OPTIMIZATION OF XANTHONE YIELD IN MANGOSTEEN PERICARP CRUDE EXTRACT OBTAINED BY MICROWAVE-ASSISTED EXTRACTION (MAE) METHOD

By

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ABSTRACT

OPTIMIZATION OF XANTHONE YIELD IN MANGOSTEEN PERICARP CRUDE EXTRACT OBTAINED BY MICROWAVE-ASSISTED EXTRACTION (MAE) METHOD

Hiew Choi Wen

Mangosteen pericarp was studied because of its rich xanthones content which exhibit antioxidant properties, proved from previous studies. Thus, response surface methodology was used to identify the optimized parameters of the sample pre-leaching time, solvent-to-feed ratio (S/F) ratio and extraction time of mangosteen pericarp in microwave-assisted extraction (MAE) method. Effect of sample pre-leaching time, S/F ratio and extraction time of MAE procedure were investigated on the mangosteen pericarp crude extract yield, total xanthone yield, total phenolic content (TPC) and total antioxidant capacity using relevant spectrophotometric assay. Experiment was designed according to the Box-behnkan design using JMP software. The results of total xanthone and TPC fit the generated model with R^2 of 0.83 and 0.91 whereas the results of DPPH scavenging activity did not fit to the model with R^2 of 0.63. The results showed that pre-leaching time and S/F ratio significantly affected the extraction yield of total xanthones and TPC ($p < 0.05$). In addition, the results indicated that interactive effect of S/F ratio and extraction time

significantly ($p<0.05$) impacted the yield of TPC. The yield of TPC was increased as S/F ratio increased from 10:1 to 20:1 with increased extraction time from 3 to 9 minutes. These results indicated that the optimal condition for MAE of total xanthone yield and TPC were 0 hour of pre-leaching time, 20:1 of solvent-to-feed ratio and 9 minutes of extraction time. Under the optimum MAE conditions, the optimized extraction yield of total xanthone and TPC were peridcted to be 46.62 ± 5.35 mg α-mangostin/g of crude extract and 46.30 ± 3.95 mg GAE/g of crude extract which were validated by the results of the validation test under the optimized conditions obtained from response surface methodology (RSM).

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Last but not least, a sincere thanks to my family members for their encouragement in helping to complete this final year project.

DECLARATION

I hereby declare the project is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

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HIEW CHOI WEN

APPROVAL SHEET

This project report entitled "**OPTIMIZATION OF XANTHONE YIELD IN MANGOSTEEN PERICARP CRUDE EXTRACT OBTAINED BY MICROWAVE-ASSISTED EXTRACTION (MAE) METHOD**" was prepared by HIEW CHOI WEN and submitted as partial fulfilment of the requirement for the degree of Bachelor of Science (Hons) Food Science at University Tunku Abdul Rahman.

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(HIEW CHOI WEN)

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LIST OF ABBREVIATIONS

%	Percentage
μl	Microlitre
μg	Microgram
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt
BBD	Box- behnkan design
DPPH	2, 2-Diphenyl-1-picrylhydrazyl
FC reagent	Folin-Ciocalteu reagent
GAE	Gallic acid equivalent
mM	Milli Molarity
MAE	Microwave-assisted extraction
mg	Milligram
ml	Millilitre
nm	Nanometer
Na_2CO_3	Sodium carbonate
^oC	Temperature
R^2	Linear regression
RSM	Response surface methodology
RT	Room temperature
SD	Standard deviation
S/F ratio	Solvent-to-feed ratio
TEAC	Trolox equivalent antioxidant capacity
Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic

	acid
TPC	Total phenolic content
UV	Ultraviolet
vis	Visible
w/v	Weight per volume
w/w	Weight per weight

CHAPTER 1

INTRODUCTION

1.1 Background Study

Garcinia Mangostana L. is a type of fruit, consumed broadly especially within tropical regions and also called as the "Queen of fruits" (Makhonpas, et al., 2015). Mostly the fruit rinds are considered as waste and discarded. Therefore, efforts have made to utilize the waste products to reduce the waste and transform it into valuable food product (Laufenberg, Kunz and Nystroem, 2003). The mangosteen pericarp is used generally for healing wounds and skin infection and to ease diarrhea in Thailand (Mahabusarakam, Wiriyachitra and Taylor, 1987). Thus, the fruit rinds are considered as sources of nutraceutical product and healthy supplement which contains several beneficial compounds that exhibit biological activities (Karim and Azlan, 2012). The biologically-active compound of interest such as phenolic acid and their derivatives namely the anthocyanin, tannin and xanthones were discovered in mangosteen fruit (Sivakumari, et al, 2016). The secondary metabolite found in greatest amount in mangosteen rind is xanthone compound (Sivakumari, et al, 2016). The major xanthone-type compounds found are α-mangostin, γ-mangostin, and β-mangostin (Chin and Kinghorn, 2008). The α-mangostin ($C_{24}H_{26}O_5$) is yellow crystalline powder, extracted from mangosteen pericarps. These isolated secondary metabolite compounds have antioxidant, antimicrobial and anti-inflammatory effects (Chen, Yang and Wang, 2008; Sutono, 2013; Tjahjani, et al., 2014). Therefore, mangosteen pericarp was supplied by Mao Shan Wang company from their own orchard with their's interest in the study of

mangosteen pericarp. Besides, chemical substance, α-mangostin reference compound was also contributed by the company in order to study the xanthone yield obtained from mangosteen pericarp under extraction method.

1.2 Problem Statement

In the mangosteen pericarp, rich content of xanthones has been discovered with comparison to the aril part of the mangosteen fruits where xanthone exhibit strong antioxidant properties (Karim and Azlan, 2012). Thus, many studies were done to find more effective extraction methods to extract the bioactive compound in mangosteen pericarp. Satong-aun, Assawarachan and Noomhorm (2011) proved that microwave-assisted extraction (MAE) exhibit highest extraction yield of α-mangostin compared to shaking water bath extraction and soxhlet extraction. In addition, Pan, Niu and Liu (2002) proved that extraction of tanshinone compounds from *Salvia miltiorrhiza bunge* by MAE was equivalent with and, in fact higher than that of conventional extraction methods. These studies suggested that MAE is an enhanced method that could shorten extraction time and lessen the amount of solvent required thus rendering the method as environmentally friendly. Less degradation of bioactive compounds were also observed in the microwave-assisted extraction of other natural food products (Satong-aun, Assawarachan and Noomhorm, 2011; Tatke and Jaiswal, 2011). However, the extraction parameters used are found to be a fundamental factor in the MAE in order to perform extraction effectively. Since different combination of extraction parameters performed could produce different extraction yield of bioactive compound, therefore, more studies are needed for mangosteen pericarp extraction using MAE to determine the factors that affect

greatly on the yield of xanthone. In the same time, response surface methodology (RSM) is used to optimize complex processes by reducing the required experiment number (Ferreira, et al., 2007; Francis, et al., 2003).

1.3 Objectives

Therefore, in order to overcome the problems stated above, this project was conducted for the following purposes:

1. To investigate the effect of sample pre-leaching time, solvent-to-feed (S/F) ratio and extraction time of microwave-assisted extraction procedure on the yield of mangosteen pericarp crude extract, total xanthone yield, total phenolic content (TPC) and total antioxidant capacity.
2. Using RSM to identify the optimized parameters for the sample pre-leaching time, solvent-to-feed (S/F) ratio and extraction time of mangosteen pericarp extraction with microwave-assisted extraction (MAE) method.

CHAPTER 2

LITERATURE REVIEW

2.1 Mangosteen Fruits

2.1.1 Origin

The mangosteen was originated from Sunda Islands, the Moluccas and Kemaman, Malaya (Lim, 2012). The mangosteen tree was initially cultivated in Thailand which located in tropical area (Lim, 2012).

2.1.2 Anatomy

Figure 2.1: Dried mangosteen pericarp

The fruit is round in shape with dark purple colour, smooth surface externally and its rind is red in colour (Lim, 2012). These mangosteen fruits may have seeds or be seedless with soft flesh which slightly acidic in flavours (Lim, 2012). However, mangosteen pericarp turned from purple colour to brown colour after heating as shown in Figure 2.1.

2.1.3 Nutritional Value of Mangosteen Pericarp

Mangosteen (*Garcinia Mangostana* L.) consists of carbohydrates, dietary fibre, protein, fats and sodium. It is low in calories, high in fibre, free of saturated fats, contains powerful antioxidants such as xanthones, B-complex vitamins, vitamin C, potassium, sodium, manganese and magnesium. Secondary metabolites, phenolic compounds were mainly concerned in mangosteen pericarp in recent trend of studies.

2.2 Phenolic Compound

Polyphenols are phytochemicals, found abundantly as widely distributed secondary metabolites in natural plant food sources and it mainly consists of a hydroxyl group, directly bonded to an aromatic hydrocarbon group (Pandey and Rizvi, 2009). These compounds are developed through metabolic pathways include phenylpropanoid pathway and shikimic acid pathway. In addition, they are categorized into four main groups such as terpenoids, phenolic and polyphebolics, nitrogen containing alkaloids, sulphur containing compounds (Morales González, 2013). In many studies, food phenolic compounds consist nutritional interest that promoting effects of human health through the prevention against diseases, inflammation and allergies via antioxidant and proteins neutralization mechanisms, used for therapeutic purposes (Pandey and Rizvi, 2009).

2.2.1 Categorization of Phenolic Compounds

Due to different functionality of number of phenol rings, phenolic compounds can be classified into dissimilar groups. Phenolic compound can be classified

into flavonoids, tannins, chalcones and coumarins, phenolic acids and etc. In flavonoids, it can be further categorized into anthocyanin, flavonols, flavonones, flavones, flavonols and isoflavones. However, the main focus of polyphenol compound is on flavonoids compounds as they are tremendously increased interest in the study on flavonoids from food sources due to their health benefits. Furthermore, it also had been proved in many researches that flavonoids have antioxidant properties and antiviral activities on prevention of heart disease, anticancer activities and anti-inflammatory.

2.2.2 Antioxidant Properties of Mangosteen Pericarps

Free radicals contain an unpaired electron that capable of independent existence, are unstable and highly reactive by accepting an electron or donating an electron (Lobo, et al., 2010). The majority of free radicals are oxygen-free radicals and known as “reactive oxygen species” (Birben, et al., 2012). These oxygen free radicals are reacted actively in the nucleus and cell membrane structure such as proteins, carbohydrates, and lipids by changing its functions (Rahman, 2007).

Free radicals involved in radical addition and substitution which indicated for chain reactions. Initiation, propagation, and termination process are involved in chain reaction. Firstly, initiation reactions result in increase of free radicals. Secondly, propagation reactions involved free radicals in which free radicals remains the same numbers. Lastly, a more stable species is formed where termination reactions involved the combination of the two free radicals.

Premature aging of cells is associated with oxidative stress with, lead to tissue inflammation and eventually cause cell death (Klein and Ackerman, 2003). Oxidative damage by free radicals causes harmful effects on human organs (Rahman, et al., 2012). Besides, Lobo, et al. (2010) showed that the oxidative damage is subjected to cell dysfunctional and diseases to cause inflammatory condition, cancers, and aging problem.

The fundamental bioactive compounds found in mangosteens are prenylated xanthone derivatives, phenolic acid, and anthocyanins (Chaovanalikit, et al., 2012). The main phenolic acid in the peel and rind is protocatechuic acid, whereas the main phenolic acid in the aril part is p-hydroxybenzoic acid (Haponiuk, Pieńkowska and Zadernowski, 2007). The mangosteen fruit contains various phenolic compounds such as tannins, flavonoids and xanthones, supporting its medical properties (Chaovanalikit, et al., 2012; Pothitirat, et al., 2009).

Xanthones are categorized into various groups such as xanthonolignoids, xanthone glycosides, prenylated xanthones and simple oxygenated xanthones. Three ring system including methoxy, isoprene and phenyl groups are the chemical structure of a xanthone (Shan, et al., 2011). Functional hydroxyl groups exhibit their antioxidant effects by chelating metal ions and scavenging free radicals.

However, the mangosteen has been noticed to contain rich source of xanthones typically in pericarps and the main concern of this study was focused on

xanthone compound and its derivative, γ-mangostin and α-mangostin. Moreover, structure of xanthones exhibit its functionality as antioxidant, anti-inflammatory and anti-cancer effects in many studies (Bumrungpert, et al., 2010; Johnson, et al., 2012). The major xanthone, α-mangostin was found mainly in mangostern fruit rind to exhibit antioxidant activity (Pothitirat, et al., 2012). The molecular formula of α-mangostin is $C_{24}H_{26}O_6$. It is an aromatic compounds containing xanthene conjugated to a ketone group at carbon 9.

2.2.3 Total phenolic content (TPC) Assays

TPC is generally measured using the Folin-Ciocalteu (FC) method. The reagent usually includes sodium molybdate and sodium tungstate. A blue colour solution is formed during transportation electrons from phenolic compounds to phosphomolybdic acid complexes (Everette, et al., 2010). Once the mixture reagent reacts with phenols in sample, it forms a blue color solution in the end. The FC assay used to measure total phenolic in natural products with oxidation and reduction reaction. The absorbance was determined at 765 nm by spectrophotometer.

2.2.4 Antioxidant Assays

Two modes of action such as hydrogen atom transfer and single electron transfer mainly applied in antioxidant capacity and both mechanisms are used for detection of antioxidant action (Moharram and Youssef, 2014; Rivero-Pérez, Muñiz and González-Sanjosé, 2007).

The HAT-based assays such as ABTS radical scavenging measure the capability of an antioxidant by hydrogen atom donation whereas the SET-based assays such as DPPH free radical scavenging, used to estimate the capacity of antioxidant compound in plant food matrix (Moharram and Youssef, 2014). DPPH free radical scavenging assay is rapid, simple and inexpensive compared to other test models such as ABTS radical scavenging (Alam, Bristi and Rafiquzzaman, 2013).

DPPH Free Radical Scavenging Assay

DPPH is used to determine the occurrence of a purple colour, with maximum absorption at 517 nm because of the delocalization of the electron over molecule (Pisoschi and Negulescu, 2012). When DPPH is mixed with substrate that acts as hydrogen donor, the reduced form of solution would form with the loss of violet colour by changing into yellow colour (Alam, Bristi and Rafiquzzaman, 2013). The absorbance depends on the antioxidant concentration of food products. Therefore, the optical density of DPPH radicals is observed to evaluate the antioxidant potential. Trolox is used as standard antioxidant to compare with sample product and DPPH scavenging values are expressed as Trolox equivalent in purpose for tabulation of data (Zou, et al., 2011).

2.3 Microwave-assisted Extraction (MAE)

MAE is a heating process involved solvent in contact with a solid particle of sample using microwave energy in purpose to division analysts from bioactive compound into the solvent (Sparr Eskilsson and Björklund, 2000).

2.3.1 Mechanism of microwave-assisted extraction (MAE)

MAE process exhibits electromagnetic waves to change the cell structure. In MAE, acceleration of process and higher extraction yield might be because of same direction interactive combination of mass and heat gradients (Veggi, Martinez and Meireles, 2012). In contrast, the conventional extraction relied on interactive opposite direction combination of heat and mass gradients.

A series of separation steps occur during the interaction between solid particles and solvent. The first step is entry of the solvent into the solid matrix, followed by disruption of components. Then, the following step is the solute is transferred out of the solid matrix, followed by moving out the extracted solute from solid into solution (Veggi, Martinez and Meireles, 2012). The last step is segregation and release of the extract and solid.

Two mechanisms include dipole rotation and ionic conduction are carried out throughout microwave heating process and these two mechanisms occurred simultaneously dipoles (Veggi, Martinez and Meireles, 2012). Electrophoresis migration occurs when electromagnetic field is applied. The friction result heating of the solution and dipole rotation occurs to cause rearrangement of dipoles (Veggi, Martinez and Meireles, 2012).

2.3.2 Importance of microwave-assisted extraction (MAE) Parameters

A) Pre-leaching Time with Constant Agitation

Leaching is defined as contact of solid and liquid where the solute can diffuse into the solvent, results in segregation of the components. The agitation used in MAE fastens the extraction by improved dissolution and desorption of active compounds between solvent and sample matrix (Veggi, Martinez and Meireles, 2012). The disadvantages of using low S/F ratio can be reduced with sufficient solvent through stirring mode of action.

Yashwant Malode, et al. (2013) showed that pre-leaching at RT increased the extraction rate of tanshinones and reach maximum yield at pre-leaching of 45 minutes. Apart from that, both studies indicated that 20 minutes of pre-leaching time allows enough swelling efficiency of the plant matrix on the extraction performance on of mangiferin content and bioactive flavonolignan - silybinin (Kullu, et al., 2013). Furthermore, a research showed that the extraction of polyphenols and caffeine was increased when the pre-leaching time was reached 90 min at room temperature (Pan, Niu and Liu, 2003). Therefore, it showed that pre-leaching time could be one of the factors that affect the extraction of a bioactive compound and it acts dependently or independently with others extraction parameters.

B) Extraction Time

The time of microwave heating is another influential parameter in MAE. Extraction times in MAE are relatively short with comparison to others extraction techniques such as Soxhlet extraction. It is because of the dielectric

properties of the solvent used in extraction (Veggi, Martinez and Meireles, 2012). Solvents may heat up enormously on greater exposure, thus originating possible thermal degradation and oxidation of target bioactive compound if exceeds 30 minutes of heating. Thus, the extraction time usually differ from range of 3 minutes to maximum of 30 minutes (Veggi, Martinez and Meireles, 2012).

According Fang, et al. (2011), the best conditions for xanthones extraction by MAE were using 10 minutes of extraction time in combination of other parameters. Besides, other studies such as Bai, et al. (2007) and Yoswathana and Eshtiaghi (2015) concluded that the optimum conditions for total yield triterpenoids (TTP) and xanthone were using 30 minutes of extraction time in combination of other parameters.

C) Use of Solvent and Solvent-to-feed (S/F) Ratio

The fundamental important parameters that impact MAE process is choice of solvent used because proper solvent can enhance the efficiency of extraction process. The solubility of the compounds of interest and dielectric constant are factors for selection of solvent (Veggi, Martinez and Meireles, 2012). The solvent choice depends on the capacity of the solvent for absorption of the microwave energy. Solvents such as hexane does not produce heat when heat is submitted to them due to its transparency to electromagnetic waves whereas ethanol is considered as a good microwave absorbing solvent compared to other solvent such as water and methanol. Polar solvents can be added into poor microwave absorbers to enhance the extraction efficiency because

microwave energy can sufficiently heat the solvent which is not transparent as hexane.

Moreover, the solvent volume must be enough to cover the sample in which the sample is soaked in the solvent throughout the microwave process. Apart from that, solvent volume is an essential factor because high S/F ratio indicates greater energy and time are needed to remove solvent used by purification procedure. It caused indirectly increasing electricity and labour cost. However, low S/F ratio might result lower recovery due to non-uniform exposure to microwave heating.

Ratio range of 10:1 to 20:1 was considered to obtain best condition for extraction in MAE. Fang, et al. (2011) illustrated the best conditions for xanthones extraction by MAE were using ratio of 10:1 of S/F ratio. Besides, Bai, et al. (2007) concluded that the optimum conditions for the ratio of triterpenoids in total yield were using ratio of 15:1 of S/F ratio.

2.3.3 Advantages of microwave-assisted extraction (MAE) Compared to Other Conventional Extraction

Conventional extractions such as soxhlet and reflux method requires large volume of solvent to increases the extraction recovery and very time consuming since it need longer extraction time compared to MAE. The used of greater volume of solvent and greater extraction time not only lead to environmental problems but also increase operating costs. Soxhlet extraction generally required whole day for extraction, certain amount of waste was

produced and thermolabile compounds degradation (Blicharski and Oniszczuk, 2017). However, MAE gives greater rate of extraction performance and thermo-labile constituents' protection in contrast to those conventional extraction techniques (Tatke and Jaiswal, 2011). MAE is defined as green technology that can reduce the environmental problem that is created by conventional extraction.

However, soxhlet extraction also has its advantages such as high extraction temperature are maintained, no filtration requirement after extraction and it is very simple and cheap to operate (Wang and Weller, 2006).

2.3.4 Drying Treatment in Sample Preparation

Drying or dehydration treatment refers to the application of heat to food sample to remove moisture to stay in stable form. Drying treatment used in sample preparation generally includes, air-drying, freeze drying, microwaving, baking, grilling and roasting. Fresh sample such as medical herbs and fruits are usually required for dehydration and long time storage during production.

Freeze-drying is the better method to retain high quality of final product by removing water compared to other drying methods such as air drying and vacuum drying. Previous study showed that freeze-drying method obtained higher extraction of biologically active compounds of different food compound comparison to others dehydration methods (Dorta, Lobo and González, 2012). The mechanism of freeze-drying is drying followed by sublimation process of

food. However, freeze-drying is an expensive process compared to conventional drying.

Satong-aun, Assawarachan and Noomhorm (2011) concluded that the suitable temperature for drying mangosteen pericarp powder was 65 ℃ in order to obtain highest retention yield of mangosteen pericarp crude extract and α-mangostin content was obtained. It indicated that greater exposure of drying temperature and time might cause degradation of α-mangostin content in mangosteen pericarp. In addition, Zarena and Udaya Sankar (2009) used temperature range of 40 to 52 ℃ to dry the fresh pericarp of mangosteen.

Drying is a method used to protect phytochemical in way to preserve food quality and bioactivity of food products (Mediani, et al., 2014). Furthermore, this process can reduce the cost of transportation and storage costs due to reduction of water content (Mediani, et al., 2014). Dehydration inactivates the enzymes in the mangosteen pericarps as some enzyme-catalysed reaction would lead to unacceptable change to food such as flavour changing and cause degradation by polyphenol oxidases in term of storage. Besides, drying over certain temperature can inactivate pathogenic or spoilage microorganisms due to insufficient of moisture content to support growth of microorganism in mangosteen pericarps. Drying also increases the shelf life but also retains the nutrients adequately, especially biologically active compound in food matrix (Agoreyo, et al., 2011). Thus, drying is concluded as a thermal process with its purpose to inactivate bioactive components, as well as maintain nutrients and quality attributes (Agrahar-Murugkar and Jha, 2010).

However, over-heating causes destruction of biologically active compound and antioxidant activity in the fresh food matrix. Protein losses its biological activity if the heating temperature exceeds its optimum temperature. Researchers have shown nutrient loss during heat treatment of food as well as colour alteration (Wojdyło, et al., 2013). Over processing can cause irreversible structure changing to the cell wall polysaccharides (Manjarres-Pinzon, Cortes-Rodriguez and Rodríguez-Sandoval, 2017).

2.4 Response Surface Methodology (RSM)

RSM, a mathematical model from industrial perspective is useful to optimize complex processes includes many extraction parameters and its interactions affect those responses to optimize condition and maximize the result obtained (Ferreira, et al., 2007; Francis, et al., 2003). Besides, RSM is also used to study the correlation between responses and factors to reduce required experiment number. Therefore, RSM is subjected to identify the optimum conditions of variables that impact on response over certain of aspect (Baş and Boyacı, 2007). This optimum condition requires validation support of good fitting model (Baş and Boyacı, 2007).

2.4.1 Box- behnkan design (BBD)

The use of the BBD needs three levels for each factor with transformed value of −1, 0, 1 with 3*k* factorial design. This design can reduce the number of experiment conducted with combination of three extraction parameters.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant Materials

Mangosteen pericarp of mixed species such as *Garcinia Mangostana* L. and *Garcinia Mangostana 'Mesta'* was supplied by Mao Shan Wang Durian Sdn. Bhd. Company from their own orchard.

3.1.2 Chemicals

Table 3.1 showed all the chemicals used.

Table 3.1: List of chemicals and reagents used.

Chemicals and reagents	**Manufacturer**
FC reagent	Merck
2, 2-Diphenyl-1-picrylhydrazyl (DPPH)	SIGMA-ALDRICH
6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX)	SIGMA-ALDRICH
99% methanol	SIGMA-ALDRICH
α-mangostin reference compound	Tokyo Chemical Industry Co., Ltd.
Gallic acid	R & M Chemicals
Sodium carbonate anhydrous	R & M Chemicals

3.1.3 Equipment

Table 3.2 showed all the equipments used.

Table 3.2: List of equipments used.

Equipments	Company
Anaytical balance	Mettler-Toledo Inc.
Centrifuge machine	Sartorius
Domestic microwave oven	Electrolux
Drying oven	BINDER
Grinder	M2 Scientifics
Micropipette	Mettler-Toledo Inc.
Moisture analyzer	Mettler-Toledo Inc.
Refrigerator	Samsung
Shaking incubator	Jenawi Scientific Sdn. Bhd.
UV-Vis Spectrophotometer	Thermo Scientific
Vortex mixer	Scientific Industries Inc.

3.2 Methods

3.2.1 Sample Preparation

The fresh mangosteen pericarps were stored at -20 ^{o}C refrigerators. The softer part of the mangosteen exocarps were taken out using common kitchen utensils and the hardened outer layer was discarded. The fresh mangosteen pericarps were then crushed into smaller pieces using common kitchen processor. The soft mangosteen pericarps were dried at 60 ^{o}C using drying oven for 3 hours regarding to the modified condition as described by Satong-aun, Assawarachan and Noomhorm (2011) to obtain 10 to 15 % moisture content. After drying, the

dried mangosteen pericarps were stored in dry, dark and cool place until further use.

3.2.2 Microwave-assisted Extraction

Before extraction, the dried mangosteen pericarps were ground into powder of particle size <1mm using an analytical grinder. In this experiment, there were 3 different parameters tested in the arrangement as below:

- Sample pre-leaching time: 0, 1 and 2 hours
- Solvent-to-Feed (S/F) ratio: 10:1, 15:1, 20:1
- Extraction time: 3, 6 and 9 minutes

In each test, an amount of mangosteen pericarp powder was mixed with 18 ml distilled water to obtain the S/F ratio as set above and transferred to a 50 ml conical flask. After that, the mixture was gently agitated on a shaker incubator at RT and 250 rpm according to the set pre-leaching time as above. After incubation, the mixture was transferred to a 50 ml centrifuge tube. Extraction was then carried out in a domestic microwave under the different extraction time as set above with 270 W of power setting. After the microwave process, the centrifuge tubes were let to cool down by standing those tubes with rack and cool with ice water. The homogenates were then centrifuged at 6000 rpm and 25 ^{o}C for 15 minutes. After centrifugation, supernatant was collected and filtered using Whatmann filter paper and through a funnel. The filtrate was collected in a petri dish and put into drying oven at 40 ^{o}C to evaporate the water. The dried crude extract obtained was stored in the -20 ℃ freezer prior to further analysis.

3.3 Analysis of Result

3.3.1 Determination of Crude Extract Yield

The dried crude extract of the mangosteen pericarps was weighed. Then, the weight of the dried crude extract from each sample was recorded and calculated as gram of crude extract per gram of sample mass. The results were reported in percentage. The formula used was as following:

Yield of extraction (%) = (amount of dried crude extract/sample mass) × 100 %

3.3.2 Determination of Xanthone Content

The xanthones content in the mangosteen pericarp sample was analyzed following the method used by Aisha, et al. (2013). A stock solution of α-mangostin reference compound was first prepared in methanol at 100 μg/mL and was further diluted to obtain the standard solutions at 16, 12, 8, 4, and 1 μ g/ml. Linearity was determined by using the alpha-mangostin reference compound at 1 to 16 μg/ml. The calibration curve was constructed by plotting the optical density versus concentration and regression analysis was employed to determine the linearity of calibration curves. Results were reported in α-mangostin equivalent.

Optical density of dried crude extracts was taken at 16 µg/ ml by dissolving the dried crude extract into methanol. The absorbance was then measured at 244 nm. Samples were done in triplicates for a more reliable result. The concentration of xanthones contained in the crude extract was then read from α-mangostin calibration curves and was reported in mg alpha-mangostin/ g crude extract used.

3.3.3 Determination of total phenolic content (TPC)

The phenolic content in the mangosteen pericarp sample was analyzed using a modified FC colorimetric method used by Chai and Wong (2012). A stock solution of Gallic acid reference compound was first prepared in methanol at 1 mg/ml, diluted to 100 μg/ml and was further diluted to obtain 10, 20, 30, 40, 60 and 80 μg/ml. Linearity was determined by using the Gallic acid reference compound at 10 to 100 μg/ml. The calibration curve was constructed by plotting the optical density versus concentration and regression analysis was employed to determine the linearity of calibration curves. Results were reported in Gallic acid equivalent (GAE).

First, a mixture of 0.2 ml of sample extract, 0.8 ml of deionized water and 0.1 ml of FC reagent was initially incubated at RT for 3 minutes. Then, 0.3 ml of 20 % w/v Na_2CO_3 was added into the mixture and incubated for 2 hours. The reaction mixture was mixed thoroughly to ensure the FC reagent reacted completely with the oxidised phenolates in the sample. After 2 hours of incubation, the intensity of blue colour was then measured at 765 nm using UV-vis spectrophotometer. Samples were done in triplicated for a more reliable result. The phenolic content in the crude extract was then read from Gallic acid calibration curves and was reported in mg GAE/ g crude extract used.

3.3.4 Determination of DPPH Scavenging Activity

This assay followed the procedure of Chai and Wong (2012) with some modifications. The amount of DPPH, crude extract and stock solution added

were changed from 1 ml to 0.5 ml. A stock solution of Trolox reference compound was first prepared in methanol at 1 mg/ml, diluted to 100 μg/ml and was further diluted to obtain 16, 12, 8, 4, and 1 μg/ml. Linearity was determined by using the Trolox reference compound at 1 to 16 μg/ml. The calibration curve was constructed by plotting the optical density versus concentration and regression analysis was employed to determine the linearity of calibration curves. Results were reported in Trolox equivalent antioxidant capacity (TEAC).

First, 0.5 ml of crude extract and 0.5 ml of DPPH solution (0.10 mM in methanol) were mixed together. Before measurement of absorbance at 517 nm, the mixture was shaken vigorously and incubated at RT for 30 minutes in the dark. A blank was prepared for each sample in which the DPPH solution was replaced with methanol. A control was prepared for each sample in which the crude extract was replaced with methanol. Triplicate for each sample and control were done for a more consistent and accurate result. The antioxidant capacity in the crude extract was then read from Trolox calibration curves and was reported in mg TEAC/g crude extract used. DPPH radical scavenging ability (%) was calculated as follows:

DPPH radical scavenging ability (%) = $(1 - [A_{sample}/ A_{control}]) \times 100$

3.4 Statistical Analysis

Statistical analyses were conducted with JMP software. Data were shown as means with standard deviations. Tukey t-test and pair t-test were performed followed by ANOVA. The data showed significant differences when $p < 0.05$.

The reliability of the results obtained was then analyzed. All the data were presented in table format with support of figure for interpretation.

3.5 Experimental Design for Optimization

RSM software was applied *via* JMP software with a BBD. The JMP was entitled to build 2D and 3D plots graph of the responses. Regarding to the Table 3.3, the optimization of responses with three extraction parameters at three levels are 15 runs with 3 central points. Experimental data were subjected to the equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3$$

where b_0 = intercept; b_1, b_2 and b_3 = linear coefficients; b_{11}, b_{22} and b_{33} = squared coefficients; b_{12}, b_{13} and b_{23} = interaction coefficients.

The optimized parameters were then found evaluated using RSM. The validation test was tested after the extraction parameters were optimized and obtained from RSM. Then, triplicate of experimental data were obtained to validate predicted values under optimized conditions.

Table 3.3: Independent variables with levels in Box- behnkan design.

Independent variable	**Levels**		
	Low	**Medium**	**High**
Pre-leaching time (hr) (X_1)	0	1	2
Solvent-to-Feed (S/F) ratio (X_2)	10:1	15:1	20:1
Extraction time (min) (X_3)	3	6	9
Transform value	-1	0	+1

Table 3.4: Composition of parameters and responses according to Box-behnkan design (BBD).

Parameters	Independent variable			Response		
	X_1*	X_2*	X_3*	Yield of total xanthone (Y_1)	Total phenolic content (Y_2)	DPPH scavenging activity (Y_3)
1	0	10:1	6			
2	2	15:1	6			
3	0	20:1	6			
4	2	20:1	6			
5	0	15:1	3			
6	2	15:1	3			
7	0	15:1	9			
8	2	15:1	9			
9	1	10:1	3			
10	1	20:1	3			
11	1	10:1	9			
12	1	20:1	9			
13	1	15:1	6			
14	1	15:1	6			
15	1	15:1	6			

*X_1: pre-leaching time, (hr), X_2: S/F ratio and X_3: extraction time, (min)

CHAPTER 4

RESULT

4.1 Determination of Crude Extract Yield

The yield of crude extract according to the combination of different experimental conditions was shown in Table 4.1. The highest crude extract yield of mangosteen pericarp obtained was 19.43 ± 0.56 % under 0 hour of per-leaching time, S/F ratio of 20:1 and extraction time of 6 minutes of extraction conditions followed by 18.92 ± 0.54 % of 0 hour of per-leaching time, S/F ratio of 15:1 and extraction time of 9 minutes, 18.42 ± 0.51 % of 0 hour of per-leaching time, S/F ratio of 15:1 and extraction time of 3 minutes and 18.03 ± 0.93 % of 1 hour of per-leaching time, S/F ratio of 20:1 and extraction time of 9 minutes.

The lowest crude extract yield of mangosteen pericarp obtained was 14.25 ± 0.66 % under 1 hour of per-leaching time, S/F ratio of 10:1 and extraction time of 9 minutes of extraction conditions, followed by 14.39 ± 0.37 % of 2 hours of per-leaching time, S/F ratio of 10:1 and extraction time of 6 minutes, 14.53 ± 0.58 % of 1 hour of per-leaching time, S/F ratio of 10:1 and extraction time of 3 minutes and 14.89 ± 0.61 % of 2 hours of per-leaching time, S/F ratio of 15:1 and extraction time of 9 minutes.

Table 4.1: Extraction yield (%) of mangosteen pericarp crude extract.

Run	Experimental condition			Extraction yield (%)
	X_1*	X_2*	X_3*	
1	0	10:1	6	15.81 ± 0.14^{a}
2	2	10:1	6	14.39 ± 0.37^{b}
3	0	20:1	6	19.43 ± 0.56^{c}
4	2	20:1	6	15.06 ± 1.30^{ab}
5	0	15:1	3	18.42 ± 0.51^{c}
6	2	15:1	3	16.01 ± 0.80^{a}
7	0	15:1	9	18.92 ± 0.54^{c}
8	2	15:1	9	14.89 ± 0.61^{b}
9	1	10:1	3	14.53 ± 0.58^{b}
10	1	20:1	3	17.76 ± 0.52^{d}
11	1	10:1	9	14.25 ± 0.66^{b}
12	1	20:1	9	18.03 ± 0.93^{c}
13	1	15:1	6	15.43 ± 0.78^{ab}
14	1	15:1	6	16.07 ± 0.89^{a}
15	1	15:1	6	17.27 ± 0.10^{d}

*X_1: pre-leaching time; X_2: S/F ratio; X_3: extraction time

Note: Data are shown as mean of triplicate values with standard deviations.

Note: Data that are not sharing the same superscript across row are significantly different ($p < 0.05$).

4.2 Optimization of Microwave-assisted Extraction Condition

4.2.1 Modelling and Model Fitting using Response Surface Methodology

Determination of model fitting of the total xanthones yield, total phenolic content (TPC) and DPPH scavenging activity were shown in Table 4.2. The result showed that the model of DPPH scavenging activity was not adequate to predict the value since Prob > F value is less than 0.05 whereas the values of total xanthone yield and total phenolic content (TPC) were fit to the model since Prob > F value was more than 0.05.

Table 4.2: Determination of model fitting of total xanthone yield, total phenolic content (TPC) and DPPH scavenging activity.

	Total xanthone yield	**TPC**	**DPPH scavenging activity**
R^2	0.830689	0.912167	0.631381
Adjusted R^2	0.787152	0.889582	0.536594
F ratio of Model	0.7804	1.7668	5.9326
Prob > F	0.5136	0.1733	0.0025

Note: Prob > F value less than 0.05 indicates the value does not fit the model.

A) Total Xanthone Yield Model

Alpha-mangostin equivalent value with R^2 = 0.9997 was used to report total xanthone of the mangosteen pericarp extract. The experimental and predicted values obtained through response surface methodology (RSM) for total xanthone yield was shown in Table 4.3. Figure 4.1 illustrated the relationship between actual and predicted values of total xanthone. In Table 4.3, the combination of experimental conditions that obtained the highest total xanthone yield from predicted value was 44.95 mg α-mangostin/g of crude

extract under 0 hour of pre-leaching time, S/F ratio of 15:1 and extraction time of 9 minutes of extraction conditions. However, the combination of experimental conditions that obtained the lowest total xanthone yield from predicted value was 21.13 mg α-mangostin/g of crude extract under 2 hours of pre-leaching time, S/F ratio of 10:1 and extraction time of 6 minutes of extraction conditions.

The combination of experimental conditions that obtained the highest total xanthone yield from experimental values was 45.01 ± 3.79 mg α-mangostin/g of crude extract under 0 hour of pre-leaching time, S/F ratio of 20:1 and extraction time of 6 minutes of extraction conditions, followed by 43.84 ± 4.01 mg α-mangostin/g of crude extract of 0 hour of pre-leaching time, S/F ratio of 15:1 and extraction time of 9 minutes, 42.18 ± 1.19 mg α-mangostin/g of crude extract of 0 hour of pre-leaching time, S/F ratio of 15:1 and extraction time of 3 minutes, 40.64 ± 7.13 mg α-mangostin/g of crude extract of 0 hour of pre-leaching time, S/F ratio of 10:1 and extraction time of 6 minutes. However, the combination of experimental conditions that obtained the lowest total xanthone yield from experimental value was 20.67 ± 4.06 mg α-mangostin/g of crude extract under 2 hours of pre-leaching time, S/F ratio of 10:1 and extraction time of 6 minutes of extraction conditions.

Predicted value of various combinations of experimental extraction conditions are mostly in the range of experimental value obtained. The highest and lowest of experimental values and predicted values were matched to each other.

The equations could be fit into the model as below:

$$\mathbf{Y}_{\mathbf{Total\ xanthone}} = 28.224444 - 8.1975X_1 + 3.5170833X_2 + 0.48625X_3 + 0.7266667X_1X_2 - 0.398333X_1X_3 + 0.7225X_2X_3 + 7.1890278\,X_1^2 - 1.845139X_2^2 + 0.4581944X_3^2$$

where X_1, X_2 and X_3 are the coded variables for pre-leaching time and S/F ratio and extraction time respectively.

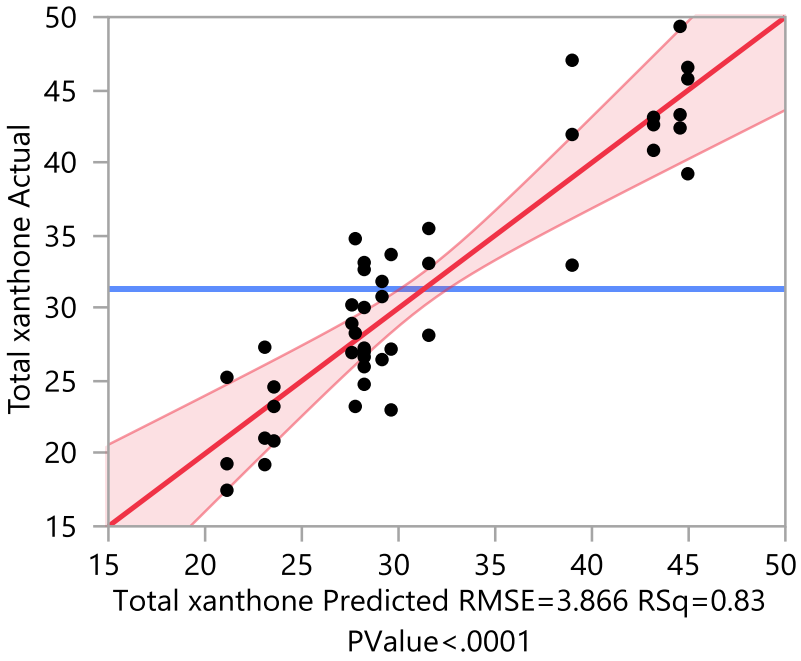

Figure 4.1: The relationship between actual and predicted values of total xanthone yield.

Table 4.3: Experimental results and predicted results of total xanthone yield of various experimental conditions obtained from mangosteen pericarp crude extract.

Run	Experimental conditions			Experimental value	Predicted value
	X_1*	X_2*	X_3*	Total xanthone (mg α-mangostin/g of crude extract)	
1	0	10:1	6	40.64 ± 7.13^{a}	38.98
2	2	10:1	6	20.67 ± 4.06^{c}	21.13
3	0	20:1	6	45.01 ± 3.79^{a}	44.56
4	2	20:1	6	27.95 ± 5.38^{b}	29.61
5	0	15:1	3	42.18 ± 1.19^{a}	43.18
6	2	15:1	3	28.70 ± 1.65^{b}	27.59
7	0	15:1	9	43.84 ± 4.01^{a}	44.95
8	2	15:1	9	28.76 ± 5.78^{b}	27.76
9	1	10:1	3	22.90 ± 1.87^{bc}	23.56
10	1	20:1	3	29.69 ± 2.85^{b}	29.15
11	1	10:1	9	22.53 ± 4.23^{bc}	23.08
12	1	20:1	9	32.22 ± 3.74^{b}	31.56
13	1	15:1	6	29.89 ± 2.82^{b}	28.22
14	1	15:1	6	26.82 ± 0.37^{b}	28.22
15	1	15:1	6	27.95 ± 4.53^{bc}	28.22

*X_1: pre-leaching time (hr); X_2: S/F ratio (ml/g); X_3: extraction time (min)
Note: Data are shown as mean of triplicate values with standard deviations.
Note: Data that are not sharing the same superscript across row are significantly different ($p < 0.05$).

B) Total phenolic content (TPC) Model

Gallic acid equivalent value with $R^2 = 0.9994$ was used to report total phenolic content (TPC) obtained from mangosteen pericarp crude extract. The experimental and predicted values obtain through response surface methodology (RSM) for total phenolic content (TPC) was shown in Table 4.4. Figure 4.2 indicated the relationship between actual and predicted values of total phenolic content (TPC). The combination of experimental conditions that obtained the highest TPC from predicted value was 43.62 mg GAE/g of crude extract under 0 hour of pre-leaching time, S/F ratio of 15:1 and extraction time of 9 minutes of extraction conditions. However, the combination of experimental conditions that obtained the lowest TPC from predicted value was 15.70 mg GAE/g of crude extract under 2 hours of pre-leaching time, S/F ratio of 10:1 and extraction time of 6 minutes of extraction conditions.

The combination of experimental conditions that obtained the highest TPC from experimental value was 45.40 ± 2.57 mg GAE/g of crude extract under 0 hour of pre-leaching time, S/F ratio of 15:1 and extraction time of 9 minutes of extraction conditions. However, the combination of experimental conditions that obtained the lowest TPC from experimental value was 16.23 ± 1.19 mg GAE/g of crude extract under 2 hours of pre-leaching time, S/F ratio of 10:1 and extraction time of 6 minutes of extraction conditions.

Predicted value of various combinations of experimental extraction conditions are mostly in the range of experimental value obtained. The highest and lowest of experimental values and predicted values were matched to each other.

The equations could be fit into the model as below:

$$\mathbf{Y_{Total\ Phenolic\ Content\ (TPC)}} = 23.89778 - 9.395833X_1 + 2.7866667X_2 + 1.005X_3 + 1.0158333X_1X_2 - 0.395833\ X_1X_3 + 2.3625X_2X_3 + 6.4548611X_1^2 - 1.450139X_2^2 + 2.4715278X_3^2$$

where X_1, X_2 and X_3 are the coded variables for pre-leaching time and S/F ratio and extraction time respectively.

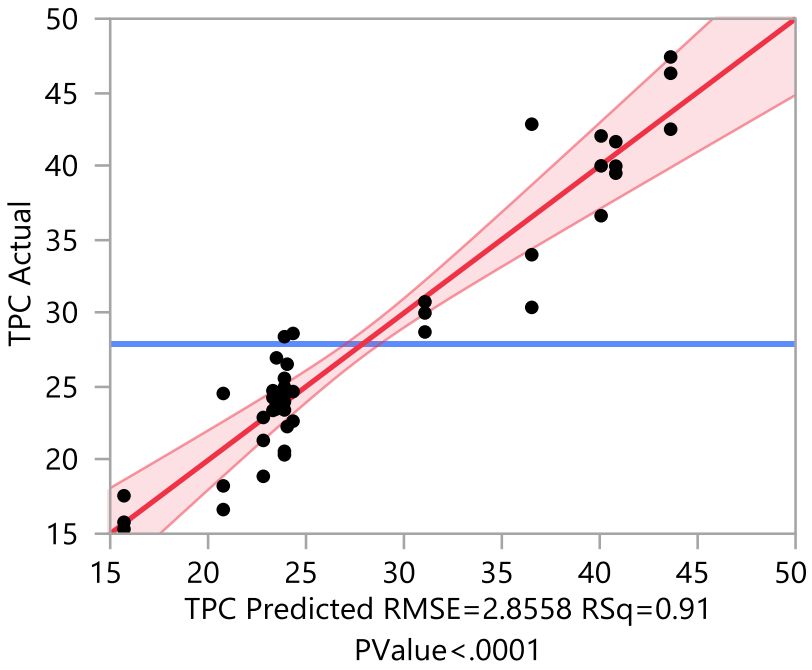

Figure 4.2: The relationship between actual and predicted values of total phenolic content (TPC).

Table 4.4: Experimental results and predicted results of total phenolic content (TPC) of various experimental conditions obtained from mangosteen pericarp crude extract.

Run	Experimental conditions			Experimental result	Predicted value
	X_1*	X_2*	X_3*	TPC (mg GAE/ mg crude extract)	
1	0	10:1	6	35.72 ± 6.41^{ab}	36.53
2	2	10:1	6	16.23 ± 1.19^{c}	15.70
3	0	20:1	6	39.54 ± 2.74^{a}	40.07
4	2	20:1	6	24.12 ± 0.68^{b}	23.31
5	0	15:1	3	40.37 ± 1.13^{a}	40.82
6	2	15:1	3	21.05 ± 2.02^{bc}	22.82
7	0	15:1	9	45.40 ± 2.57^{d}	43.62
8	2	15:1	9	24.48 ± 2.13^{b}	24.04
9	1	10:1	3	24.74 ± 1.92^{b}	23.49
10	1	20:1	3	25.31 ± 1.04^{b}	24.34
11	1	10:1	9	19.81 ± 4.17^{bc}	20.78
12	1	20:1	9	29.82 ± 1.04^{b}	31.07
13	1	15:1	6	25.61 ± 2.42^{b}	23.90
14	1	15:1	6	24.11 ± 1.26^{b}	23.90
15	1	15:1	6	21.97 ± 2.59^{b}	23.90

*X_1: pre-leaching time (hr); X_2: S/F ratio (ml/g); X_3: extraction time (min)
Note: Data are shown as mean of triplicate values with standard deviations.
Note: Data that are not sharing the same superscript across row are significantly different ($p < 0.05$).

C) DPPH scavenging activity Model

Trolox equivalent antioxidant capacity value with R^2 = 0.9859 was used to report DPPH scavenging activity of the mangosteen pericarp extract. Table 4.5 illustrated the experimental and predicted values obtain through response surface methodology (RSM) for DPPH scavenging activity. Figure 4.3 indicated the relationship between actual and predicted values of DPPH scavenging activity. The combination of experimental conditions that obtained highest total phenolic content (TPC) from predicted value was 510.73 mg TEAC/g of crude extract under 0 hour of pre-leaching time, S/F ratio of 15:1 and extraction time of 3 minutes of extraction conditions. However, the combination of experimental conditions that obtained lowest total phenolic content (TPC) from predicted value was 214.37 mg TEAC/g of crude extract under 1 hour of pre-leaching time, S/F ratio of 15:1 and extraction time of 6 minutes of extraction conditions.

The combination of experimental conditions that obtained the highest total phenolic content (TPC) from experimental value was 584.12 $\pm$ 6.74 mg TEAC/g of crude extract under 0 hour of pre-leaching time, S/F ratio of 15:1 and extraction time of 3 minutes of extraction conditions. However, the combination of experimental conditions that obtained the lowest total phenolic content (TPC) from experimental value was 118.61 $\pm$ 15.77 mg TEAC/g of crude extract under 1 hour of pre-leaching time, S/F ratio of 15:1 and extraction time of 6 minutes of extraction conditions.

The highest and lowest of experimental values and predicted values were matched to each other. Predicted values of various combinations of experimental extraction conditions were mostly out of the range of experimental value obtained and the range of accepted predicted value and experimental was wider than total xanthone yield and total phenolic content (TPC).

The equations could be fit into the model as below:

$$\mathbf{Y_{DPPH\ scavenging\ activity}} = 214.36889 - 75.65083X_1 - 7.248333X_2 - 62.18167X_3 - 31.8275X_1X_2 - 25.7225\ X_1X_3 + 2.3425X_2X_3 + 108.97764X_1^2 + 59.500972X_2^2 + 75.269306X_3^2$$

where X_1, X_2 and X_3 are the coded variables for pre-leaching time and S/F ratio and extraction time respectively.

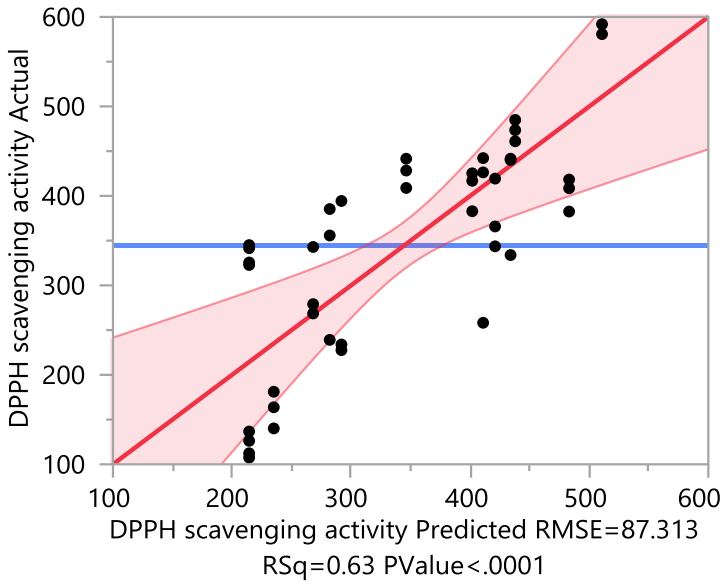

Figure 4.3: The relationship between actual and predicted values of DPPH scavenging activity.

Table 4.5: Experimental results and predicted results of DPPH assay of various experimental conditions obtained from mangosteen pericarp crude extract.

Run	Experimental conditions			Experimental result	Predicted value
	X_1*	X_2*	X_3*	DPPH scavenging activity (mg TEAC/ mg crude extract)	
1	0	10:1	6	405.19 ± 61.75[a]	433.92
2	2	10:1	6	426.94 ± 17.40[a]	346.27
3	0	20:1	6	403.10 ± 18.49[a]	483.08
4	2	20:1	6	296.85 ± 40.23[b]	268.12
5	0	15:1	3	584.12 ± 6.74[d]	510.73
6	2	15:1	3	442.22 ± 15.97[a]	410.87
7	0	15:1	9	473.13 ± 11.98[a]	437.81
8	2	15:1	9	161.67 ± 20.57[c]	235.06
9	1	10:1	3	376.25 ± 38.91[a]	420.91
10	1	20:1	3	408.31 ± 22.44[a]	401.73
11	1	10:1	9	285.28 ± 94.47[ab]	291.86
12	1	20:1	9	326.71 ± 77.30[ab]	282.05
13	1	15:1	6	188.06 ± 119.28[bc]	214.37
14	1	15:1	6	336.44 ± 11.95[b]	214.37
15	1	15:1	6	118.61 ± 15.77[c]	214.37

*X_1: pre-leaching time (hr); X_2: S/F ratio (ml/g); X_3: extraction time (min)
Note: Data are shown as mean of triplicate values with standard deviations.
Note: Data that are not sharing the same superscript across row are significantly different ($p < 0.05$).

4.2.2 Response Surface Analysis

A) Effect of Extraction Parameters on Total Xanthone Yield

Analysis of variance on total xanthone yield was shown in Table 4.6. It indicated that parameters such as b_1, b_2 and b_{11} had significance effect on the total xanthone yield. Other parameters did not indicate significantly difference on the total xanthone yield. It indicated that pre-leaching time and S/F ratio had markedly effect on total xanthone yield.

Interaction profiler of pre-leaching time, solvent-to-feed (S/F) ratio and extraction time toward total xanthone yield was shown in Figure 4.4. Figure 4.5 showed that increasing of S/F ratio and extraction time gave higher yield of total xanthone. Figure 4.6 illustrated that decreasing of S/F ratio with increasing pre-leaching time gave lower yield of total xanthone. In Figure 4.7, it indicated that increasing extraction time with decreasing pre-leaching time could increase the yield of total xanthone.

Table 4.6: Analysis of variance on total xanthone yield.

Parameters	**Total xanthone yield**	
	Estimate	**Prob >|t|**
b_0**	28.224444	<0.0001*
b_1**	-8.1975	<0.0001*
b_2**	3.5170833	<0.0001*
b_3**	0.48625	0.5418
b_{12}**	0.7266667	0.5192
b_{13}**	-0.398333	0.7233
b_{23}**	0.7225	0.5216
b_{11}**	7.1890278	<0.0001*
b_{22}**	-1.845139	0.1212
b_{33}**	0.4581944	0.6956

*Parameter that is $p < 0.05$ is significantly different.

**b_0 = Intercept; b_1, b_2, and b_3 = Linear regression coefficients for pre-leaching time, S/F ratio and extraction time; b_{12}, b_{13}, and b_{23} = Regression coefficients for interaction between pre-leaching time × S/F ratio, pre-leaching time × extraction time and S/F ratio × extraction time; b_{11}, b_{22}, and b_{33} = Quadratic regression coefficients for pre-leaching time × pre-leaching time, S/F ratio × S/F ratio and extraction time × extraction time.

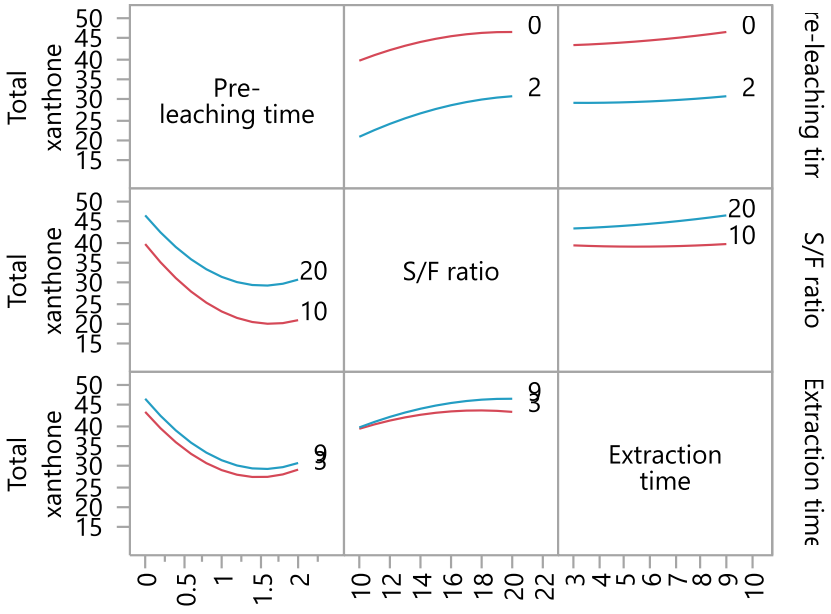

Figure 4.4: Interaction profiler of pre-leaching time, solvent-to-feed (S/F) ratio and extraction time toward total xanthone yield.

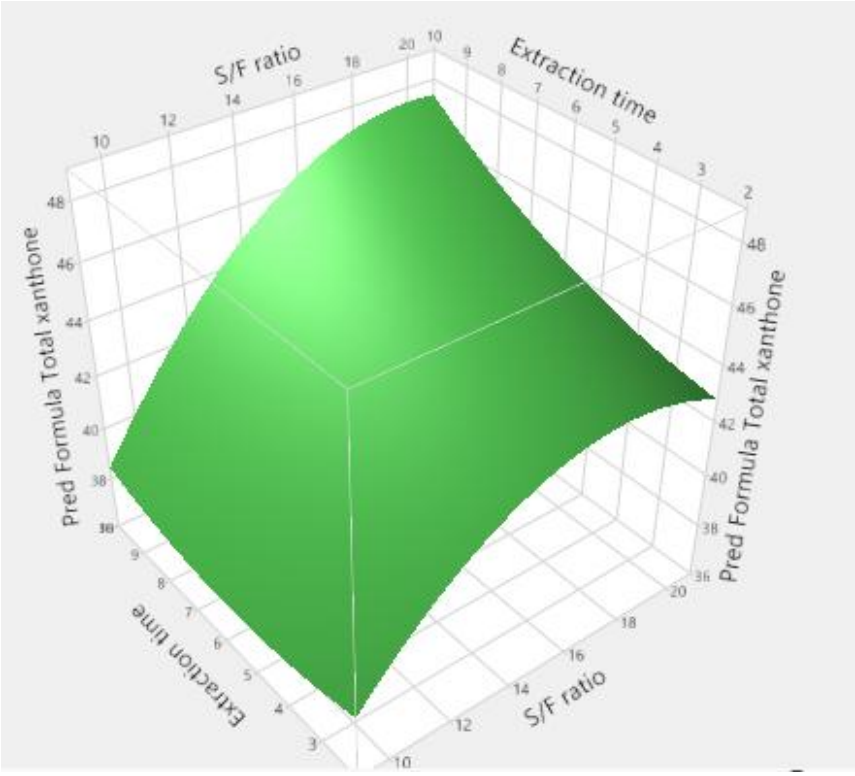

Figure 4.5: Response surface plot that showed impact of solvent-to-feed (S/F) ratio (10:1-20:1) and extraction time (3-9 minutes) on total xanthone yield.

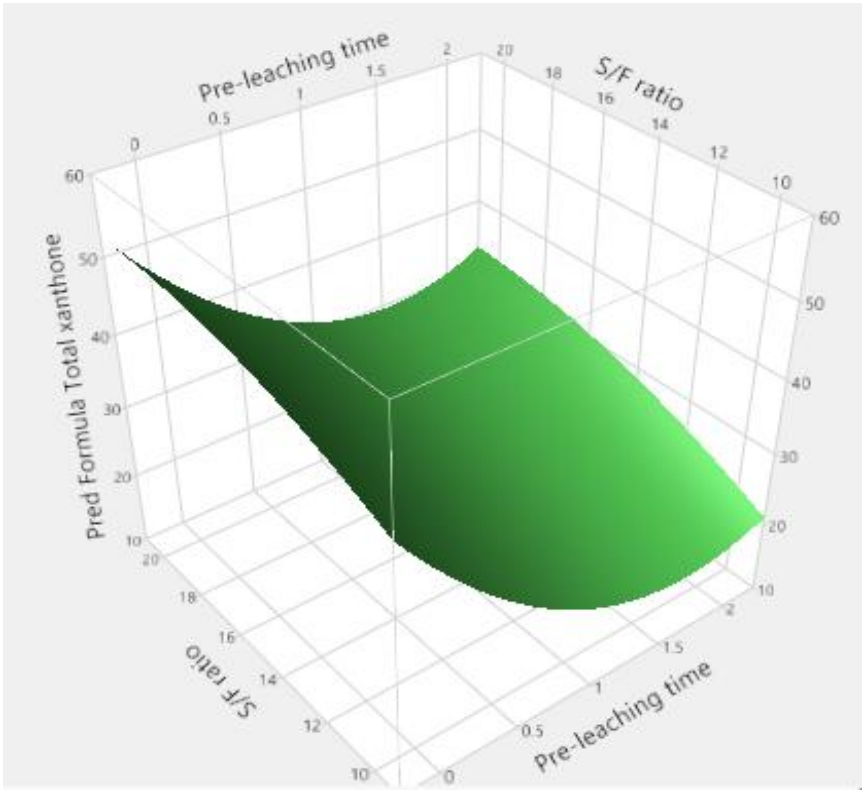

Figure 4.6: Response surface plot that showed impact of pre-leaching time (0-2 hours) and solvent-to-feed (S/F) ratio (10:1-20:1) on total xanthone yield.

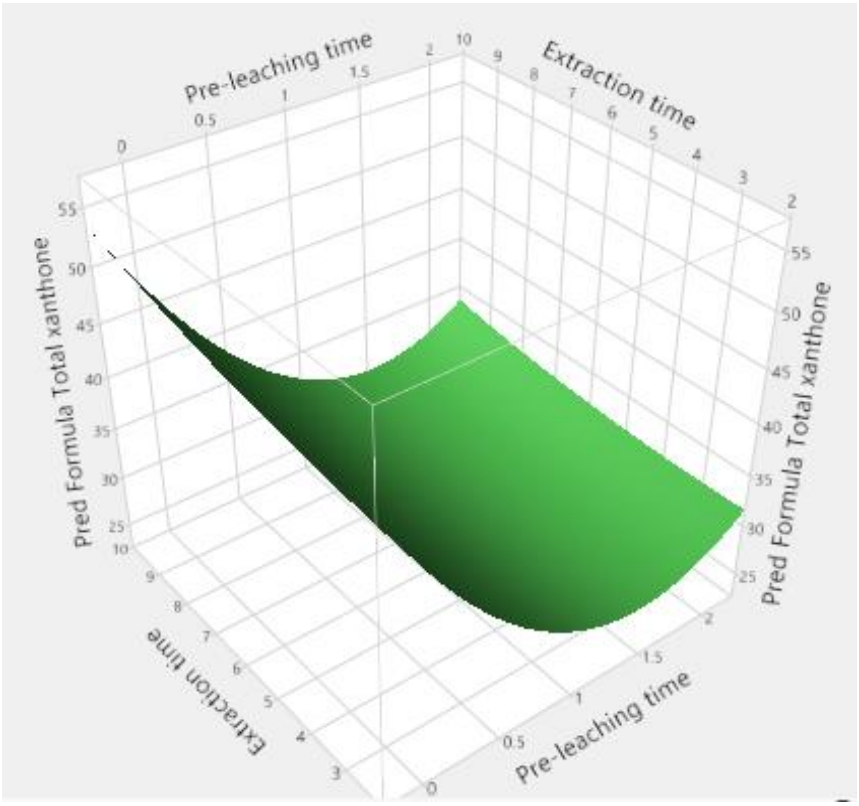

Figure 4.7: Response surface plot that showed impact of pre-leaching time (0-2 hours) and extraction time (3-9 minutes) on total xanthone yield.

B) Effect of Extraction Parameters on Total Phenolic Content (TPC)

Analysis of variance on total phenolic content (TPC) was shown in Table 4.7. It indicated that parameters of b_1, b_2, b_{23}, b_{11}, b_{33} had significance effect on total phenolic content (TPC). Other parameters did not indicate significantly difference on TPC. It also indicated that pre-leaching time and solvent-to-feed (S/F) ratio had markedly effect on total phenolic content (TPC).

Interaction profiler of pre-leaching time, solvent-to-feed ratio and extraction time toward TPC was shown in Figure 4.8. Figure 4.9 showed that increasing of S/F ratio and extraction time gave higher value of total phenolic content (TPC). Figure 4.8 and 4.10 illustrated that decreasing of solvent-to-feed (S/F) ratio with increasing pre-leaching time gave lower value of total phenolic content (TPC). In Figure 4.8 and 4.11, it indicated that increasing extraction time with decreasing pre-leaching time could increase total phenolic content (TPC).

Table 4.7: Analysis of variance on total phenolic content (TPC).

Parameters	**Total phenolic content (TPC)**	
	Estimate	**Prob >|t|**
b_0**	23.897778	<0.0001*
b_1**	-9.395833	<0.0001*
b_2**	2.7866667	<0.0001*
b_3**	1.005	0.0935
b_{12}**	1.0158333	0.2261
b_{13}**	-0.395833	0.6341
b_{23}**	2.3625	0.0070*
b_{11}**	6.4548611	<0.0001*
b_{22}**	-1.450139	0.0999
b_{33}**	2.4715278	0.0067*

*Parameter that is $p < 0.05$ is significantly different.

**b_0 = Intercept; b_1, b_2, and b_3 = Linear regression coefficients for pre-leaching time, S/F ratio and extraction time; b_{12}, b_{13}, and b_{23} = Regression coefficients for interaction between pre-leaching time × S/F ratio, pre-leaching time × extraction time and S/F ratio × extraction time; b_{11}, b_{22}, and b_{33} = Quadratic regression coefficients for pre-leaching time × pre-leaching time, S/F ratio × S/F ratio and extraction time × extraction time.

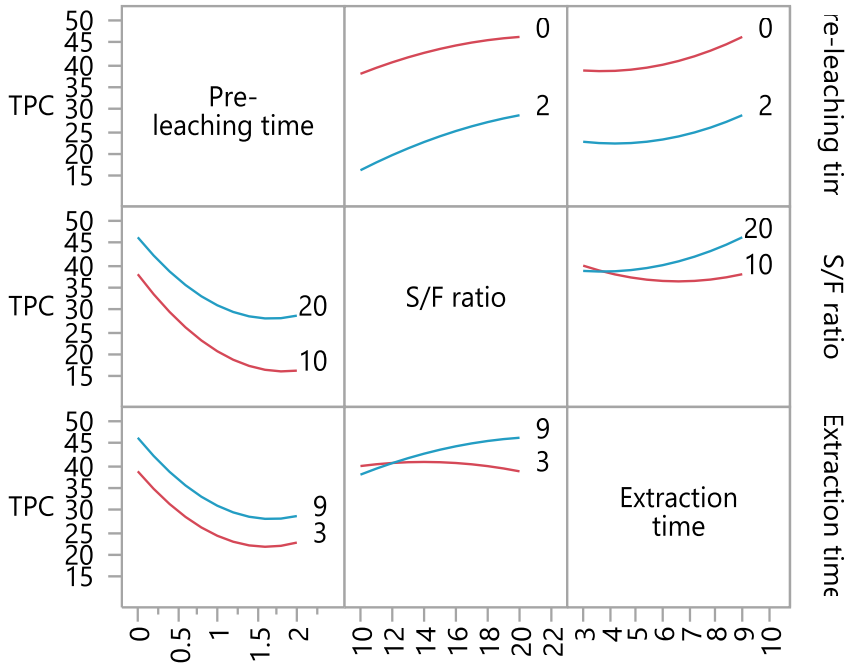

Figure 4.8: Interaction profiler of pre-leaching time, solvent-to-feed (S/F) ratio and extraction time toward total phenolic content (TPC).

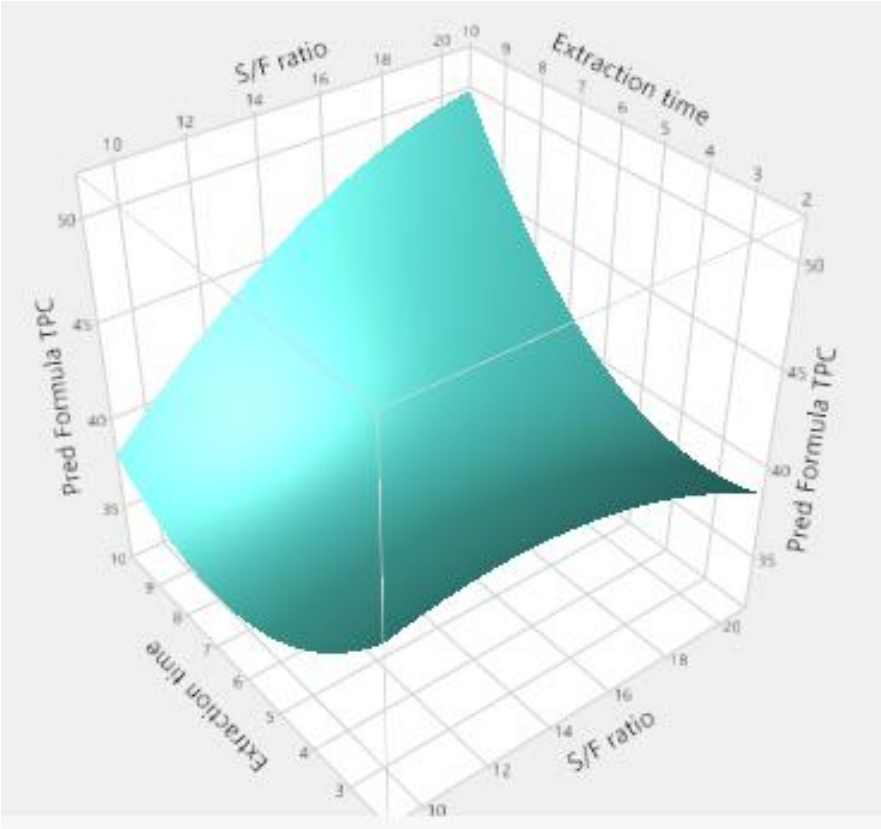

Figure 4.9: Response surface plot that showed impact of solvent-to-feed (S/F) ratio (10:1-20:1) and extraction time (3-9 minutes) on total phenolic content (TPC).

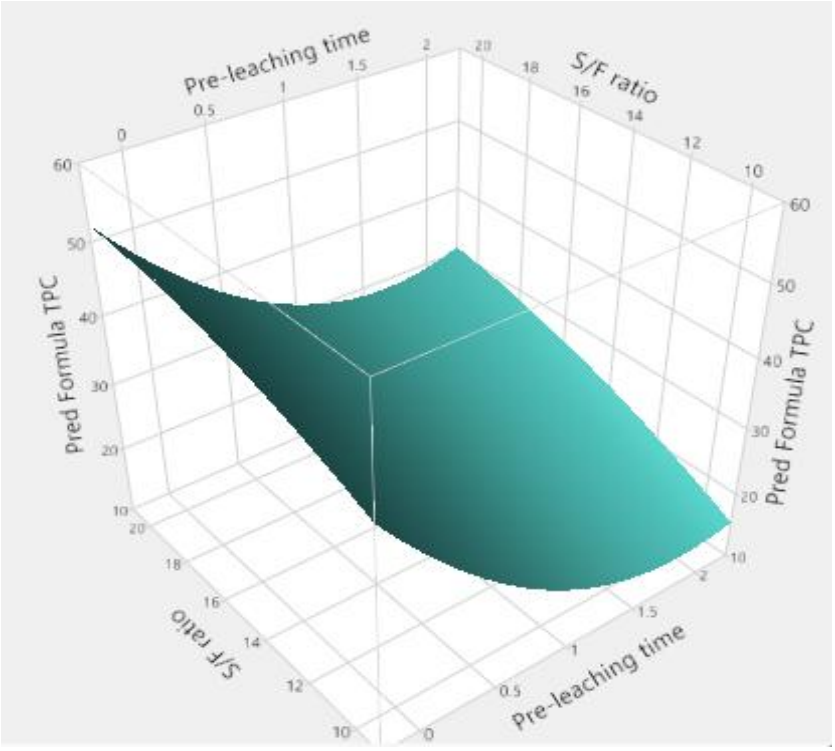

Figure 4.10: Response surface plot that showed impact of pre-leaching time (0-2 hours) and S/F ratio (10:1-20:1) on total phenolic content (TPC).

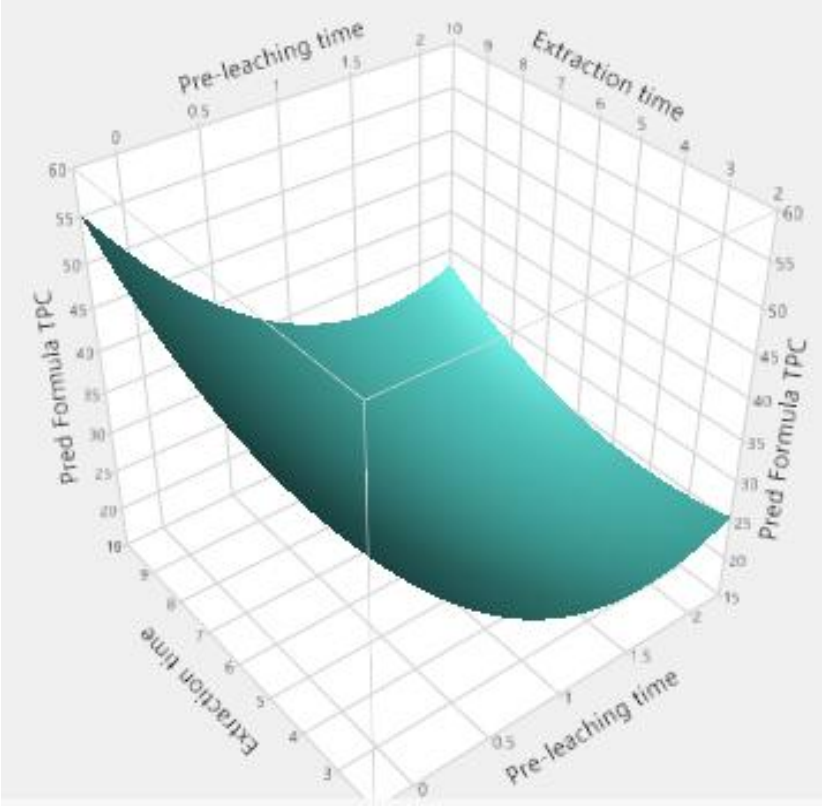

Figure 4.11: Response surface plot that showed impact of pre-leaching time (0-2 hours) and extraction time (3-9 minutes) on total phenolic content (TPC).

4.3.3 Validation of Total Xanthone Yield and Total Phenolic Content (TPC)

Figure 4.12 showed the optimized conditions to obtain maximum yield of total xanthone and TPC. Table 4.8 showed the experimental values of total xanthone yield and TPC obtained under optimized extraction conditions with comparison to the predicted values. These results indicated that the optimal conditions for extraction of total xanthone yield and total phenolic content (TPC) by microwave-assisted extraction (MAE) were 0 hour of pre-leaching time, 20:1 of S/F ratio and 9 minutes of extraction time. Under the optimum microwave-assisted extraction (MAE) conditions, the desirability obtained was 0.88 and the total xanthone yield and total phenolic content (TPC) were 45.71 $\pm$ 0.67 mg α-mangostin/g of crude extract and 46.16 $\pm$ 0.18 mg GAE/g of crude extract, respectively.

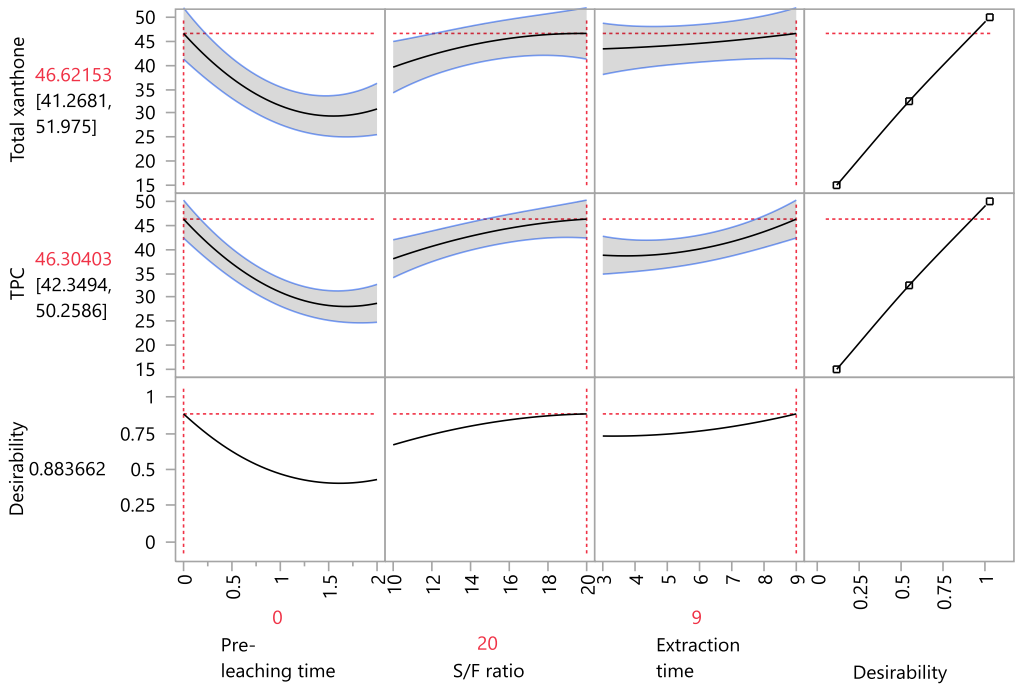

Figure 4.12: Optimization of pre-leaching time (0-2 hrs), S/F ratio (10:1-20:1) and extraction time (3-9 min) on total xanthone and total phenolic content (TPC) with desirability.

Table 4.8: Validation of the experimental values by predicted values obtained from JMP under optimized extraction conditions for total xanthone yield and total phenolic content (TPC).

	Experimental value			Mean	Predicted value
	1	2	3		
Total xanthone (mg α-mangostin/g crude extract)	45.27	45.39	46.48	45.71 ± 0.67^{a}	46.62 ± 5.35^{a}
Total phenolic content (mg GAE/g crude extract)	45.95	46.28	46.24	46.16 ± 0.18^{a}	46.30 ± 3.95^{a}

Note: Data are shown as mean of triplicate values with standard deviations.
Note: Data that are not sharing same superscript across column are significantly different ($p < 0.05$).

CHAPTER 5

DISCUSSION

5.1 Determination of Crude Extract Yield

The highest crude extract yield of mangosteen pericarp was obtained under no pre-leaching condition while the lowest crude extract yield of mangosteen pericarp was obtained under 1 hour or 2 hours of pre-leaching condition by analysis from Table 4.1. In a study done by Kullu, et al. (2013), increasing pre-leaching time was shown to obtain higher yield of mangiferin as increased hydrated status of sample could help the rupture of the cell wall. Thus, enlargement of the cellular pores and internal thermal stress would facilitated leaching of the target bioactive compound into the extraction solvent. Dahmoune, et al. (2014) also showed that increased pre-leaching time could increase the extraction of phenolic content. However, the result of this experiment did not exhibit the same trend as the two studies mentioned above since the highest yield of crude extract was obtained by the no pre-leaching condition. This result could be explained by the type of extraction solvent used in this experiment which is the distilled water. Bioactive compound from mangosteen pericarp could not fully soluble into distilled water since the polyphenol compounds mainly dissolved in the organic solvent such as ethanol or methanol due to its polarity (Suttirak and Manurakchinakorn, 2012). Moreover, longer pre-leaching time in room temperature caused degradation of bioactive compounds in mangosteen pericarp because bioactive compounds in mangosteen pericarp was relatively unstable and substantially lost to the surrounding when stayed in solution formed instead of solid powder form.

Bioactive compounds in mangosteen pericarp stayed in solution form were in active form which enhanced oxidation reaction, thus causing lost of bioactive compound to surrounding (Chaovanalikit, et al., 2012).

Besides, solvent-to-feed (S/F) ratio of 15:1 resulted in greater crude extract yield of mangosteen pericarp whereas S/F ratio of 10:1 resulted in lower crude extract yield of mangosteen pericarp. An increased of solvent-to-feed (S/F) ratio could increase the extraction yield of mangosteen pericarp crude extract. The difference in extraction yield was due to density of solute in solvent during extraction. The different density of solute in solvent could greatly affect on the solubility of solute in solvent. Higher solvent-to-feed (S/F) ratio which means lower density of solute in the extraction solvent would then give higher extraction efficiency (Kislik, 2012).

The third extraction parameters examined for the crude extract yield was the extraction time which found did not have significant effect on the crude extract yield as referred from Table 4.1. This phenomenon could be explained by insufficient extraction time for bursting most of the bioactive compound from the plant matrix of mangosteen pericarp. It also could be said that the operation of extraction period did not reach optimum extraction time to show greater differences because longer extraction time, the larger water molecules were provided to extract phenolic compound, and the longer water molecules contacted with the samples (Ash Shiddiqi, et al., 2014). Thus, the time for extraction needed to be extended to further investigate the effect of extraction time on the crude extract yield of mangosteen pericarp.

5.2 Optimization of Microwave-assisted Extraction Condition

5.2.1 Modelling and Model Fitting using Response Surface Methodology

A) Total Xanthone Yield Model

The experimental results of total xanthone yield were found to fit the mathematical model since Prob > F value was more than 0.05 as shown by Table 4.2. This means that the model was adequate to predict the value which exhibits a good fit to the true behaviour. Thus, the model of total xanthone yield could be applied for maximum recovery of antioxidant properties and polyphenol compounds from mangosteen pericarps since the predicted conditions were valid. As seen from Figure 4.1, the predicted values for total xanthone yield were linear to their actual values, with R^2 of 0.83, indicating a close relationship and further supporting that the mathematical models were reliable and also appropriate for the prediction of the total xanthone yield values in the current study.

B) Total Phenolic Content (TPC) Model

The experimental results for total phenolic content (TPC) were also found to fit the model since Prob > F value was more than 0.05 as shown by Table 4.2. This showed that the mathematical model was adequate to predict the value which exhibited a good fit to the true behaviour. Thus, the model of total phenolic content (TPC) could apply for maximum recovery of antioxidant properties and polyphenol compounds from mangosteen pericarps since the predicted conditions were valid. As seen from Figure 4.2, the predicted values for total phenolic content (TPC) were linear to their actual values, with R^2 of 0.91, indicating a close relationship and further supporting that the

mathematical models were reliable and also appropriate for the prediction of the total phenolic content (TPC) values in the current study.

C) DPPH Scavenging Activity Model

The model for DPPH scavenging activity was found to be inadequate to predict the value since Prob > F value is less than 0.05 as shown by Table 4.2. The mathematical model did not show a good fit to the true behaviour. As seen from Figure 4.3, the predicted values for DPPH scavenging activity values were not linear to their actual values, with R^2 of 0.63. The linear regression of this model indicated a relationship that did not further support the mathematical models were reliable and could not predict DPPH scavenging activity values in the current study. Thus, the response surface analysis of effect of extraction parameters on DPPH scavenging activity could not be carried out as the DPPH scavenging activity values were not fit to the model.

The reason why the model was unfit for DPPH scavenging activity could probably be due to the inconsistent quality of the crude extracts used to determine the DPPH scavenging activity which were stored in different durations ranging from two days to over a week from the time of extraction to analysis. The biologically active compounds in the crude extract may be subjected to degradation over storage time and light penetration (Patras, et al., 2009).

5.2.2 Response Surface Analysis

Effect of Extraction Parameters on Total Xanthone Yield and Total Phenolic Content (TPC)

Several studies showed that total phenolic content (TPC) was correlated with the antioxidant activity content (Almeida, et al., 2011). Therefore, antioxidant properties were always associated with polyphenolic compounds as fundamental quality parameters in plant-based food. In this experiment, the yield of crude extract is directly correlated to the total xanthone yield and total phenolic content (TPC) as shown in Table 4.1, 4.3 and 4.4.

A) Effect of Pre-leaching Time with Constant Agitation & Interactive effect of Pre-leaching time and extraction time

The yield of total xanthone and total phenolic content (TPC) were significantly affected by pre-leaching time as shown in Table 4.6 and 4.7. Pre-leaching time is one of the main extraction parameters, affected greatly on the total xanthone yield and total phenolic content (TPC). 0 hour of pre-leaching conditions produced higher yield of total xanhone and total phenolic content (TPC) as increased of pre-leaching time would decreased the total xanthone yield and total phenolic content (TPC). However, pre-leaching time was found to improve the yield of mangiferin, caffeine and tanshinones in previous studies (Kullu, et al., 2013; Pan, Niu and Liu, 2002; Yashwant Malode, et al., 2013). The results of the experiment did not reflect the same trend as obtained in the above findings. Thus, this phenomenon could be explained by the over-exposure to microwave radiation after greater pre-leaching time (Chemat and Cravotto, 2013). Greater pre-leaching time caused bioactive compound,

leaching out of the food matrix before extraction at low operating power (Chemat and Cravotto, 2013). Thus, the significantly decreased on yield of total xanthones over pre-leaching time in this study could be due to loss of chemical structure of the active compounds.

The adverse effect of pre-leaching could also be explained by the type of extraction solvent used in the pre-leaching procedures as type of solvent used was the main factor to affect the extraction yield of total xanthone and total phenolic content (TPC). Most of the studies showed that increasing pre-leaching time with organic solvent such as methanol and ethanol obtained higher extraction yield compared to distilled water due to the polarity of solvent used (Suttirak and Manurakchinakorn, 2012). Azmir, et al. (2013) indicated that the polarity of the targeted compound was the most important factor for choice of solvent and extraction efficiency mainly depends on the type of solvents used. A study showed that ethanol could enhance an increased in extraction efficiency while water favourably increased the contact surface area between sample and solvent by facilitating swelling of cell wall, thus resulting in an increased extraction yield (Ghasemzadeh, et al., 2017). Therefore, the use of distilled water individually was not enough to facilitate increased of extraction yield without the use of organic solvent. Xanthone compound was mainly dissolved in medium polar and non-polar solvent and mostly secondary metabolites were extracted by methanol instead of distilled water in many studies (Rybczynski, Davey and Mikula, 2015).

Moreover, another reason as to why longer pre-leaching time in room temperature caused degradation of bioactive compounds in mangosteen pericarp could be because bioactive compounds in mangosteen pericarp was relatively unstable and substantially lost to the surrounding when stayed in solution formed instead of solid powder form. Bioactive compounds in mangosteen pericarp stayed in solution form were in active form which enhanced oxidation reaction, thus causing lost of bioactive compound to surrounding (Chaovanalikit, et al., 2012).

In addition, a research showed that constant agitation during extraction could overcome the adverse effect of solvent-to-feed (S/F) ratio since agitation aids to eliminate the barrier for mass transportation of polyphenol compound to the solvent (Ameer, Shahbaz and Kwon, 2017). The reason was the contribution temperature on the solid particle and solvent from electromagnetic microwave was evenly in case agitation applied during extraction compared to before extraction (Azwanida, 2015). Thus, it specified that agitation before extraction and during extraction showed different influences on the extraction yield of polyphenol compounds.

As shown in Figure 4.7 and 4.11, increasing the pre-leaching time from 0 to 2 hours resulted gradual decrease in extraction yield of total xanthone and total phenolic conent (TPC), followed by a slight rise with a further increase in extraction time. As pre-leaching time increased from 0 to 2 hours with extraction time of 3 minutes, the lowest recovery of total xanthone yield and total phenolic content (TPC) was obtained. Apart from that, the yield of total

xanthone with 0 hour of pre-leaching time with 9 minutes of extraction time exhibited highest recovery of total xanthone yield and total phenolic content (TPC). These total xanthone yield and total phenolic conent (TPC) trend could be attributed to the increase in extraction time, accelerating release of bioactive compound due to internal pressure, rupturing the plant matrix cell wall (Ghasemzadeh, et al., 2017). However, the interactive effect of pre-leaching time and extraction time did not show significantly effect on total xanthone yield and total phenolic content (TPC) as shown in Table 4.6 and 4.7.

B) Effect of Solvent-to-feed (S/F) Ratio & Interactive Effect of Pre-leaching time and Solvent-to-feed (S/F) Ratio

The total xanthone yield and total phenolic content (TPC) were significantly affected by solvent-to-feed (S/F) ratio as shown in Table 4.6 and 4.7. The one of the fundamental extraction parameters that significantly affected the total xanthone yield and total phenolic content (TPC) in this experiment was solvent-to-feed (S/F) ratio. As solvent-to-feed (S/F) ratio increased from 10:1 to 20:1, the extraction yield of total xanthone and total phenolic content (TPC) were also increased. This finding was agreed to the previous finding from Bhuyan, et al. (2015). It could be explained by increasing in density of the solvent result the lower extraction efficiency. Besides, high solvent-to-feed (S/F) ratio could increase the contact surface of solid particles to solvent in order to dissolve sample matrix thoroughly into solvent.

However, Fang, et al., (2011) stated that yield of total xanthone at different solvent-to-feed (S/F) ratio showed no significant differences as the solvent-to-

feed (S/F) ratio used was 6:1, 8:1 and 10:1. The difference of the result obtained could be explained by narrow range of solvent-to-feed (S/F) ratio of tested condition (6:1, 8:1 and 10:1) compared to this experimental condition (10:1, 15:1 and 20:1).

Furthermore, as shown in Figure 4.6 and 4.10, as pre-leaching time increased from 0 to 2 hours at 10:1 of solvent-to-feed (S/F) ratio, the lowest total xanthone yield and total phenolic content (TPC) was obtained. It depicted the correlation between pre-leaching time and solvent-to-feed (S/F) ratio. However, as pre-leaching time increased from 0 to 2 hours at 20:1 of solvent-to-feed (S/F) ratio, the total xanthone yield and total phenolic content (TPC) obtained was higher than at 10:1 of solvent-to-feed (S/F) ratio. Ameer, Shahbaz and Kwon (2017) found that pre-leaching improved the extraction yield of bioactive compounds by reducing the adverse effect of low solvent-to-feed (S/F) ratio used in experiment. This finding was agreed with the experimental results obtained. However, as shown in Table 4.6 and 4.7, the interactive effect of pre-leaching time and solvent-to-feed (S/F) ratio was not significantly affect the total xanthone yield and total phenolic content (TPC).

C) Effect of Extraction Time & Interactive Effect of Solvent-to-feed (S/F) Ratio and Extraction Time

Table 4.6 and 4.7 showed that total xanthone yield and total phenolic content (TPC) were not significantly affected by extraction time. As extraction time increased, the total xanthone yield and total phenolic content (TPC) were increased. However, Fang, et al. (2011) stated that the yield of xanthone

decreased as extraction time increased because xanthone compound could be destroyed with increasing irradiation time. The extraction time used in Fang, et al. (2011) was 10, 15 and 20 minutes. Thus, differ of the result obtained could be clarified by narrow range of extraction time used in this experiment were 3, 6 and 9 minutes with only difference of 3 minutes. The operation of extraction time did not reach optimum extraction time to show greater differences since it did not show decreased of the total xanthone yield and total phenolic content (TPC) until 9 minutes of extraction. Thus, it indicated that extraction time could be extended to further investigate the impact of extraction time on the yield of total xanthone and total phenolic content (TPC).

Furthermore, Padmapriya, et al. (2011) illustrated that increasing microwave power from 350 to 500 W of power setting could significantly improve the extraction efficiency with only 20 seconds of irradiation time of microwave. Thus, the adverse effect of shorter extraction time could be eliminated by increasing the microwave power used in the experiments condition as the microwave power used in the experiment was only 270 W of power setting. Apart from that, another study also indicated that increasing microwave power can accelerate extraction on polyphenolic compound because the more microwave energy applied, the greater ionic conduction and dipole rotation in purpose to generate molecular movement and heating on the sample matrix (Shao, et al., 2011).

As shown in Figure 4.5 and 4.9, the best recovery of total xanthone yield and total phenolic content (TPC) was obtained using an extraction time of 9

minutes with solvent-to-feed (S/F) ratio of 20:1. It depicted the correlation between extraction time and solvent-to-feed (S/F) ratio. It showed that the extraction yield of total phenolic content (TPC) increased as extraction time increased from 3 to 9 minutes at solvent-to-feed (S/F) ratio of 20:1 as shown in Figure 4.9. The total phenolic content (TPC) was significantly affected by interactive effect of extraction time and solvent-to-feed (S/F) ratio but not for total xanthone yield as shown in Table 4.6 and 4.7.

This finding did not agree to the previous finding from Dailey and Vuong (2015). This finding indicated the interactive effect of solvent-to-feed (S/F) ratio and extraction time did not markedly affect the yield of total phenolic content (TPC). The differing results could be explained by the greater effect of differences of solvent-to-feed (S/F) ratio used in this experimental condition (10:1, 15:1 and 20:1). The increased of solvent-to-feed (S/F) ratio could accelerate the extraction of total phenolic content (TPC), followed by increasing trend of extraction time due to higher heating efficiency under microwave conditions (Ghasemzadeh, et al., 2017).

Therefore, analysis of this experiment showed the extraction parameters of a single factor, pre-leaching time and solvent-to-feed (S/F) ratio significantly impacted in the total xanthone yield and total phenolic content (TPC) as decreasing of pre-leaching time and increasing of solvent-to-feed (S/F) ratio would give higher recovery of total xanthone yield and total phenolic content (TPC). Besides, the quadratic effect of solvent-to-feed (S/F) ratio and extraction time was significantly affected the total phenolic content (TPC) but

total xanthone yield was not. As solvent-to-feed (S/F) ratio rose from 10:1 to 20:1, total phenolic content (TPC) in mangosteen pericarp gradually increased, followed by increased of extraction time from 3 to 9 minutes.

5.2.3 Validation of Total Xanthone Yield and TPC

A verification experiment was carried out under optimum condition within the experimental range to validate the accuracy of the model equation. Therefore, the maximum yield of total xanthone and TPC obtained from experiment are 45.71 ± 0.67 mg α-mangostin/g of crude extract and 46.16 ± 0.18 mg GAE/g of crude extract under the optimized condition of 0 hour of pre-leaching time, 20:1 of S/F ratio and 9 minutes of extraction time with 0.88 of desirability. The predicted values were in close agreement with experimental values and there are no significant difference ($p > 0.05$) using paired t-tests. It proved that the optimized condition is valid to obtain maximum total xanthone yield and TPC.

5.3 Further Studies

Mangosteen contains plenty of antioxidant compounds especially in pericarp and xanthone is the main antioxidant compounds that contribute high antioxidant properties. Therefore, further studies on the identification of composition in mangosteen pericarp are essential as the antioxidant capacity of it is greatly affected by polyphenol compounds. Besides, further studies on the others antioxidant assay such as ABTS antioxidant capacity and modification of the method of analysis using DPPH scavenging activity is important in order to understand its relationship related to total xanthone yield and total phenolic content (TPC).

CHAPTER 6

CONCLUSION

The results of the study indicated that microwave-assisted extraction (MAE) parameters of pre-leaching time and solvent-to-feed (S/F) ratio significantly affected the extraction yield of mangosteen pericarp crude extract, total xanthones and the total phenolic content (TPC) but the extraction time parameter did not. The effect of the extraction parameters on the antioxidant activity could not be defined as the collected data could not yield a fitting model (R^2=0.63). The highest yield of crude extract obtained was 19.43 $\pm$ 0.56 % (w/w) under 0 hour of pre-leaching time, S/F ratio of 20:1 and extraction time of 6 minutes of extraction conditions. The interactive effect of S/F ratio and extraction time markedly impacted the extraction of TPC from mangosteen pericarp crude extract with increasing yield of TPC seen as S/F ratio was increased from 10:1 to 20:1 and extraction time from 3 to 9 minutes. The optimal MAE conditions to obtain the maximum total xanthone yield and TPC from mangosteen pericarp crude extract using distilled water as solvent were the pre-leaching time of 0 hour, S/F ratio of 20:1 and the extraction time of 9 minutes as predicted using response surface methodology (RSM). Under these conditions, the RSM model predicted a total xanthone yield and TPC of 46.62 ± 5.35 mg alpha-mangostin equivalent/ g of crude extract and 46.30 ± 3.95 mg GAE/g of crude extract respectively. These values were validated by the result of the validation test where total xanthones of 45.71 ± 0.67 mg alpha-mangostin equivalent/ g of crude extract and TPC of 46.16 ± 0.18 mg GAE/g of crude extract were obtained.

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APPENDICES

Appendix A

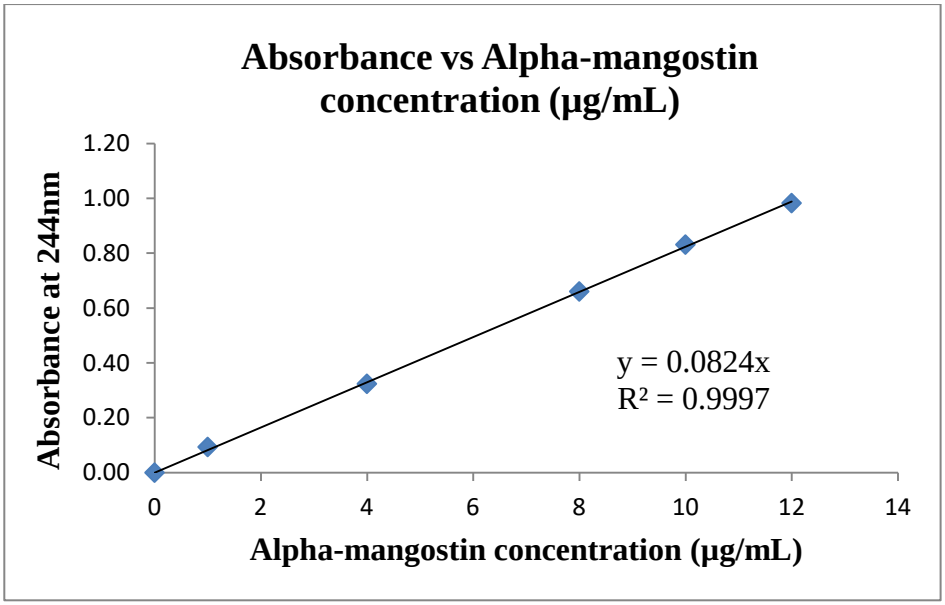

Figure 1: Absorbance against concentration of Alpha-mangostin for total xanthone analysis.

Appendix B

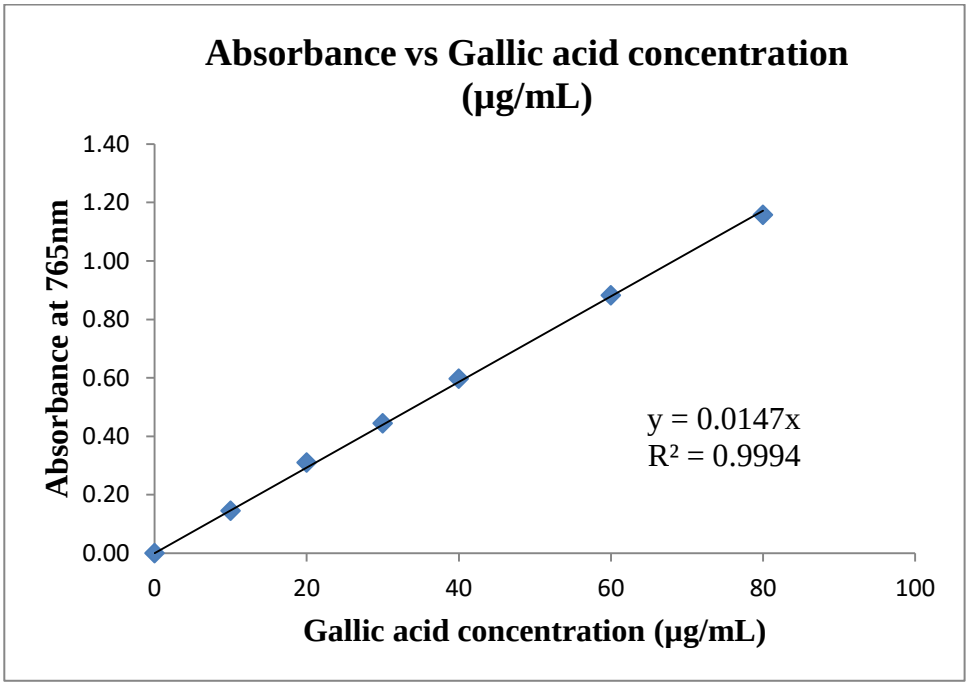

Figure 2: Absorbance against concentration of Gallic acid for TPC analysis.

Appendix C

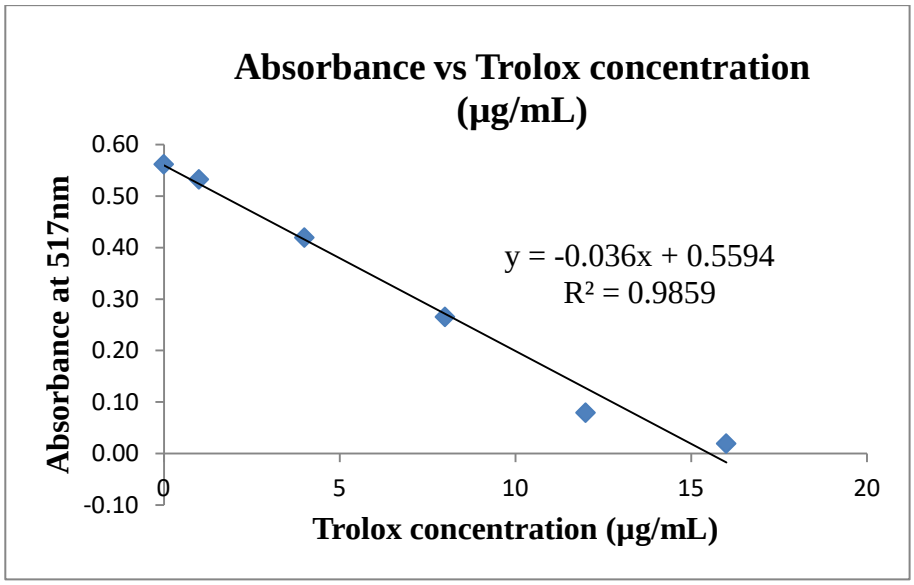

Figure 3: Absorbance against concentration of Trolox for DPPH assay.