0.71	2.20	1.33
Aloe vera leaves extraction solvents										
T	Water		Ethanol		Mixture		Water	Ethanol	WL	PD
°C	έ	ε″	έ	ε″	Έ	ε″	tan δ	tan δ	(cm)	(cm)
25	76.80	11.64	14.84	10.31	46.13	14.84	0.15	0.69	1.79	1.20
70	61.69	4.03	14.36	10.21	38.26	14.36	0.06	0.71	1.97	1.69

4.6.2 Kinetic Modelling

The kinetic studies in an extraction would allow a better understanding of the extraction mechanism. Table 4.10 and 4.11 show the first and second-order kinetic reactions for fenugreek seed and Aloe vera leaves, respectively. The kinetic studies were carried out for yield of extraction, TSC and TPC of both targeted plants. It is seen in both tables that for the first-order kinetic model, plots of log (Cs - Ct) versus t was used for the study (see Appendix C). Based on the derived equations, linearization of the individual plot made it easy to determine the values of coefficient of determination (R^2), k_1 and C_s. These were done by minimizing the sum of square errors of the predicted data from the first-order kinetic model and experimental data. On an overall, it can be observed in Table 4.10 that the coefficient of determination obtained for the extraction of fenugreek seed and Aloe veral leaves using MAE was relatively low, the obtained R^2 for yeild of extraction, TSC and TPC were 0.9457, 0.9354 and 0.9321, and for Aloe vera recorded at 0.9578, 0.9726 and 0.9617, respectivly. Hence, it can be claimed that a first-order kinetic model does not well describe the experimental results obtained from MAE of plants. Compared with the first order model, the coefficient of determinations (R^2) were higher in second-order kinetic models as seen in Table 4.11 the R^2s were 0.9561, 0.9512 and 0.9489 for fenugreek seed and 0.9608, 0.9738 and 0.9656 for Aloe vera, respectivly , which can be used to determine the extraction process. In addition, in both kinetic models, Aloe vera indicated higher coefficient of determination compared to fenugreek seed. This may be due to the suitability of the model for Aloe vera extraction.

Table 4.10 First order kinetic reaction.

Plants	Slope	Intercept	$K_1(min^{-1})$	R^2
Fenugreek Seed				
Yield	-0.0298	3.3158	0.0264	0.9457
TSC	-0.0334	5.0989	0.0296	0.9354
TPC	-0.0407	4.2273	0.0356	0.9321
Aloe vera leaves				
Yield	-0.025	3.0765	0.0223	0.9578
TSC	-0.0149	3.893	0.0136	0.9726
TPC	-0.0201	3.9728	0.0182	0.9617

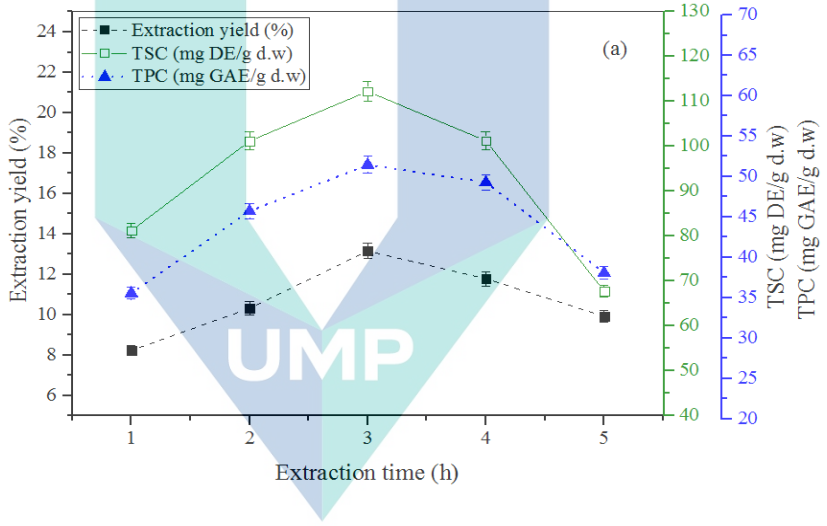
Table 4.11 Second order kinetic reaction.

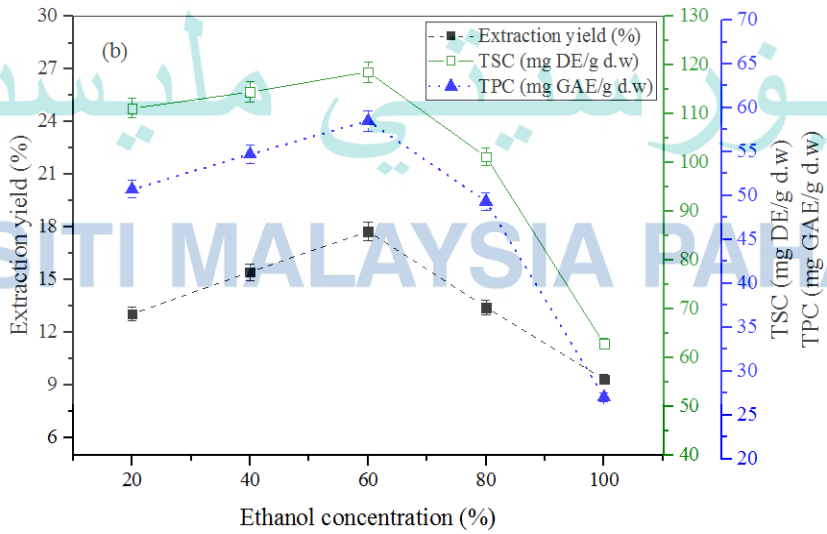
Plants	Slope	Intercept	K_2(mL/mg.min)	R^2
Fenugreek Seed				
Yield	0.0013	0.0356	0.013	0.9561
TSC	0.0003	0.006	0.001	0.9512
TPC	0.0008	0.0141	0.003	0.9489
Aloe vera leaves				
Yield	0.0014	0.0455	0.02	0.9608
TSC	0.0003	0.0203	0.014	0.9738
TPC	0.0004	0.0187	0.01	0.9656

4.7 Effect of Soxhlet Extraction Factors

The Soxhlet extraction factors including extraction time, ethanol concentration and feed-to-solvent ratio were evaluated on the recoveries of extraction yield, TSC and TPC of fenugreek seed and Aloe vera leaves as shown in Figures 4.15 and 4.16. The evaluation of factors effect in Soxhlet extraction has been carried out using one-factor-at-a-time (OFAT) experiment. The extractions were carried out at extraction time of 1, 2, 3, 4 and 5 h, ethanol concentrations 20, 40, 60, 80 and 100%, and feed-to-solvent ratio of 1:14, 1:18, 1:20, 1:22, and 1:24 g/mL. As seen in Figures, the maximum recoveries of extraction yield, TSC and TPC for fenugreek seed and Aloe vera leaves were carried out at 3 h of extraction time, 60 % ethanol concentration and 1:20 g/mL F:S ratio which were 19.35±0.75%, 125.04±1.55 mg DE/g d.w. and 60.13±2.04 mg GAE/g d.w. for fenugreek seed and 22.45±0.76%, 44.78±1.01 mg OAE/g d.w. and 49.99±0.56 mg GAE/g d.w. for Aloe vera leaves, respectively. This finding is in a good agreement to a study carried out by Chan et al. (2013) at similar extraction condition for the extraction of saponin and phenolic compounds extracted from rice bran. In current study, higher extraction time, ethanol concentration and F:S ratio more than 3 h for the extraction of fenugreek seed and Aloe vera resulted to the reduction of yield, TSC and TPC (Figure 4.15a and 4.16a). This might be due to longer extraction time and solvent boiling inside the solvent flask which leads to the degradation of plant matrix. The drawback of this method is the longer extraction time which heats up the sample continuously for longer time, hence results to the lower yield. However, extraction time in Soxhlet is shorter than maceration (Cheok

et al., 2014). As can be seen in Figures 4.15 and 4.16 (b and c), increasing the ethanol concentration and F:S ratio more than 60% and 1:20 g/mL resulted to a significant decline in yields of extraction, TSC and TPC. Studies revealed that combination of ethanol and water results to higher extraction yields and efficiency. Water and ethanol alone are not capable to extract phytochemicals from plant matrix. Hence, a proper formulation for solvent combination is required (Cheok et al., 2014; Wang et al., 2011). Soxhlet extraction has been used for long time, however, currently it is considered as conventional extraction methods, since the non-conventional extraction techniques specially MAE is widely used technique due to shorter extraction time and high extraction yield and efficiency (Karami et al., 2015).





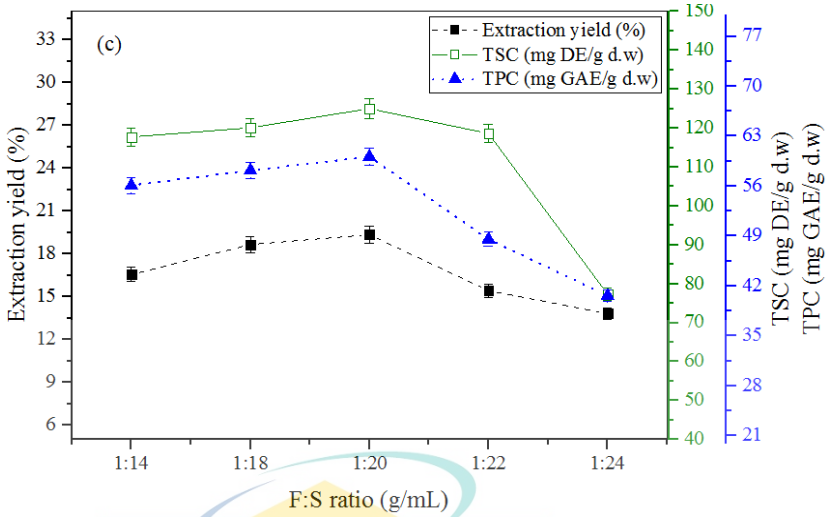
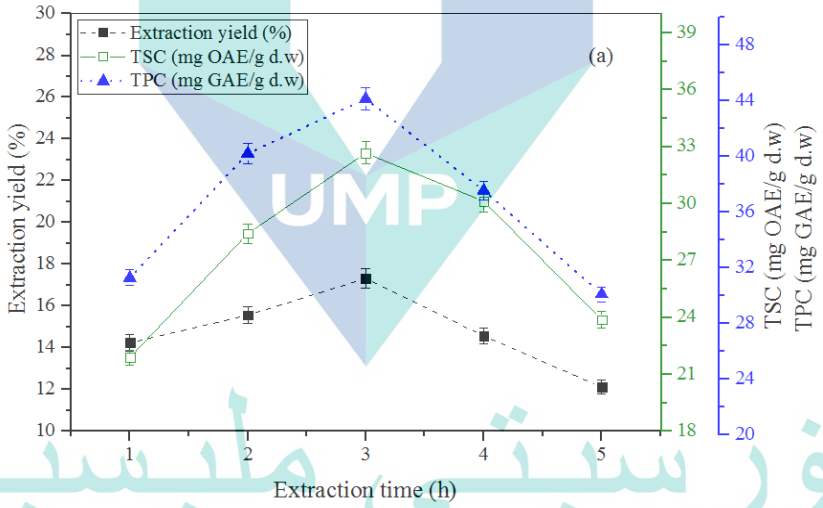


Figure 4.15 Effect of extraction time (a); ethanol concentration (b) and feed-to-solvent ratio (c) on extraction yield, TSC and TPC of Fenugreek seed extracted via Soxhlet using OFAT experiment.



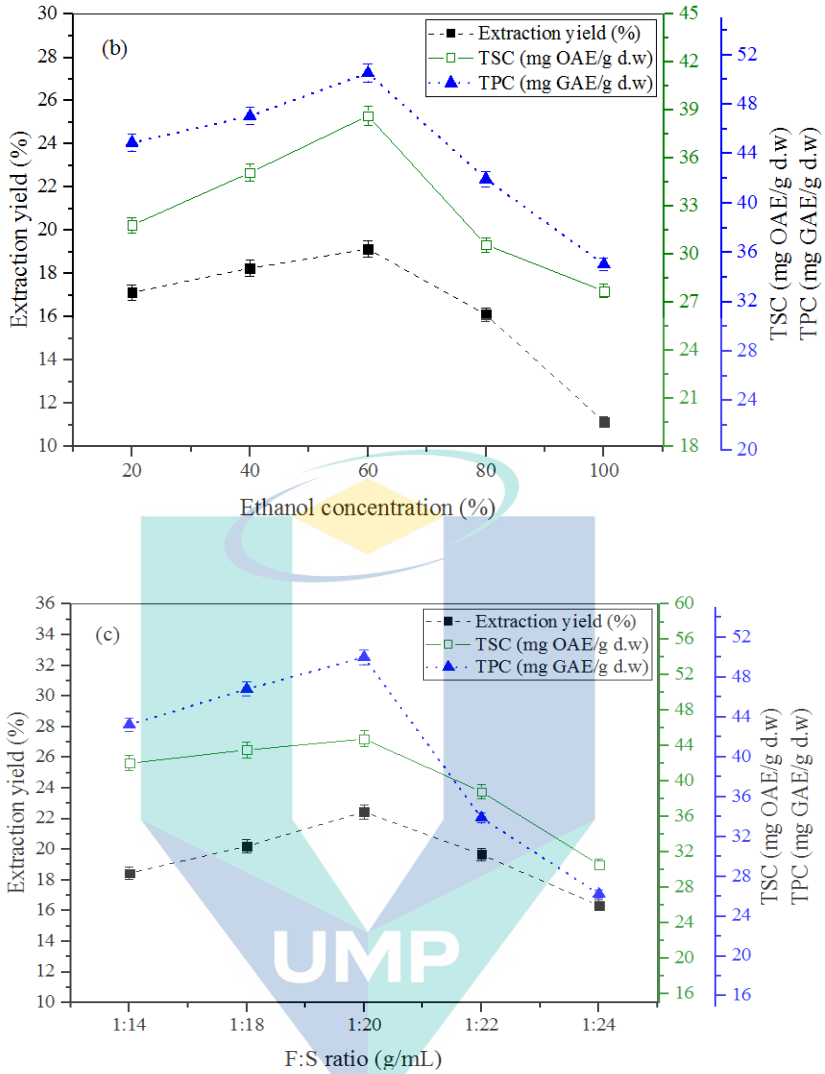


Figure 4. 16 Effect of extraction time (a); ethanol concentration (b) and feed-to-solvent ratio (c) on extraction yield, TSC and TPC of Aloe vera extracted via Soxhlet using OFAT experiment.

4.8 Characterization and Analysis of the Samples

Characterization of the fenugreek seed and Aloe vera leaves samples were caried out using various characterization, analysis and testing methods such as DPPH and ABTS antioxidant capacity analysis, LC-QTOF-MS alanysis, SEM, FTIR, wettability, surface tension, foaming and emulsification testing. Each of them are described in the following subsections.

4.8.1 Analysis of Antioxidant Capacity

Plants with antioxidant properties have achieved a great attention in health and food products. Additionally, some of the phytochemicals such as phenols and flavonoids are known as natural antioxidant which can be hydrogen donor for human body. The hydroxyl (OH) group in phenolic compounds are responsible to act as antioxidant and inhibit the oxidation reaction in human body (Altemimi et al., 2017; Paj et al., 2019). Plants phytochemicals demonstrated potential health benefits to fight many disease such as tumor, cancer, diabetes, heart problems and many types of infections (Riasat et al., 2018). The antioxidant activities of fenugreek seed and Aloe vera leaves were evaluated using DPPH and ABTS free radical scavenging assays. The half maximal inhibitory concentration (IC50) of the samples was also determined. Table 4.12 shows the free radical scavenging activities of fenugreek seed and Aloe vera leaves extracts via MAE and Soxhlet extraction methods against ascorbic acid (see Appendix B). As seen, the TSC and TPC, DPPH and ABTS values of the extracts obtained by MAE is higher than those obtained via Soxhlet extraction method. It is also seen that, the IC50 values of DPPH in fenugreek seed extract (195.27 ± 0.56, MAE; 224.47 ± 0.77 Soxhlet) and Aloe vera leaves extract (275.9 ± 1.45, MAE; 305.79 ± 0.66, Soxhlet) are higher when compared to IC50 values of ABTS assay (157.92 ± 1.11, MAE; 199.67 ± 0.96, Soxhlet) and (215.58 ± 0.57, MAE; 263.29 ± 1.21, Soxhlet), respectively. The proton free radicals of ABTS and DPPH compounds can be reduced when exposed to proton radical scavengers (K. J. Lee et al., 2015). Previous findings suggested that the capacity of antioxidant activities in most of the plants was better with ABTS rather than with DPPH, and the reason is the sensitivity of ABTS assay which increases the rate of kinetic reaction (Chan et al., 2014; K. J. Lee et al., 2015). In addition, fenugreek seed extract indicated higher DPPH and ABTS free radical scavenging activity compared to Aloe extract, while ascorbic acid showed the lowest. This might be due to higher TPC content in fenugreek seed extract. As reported, phenolic and flavonoids also act as antioxidant or hydrogen donor, which is a chemical reaction inside human body, specifically, phenolic compounds due to the presence of hydroxyl (-OH) group. The free -OH group as an antioxidant is responsible to control the oxidative damage by inhibiting the oxidation reaction caused by reactive oxygen species (ROS) in foods (Altemimi et al., 2017; Paj et al., 2019).

Table 4.12 TSC, TPC, DPPH and ABTS antioxidant activities of fenugreek seed and Aloe vera leaves extract.

Extraction technique	TSC (mg DE/g d.w)/ (mg OAE/g d.w)	TPC (mg GAE/g d.w)	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)
Fenugreek seed extract				
MAE	195.89 ± 1.07	81.85 ± 0.61	195.27 ± 0.56	157.92 ± 1.11
Soxhlet	125.04±1.55	60.13±2.04	224.47 ± 0.77	199.67 ± 0.96
Aloe vera leaves extract				
MAE	65.89 ± 0.77	73. 05 ± 1.05	275.9 ± 1.45	215.58 ± 0.57
Soxhlet	44.78±1.01	49.99±0.56	305.79 ± 0.66	263.29 ± 1.21
Ascorbic Acid			129.89 ± 1.33	70.57 ± 0.78

4.8.2 LC-QTOF-MS Analysis of Extracted Compounds

Saponin and phenolic bioactive compounds are among the secondary metabolites that are known to be useful for the treatment of many diseases and are commonly found in many plants. Saponins are found in either steroid or triterpenoid glycosides. In terms of health benefit, both saponins and phenolic compounds existed in plants have demonstrated potential medicinal values for the treatment of numerous diseases such as cancers, heart-related illnesses, tumor, infections, and diabetes (Chan et al., 2014).

The identification of saponin and phenolic bioactive compounds of fenugreek seed and Aloe vera leaves extracts were performed using LC-QTOF-MS analysis. A total of 58 and 27 saponin and phenolic compounds were identified in optimized fenugreek seed extracted via MAE in both positive and negative ion modes, respectively (Table 4.10). The obtained biologically active compounds are responsible for many activities in human body such as antioxidant, anti-inflammatory, antidiabetic, and anticancer properties (Lidia et al., 2017). As seen in Table 4.13, that the saponin components are belonging to steroid and terpenoid saponins. It is also seen that different types of phenolic compounds such as simple phenols, phenolic aldehydes, phenolic acids and polyphenols

were presented in the extract of fenugreek seed. Phenolic compounds such as protocatechuic, cistanoside C, campneoside, forsythoside E, and saponins such as terrestrosin, timosaponin, markogenin, protodiosgenin, yamogenin, and epi-Smilagenin have been reported to possess anti-cancer, anti-diabetic, anti-inflammatory, antibiotic, antioxidant, hormone balancing, and antidepressant properties (B. Lee et al., 2009; Lidia et al., 2017; Wei et al., 2014). Cistanoside C is used to repair DNA damage (Sperandio et al., 2002). Figures. 4.17 (a and b) show the identified compounds of fenugreek seed through LC-QTOF-MS analysis based on retention time and observed intensity of positive and negative ion modes, respectively. It is seen in Figure 4.17 and Table 4.13 that most of the saponins are observed in positive ion modes, while phenolic compounds tended to be visible in negative ion modes. This may be due to the high affinity of alkali cations in saponin compounds therefore, most of the saponins detected in the positive ion mode and charged hydrogen, sodium and even potassium adducts of the molecules [+H, +Na and +K]. Relatively, most of the phenolic compounds detected in the negative ion mode and charged hydrogen adducts of the molecules [-H] (Bahrami et al., 2014).

Likewise, the LC-QTOF-MS results of Aloe vera leaves extract in optimized condition of MAE in both positive and negative ion modes are shown in Figure 4.18 (a and b) and Table 4.14, respectively. A total of 29 saponins and 32 phenolic compounds have been identified in Aloe extract. The extracted saponin compounds have shown different activities and health benefits. Saponin compounds such as Esculentoside J, Timosaponin B1, Gentiopicroside, Prosapogenin A, soyasaponin βg and Esculentoside A and phenolic compounds such as Quercetin-3-O-neohesperidoside, Salidroside, Apocynin B are reported to be useful for the treatment of diseases related to inflammation, cancer, tumor, kidney, infections and central nervous systems (Chen et al., 2017; Jang et al., 2018; Kumarasamy et al., 2003; Wang et al., 2013; Yi & Dai, 1991; Zhang et al., 2014; Zhong et al., 2018). In addition, soyasaponin βg has also demonstrated a potent efficacy as a scavenger of reactive oxygen species (ROS) (Sagratini et al., 2009). It can be seen in Figure 4.18 and Table 4.14 that most of the saponins are observed in positive ion modes, while phenolic compounds tended to be visible in negative ion modes. This may be due to the high affinity of alkali cations in saponin compounds therefore, most of the saponins detected in the positive ion mode and charged hydrogen, sodium and even

potassium adducts of the molecules [+H, +Na and +K]. Relatively, most of the phenolic compounds detected in the negative ion mode and charged hydrogen adducts of the molecules [-H] (Bahrami et al., 2014).

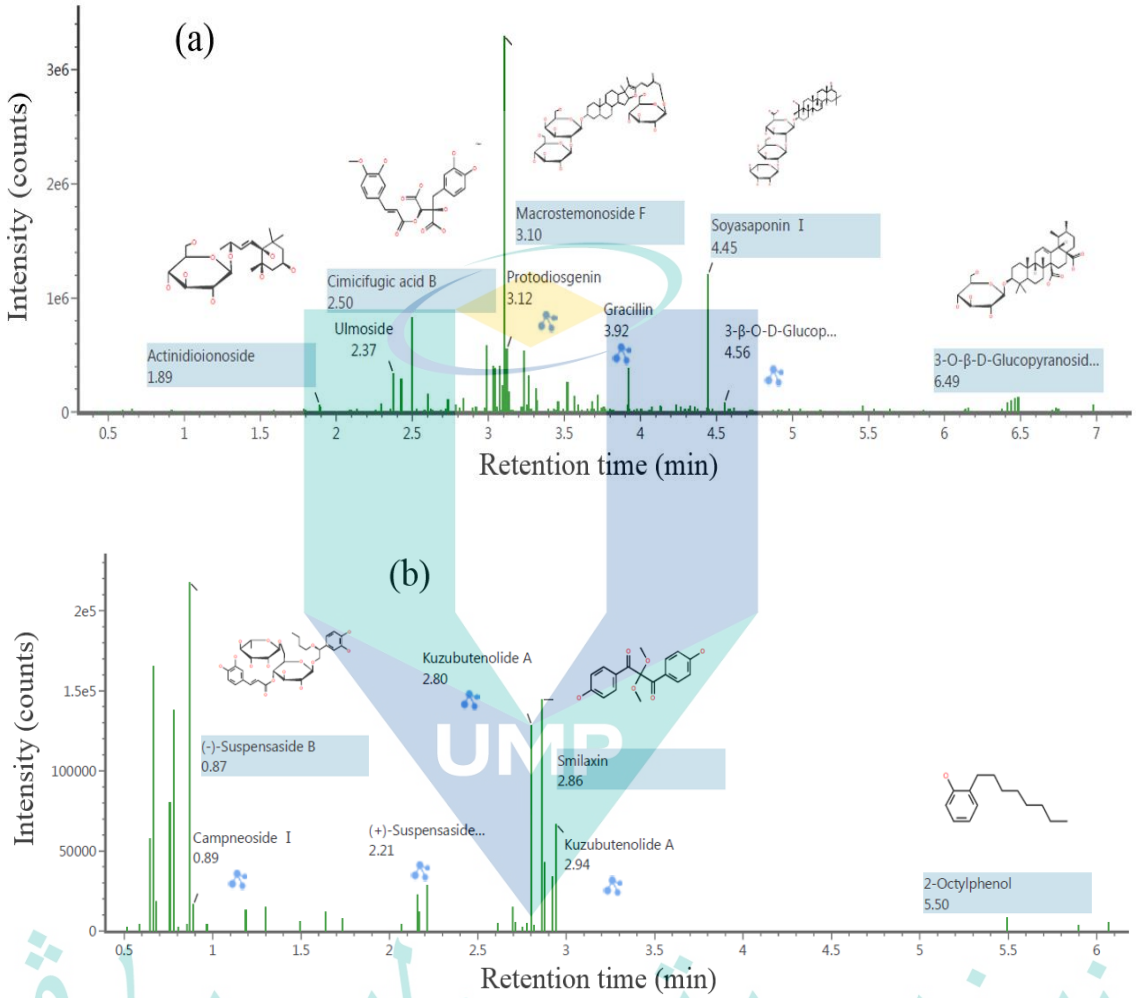


Figure 4.17 LC-QTOF-MS chromatogram of tentative assigned compounds of fenugreek seed extract in positive ion mode (a) and negative ion mode (b).

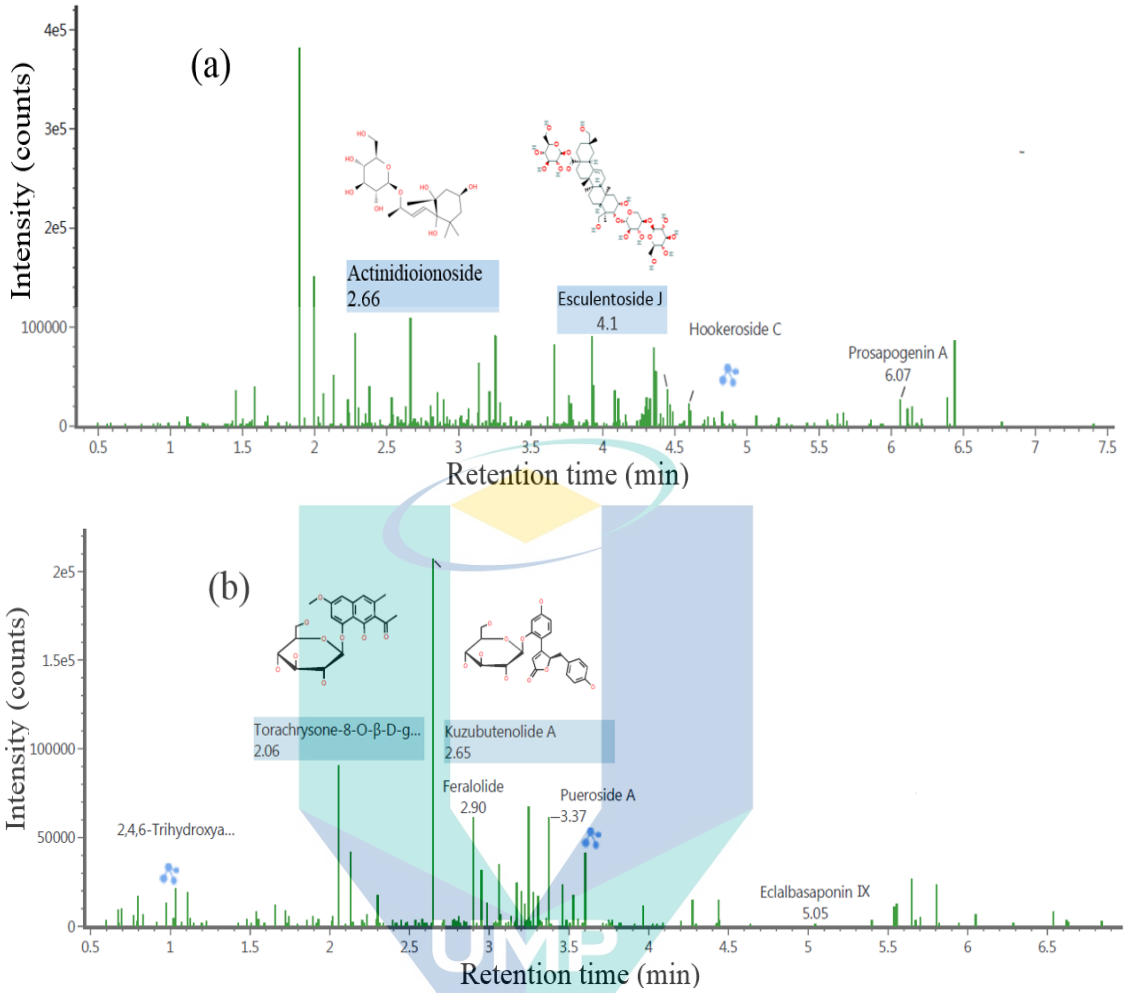


Figure 4. 18 LC-QTOF-MS chromatogram of tentative assigned compounds of Aloe vera leaves extract in positive ion mode (a) and negative ion mode (b).

Table 4.13 Saponin and phenolic compounds of fenugreek seed extract identified by LC-QTOF-MS in positive and negative ion modes.

	Component name	Formula	Observed neutral mass (Da)	Observed m/z	Observed RT (min)	Response	Adducts	Total Fragment
Saponin compounds								
1	Gentiopicroside	$C_{16}H_{20}O_9$	356.1127	395.0759	2.43	22086	+K	10
2	Periplocoside L	$C_{28}H_{46}O_7$	494.3232	517.3125	2.93	1716	+Na	12
3	Timosaponin D	$C_{45}H_{74}O_{19}$	918.482	919.4892	2.99	20828	+H	66
4	25(S)-Ruscogenin	$C_{27}H_{42}O_4$	430.309	431.3163	3	6952	+H	12
5	Prosapogenin 2	$C_{32}H_{48}O_8$	560.3351	599.2983	3	1192	+H	19
6	Zingiberogenin	$C_{27}H_{42}O_4$	430.3092	431.3165	3.08	7355	+H	17
7	Markogenin	$C_{27}H_{44}O_4$	432.3237	433.331	3.12	62957	+H	28
8	Protodiosgenin	$C_{33}H_{54}O_9$	594.3764	595.3837	3.12	552505	+H	42
9	Tigogenin-3-O-β-Dglucopyranosyl(1 4)-β-D-galactopyranoside	$C_{39}H_{64}O_{13}$	740.4347	741.442	3.1	113363	+H	18
10	Terrestrosin A	$C_{45}H_{74}O_{18}$	902.4878	903.4951	3.24	537223	+H	69
11	Timosaponin B2	$C_{45}H_{76}O_{19}$	920.498	943.4872	3.24	16023	+Na	68
12	Ophiopogonin C'	$C_{39}H_{62}O_{12}$	722.424	723.4313	3.25	57789	+H	25
13	Yamogenin	$C_{27}H_{42}O_3$	414.3131	415.3204	3.25	11482	+H	5
14	epi-Smilagenin	$C_{27}H_{44}O_3$	416.3277	417.335	3.25	16045	+H	25
15	Trigoneoside Ⅱa	$C_{44}H_{74}O_{18}$	890.4865	913.4757	3.31	8245	+Na	37
16	Quinatoside D	$C_{39}H_{60}O_{11}$	704.4144	705.4217	3.43	10962	+H	6

Table 4.13 Continued

	Component name	Formula	Observed neutral mass (Da)	Observed m/z	Observed RT (min)	Response	Adducts	Total Fragment
17	Timosaponin A2	$C_{39}H_{64}O_{14}$	756.4311	779.4203	3.44	10412	+Na	16
18	Cimifoetiside Ⅶ	$C_{43}H_{70}O_{16}$	842.4668	843.474	3.48	1218	+H	45
19	Timosaponin G	$C_{39}H_{64}O_{14}$	756.4304	757.4377	3.53	14169	+H	40
20	Abrisaponin Ⅰ	$C_{48}H_{74}O_{20}$	970.4773	971.4845	3.53	260947	+H	45
21	Diosgenone	$C_{27}H_{40}O_3$	412.2978	413.305	3.53	4485	+H	7
22	Macrostemonoside F	$C_{45}H_{74}O_{18}$	902.4882	903.4954	3.59	62506	+H, Na	66
23	Terrestroside F	$C_{33}H_{54}O_7$	562.3877	563.395	3.6	2686	+H	15
24	Soyasaponin βg	$C_{47}H_{74}O_{17}$	910.4909	911.4982	3.67	24485	+H	56
25	3-O-β-D-Galactopyranosyl-(1 2)-β-Dglucuronopyranosyl gypsogenin	$C_{42}H_{64}O_{16}$	824.4205	825.4277	3.68	5161	+H	53
26	Ophiogenin	$C_{27}H_{42}O_5$	446.3032	447.3105	3.69	1702	+H	11
27	Trigoneoside Ⅲb	$C_{45}H_{76}O_{18}$	904.5044	927.4936	3.7	27683	+K, +Na	61
28	Neohecogenin-3-O-β-D-glucopyranoside	$C_{33}H_{52}O_9$	592.3605	615.3498	3.71	1351	+Na	12
29	Atroposide D	$C_{39}H_{62}O_{13}$	738.4185	739.4258	3.72	147331	+H	38
30	Δ3,5-Deoxytigogenin	$C_{27}H_{40}O_2$	396.3033	397.3106	3.79	4616	+H	13
31	Gracillin	$C_{45}H_{72}O_{17}$	884.4773	885.4846	3.92	378625	+H, + Na	63
32	Toosendanin	$C_{30}H_{38}O_{11}$	574.243	575.2503	3.95	2266	+H	16

Table 4.13 Continued

	Component name	Formula	Observed neutral mass (Da)	Observed m/z	Observed RT (min)	Response	Adducts	Total Fragment
33	Kingianoside D	$C_{39}H_{60}O_{14}$	916.4653	917.4725	4.07	38900	+H	49
34	Atroposide B	$C_{33}H_{52}O_{8}$	576.3661	577.3733	4.14	42670	+H	17
35	Hecogenone	$C_{27}H_{40}O_{4}$	428.2938	429.301	4.19	3752	+H	3
36	Picfeltarraenin Ⅳ	$C_{47}H_{72}O_{18}$	924.4726	925.4798	4.19	5948	+H	31
37	Ophiopogonin E	$C_{38}H_{60}O_{13}$	724.4046	725.4119	4.24	18991	+H, Na	25
38	Hookeroside C	$C_{38}H_{62}O_{15}$	758.4093	781.3985	4.29	19378	+Na	32
39	Timosaponin A1	$C_{33}H_{54}O_{8}$	578.3821	579.3894	4.3	17525	+H	17
40	Kingianoside B	$C_{39}H_{60}O_{13}$	736.4019	737.4092	4.38	21728	+H	33
41	Azukisaponin Ⅱ	$C_{42}H_{68}O_{14}$	796.4612	797.4685	4.4	66788	+H	146
42	Azukisaponin Ⅰ	$C_{42}H_{68}O_{13}$	780.4663	781.4736	4.45	26138	+H	143
43	Soyasaponin Ⅰ	$C_{48}H_{78}O_{18}$	942.5201	943.5273	4.45	1202823	+H, +Na, +K	160
44	3-O-(β-DGlucuronopyranosyl)-soyasapogenol B	$C_{36}H_{58}O_{9}$	634.4083	635.4156	4.45	26648	+H	129
45	Achyranthoside II	$C_{42}H_{68}O_{13}$	780.4663	781.4736	4.45	3564	+H	29
46	(25R)-Ruscogenin-1-O-β-D-xylopyranosyl(1 3)-β-Dfucopyranoside	$C_{38}H_{60}O_{12}$	708.4102	709.4175	4.49	7368	+H	13
47	Kaikasapomin Ⅲ	$C_{48}H_{78}O_{17}$	926.524	949.5132	4.58	23277	+Na	12

Table 4.13 Continued

	Component name	Formula	Observed neutral mass (Da)	Observed m/z	Observed RT (min)	Response	Adducts	Total Fragment
48	Esculentoside A	$C_{42}H_{66}O_{16}$	826.4348	827.442	4.71	5466	+H	23
49	Su-diosgenin-3-O-α-Lrhamnosus(1 2)-O-[α-L-rhamnopyranosyl(1 4)]-β-Dglucopyranoside	$C_{45}H_{72}O_{16}$	868.4814	869.4887	4.72	15860	+H	4
50	Celosin C	$C_{42}H_{66}O_{13}$	778.4518	779.4591	4.74	1293	+H	37
51	Esculentoside J	$C_{47}H_{76}O_{20}$	960.4934	983.4826	4.78	2535	+Na	94
52	Eleutheroside K	$C_{41}H_{66}O_{11}$	734.4592	735.4664	4.87	10546	+H	16
53	Picfeltarraenin IA	$C_{41}H_{62}O_{13}$	762.4208	763.4281	5.19	9037	+H	4
54	Esculentoside B	$C_{36}H_{56}O_{11}$	664.383	687.3722	5.53	2049	+H	15
55	23,27-Dihydroxypennogenin	$C_{27}H_{42}O_6$	462.2969	463.3042	6.29	5218	+H	5
56	Pulchinenoside A	$C_{41}H_{66}O_{12}$	750.4535	751.4607	6.71	14882	+H	17
57	Eclalbasaponin Ⅹ Ⅲ	$C_{37}H_{58}O_{10}$	662.4012	663.4084	6.73	29031	+H	8
58	Smilaxin	$C_{17}H_{16}O_6$	316.0948	315.0875	2.86	144596	-H	10
Phenolic compounds								
1	Cimicifugic acid B	$C_{21}H_{20}O_{11}$	448.1003	449.1076	1.79	21004	+Na	14
2	E-p-Coumatic acid	$C_9H_8O_3$	164.0473	165.0545	0.68	7808	+H	3
3	Auraptenol	$C_{19}H_{22}O_4$	314.1535	337.1427	1.06	3237	+Na	1
4	Actinidioionoside	$C_{19}H_{34}O_9$	406.2198	429.209	1.89	65198	+Na	6

Table 4.13 Continued

	Component name	Formula	Observed neutral mass (Da)	Observed m/z	Observed RT (min)	Response	Adducts	Total Fragment
5	Cichorioside B	$C_{21}H_{28}O_{10}$	440.1681	463.1573	1.41	5223	+Na	0
6	Quercetin-3-Oneohesperidoside	$C_{27}H_{30}O_{16}$	610.153	611.1603	1.79	16702	+H,+Na	10
7	Sweroside	$C_{16}H_{22}O_9$	358.1263	381.1155	1.57	6491	+Na	0
8	Onitin-2′-O-β-Dallopyranoside	$C_{21}H_{30}O_8$	410.1951	433.1843	3.79	8673	+Na	3
9	Estrone	$C_{18}H_{22}O_2$	270.16	293.1492	2.09	13866	+Na	1
10	Ulmoside	$C_{21}H_{32}O_{14}$	508.1812	547.1443	2.37	339575	+Na	47
11	5,7,2',5'-Tetrahydroxyflavone	$C_{15}H_{10}O_6$	286.0479	287.0551	2.74	7172	+H	1
12	Oleuropein	$C_{25}H_{32}O_{13}$	540.1862	579.1494	3.28	23590	+H	12
13	6′-O-Galloylhomoarbutin	$C_{20}H_{22}O_{11}$	438.1162	437.109	0.51	2365	-H	1
14	Meliadanoside B	$C_{15}H_{20}O_8$	328.1155	327.1082	0.59	4384	-H	2
15	Protocatechuic aldehyde	$C_7H_6O_3$	138.0322	137.0249	0.59	2326	-H	0
16	2,4,6Trihydroxyacetophenone-2,4-di-O-β-Dglucopyranoside	$C_{20}H_{28}O_{14}$	492.1487	491.1415	0.76	80020	-H	23
17	Cistanoside C	$C_{30}H_{38}O_{15}$	638.222	637.2147	0.78	137579	-H	15
18	(-)-Suspensaside B	$C_{33}H_{44}O_{16}$	696.2635	695.2563	0.87	217570	-H	29
19	Campneoside Ⅰ	$C_{30}H_{38}O_{16}$	654.2163	653.209	0.89	16268	-H	34
20	Osmanthuside H	$C_{19}H_{28}O_{11}$	432.164	431.1567	1.3	14481	-H	0

Table 4.13 Continued

	Component name	Formula	Observed neutral mass (Da)	Observed m/z	Observed RT (min)	Response	Adducts	Total Fragment
21	Decaffeoylacteoside	$C_{20}H_{30}O_{12}$	462.1744	461.1671	1.5	6002	-H	8
22	Forsythoside E	$C_{20}H_{30}O_{12}$	462.1727	461.1654	1.64	11463	-H	1
23	(+)-Suspensaside A	$C_{29}H_{34}O_{15}$	622.1905	621.1833	2.16	22531	-H	6
24	Erigoster A	$C_{27}H_{26}O_{13}$	558.1377	557.1304	2.7	15171	-H	45
25	Kuzubutenolide A	$C_{23}H_{24}O_{10}$	460.1376	459.1304	2.8	128449	-H	25
26	Dihydroresveratrol	$C_{14}H_{14}O_3$	230.0945	229.0872	2.82	3188	-H	1
27	2,3,5,4'-Tetrahydroxystilbene-2,3-O-β-Dglucopyranoside	$C_{26}H_{32}O_{14}$	568.1801	567.1728	2.92	33603	-H	32

Table 4.14 Saponin and phenolic compounds of Aloe vera extract identifed by LC-QTOF-MS in positive and negative ion modes.

	Component name	Formula	Observed neutral mass (Da)	Observed m/z	Observed RT (min)	Response	Adducts	Total Fragment
Saponin compounds								
1	Gentiopicroside	$C_{16}H_{20}O_9$	356.1105	379.0997	1.14	1900	+Na	
3	Esculentoside J	$C_{47}H_{76}O_{20}$	960.4914	961.4987	4.1	27477	+H	18
4	Timosaponin B1	$C_{46}H_{78}O_{19}$	934.512	935.5193	4.31	9810	+H	21
5	Hookeroside C	$C_{38}H_{62}O_{15}$	758.407	759.4143	4.35	79213	+H	56
6	Curculigo saponin C	$C_{41}H_{68}O_{13}$	768.4645	791.4537	4.57	1344	+Na	6
7	Kingianoside A	$C_{39}H_{60}O_{14}$	752.3994	753.4067	4.7	1420	+H	48
8	Dendronobilin G	$C_{15}H_{26}O_3$	254.1898	277.179	4.73	1372	+Na	1
9	Esculentoside A	$C_{42}H_{66}O_{16}$	826.436	849.4252	4.77	7887	+Na	20
10	Clinopodiside A	$C_{48}H_{78}O_{19}$	958.5138	981.503	5.31	1141	+Na	10
11	Soyasaponin βg	$C_{47}H_{74}O_{17}$	910.4937	933.4829	5.46	2812	+Na	9
12	Esculentoside E	$C_{35}H_{54}O_{11}$	650.3661	651.3733	5.56	5803	+H	11
13	24-O-Acetylcimigenol-3-O-β-Dxylopyranoside	$C_{37}H_{60}O_{11}$	680.4123	681.4196	5.86	5753	+H	5
14	Prosapogenin 2	$C_{32}H_{48}O_8$	560.3354	583.3247	5.92	1293	+Na	2
15	Daturametelin D	$C_{28}H_{36}O_4$	436.2633	459.2525	5.94	1720	+Na	2
16	Prosapogenin A	$C_{39}H_{62}O_{12}$	722.4224	745.4116	6.07	26938	+Na	8
17	Cornutaside C	$C_{41}H_{66}O_{13}$	766.4489	789.4381	6.11	16592	+Na, +H, + K	7
18	Cimicifugoside H2	$C_{35}H_{54}O_{10}$	634.3731	657.3624	6.12	1488	+Na	4
19	Akeboside Std	$C_{41}H_{66}O_{13}$	766.4484	767.4557	6.15	19230	+H	7
20	Koryoginsenoside R1	$C_{46}H_{76}O_{15}$	868.5171	869.5244	6.39	28431	+H	18

Table 4.14 Continued

	Component name	Formula	Observed neutral mass (Da)	Observed m/z	Observed RT (min)	Response	Adducts	Total Fragment
21	7,8- Didehydrocimigenol-3- O-β-D-xylopyranoside saponin	$C_{35}H_{54}O_9$	618.3758	619.3831	6.77	5425	+H	2
22	Ocotillol acetate	$C_{32}H_{54}O_4$	502.4034	503.4106	7.41	2041	+Na	0
23	Mudanpioside J	$C_{31}H_{34}O_{14}$	630.1949	629.1876	1.07	2315	-H	13
24	Gentiopicroside	$C_{16}H_{20}O_9$	356.1104	355.1031	2.02	5655	-H	12
25	Picfeltarraenin IB	$C_{42}H_{64}O_{14}$	792.4316	791.4243	2.3	1227	-H	48
26	Trigoneoside Ia	$C_{44}H_{74}O_{19}$	906.482	905.4748	3.04	2804	-H	6
27	Ginsenoside Re	$C_{48}H_{82}O_{18}$	946.5499	945.5426	3.72	1534	-H	3
28	Terrestrosin B	$C_{45}H_{74}O_{17}$	886.4915	885.4842	3.97	11051	-H	9
29	Eclalbasaponin IX	$C_{36}H_{62}O_{11}$	702.4028	701.3955	5.05	1121	-H	1
Phenolic compounds								
1	3,4-O-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	516.125	539.1142	0.5	2594	+Na	2
2	2,4,6-Trihydroxyacetophenone-2,4-di-O-β-Dglucopyranoside	$C_{20}H_{28}O_{14}$	492.1485	515.1377	1.06	4178	+Na, +K	2
3	Salidroside	$C_{14}H_{20}O_7$	300.1213	323.1105	1.68	10560	+Na	0
4	Quercetin-3-Oneohesperidoside	$C_{27}H_{30}O_{16}$	610.1547	611.162	1.83	1921	+H	0
5	Meliadanoside B	$C_{15}H_{20}O_8$	328.116	351.1052	1.93	7631	+Na	0
6	Actinidioionoside	$C_{19}H_{34}O_9$	406.2183	407.2256	2.66	109154	+H	5
7	Apocynin B	$C_{24}H_{20}O_{10}$	468.1065	469.1138	3.29	23513	+H	17
8	(R)-Prechrysophanol	$C_{15}H_{14}O_4$	258.0889	259.0962	4.45	2779	+H	4
9	Periplocin	$C_{36}H_{56}O_{13}$	696.3727	719.3619	5.22	7767	+Na	27
10	5,7,2',5'-Tetrahydroxy-flavone	$C_{15}H_{10}O_6$	286.0476	285.0403	0.67	9075	-H	0
11	4-Hydroxyacetophenone	$C_8H_8O_2$	136.0524	135.0452	0.92	2180	-H	0

Table 4.14 Continued

	Component name	Formula	Observed neutral mass (Da)	Observed m/z	Observed RT (min)	Response	Adducts	Total Fragment
12	E-p-Coumatic acid	$C_9H_8O_3$	164.0474	163.0401	0.98	12735	-H	1
13	Odoriflavene	$C_{17}H_{16}O_5$	300.0991	299.0918	1.02	4127	-H	7
14	Methyl caffeate	$C_{10}H_{10}O_4$	194.0581	193.0508	1.12	4321	-H	1
15	(+)-Suspensaside A	$C_{29}H_{34}O_{15}$	622.1883	621.181	1.54	1546	-H	9
16	3'-O-Methylbrazilin	$C_{17}H_{16}O_5$	300.0996	299.0923	1.54	1511	-H	3
17	Tubuloside E	$C_{31}H_{38}O_{15}$	650.2214	649.2141	1.56	2165	-H	11
18	Forsythoside B	$C_{34}H_{44}O_{19}$	756.2478	755.2406	1.59	1230	-H	19
19	Meliadanoside B	$C_{15}H_{20}O_8$	328.1149	327.1076	1.91	1767	-H	4
20	Paeonol	$C_9H_{10}O_3$	166.0628	165.0555	1.92	1032	-H	2
21	Torachrysone-8-O-β-Dglucopyranoside	$C_{20}H_{24}O_9$	408.142	407.1347	2.06	10895	-H	7
22	Torachrysone-8-O-β-Dglucopyranoside	$C_{20}H_{24}O_9$	408.142	407.1347	2.06	10895	-H	7
23	Cearoin	$C_{14}H_{12}O_4$	244.0729	243.0656	2.06	2711	-H	5
24	Onitin-2′-O-β-Dglucopyranoside	$C_{21}H_{30}O_8$	410.1943	409.1871	2.3	1128	-H	9
25	Taraxacolide-1-O-β-Dglucopyranoside	$C_{21}H_{34}O_9$	430.2207	429.2134	2.34	1584	-H	5
26	Cimicifugic acid B	$C_{21}H_{20}O_{11}$	448.1008	447.0935	2.45	3577	-H	6
27	Syringaldehyde	$C_9H_{10}O_4$	182.0579	181.0506	2.48	1294	-H	1
28	Kuzubutenolide A	$C_{23}H_{24}O_{10}$	460.1374	459.1301	2.65	206977	-H	13
29	Feralolide	$C_{18}H_{16}O_7$	344.0892	343.0819	2.9	60860	-H	17
30	Cistanoside C	$C_{30}H_{38}O_{15}$	638.222	637.2147	2.95	2162	-H	13
31	Nudol	$C_{16}H_{14}O_4$	270.0888	269.0815	3.11	1808	-H	7
32	Pueroside A	$C_{29}H_{34}O_{14}$	606.1953	605.1881	3.37	61178	-H	19

4.8.3 Scanning Electron Microscopy

Scanning electron microscopy (SEM) analysis was performed for conspicuous visualization of fenugreek seed and Aloe vera leaves powder before (raw) and after (residue) extraction using MAE and Soxhlet extraction methods. The morphological analyses of the fenugreek seed and Aloe vera leaves samples before and after MAE and Soxhlet extraction are shown in Figure 4.19 (a to f), respectively. As it can be seen the structural alteration is obvious in samples before and after extraction specially those extracted by microwave-assisted extraction (Figures 4.19 b and e). The surface of raw fenugreek powder and Aloe vera leaves (Figure 4.19 a and d) are observed to be with wrapped pores attached together. While, the residues after MAE (Figure 4.19 b and e) were appeared to be rough with large caves and pores, showing that MAE efficiently destroyed the vegetal cell wall. This is due to the breakage of seed and leaves matrix and bonding while exposed to microwave heating extraction (Dahmoune et al., 2015).

However, fenugreek seed and Aloe vera leaves extracted via Soxhlet extraction method, indicated not much difference before and after extraction (Figure 4.19 c and f). Indicating that Soxhlet extraction was not properly able to break the textures of fenugreek seed and Aloe vera leaves and increase the extraction efficiency. This also indicated that comparing to MAE, Soxhlet extraction provided poor heating during the extraction process. In fact, the heating mechanism between Microwave and Soxhlet extraction is different. In a MAE, the heat passes to the samples from few seconds to few minutes via the mechanisms of ionic conduction and dipole rotation (Zhang et al., 2011). However, in Soxhlet extraction, the sample is heated for a long time and usually it takes hours to complete a cycle. This may be due to the mechanisms involved in a Soxhlet extraction and hence the sample is heated through convection, conduction and radiation which is a time consuming process (Veggi et al., 2013).

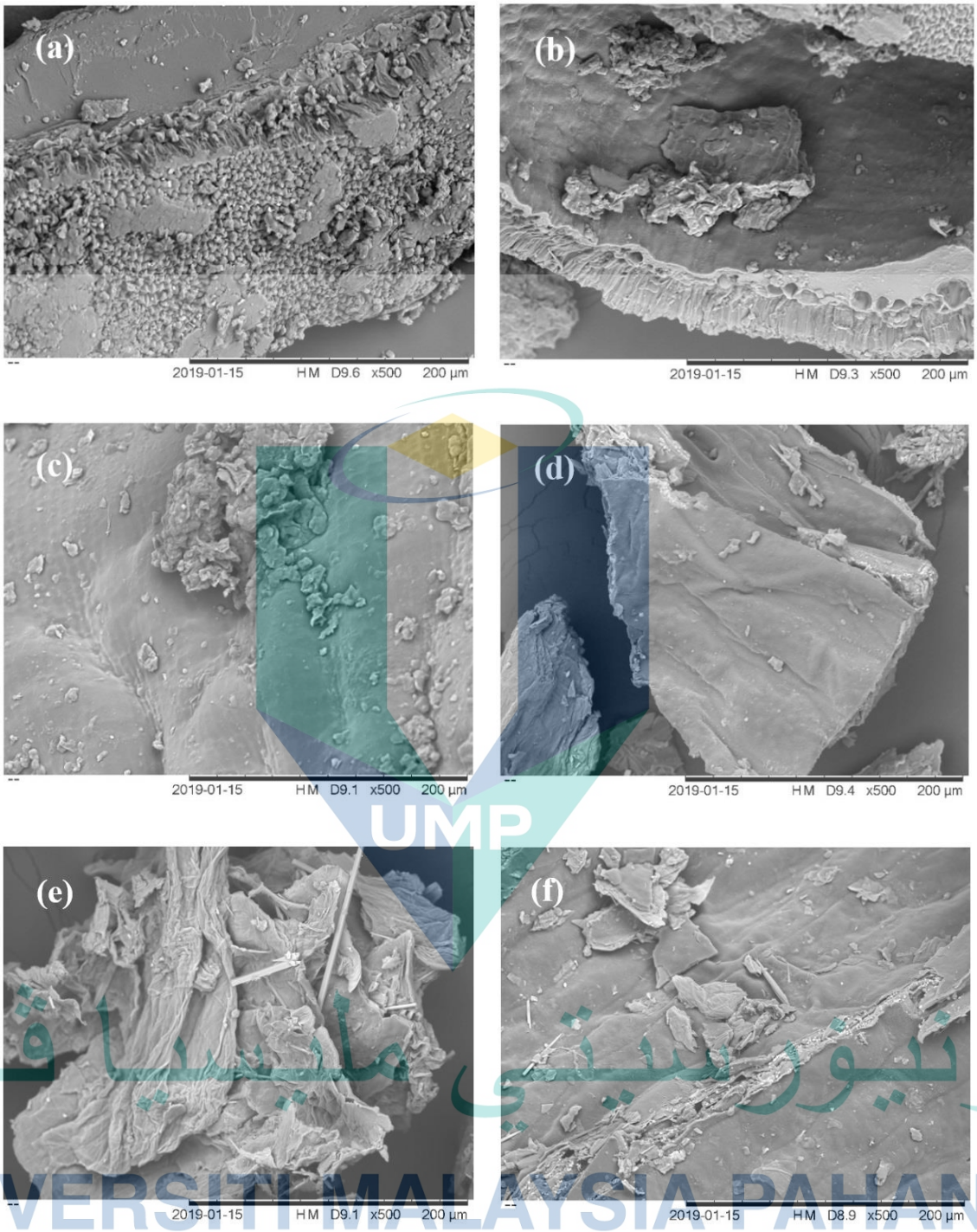


Figure 4.19 Morphological structure of fenugreek seed before extration (a), fenugreek seed after optimized MAE (b), fenugreek seed extracted via Soxhlet (c); Aloe vera leaves before extraction (d) Aloe vera leaves after optimized MAE (e) and Aloe vera leaves after Soxhlet extraction (f).

4.8.4 Fourier Transform Infrared Analysis

FTIR is a useful tool for analysing the structural bonding and identifying the functional groups of compounds. FTIR spectra of fenugreek seed extract at optimized MAE and Soxhlet extract is shown in Figure 4.20. The peaks lied on the wavelength of 500-4000 cm^{-1}. As seen in Figure 4.20a, a total of 12 peaks were identified in optimized MAE of fenugreek seed. The depicted peaks ranged from 3277 cm^{-1} to 837 cm^{-1} (Figure 4.20a). The peak at 3277.87 cm^{-1} is attributed to the existence of hydroxyl group (O-H) which indicate the presence of phenolic in the extract. The peaks at 2928.37 cm^{-1} could be associated to -CH stretching corresponds the presence of saponin glucosides. The observed peaks at 2104.87 cm^{-1} and 1638.01-1284.56 cm^{-1} corresponded to C=C, C=O, CH_2 and CH_3 which can be attributed to the presence of aromatic ring, aldehyde, carboxyl, phenols, flavonoids, saponin glycosides, and amino acids (Alara et al., 2018; Almutairi & Ali, 2014). The peaks at 1034.30-837.34 cm^{-1} also represents the presence of glycosidic bonds which indicates the existence of sugar chains belonging to saponins (Gil-ramirez et al., 2018). Figure 4.20b indicates the FTIR spectra of fenugreek seed extract obtained via Soxhlet extraction. A total of 6 peaks were identified and ranged from 3327.74 cm^{-1} to 1040.41 cm^{-1}. The peak at 3327.74 cm^{-1} shows the stretching vibration of O-H groups which indicates the phenolic compounds. Likewise, the peaks from 1637.92 to 1040.41 cm^{-1} corresponded to CH_2 and CH_3 compounds belonging to aldehyde, carboxyl, phenols, flavonoids, saponin glycosides, and amino acids (Alara et al., 2018; Almutairi & Ali, 2014).

The identification of FTIR spectra of optimized Aloe extract via MAE and Soxhlet extraction is shown in Figure 4.21 (a and b). It is seen that a total of 11 peaks were detected for optimized Aloe vera extract via MAE (Figure 4.21a). The peaks observed at the wavelength of 500-4000 cm^{-1}. The depicted peaks ranged from 3344.64 cm^{-1} to 990.53 cm^{-1} (Figure 4.21a). The peak at 3344.64 cm^{-1} is attributed to the presence of O-H group which indicates the presence of phenolic compounds in the extract. The observed peaks at 2099.93 cm^{-1} and 1728.84-1106.66 cm^{-1} corresponded to C=C, C=O, CH_2 and CH_3 which can be attributed to the presence of aromatic ring, aldehyde, carboxyl, phenols, flavonoids, saponin glycosides, and amino acids (Keshari et al., 2018). The peaks at

1077.34 and 990.53 cm^{-1} also represents the presence of glycosidic bonds which indicates the existence of sugar chains belonging to saponins (Samal et al., 2017). Figure 4.21b indicates the FTIR spectra of Aloe vera extract obtained via Soxhlet extraction. A total of 6 peaks were identified and ranged from 3257.52 cm^{-1} to 1106.66 cm^{-1}. The peak at 3257.52 cm^{-1} shows the stretching vibration of O-H groups which indicates the phenolic compounds. Likewise, the peaks from 2112.29 to 1106.66 cm^{-1} corresponded to CH_2 and CH_3 compounds belonging to aldehyde, carboxyl, phenols, flavonoids, saponin glycosides, and amino acid (Keshari et al., 2018).

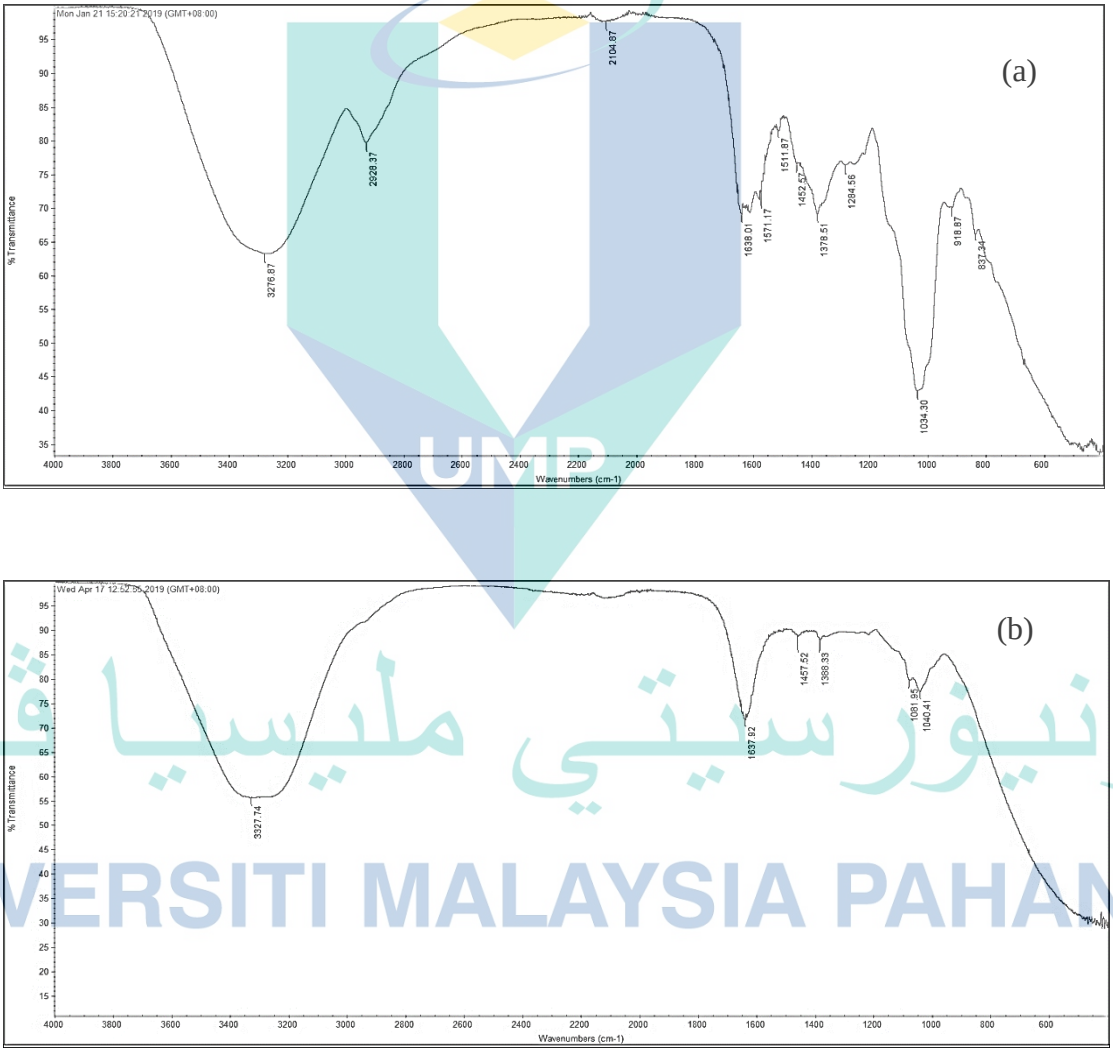


Figure 4.20 FTIR spectra of fenugreek seed extract at optimized MAE (a), and the extract obtained via Soxhlet extraction (b).

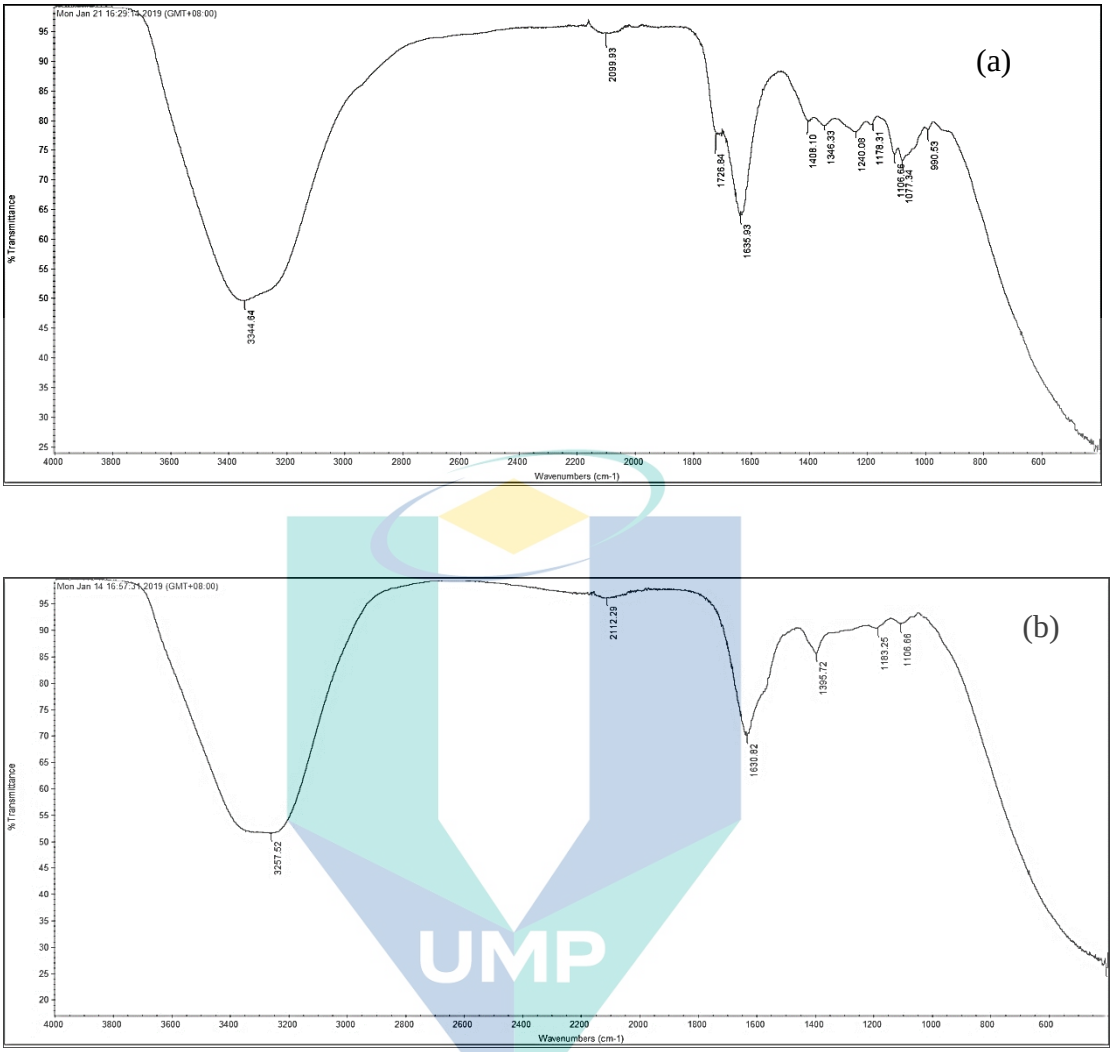


Figure 4.21 FTIR spectra of Aloe vera leaves extract at optimized MAE (a), and the extract obtained via Soxhlet extraction (b).

4.8.5 Surface Tension and Foaming Properties

The surface tension of optimized fenugreek seed and Aloe vera leaves extracts were tested to understand the air/extract solution interface. Fenugreek seed extract was able to reduce the surface tension of water from 70 mN/m to 39.85 mN/m. Aloe extract also indicated surface tension reduction of 42.23 mN/m. Tween 80 reduced the surface tension of water to 38.33 mN/m. The reason behind this reduction is the breaking of hydrogen bonds caused at the air/solution interface (Pradhan & Bhattacharyya, 2017). The reduction of surface tension with surfactants are also reported by (Feng et al., 2006). Table 4.15 shows the foaming capacity and foam stability of fenugreek seed and Aloe

vera leaves extract against Tween 80. As seen, fenugreek seed exhibited a good foaming capacity (26%) and foaming stability during 1 hour of observation. The foam stability of fenugreek seed was 100% just after the agitation, while it reduced to 38.46 % in 60 min. At the same time, the foaming capacity and stability of Aloe extract were significantly low comparing to fenugreek seed extract and Tween 80. At the beginning, the foam stability was 100%, however, after 60 min it declined to 16.67%. Aloe extract indicated 12% foaming capacity. The reason behind the fast reduction of foam stability and low foaming capacity of Aloe extract might be the low content of saponin. The ability of foam formation by saponins is due to the existence of nonpolar sapogenin and water soluble side chain which in saponins which is similar to the property of surface active agents containing both hydrophilic and lipophilic molecular parts (Pradhan & Bhattacharyya, 2017). *Quillaya Saponaria*, is a valuable source of saponin in Chile. The bark of the tree is used as a shampoo for hundreds of years in Chile. Commonly, *Yucca* and *Quillaya* extracts are used in some beverages as a foaming agent. In Japan, the *Quillaya* saponin is used as a natural emulsifier in food, cosmetic and pharmaceutical products (Oleszek & Hamed, 2010a). In regard to Tween 80, it showed the highest foaming capacity (30%) and foam stability of 46.67% after 60 min, which is in a good agreement with the study found by Kothekar et al. (2007). The R5 value which represents the ratio of the foam height at 5 min to that at the initial stage, generally it shows the foam stability. Foam with R5 value greater than 50% indicates a metastable system with high stability (Pradhan & Bhattacharyya, 2017). As seen fenugreek seed, Aloe extract and Tween 80 represented a R5 values of 76.92%, 66.67% and 93.33%, respectively.

Table 4.15 Foaming capacity and foam stability of the extracts

Samples	Foaming capacity (%)	Foam stability (%)							
		0 min	5 Min	10 min	15 min	20 min	25 min	30 min	60 min
Fenugreek extract	26	100	76.92	76.92	69.23	53.85	38.38	38.46	38.46
Aloe extract	12	100	66.67	66.67	50.00	33.33	33.33	16.67	16.67
Tween 80	30	100	93.33	86.67	73.33	66.67	46.67	46.67	46.67

R5 is measured based on 5 min.

4.8.6 Wettability and Contact Angle Analysis

The contact angle is the visual measurement of wettability for a liquid solution or a solid substrate. Figure 4.22 shows the contact angle and wettability measurement of fenugreek seed extract and Aloe vera leaves extract solutions. The contact angle of Tween 80 solution was also measured to compare the results. As seen, the contact angle of water is more than 90°, while the solutions of fenugreek seed extract, Aloe vera leaves extract and Tween 80 indicated significant reduction of 48.5°, 56.3° and 44.5°, respectively. Reduction of contact angle is one of the most important characteristics of surfactants due to their surface-active property. The contact angle also indicates the wettability property of a surfactant. The smaller the contact angle, the better the wettability (Bracco & Holst, 2013). In fact, fenugreek seed and Aloe vera leaves extract were able to reduce the contact angle of the water, this is due to their properties as natural surfactants, which makes them a good co-emulsifier for food, cosmetic and pharmaceutical products despite of having potential health benefits for these products.

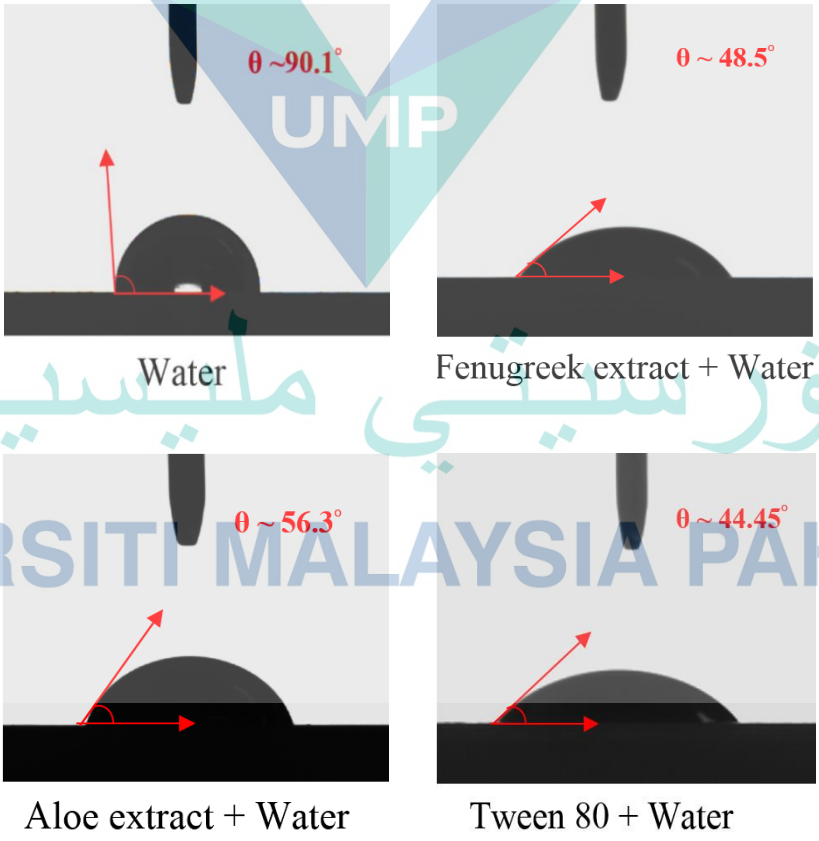


Figure 4.22 Contact angle and wettability measurement of extracts and Tween 80.

4.8.7 Emulsification Property of the Extracts

The emulsification property of fenugreek seed and Aloe vera leaves optimized extracts obtained via MAE were evaluated to understand the emulsification ability of the extract as a surfactant or co-surfactant in preparing an oil-in-water (O/W) emulsion at ratio of 40-60%v/v at room temperature. Table 4.16 shows the emulsification index of fenugreek seed, Aloe vera leaves and Tween 80 a commerical surfactant, which was used to compare the results. As indicated, fenugreek seed extract provided a very good emulsification abality and followed by Aloe vera leaves extract. The emulsification index after 24 hours of observation for the extracted samples were achived at 44% and20%, respectively. The emulsification index of optimized fenugreek seed were 100% for the first 10 min, and then reduced to 44% by the next 24 hours.

However, Aloe vera leaves extract did not show high emulsification activity as fenugreek seed. This may be due to the high content of saponin in fenugreek seed. Since, saponins has also been used as biosurfactant in food, cosmetic and pharmaceutical industries (Guclu-Ustundag & Mazza, 2007). In addition, Tween 80 indicated better stability and emulsification index compared to fenugreek seed and Aloe vera leaves extract. Relativley, according to US Food and Drug Administration, the use of Tween 80 in food, pharmaceutical and cosmetic products are safe when it is used at less than 1% concentration (Nielsen et al., 2016). A study found that addition of 1% Tween 80 in drinking water caused to inflamaltion and adiposity in mice (Chassaing et al., 2015). Therefore, the extracts of fenugreek seed and Aloe vera can be recommended as co-surfactant to reduce the volume of synthetic surfactats in health related products.

Table 4.16 Emulsification index of the extracts

Extracts	Emulsification index (%)						
	$E_{10\ min}$	E_{1h}	E_{2h}	E_{3h}	E_{4h}	E_{5h}	E_{24h}
Fenugreek seed extract	100	100	80	50	48	48	44
Aloe vera extract	100	88	56	52	42	40	20
Tween 80	100	100	100	100	50	50	46

E = emulsification index

4.9 Summary

Fenugreek seed and Aloe vera leaves extracts were obtained via microwave-assisted extraction and Soxhlet extraction. The extraction was first carried out using OFAT at different ranges of extraction parameters such as extraction time, microwave power, ethanol concentration, feed-to-solvent ratio and extraction temperature. Results indicated that higher yields of extraction, TSC and TPC can be achieved via MAE compared to Soxhlet extraction. Based on the results of OFAT, MAE was selected for factor screening and optimization. The ranges of parameters for optimization process were obtained based on the results of OFAT. Prior to optimization, a factor screening was performed via two-level factorial design to understand the significant factors in achieving high recovery of yields. Optimization of TSC and TPC were carried out using RSM. The optimized extracts were evaluated for antioxidant activities using DPPH and ABTS essays, chemical compositions via LC-QTOF-MS, bonding and structural analysis using FTIR, morphological studies using SEM, and surfactant properties using wettability, surface tension, foaming and emulsification properties. Results indicated that the extracts obtained via MAE have the potential to be used as a food, cosmetic and pharmaceutical industries as an active ingredient. Further summary of the results is discussed in conclusion.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusions

Recently, bioactive compounds extracted from plants have achieved potential interest as an ingredient in food, cosmetic and pharmaceutical products. This study focused on the extraction of bioactive compounds of total saponin and phenolics from fenugreek seed and Aloe vera leaves via MAE and Soxhlet extraction. In this study, initially the extraction of the seed and leaves were carried out using OFAT and the obtained results showed that the range for MAE parameters were extraction time 2-4 min, microwave power 500-700 W and 400-600 W, ethanol concentration 40-80% and 20-60%, feed-to-solvent ratio 1:8-1:10 g/mL and 1:18-1:22 g/mL and extraction temperature 60-80°C, respectively. The selected ranges were applied for screening purpose and optimization process. While, for OFAT experiment in Soxhlet extraction the parameters were time (1-5 h), ethanol concentration (20-100%) and feed-to-solvent ratio (1:14-1:24 g/mL) were applied. The OFAT experiment indicated that MAE provided higher recovery of extraction yields, TSC and TPC compared to Soxhlet extraction for fenugreek seed and Aloe vera leaves at 27.98%, 193.02 mg DE/g d.w and 81.78 mg GAE/ g d.w. for fenugreek seed, and 35.65%, 66.95 mg OAE/g d.w and 71.21 mg GAE/g d.w. for Aloe vera leaves, respectively, and for Soxhlet it was achieved at 19.35%, 125.04 mg DE/g d.w. and 60.13 mg GAE/g d.w. for fenugreek seed, and 22.45%, 44.78 mg OAE/g.d.w, and 49.99 mg GAE/g.d.w. for Aloe vera leaves, respectively. After OFAT, a factor screening was performed using two-level factorial design for MAE, and the results showed that all extraction parameters were significant in obtaining high amount of yield, except extraction temperature. Additionally, ethanol concentration was found to be the most effective and significant extraction factor.

The optimization of MAE parameter for fenugreek seed showed that the optimal conditions of parameters were at 2.84 min, 572.50 W, 63.68%, and 1:9 g/mL. Based on the optimum condition, the responses of extraction yield, TSC and TPC of fenugreek seed were 26.04%, 195.89 mg DE/g d.w., 81.85 mg GAE/g d.w, respectively. Likewise, the suggested optimum condition for Aloe vera leaves extracts in MAE were 2.79 min, 478.95 W, 43.38% ethanol, and 1:19 g/mL. Where, based on these conditions, the extraction yield, TSC and TPC of Aloe vera leaves extract were 36.17%, 65.89 mg OAE/g d.w and 73. 05 mg GAE/g d.w, respectively.

The dielectric properties and kinetic studies were also carried out to better understand the mechanism of extraction. It was sound that the dielectric constant and loss of water were much higher compared to ethanol at 25°C (76.80; 11.64) and (14.84; 10.31) respectively. However, by increasing the temperature to 70°C, the dielectric properties of both solvents reduced to (61.69; 4.03) and (14.36; 10.21), respectively. The kinetic studies also revealed that the second-order kinetic model was more suitable for this study compared to first-order kinetic model with a coefficient of determination R^2>0.95.

The LC-QTOF-MS analysis of the extracts confirmed the presence of saponin and phenolic compounds in fenugreek seed and Aloe vera leaves. A total of 58 and 27 saponin and phenolic compounds were identified in optimized fenugreek seed extracted via MAE in both positive and negative ion modes, respectively. Likewise, a total of 29 saponins and 32 phenolic compounds were identified in Aloe vera leaves extract. FTIR results also confirmed the presence of saponins, phenolic, flavonoid and alkaloid compounds inside the extracts. The extracts of fenugreek seed and Aloe vera leaves obtained via MAE showed 12 and 11 identified peaks, however, extract obtained from Soxhlet extraction indicated 6 identified peaks for both plants. The SEM morphological analysis also indicated MAE has the potential the break down the plant texture properly and increase the yields. While, Soxhlet extraction was not very able to break the cells and texture of the plants during the extraction process. Therefore, the maximum yields were obtained via MAE with a very significant difference compared to Soxhlet extraction.

The antioxidant activities of fenugreek seed and Aloe vera leaves were evaluated using DPPH and ABTS free radical scavenging assays. The half maximal inhibitory concentration (IC50) of the samples was also determined. The TSC, TPC, DPPH and ABTS values of the extracts obtained by MAE is higher than those obtained via Soxhlet extraction method. It is also seen that, the IC50 values of DPPH in fenugreek seed extract (195.27 ± 0.56, MAE; 224.47 ± 0.77 Soxhlet) and Aloe vera leaves extract (275.9 ± 1.45, MAE; 305.79 ± 0.66, Soxhlet) are higher when compared to IC50 values of ABTS assay (157.92 ± 1.11, MAE; 199.67 ± 0.96, Soxhlet) and (215.58 ± 0.57, MAE; 263.29 ± 1.21, Soxhlet), respectively, which shows a strong antioxidant activity for both plants.

The wettability, surface tension, foaming and emulsification of optimized extracts of fenugreek seed and Aloe vera leaves were also evaluated. The contact angle, surface tension, foaming capacity and emulsification index (E_{24}) of fenugreek seed extract were 48.5°, 39.85 mN/m, 26% and 44%, respectively, and for Aloe vera leaves were 56.3°, 42.23 mN/m, 12% and 20%, respectively. In addition, Foam with R5 value greater than 50% indicates a metastable system with high stability. As seen fenugreek seed and Aloe vera leaves extract compared to commercial surfactant Tween 80 represented a R5 values of 76.92%, 66.67% and 93.33%, respectively. Results indicated that fenugreek seed extract has good surfactant property compared to Aloe vera extract. Hence, the results suggest that fenugreek seed and Aloe vera leaves extract obtained via MAE can be used as a good source of saponin, phenolic, antioxidants and natural co-emulsifier in food, cosmetic and pharmaceutical industries.

5.2 Recommendation

Taking the results into account, the recommendations for future works are as follow:

i. The results of this research showed that fenugreek seed and Aloe vera leaves extracts are a good source of saponin, phenolics and antioxidants which are very essential for the treatment of diseases such cancer, diabetes, skin problems, hearth

problems and inflammation. Therefore, further application of these extracts for clinical or animal testing should be carried out.

ii. Production scale up can be suggested after a demand and market survey.

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APPENDIX A
PREPARATION OF DIOSGENIN, OLEANOLIC ACID AND GALLIC ACID STANDRAD CALIBRATION CURVE

The standard calibration curve for diosgenin and oleanolic acid stock solution was prepared at 1000 mg/L and different concentrations (100-600 mg/L) the absorbance was taken at 544 nm. The stock solution of gallic acid was prepared at 5000 mg/L) and the absorbance was taken at 765 nm. The standards were used for the measurement of TSC and TPC of fenugreek seed and Aloe vera leaves and results were expressed as mg of standard equivalent per gram of dry weight, respectively. The stock solution was prepared using the dilution equation as follows:

$$C_1V_1 = C_2V_2 \qquad A1$$

Where C_1 indicates the concentration of stock solution, V_1 shows the volume of stock solution (standard solution), V_2 represents the final volume of the solution and C_2 is the final concentration of the stock solution need to be prepared. Table A.1 and A.2 represent the diosgenin and oleanolic acid solution prepared at different concentrations for standard curve.

Table A.1 Prepared stock solution of diosgenin for standard curve at different concentrations.

Concentration (mg/L)	Volume of stock solution (diosgenin) (µL)	Volume of methanol (µL)
100	100	900
200	200	800
300	300	700
400	400	600
500	500	500
600	600	400

Table A.2 Prepared stock solution of oleanolic acid for standard curve at different concentrations.

Concentration (mg/L)	Volume of stock solution (Oleanolic acid) (µL)	Volume of methanol (µL)
100	100	900
200	200	800
300	300	700
400	400	600
500	500	500
600	600	400

Table A.3 Prepared stock solution of gallic acid for standard curve at different concentrations.

Concentration (mg/L)	Volume of stock solution (gallic acid) (µL)	Volume of methanol (µL)
100	20	980
200	40	960
300	60	940
400	80	920
500	100	900
600	120	880

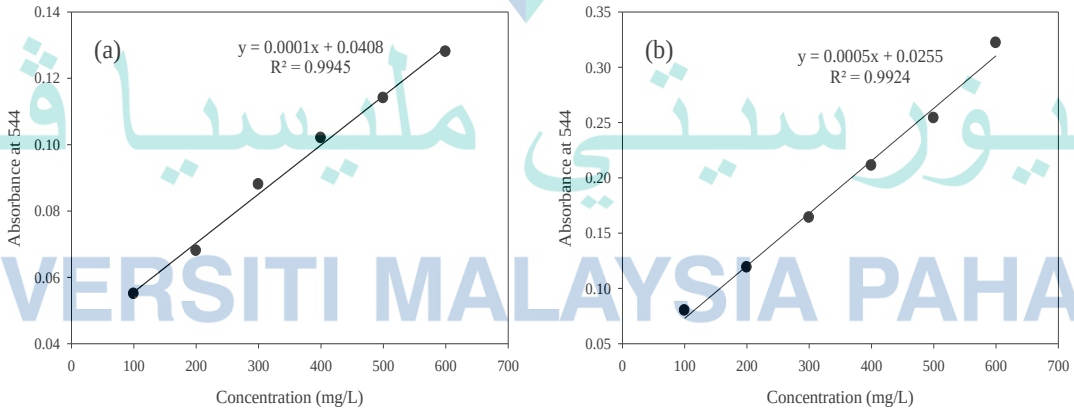


Figure A.1 Calibration curve of diosgenin (a) and oleanolic acid (b).

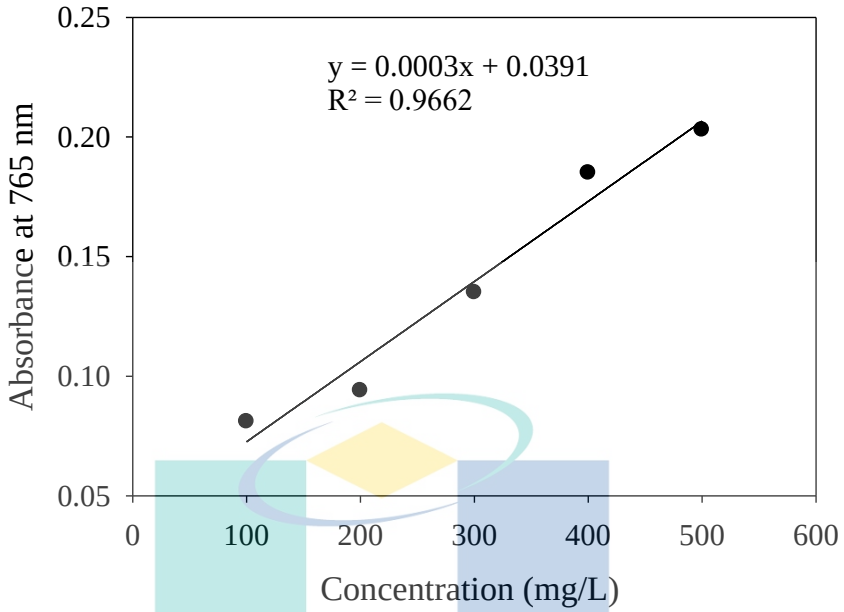


Figure A.2 Calibration curve of gallic acid.

APPENDIX B
CALIBRATION CURVES FOR ANTIOXIDANT ACTIVITIES

The calibration curves of DPPH, ABTS and Ascorbic acid are presented in Figure B.1 and B.2 (a-d). The calibration curves of extracts/ascorbic acid were prepared at different concentrations of (100-500 µg/mL). The stock solutions were prepared at 1000 mg/L) as seen in Table B.1.

Table B.1 Prepared stock solution of ascorbic acid for standard curve at different concentrations for antioxidant activities.

Concentration (mg/L)	Volume of stock solution (extract/ascorbic acid) (µL)	Volume of methanol (µL)
100	100	900
200	200	800
300	300	700
400	400	600
500	500	500

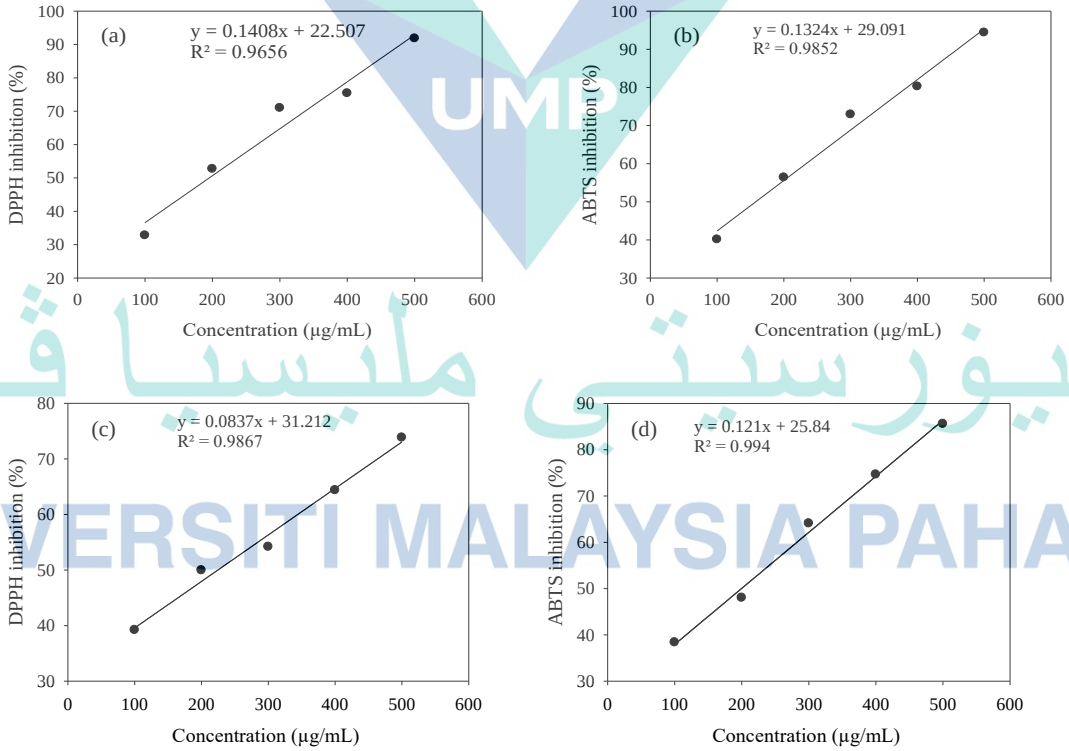


Figure B.1 Calibration curve of DPPH and ABTS antioxidant activities of fenugreek seed extracted via MAE (a) and (b), respectively. Calibration curves of DPPH and ABTS antioxidant capacities of fenugreek seed extracted via Soxhlet (c) and (d), respectively.

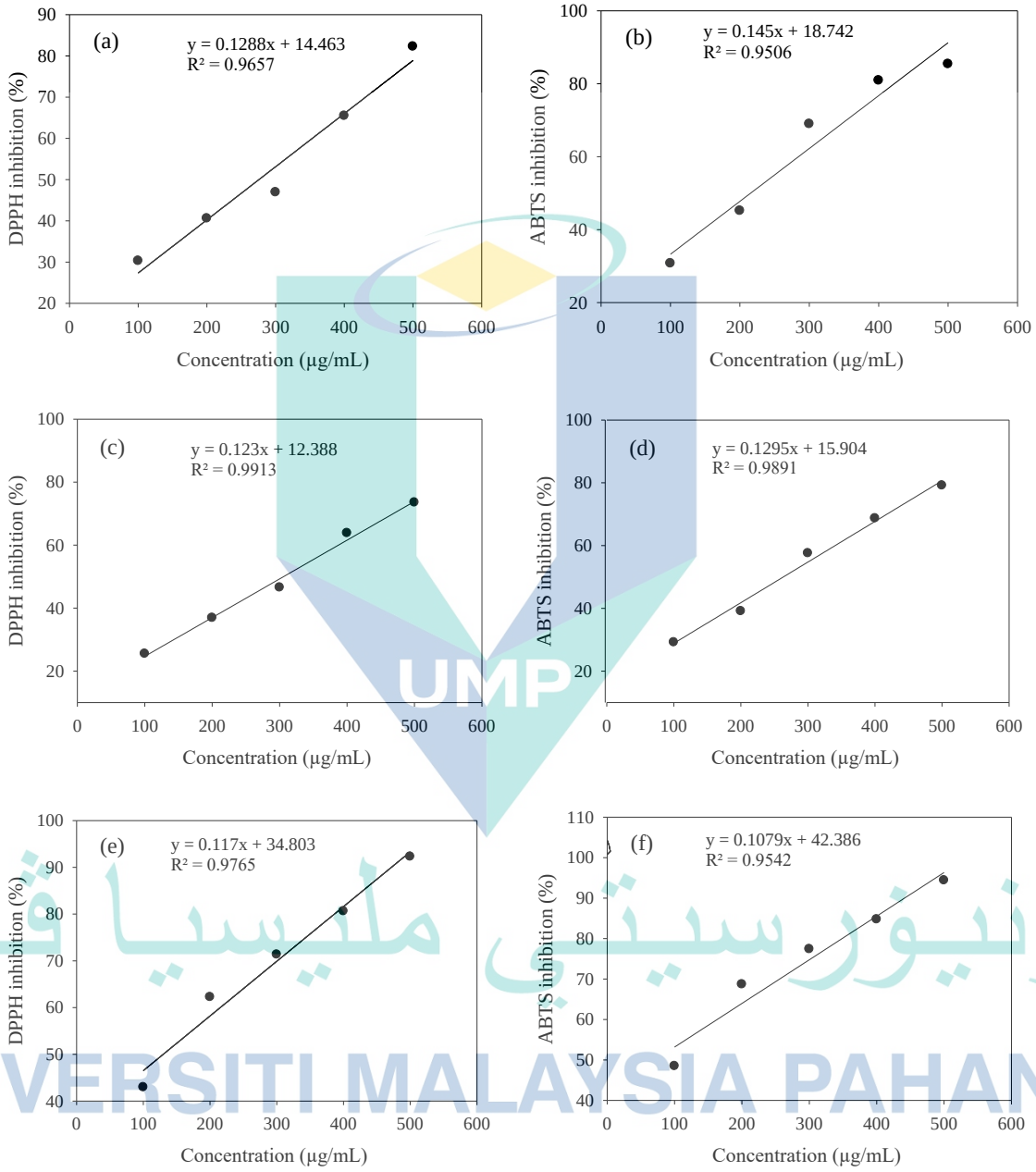


Figure B.2 Calibration curve of DPPH and ABTS antioxidant activities of Aloe vera leaves extracted via MAE (a) and (b), respectively. Calibration curves of DPPH and

ABTS antioxidant capacities of Aloe vera leaves extracted via Soxhlet (c) and (d), respectively. Ascorbic acid using DPPH (e); Ascorbic acid using ABTS (f).

APPENDIX C
DIELECTRIC PROPERTIES AND KINETIC STUDIES

Calculation of Parameters in Microwave Heating

The dielectric properties and microwave heating were calculated based on following equations.

Where, T_a is the temperature of MAE extraction in seconds, T_0 is the room temperature, and Ø is the ethanol-water volume at optimum point. Room temperature = 25°C, process temperature 70°C.

$$Q_{mix}\left(\frac{cal}{s}\cdot cm^3\right) = \rho_{mix} \times C_{p,mix}\left(\frac{dT}{dt}\right) \qquad C.1$$

$$\left(\frac{dT}{dt}\right)\left(°C\frac{}{s}\right) = \left(\frac{T_a - T_0}{T_a}\right) \qquad C.2$$

$$\rho_{mix}\left(\frac{g}{cm^3}\right) = \rho_w Ø + \rho_e(1 - Ø) \qquad C.3$$

$\rho_{e=}\, desity\ of\ ethanol = 0.789\ g/cm^3$

$$Ø = \frac{\frac{Ø_w}{\rho_w}}{\frac{Ø_w}{\rho_w} + \frac{Ø_e}{\rho_e}} \qquad C.4$$

$$C_{P,mix} = C_{p,w}Ø + C_{p,e}(1 - Ø) \qquad C.5$$

Heat capacity of hydrocarbon fractions in their liquid state can be found as follows:

$T = 25°C$

$C_{p,w} = 0.9997\ at\ 25℃$

$$C_p(kj) = \frac{1.685 + 0.0034\,T}{\sqrt{spacific\ gravity}} = (in\ cal) \qquad C.6$$

$$\acute{\varepsilon}_w = 85.2 - 0.3358T_a \qquad C.7$$

$$\ddot{\varepsilon}_w = 320.66T_a^{-1.03} \qquad C.8$$

$$\acute{\varepsilon}_{mix} = \acute{\varepsilon}_w\emptyset + \acute{\varepsilon}_e(1-\emptyset) \qquad C.9$$

$$\varepsilon'_e = \varepsilon_\infty + \frac{(\varepsilon_{s-}\varepsilon_\infty)}{1+\omega^2\tau^2} \qquad C.10$$

Where $\tau = 1/\omega$

$$\varepsilon'_e = \frac{(\varepsilon_{s+}\varepsilon_\infty)}{2} \qquad C.11$$

$$\varepsilon''_e = \frac{(\varepsilon_{s-}\varepsilon_\infty)}{2} \qquad C.12$$

$$\ddot{\varepsilon}_{mix} = \ddot{\varepsilon}_w\emptyset + \ddot{\varepsilon}_e(1-\emptyset) \qquad C.13$$

$$\lambda\,(cm) = \frac{\lambda_0}{\sqrt{\acute{\varepsilon}_{mix}}} \qquad C.14$$

$\lambda_0 = 12.2cm$

$$Tan\delta = \frac{\varepsilon''}{\varepsilon'} \qquad C.15$$

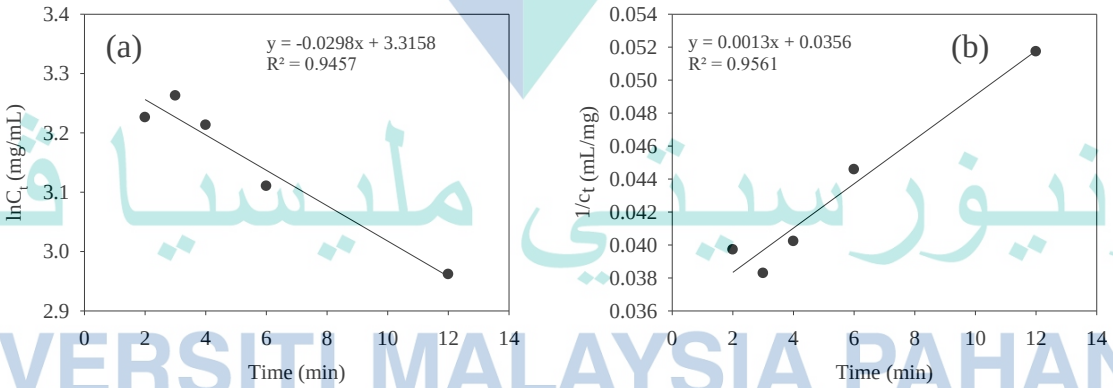
$$D_p(cm) = \frac{\lambda_0 \sqrt{\acute{\varepsilon}_{mix}}}{2\pi \ddot{\varepsilon}_{mix}} \qquad C.16$$

$\pi = 3.14$

Table C.1 Physical properties of solvent.

Properties	**Ethanol**	**Water**
Density $\boldsymbol{\rho}$ (g/cm^3)	0.789	1
Heat capacity Cp (Jg^{-1} k^{-1})	2	4.18
Density of solvent mixture $\boldsymbol{\rho}$ (g/cm^3)	0.852 for F.G	0.894 for A.V
Rate of Heat generation, Q_{mix} ($\boldsymbol{cal/s \cdot cm^3}$)	0.350 for F.G	0.426 for A.V
Volume of Phase mixture	0.309 F.G	0.505 A.V
Heat capacity of mixture, Cp, (Jg^{-1} k^{-1})	0.640 F.G	0.742 A.V
Volume fraction of solvents, V, (%) EtOH/W	60-40	40-60

F.G = Fenugreek seed; A.V = Aloe vera



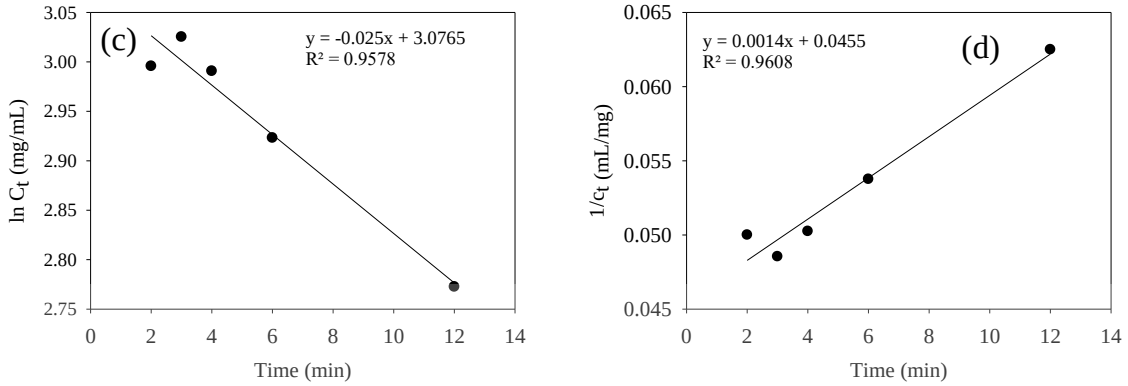


Figure C.1 First and second-order kinetic model for extraction yields of fenugreek seed (a) and (b) and Aloe vera leaves extracted via MAE (c) and (d), respectively.

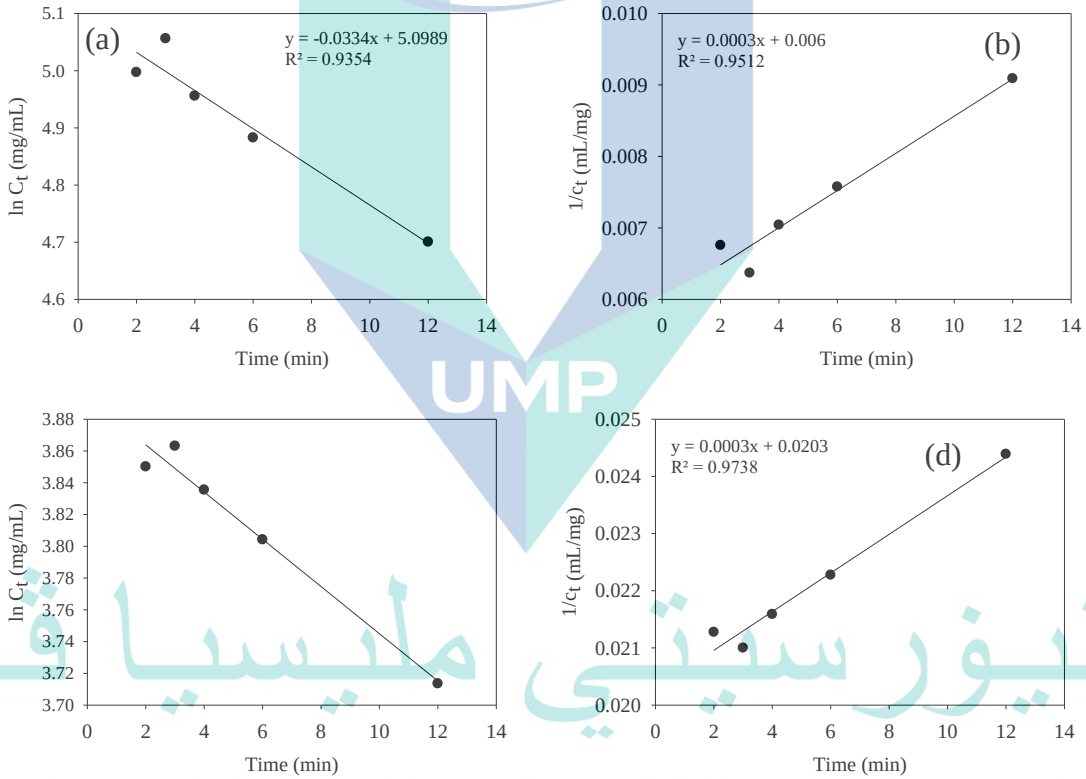


Figure C.2 First and second-order kinetic model for TSC of fenugreek seed (a) and (b) and Aloe vera leaves extracted via MAE (c) and (d), respectively.

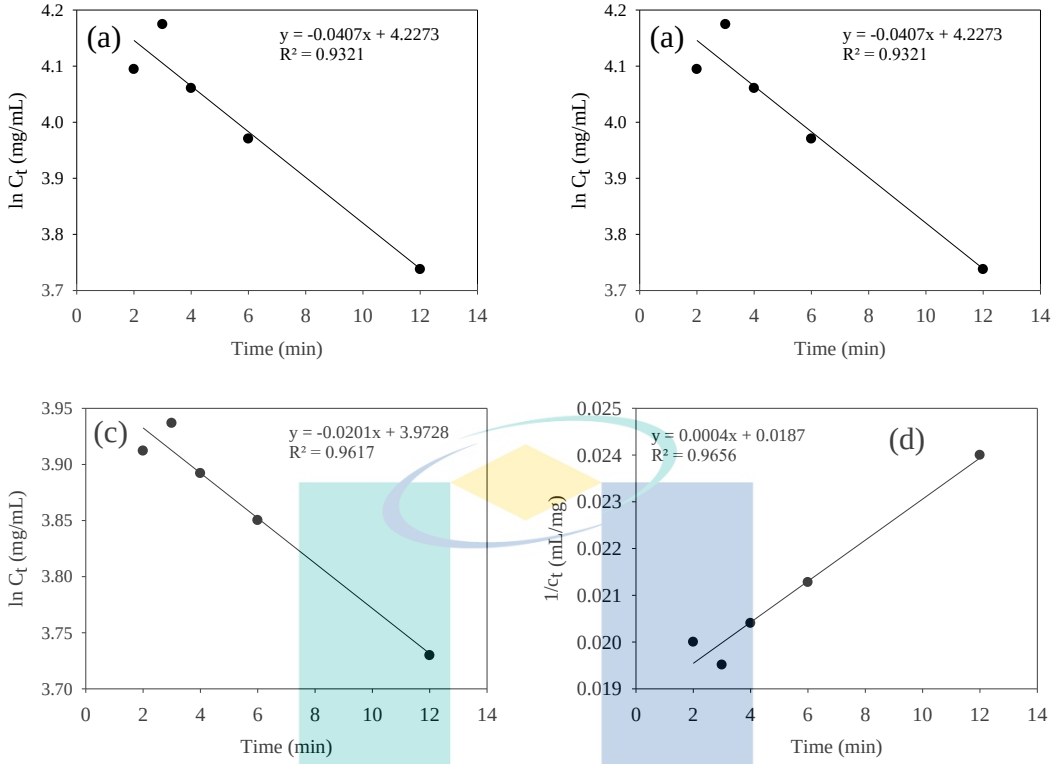


Figure C.3 First and second-order kinetic model for TPC of fenugreek seed (a) and (b) and Aloe vera leaves extracted via MAE (c) and (d), respectively.

APPENDIX D
LIST OF PUBLICATION, EXHIBITION AND CONFERENCE

Published Papers

Sweeta, A., Abdurahman, N. H., Yunus, R. M., (2020). Determination of phenolics and saponins in fenugreek seed extracted via microwave-assisted extraction method at the optimal condition. In *IOP Conference Series: Materials Science and Engineering*. IOP Publishing. **Indexed in Scopus.**

Sweeta, A., Abdurahman, N. H., & Yunus, R. M. (2019). Optimization of saponins, phenolics, and antioxidants extracted from fenugreek seeds using microwave-assisted extraction and response surface methodology as an optimizing tool. *Comptes Rendus Chimie*, *22*(11-12), 714-727. **Elsevier (IF=2.366, Q2).**

Sweeta, A., Abdurahman, N. H., Yunus, R. M., & Fayaz, F. (2019). Microwave-assisted extraction of saponin, phenolic and flavonoid compounds from Trigonella foenum-graecum seed based on two level factorial design. *Journal of Applied Research on Medicinal and Aromatic Plants*, *14*, 100212. **Elsevier (IF = 1.966, Q2).**

Sweeta, A., Abdurahman, N. H., Yunus, R. M., Alara, O. R., & Abayomi, O. O. (2019). Extraction, characterization and antioxidant activity of fenugreek (Trigonella-foenum graecum) seed oil. *Materials Science for Energy Technologies*, *2*(2), 349-355. **Elsevier indexed in (DOAJ).**

Abed, S. M., Abdurahman, N. H., Yunus, R. M., Abdulbari, H. A., & **Sweeta, A**. (2019). Oil emulsions and the different recent demulsification techniques in the petroleum industry-A review. In *IOP Conference Series: Materials Science and Engineering* (Vol. 702, No. 1, p. 012060). IOP Publishing. **Indexed in Scopus.**

Sweeta, A., Abdurahman, N. H., Yunus, R. M., Fayaz, F., & Alara, O. R. (2018). Biosurfactants—a new frontier for social and environmental safety: a mini review. *Biotechnology Research and Innovation*, *2*(1), 81-90. **Elsevier**

Olalere, O. A., Abdurahman, N. H., bin Mohd Yunus, R., Alara, O. R., & **Sweeta, A**. (2018). Evaluation of optimization parameters in microwave reflux extraction of piperine-oleoresin from black pepper (Piper nigrum). *Beni-Suef University journal of basic and applied sciences*, *7*(4), 626-631. **Elsevier**

Sweeta, A., Nour, A. H., Yunus, R. M., & Farhan, A. H. (2018). Biosurfactants as promising multifunctional agent: A mini review. *International Journal of Innovative Research and Scientific Studies*, *1*(1). **Peer-reviewed**

Under Review

Sweeta, A., Abdurahman, N. H., Yunus, R. M., (2020)**.** LC-QTOF-MS analysis of saponin and phenolic bioactive compounds extracted from Aloe vera leaves. submitted to *South African Journal of Botany*. **Elsevier (IF = 1.79, Q2).**

Exhibitions

Sweeta, A., Abdurahman, N. H., Yunus, R. M., Nor Hanuni, B, R., (2020). The potential of fenugreek seed extract for health, environment and economic extracted via microwave technology. **Creation, Innovation, Technology and Research Exposition (CITREX),** 12-13 February-2020, University Malaysia Pahang. (**Gold Medal**).

Sweeta, A., Abdurahman, N. H., Yunus, R. M., (2019). Microwave-assisted extraction of bioactive compounds from fenugreek seed and Aaloe vera Leaves. **International Festival of Innovation on Green Technology (IFINOG),** 19-21 April-2019, University Malaysia Pahang. (**Gold Medal**).

Sweeta, A., Abdurahman, N. H., Yunus, R. M., Yaseen, A., (2019). Microwave-assisted extraction of bioactive compounds from fenugreek seed. **Creation, Innovation, Technology and Research Exposition (CITREX),** 12-13 February-2019, University Malaysia Pahang. (**Silver Medal**).

Abdulrahman, H. F. F., Abdurahman, N. H, **Sweeta, A**., (2019). An environmentally friendly gum Arabic for destabilization of crude oil emulsions. **Creation, Innovation, Technology and Research Exposition (CITREX),** 12-13 February-2019, University Malaysia Pahang. (**Silver Medal**).

Sweeta, A., Abdurahman, N. H., Yunus, R. M., (2018). Characterization and formulation of bioactive compound extracted from fenugreek seed as a bio-surfactant. **International Festival of Innovation on Green Technology (IFINOG),** 20-22 April-2018, University Malaysia Pahang. (**Silver Medal**).

Conferences:

Sweeta, A., Abdurahman, N. H., Yunus, R. M. **Energy Security and Chemical Engineering Congress (ESChE, 17-19 July 2019),** Organized by University Malaysia Pahang (UMP) and CARIFF, Penang, Malaysia.

Sweeta, A., Abdurahman, N. H., Yunus, R. M. **International Conference of Chemical Engineering & Industrial Biotechnology (ICCEIB, 9-11 August-2020).** Organized by Faculty of Chemical and Process Engineering Technology, University Malaysia Pahang (UMP), Kuala Lumpur, Malaysia.