OPTIMIZATION OF ULTRASOUND ASSISTED EXTRACTION AND ANTIOXIDANT ACTIVITY OF *PHALERIA MACROCARPA*

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Especially dedicated to M, thank you for the hard work.

AS, thank you for willingly went through hard ship especially the last 10 days.

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ABSTRACT

In the past decades, markets had been flooded with herbal based products including *Phaleria macrocarpa (P. macrocarpa)* or known as Mahkota Dewa. Previous studies reported that this plant contains high amount of active compound that are responsible for various bioactivities. However, these active compounds are sensitive towards many factors; temperature, pH, light and others in the production line. Thus, this study was conducted to optimize the extraction condition using ultrasound assisted extraction (UAE), determine the primary metabolites of the optimized extract and investigate the antioxidant activities using 2, 2-diphenyl-1-picrylhydrazine radical scavenging activity and ferric reducing antioxidant power assays. The result showed that concentration of solvent, solid to solvent ratio and sonication time affected percentage yield, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activities. The optimized parameters for the extraction are 75% methanol, 1: 31 solid to solvent ratio and 4.06 minutes of sonication time. Under these conditions, *P. macrocarpa* extract yielded 18.54% of crude extract, 81.59 mg GAE/g (milligram gallic acid/gram) of TPC and 28.17 mg QUE/g (milligram quercetin/gram) of TFC. Primary metabolite assays showed that *P. macrocarpa* optimized extract contained 40.427±1.127 mg BSA/g (milligram bovine serum albumin/gram) of protein, 23.981±0.2306 mg aescin/g of saponin and 0.853±0.0452 mg glucose/g of polysaccharides. Meanwhile, quantification of phenolic compound (gallic acid) and flavonoid compounds (quercetin, rutin and kaempferol) were done using high performance liquid chromatography and it was found that all compounds were present in the optimized extract with amount of 23.908 mg/g, 6.262 mg/g, 0.393 mg/g and 0.077 mg/g for rutin, gallic acid, kaempferol and quercetin, respectively. This extract was then tested for antioxidant activities and it exhibited potent antioxidant activities in both assays due to the relatively high TPC and TFC. This study suggested that extraction using UAE under controlled parameters contributed to the high antioxidant activities in *P. macrocarpa* and therefore can improve the quality of herbal products in the market.

ABSTRAK

Sejak beberapa dekad yang lalu, pasaran telah dibanjiri dengan pelbagai produk berasaskan herba termasuk *Phaleria macrocarpa (P. macrocarpa)* atau dikenali sebagai Mahkota Dewa. Kajian sebelum ini melaporkan bahawa tumbuhan ini mengandungi sebatian aktif tersebut yang tinggi yang bertanggungjawab terhadap pelbagai bioaktiviti. Walau bagaimanapun, sebatian aktif adalah sensitif terhadap pelbagai faktor; suhu, pH, cahaya dan lain-lain dalam barisan pengeluaran. Oleh itu, kajian ini dijalankan untuk mengoptimumkan keadaan proses pengekstrakan menggunakan pengekstrakan dengan bantuan ultrasonik (UAE), menentukan metabolit utama ekstrak yang telah dioptimumkan dan menyiasat aktiviti antioksidan ekstrak tersebut menggunakan aktiviti cerakin memerangkap radikal 2, 2-difenil-1-pikrilhidrazin dan cerakin kuasa antioksidan penurunan ferik. Hasilnya menunjukkan bahawa kepekatan pelarut, nisbah pepejal kepada pelarut dan tempoh sonikasi mempengaruhi peratusan hasil, jumlah kandungan fenolik (TPC), jumlah kandungan flavonoid (TFC) serta aktiviti antioksidan. Parameter yang optimum untuk proses pengekstrakan adalah 75% kepekatan pelarut, 1: 31 nisbah pepejal kepada pelarut dan 4.06 minit tempoh sonikasi. Di bawah keadaan ini, ekstrak *P. macrocarpa* menghasilkan 18.54% peratusan hasil, 81.59 mg GAE/g (miligram asid galik/gram) TPC dan 28.17 mg QUE/g (miligram kuersetin/gram) TFC. Cerakin metabolit utama menunjukkan bahawa ekstrak *P. macrocarpa* yang optimum mengandungi 40.427±1.127 mg BSA/g (miligram albumin serum lembu/gram) protein, 23.981±0.2306 mg aesin/g saponin dan 0.853±0.0452 mg glukosa/g polisakarida. Sementara itu, pengkuantitian sebatian fenolik (asid galik) dan sebatian flavonoid (kuersetin, rutin dan kaempferol) telah dilakukan menggunakan kromatografi cecair berprestasi tinggi dan didapati bahawa semua sebatian wujud dalam ekstrak optimum dengan jumlah 23.908 mg/g, 6.262 mg/g, 0.393 mg/g dan 0.0767 mg/g masing-masing untuk rutin, asid galik, kaempferol dan kuersetin. Kemudian, ekstrak ini diuji untuk aktiviti antioksidan dan ekstrak tersebut menunjukkan aktiviti antioksidan yang kuat pada kedua-dua cerakin selari dengan kandungan TPC dan TFC yang tinggi di dalamnya. Kajian ini mencadangkan bahawa pengekstrakan menggunakan UAE di bawah parameter yang dikawal menyumbang kepada aktiviti antioksidan yang tinggi bagi *P. macrocarpa* dan dengan itu boleh meningkatkan kualiti produk herba di pasaran.

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LIST OF EQUATIONS

LIST OF SYMBOLS

%	-	Percentage
^{o}C	-	Degree Celsius
µl	-	Microliter
µg	-	Microgram
g	-	Gram
Ha	-	Alternative hypothesis
Ho	-	Null hypothesis
mg	-	Milligram
min	-	Minute
ml	-	Milliliter
nm	-	Nanometer
ppm	-	Part per million
R^2	-	Coefficient of multiple determination

LIST OF ABBREVIATIONS

ANOVA	-	Analysis of variance
BSA	-	Bovine serum albumin
C	-	Concentration
CCD	-	Central composite design
Df	-	Dilution factor
DPPH	-	2, 2-diphenyl-1-picrylhydrazine
FRAP	-	Ferric reducing antioxidant power activity
GAE	-	Gallic acid
GC-MS	-	Gas Chromatography-Mass Spectrophotometer
LC-MS	-	Liquid Chromatography- Mass Spectrophotometer
HPLC	-	High Performance Liquid Chromatography
P	-	Probability
P. macrocarpa	-	*Phaleria macrocarpa*
QUE	-	Quercetin
RSM	-	Response surface methodology
TFC	-	Total flavonoid content
TPC	-	Total phenolic content
Uv-vis	-	Ultraviolet-visible

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CHAPTER 1

INTRODUCTION

1.1 General Background

Phaleria macrocarpa (*P. macrocarpa*) is the scientific name for Mahkota Dewa and can be found throughout the year in tropical areas. It is traditionally used to treat cancer, impotency, hemorrhoids, diabetes mellitus, allergies, liver and heart diseases, kidney disorders, blood related diseases, acne, stroke, migraine, and various skin ailments (Zhang *et al.*, 2006; Hendra *et al.*, 2011a and Hendra *et al.*, 2011b). *P. macrocarpa* attracts scientists around the globe to do extensive research and revealed the bioactivity potential of this plants.

P. macrocarpa based product had been penetrating herbal market few years back and the demand towards the product kept increasing. Most products available in market nowadays claim for high antioxidant activity, that includes P. macrocarpa based product. However, compounds responsible for antioxidant may degrade in the processing line due to the sensitivity towards extreme conditions. Therefore, it is important to choose the best method of extraction and control the conditions of extraction so that the active compounds can be retained till the end product. There are several advance methods of extraction that has numerous advantages in many ways. Previous study suggested that ultrasound assisted extraction (UAE) method

can easily be employed in the existing extraction machine for manufacturing plant. UAE had been proven extracting in a short time with higher percentage yield of active compounds (Vilkhu *et al.*, 2008; Gil-Chávez *et al.*, 2013).

There are a growing number of studies regarding *P. macrocarpa* in the past few years. However, there have been no reports on optimizing extraction of *P. macrocarpa* using ultrasonic-assisted extraction method as well as comparing antioxidant activity between 2, 2-diphenyl-1-picrylhydrazil (DPPH) Radical Scavenging Activity and ferric reducing antioxidant power activity (FRAP). By employing UAE method of extraction, percentage yield of active compounds can be increased and thus, contributed to the high antioxidant activity of that particular extract. In accordance with a tendency of increasing demand to consume products rich in antioxidants, optimization extraction of the *P. macrocarpa* extract and antioxidant activity is studied in this research.

1.2 Problem Statement

Malaysian's herbal market is saturated with a lot of local herbs mainly in nutraceutical, pharmaceutical and cosmeceutical fields. Many products claimed to have lots of biological benefits, for example supplement high in antioxidants as the result of increasing awareness among consumers. However, the increasing awareness on consuming natural products leads to high demand on natural and herbal market. There is no doubt that the increasing awareness among consumers provides positive economic growth in Malaysia however, for manufacturers, high demand leads to the massive production. Production, especially the one involving extraction of active compounds needs large volume of raw material. There are a lot of challenges need to be tackled in order to meet the growing market demand. One of the most vital phases in manufacturing is the processing.

Herbal plants naturally contained high antioxidant activity due to the presence of various active compounds for example phenolic and flavonoid compounds. However, this activity is reduced with the reduction of total active compounds remain after extraction process. Some active compounds are heat sensitive, light sensitive, pH sensitive and others. These make them unstable and most likely will denature due to the unfavorable conditions during drying and extraction process. In extraction process, methods and parameters play significant role in contributing the quantity and quality of the end product; the extract. Many scholars agreed that conventional methods of extraction are no longer suitable in term of yield, environmental friendly and even production cost to meet market demand. Therefore, in the past decade, a lot of studies proposed the improvised conventional method and others provide a totally new and advance method like supercritical fluid extraction.

In order to meet consumer demand, manufacturers choose to scaling up the production. Scaling up in industrial production often involve huge amount of investment and effort, however, it does not ensuring promised returns. However, optimization processing method and conditions need to be completed prior to scaling up. Manufacturer should not only consider most suitable method. They should also consider the parameters of that particular method. The optimization parameters during extraction may help in increasing extracted active compound. Based on previous study of other plants, UAE showed a great supremacy of extracting active compounds in a short period of time. Since the extraction of active compounds increased, the antioxidant activity which was contributed greatly by the presence of active compounds will most like to increase as well. Thus, this research studied on ultrasonic-assisted extraction (UAE) method by controlling the condition of extraction. Subsequently, the percentage yield, total phenolic content (TPC) and total flavonoid content (TFC) are increased. Thereby, applying correct method and conditions of extraction is crucial to optimize percentage yield, TPC as well as TFC of *P. macrocarpa*.

1.3 Significant of the Study

With the increase of awareness among consumers regarding healthy living, the development of high antioxidant content based product had been increased rapidly for the past few years. This plant especially can be capitalized as source of antioxidant agent and can be commercialized as value-added ingredient into nutraceutical and cosmeceutical based product. At the end of this study, the optimized parameter of UAE can guide herbal related manufacturers to boost their production. The quantity and quality of extract can be increased with the employment of best extraction method. These will leads to the decrement of natural resources wastage

1.4 Objective of the Study

The objectives of this study are:

i. To optimize the yield of *P. macrocarpa* fruit extract using ultrasonic-assisted extraction (UAE) method and determine the primary metabolite of the optimized extract.

ii. To investigate antioxidant activity of *P. macrocarpa* fruit extract using antioxidant activity assay of DPPH scavenging activity and Ferric Reducing Antioxidant Power (FRAP) assay.

1.5 Scope of the Study

i. Screening of parameter conditions (percentage of solvent, %, solid to solvent ratio, g/g, sonication time, min) of extraction process using ultrasonic-assisted extraction (UAE) method and screening of selected phytochemical compounds onto the extracts.

ii. Optimization extraction of phytochemical compounds from *P. macrocarpa* using UAE method with methanol as solvent.

iii. Quantification of selected phenolic and flavonoid compounds using high performance liquid chromatography (HPLC) method.

iv. Determination of primary metabolites (total polysachharide, total glycosaponin and total protein) on the optimized extract.

v. Investigation of antioxidant activity assays of *P. macrocarpa* using 2, 2-diphenyl-1-picrylhydrazine scavenging activity (DPPH) and ferric reducing antioxidant power (FRAP) assay.

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