ALPHA MANGOSTIN OPTIMIZED EXTRACTION BY SUPERCRITICAL CARBON DIOXIDE TECHNIQUE

.

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Especially dedicated to my wonderful husband, amazing prince and princess; Mohd Fazriherni, Muhammad Asyraaf and Nur Amni Husna and both of our family.

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ABSTRACT

Recently, there is an increasing interest to include plant derived compounds both in cosmetic and nutraceutical products. However, majority of these compounds are hard to isolate and some biologically active compounds are usually in low concentration. Furthermore, conventional extraction processes are mostly time consuming with high usage of toxic solvent. Thus, carbon dioxide (CO_2) which generally recognized as safe solvent can be a promising alternative to extract the plant derived compounds. In this study, supercritical carbon dioxide (SC-CO_2) extraction was compared with conventional soxhlet extraction for the extraction of α-mangostin from pericarps of *Garcinia mangostana*. The optimization was carried out using Box-Behnken Design. Three parameters were manipulated in SC-CO_2 optimization process which included extraction pressure (20 to 30 MPa), extraction temperature (40 to 60 °C) and percentage of ethanol as co solvent (0 to 3%) at fixed sample particle size of 0.10-0.50 mm and 4 mL/min of CO_2 flow rate. The analysis was carried out by using high performance liquid chromatography (HPLC). The optimum conditions for α-mangostin extraction (58% w/w) was at 20 MPa of pressure, temperature of 45 °C and 3% of ethanol as co solvent. Further isolation of SC-CO_2 extracts using column chromatography succesfully isolated pure α-mangostin (1.42% w/w) based from spectral data of HPLC chromatogram, ^{1}H and ^{13}C nuclear magnetic resonance and mass spectrometry. It was found that SC-CO_2 was more superior in terms of less toxic solvent used and more yield of α-mangostin extracted compared with the conventional and traditional soxhlet extraction technique.

ABSTRAK

Sejak kebelakangan ini, jenis sebatian daripada tumbuhan semakin kerap digunakan dalam kosmetik dan juga dalam bidang nutraseutikal. Walau bagaimanapun, sebahagian besar dari sebatian ini adalah susah untuk diasingkan dan dalam kepekatan yang rendah. Tambahan pula, proses pengekstrakan konvensional biasanya mengambil masa yang lama dan melibatkan penggunaan pelarut yang berbahaya dan bertoksik. Dengan itu, karbon dioksida (CO_2), sebagai pelarut yang dikenali selamat adalah penyelesaian yang terbaik untuk mengekstrak sebatian ini. Dalam kajian ini, pengekstrakan dengan menggunakan karbon dioksida lampau genting (SC-CO_2) dibandingkan dengan pengekstrakan soxhlet yang konvensional untuk mengekstrak α-mangostin daripada kulit buah *Garcinia mangostana*. Proses optimum dijalankan menggunakan reka bentuk Box-behnken (BBD). Dalam proses pengoptimuman SC-CO_2, tiga parameter telah dimanipulasikan iaitu tekanan pengekstrakan (20 ke 30 MPa), suhu pengekstrakan (40 ke 60 °C) dan peratus kehadiran etanol sebagai se-pelarut (0 ke 3%) pada saiz zarah sampel yang tetap iaitu 0.10-0.50 mm dan juga kadar alir CO_2 pada 4 mL/min. Analisa telah dijalankan menggunakan kromatografi cecair prestasi tinggi (HPLC). Keadaan yang paling optimum untuk pengekstrakan α-mangostin (58% w/w) ialah pada tekanan 20 MPa, suhu pada 45 °C dan etanol sebanyak 3% sebagai se-pelarut. Pengasingan lanjut ekstrak daripada SC-CO_2 menggunakan kromatografi kolum telah berjaya mengasingkan α-mangostin (1.42% w/w) yang asli berdasarkan data spektrum daripada kromatogram HPLC, ^{1}H dan ^{13}C resonans magnetik nuklear dan spektrometri jisim. Dengan ini, SC-CO_2 adalah terbukti lebih baik dari segi pengekstrakan α-mangostin yang lebih banyak dan kurang penggunaan pelarut yang berbahaya berbanding teknik soxhlet.

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LIST OF ABBREVIATIONS

AFLP	-	Amplified Fragment Length Polymorphism
CLEAR	-	Centre of Lipid Engineering and Applied Research
HPLC	-	High Performance Liquid Chromatography
LDL	-	Low Density Lipoprotein
MAO	-	Monoamine Oxidase
MRSA	-	Methicillin-Resistant Staphylococcus Aureus
PDA	-	Photo Diode Array
RAPD	-	Random Amplified Polymorphic DNA
SC-CO_2	-	Supercritical Carbon Dioxide Extraction
SFE	-	Supercritical Fluid Extraction
TLC	-	Thin Layer Chromatography

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Plants with folkloric reputation or with traditional medicine history have becoming major interest among researchers both in cosmetic and nutraceutical field, for example mangosteen (*Garcinia mangostana Linn*). As compound obtained from isolation of these plants, may be useful directly as agents or indirectly as starting materials in producing active agents.

However, majority of the compounds were hard to isolate and some biologically active compound were usually in low concentration. These drawbacks have upsurge researchers in finding more effective and selective extraction methods in order to recover the compounds. Furthermore, commonly used traditional extraction methods usually time consuming, laborous, low selectivity and most of them resulting in low extraction yields. Moreover, these techniques employ large amounts of toxic solvents which known to harm the environment.

Therefore, supercritical fluid extraction (SFE) method is in great interest as it posses an ability to ovecome those problem mentioned. Since SFE shows great contrast when compared to traditional method because of usage of less organic solvent and low temperature separation process which prevents the degradation of chemical compounds as stated by Zarena and Udaya Sankar, (2009). Furthermore, Glisic *et al*. (2007) have proved that supercritical carbon doxide (SC-CO_2) extraction of biologically active compounds from plant parts can be used and safe for further application in food, nutraceutical and cosmetic industries.

Mangosteen fruits are the most precious part of the mangosteen plants and well knowns for the sweet and savoury taste. Consequently, mangosteen fruits even called as the ‘queen of tropical fruits’ (Lim, 1984; Ramage *et al.*, 2004). Hull of the fruits has been prescribed by traditional practitioners for years across the world, especially in Southeast Asia, as a cure for a variety of medical conditions. Over the past decades, mangosteen plant parts have been studied and shown to contain high amounts of xanthones which then learned to have potential biological activities in *in vitro* systems by Dmitriy *et al*. (2009).

Xanthones are secondary metabolites and one of the naturally occurring compounds that contain a distinctive chemical structural component which is a tricyclic aromatic ring system. The ring systems often replaced with other types of functional groups such as isoprene, phenolic, and methoxy groups which then promotes to various of possible structures (Edward, 2007). To date, fifty xanthones have been isolated from mangosteen fruit pericarp (Pedraza-Chaverri. *et al.* 2008) and the first was α mangostin which isolated in the year of 1855. Many researchers claimed that α-mangostin have shown great variety as cosmeceuticals and nutraceutical active agents including antioxidant activity and antiinflammatory activity. As stated by Williams *et al*. (1995), α-mangostin can acts as a free radical scavenger to protect the Low Density Lipoprotein (LDL) from oxidative damage and Jung *et al*. (2006) also found that α-mangostin shows a potent antioxidant activity by using authentic and morphosydnonimine-derived reoxynitrite methods.

The antiinflammatory activities of α mangostin have shown great effects both in-vitro and in-vivo systems as studied by Chen *et al*. (2008). With the great potential of α-mangostin both in nutraceutical and cosmeceutical activities, many researchers have conducted various studies on this compound especially on the potential application of the compound to acts as an antioxidant, antitumor, antiinflammatory,antiallergic, antibacterial and many more.

Therefore, promoting for high demands of good quality and quantity of α-mangostin extract upsurged researchers to find more efficient techniques for the extraction of α mangostin while at the same time reduced the thermal degradation and minimize the solvent contamination.

1.2 Problem Statement

Several studies have claimed that xanthones from mangosteen especially α-mangostin, as the major xanthone, exhibits variety of applications such as an antioxidant, antitumoral, antiinflammatory, anti-allergic, antibacterial and antifungal activities. As a result, it is mainly applied to herbal cosmetics and nutraceutical products (Pothitirat and Gritsanappan, 2009). Therefore, there is consequently a great demand for the pure compound as more research and findings being done on the application of α-mangostin in both fields.

There are several reports on extraction and isolation of α-mangostin. However, mostly were focused on the quality and not both optimized quantity and quality of the α-mangostin. Furthermore, previous study done by Kaomongkolgit *et al.* (2011) employs maceration technique which were time consuming and high amount of harmful toxic solvent used. Pothitirat and Gritsanappan, 2009 also had studied the quantitative analysis of total α-mangostin in mangosteen pericarp by using soxhlet extraction method. However, the method have proved to be high in time consumption and quite laborous due to 15 hours of extraction time.

Nowadays, supercritical carbon dioxide (SC-CO_2) extraction had received great attention among researchers in extraction of bioactive compounds from plant tissues for the short time of extraction process and high recovery of compounds (Bimakr *et al*., 2011). Studies by Liza *et al.* 2010 and Reverchon *et al.* 1993, found that supercritical fluid was advantageous when the solvent strength can be manipulated due to the change in pressure (P) and temperature (T). Carbon Dioxide (CO_2) is an inert, non-toxic and non-polar solvent in supercritical fluid extraction (SFE). Therefore for the extraction of more polar/semi polar compound for example α-mangostin, modifier need to be added during the extraction process such as the food grade solvents (ethanol) to act as co-solvent (Lang and Wai, 2001). As stated by Bimakr *et al.* (2011), there were many variables that may affect the extraction process in SFE such as pressure, time, temperature, solvent flow rate, co solvent, co solvent flow rate, sample particle size, porosity, density, bed diameter and height of the extraction vessel. Therefore, those parameters need to be analysed and optimised in order to obtain high amount of desired compound extracted.

Recently, there have been studies reported on the extraction of total xanthones from mangosteen pericarp by using SC-CO_2 by Zarena and Udaya Sankar, (2010). However in their study, all xanthones from mangosteen pericarp including α-mangostin and other xanthones compounds were extracted together. Therefore, further step is needed to isolate α-mangostin from those compounds. Furthermore, usage of 5% organic solvent were quite large in amount which may hinders the strength of the supercritical fluid to extract polar and slightly polar compound such as α-mangostin. The optimization parameters involved in their study were pressure at 18-38 MPa, temperature from 40-60 °C and time of extraction from 2-8 hours with optimum condition obtained at 28 MPa, 50 °C and 8 hours time of extraction to produce about 51% w/w of α-mangostin. However, the highest yield of α-mangostin was only obtained at the longest extraction time and the used of quite high amount of co-solvent which was at 5% requires more research to be conducted.

For the optimization technique, the conventional and classical method involving one parameter at a time was a waste as the lack of inclusion of the interactive effects among variables and therefore could not lead to optimum parameters. Hence, multivariate techniques such as Central Composite Design (CCD), Box-Behnken design (BBD) and Doehlert matrix have been frequently used (Zolgharnein *et al*., 2013).

Due to numerous potential application of α-mangostin in both cosmeceutical and nutraceutical areas, a study need to be conducted in order to identify the most efficient and less time consuming extraction technique. Therefore, the purposes of this study were to optimize the extraction process of α-mangostin with minimum presence of co solvent, optimum temperature and pressure as the variables in SC-CO_2 by using Response Surface Methodology in Design Expert software and further isolation of α-mangostin compound.

1.3 Objective of Research

1) To compare between conventional soxhlet extraction and SC-CO_2 extraction on α-mangostin yield.
2) To optimize SC-CO_2 extraction of α-mangostin using response surface methodology for the highest yield of α-mangostin content.
3) To isolate and structural elucidation of α-mangostin.

1.4 Scopes of Research

In order to achieve the objective, studies have been narrowed down and identified in this research. The scopes of the research were listed as below:-

1) Comparison between SC-CO_2 and soxhlet extraction in terms of yield of α-mangostin based from TLC and HPLC analysis and determine the best particle size range either 0.10-0.50 mm or 0.60-1.00 mm and optimum CO_2 flow rate.
2) Optimization of α-mangostin extraction conditions with parameters of pressure range from 20, 25, 30 MPa, temperature at 40, 50, 60 °C and percentage of ethanol as co solvent ranging from 0, 1.5, 3% using Box-Behnken design.
3) Isolation of α-mangostin using column chromatography and structural elucidation by HPLC, H^1 NMR, C^{13} NMR and Mass Spectrometry.

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