EXTRACTION AND CHARACTERIZATION OF OILS FROM *PHALERIA MACROCARPA* SEED

BY

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ABSTRACT

Phaleria macrocarpa (local name: *Mahkota Dewa*) is being traditionally used as an important medicinal plant for centuries in Malaysia and Indonesia. Different parts of this plant have been studied by several researchers for the pharmacological activities and bioactive compounds. Apart from the bioactive compounds, the seed of this plant contains high amount of oil that is of more than half of its dry weight. The oil content of *P. macrocarpa* seed, fatty acids composition and physicochemical properties of the oil were determined in this study. The oil of *P. macrocarpa* seed was extracted by solvent extraction as a conventional method using n-hexane and by supercritical CO_2 ($scCO_2$) extraction as a non-conventional method. The extraction conditions were optimized for both techniques using central composite design (CCD) of response surface methodology (RSM). In solvent extraction, 72°C, 8.4 h and 10.9 ml/g solvent-to-feed ratio was the optimum condition whereas 72°C, 42 MPa and 4.5 ml/min CO_2 flow rate was the optimum condition for $scCO_2$ extraction. At optimized condition, oil yields were 55.68g/100g and 52.9g/100g of dry seeds obtained by solvent extraction and $scCO_2$ extraction methods, respectively. The results have shown that 95% oil extracted without utilizing any hazardous organic solvents in a short period of time by $scCO_2$ extraction technique. The experimental and predicted data from the second order polynomial model of RSM was fitted adequately with R^2 of 0.97 and 0.99 for solvent extraction and $scCO_2$ extraction, respectively. To compare the predictive quality of RSM, a feed forward artificial neural network (ANN) based on a multi-layer perceptron (MLP) was modeled for the oil yield. ANN was proven as of better predictive model for the seed oil extraction than the RSM as it had similar R^2 value and lower absolute average deviation (AAD) compare to RSM. The fatty acid composition in terms of triglycerides of the extracted *P. macrocarpa* seed oil was analyzed by gas chromatography-mass spectroscopy (GC-MS). Five major fatty acids such as palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and eicosanoic acid (C20:1) were found in the *P. macrocarpa* seed oil. Oleic acid (18:1) was found to be the predominant fatty acid constituent with 43.84±0.52% of the total fatty acid constituents. On the other hand, the long chain fatty acid (eicosanoic acid, 0.7%) and polyunsaturated fatty acid with high percentage of linoleic acid (29.18±0.65%) were also determined in the *P. macrocarpa* seed oil. Moreover, the total unsaturated fatty acids were 73.62±1.41% whereas the saturated fatty acids were only 26.38±0.18% in this seed oil. Physicochemical properties of the *P. macrocarpa* seed oil were also determined and comparable with other claimed high grade vegetable oils such as sunflower oil, soybean oil, palm oil etc. Thus, the *P. macrocarpa* (*Mahkota Dewa*) seed oil could also be claimed as food and pharmaceutical grade oil.

خلاصة البحث

Phaleria macrocarpa (الاسم المحلي : ماهكوتا ديوا) وتستخدم تقليديا باعتبارها من النباتات الطبية الهامة لعدة قرون وخاصة في ماليزيا واندونيسيا. وقد تم دراسة أجزاء مختلفة من هذا النبات من قبل العديد من الباحثين لأثبات المركبات البيولوجية النشطة علميا فيها. وبصرف النظر عن المركبات البيولوجية النشطة ، فالبذور تحتوي على كمية عالية من الزيت الذي يمثل أكثر من نصف وزنها الجاف. CO_2 سوبركرتيكال ($scCO_2$) كوسيلة استخراج غير تقليدية . تم تحسين شروط الاستخراج لكلا تقنيات التصميم باستخدام CCD و RSM. في الاستخلاص بالمذيبات، كانت 72 درجة مئوية ، 8.4 ساعة و 10.9 مل نسبة المذيبات هي الظروف المثلى، في حين 72 درجة مئوية، 42 ميجا باسكال و 4.5 مل / دقيقة معدل تدفق CO_2 كانت الظروف المثلى لاستخراج $scCO_2$. في الحالة الأمثل، تم الحصول على زيت بنسبة 55.68 ٪ و 52.9٪ على أساس الوزن الجاف للبذور بواسطة مذيبات و $scCO_2$ على التوالي. وقد اظهرت النتائج أن حوالي 95٪ غير التقليدية باستخدام $scCO_2$ وهذا يمثل والمتوقعة لل RSM كانت R^2 لها بقيمة 0.97 و 0.99 أيضا لمقارنة جودة التنبؤية مع RSM. وقد ثبت الشبكة العصبية الاصطناعية أفضل نموذج تنبؤي لاستخراج زيت البذور مقارنة بـ RSM فقد كانت قيمة R^2 متماثلة (AAD) اقل مقارنة بـ RSM. تم تحليل تكوين الأحماض الدهنية من حيث الدهون الثلاثية في استخراج زيت بذور *P. macrocarpa* بواسطة الغاز اللوني الطيفي الشامل (GC-MS). خمسة احماض دهنية رئيسية وهم : حمض البالمتيك (C16:0)، وحمض الستريك (C18:0)، وحمض الأوليك (C18:1)، وحمض اللينوليك (C18:2) وحمض الايكوسانويك (C20:1) تم العثور عليها في زيت بذور النبات. تم العثور على حمض الأوليك (18:1) ليكون الحمض الدهني السائد مع نسبة 43.84 ± 0.52٪ من إجمالي مكونات. من ناحية أخرى، لوحظ سلسلة طويلة من الأحماض الدهنية (حمض 0.7٪) و الأحماض الدهنية غير المشبعة مع نسبة عالية من حمض اللينوليك (29.18 ± 0.65 ٪) أيضا في زيت بذور *P. macrocarp* . ومع ذلك ، كانت نسبة الأحماض الدهنية غير المشبعة التي تم العثور عليها 73.62 ± 1.41 ٪ في حين كانت الأحماض الدهنية المشبعة فقط نسبتها 26.38 ± 0.18 ٪ في زيت هذه البذور . تم تحديد الخصائص الفيزيائية والكيميائية لزيت بذور *P. macrocarpa* أيضا والتي كانت قابلة للمقارنة مع غيرها من الزيوت النباتية مثل زيت الزيتون وزيت عباد الشمس وزيت فول الصويا وزيت بذور العنب الخ، وبهذا يمكن لزيت بذور *P.macrocarpa* (ماهكوتا ديوا) أن يعد كزيت للطعام و الاستخدامات الصيدلية.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Pharmaceutical Technology.

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DECLARATION

I hereby declare that this thesis is the result of my own investigation, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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CHAPTER ONE

INTRODUCTION

1.1 PREAMBLE

Phaleria macrocarpa is a popular herbal plant to the traditional practitioners of Malaysia and Indonesia. The local name of this plant is *Mahkota Dewa* that means God's Crown. Most of the parts of this plant have been used in traditional medicine for its several anti-diseases properties like anti-diabetic, anti-hypertensive, anticancer, anti-allergic, anti-uretic, anti-rheumatic and healing properties (Harmanto, 2003). Different parts (leaves, stem, fruit and seed) of *Mahkota Dewa* have been examined for its biological and pharmacological potencies by several researchers (Ali, Atangwho, Kuar, Mohamed & Mohamed, 2012a; Katrin & Winarno, 2011; Yosie, Effendy, Sifzizul, & Habsah, 2011; Rahmawati, Dewoto & Wuyung, 2006; Sugiwati, Kardono & Bintang, 2006; Susilawati, Matsjeh, Pranowo & Anwar, 2011; Hendra, Ahmad, Sukari, Shukor & Oskoueian, 2011a). Alkaloid, lignin, saponin, terpenoid and poly-phenol are the major types of bioactive compounds in this plant. The essential oils of *P. macrocarpa* fruit flesh are octadecana tricosan, octacosan, diocthylester and tributylacetylcitrate. Three active compounds namely mahkoside A, mangiferin (xanthone glycosides), kaempferol 3-O-β-D-glucoside have been isolated from the seed part (Zhang, Xu & Liu, 2006). Apart from bioactive compounds, the seed of this plant contains high amount of oil. But no information has been found in literature concerning the amount of oil content as well as the composition and characteristics of the oil.

Fatty acids are the main components of fat or oil and they have importance in multidimensional functions such as energetic, metabolic and structural activities on living body (Elias, 1983). Some oils are used for nutritional or food purposes, some for cosmetic productions while others are necessary for producing energy like biofuel. The proper application of particular oil depends on the characteristics of that oil meanwhile the characteristics depends mainly on their composition (Ramadan & Mörsel, 2003). For example, the vegetable oils are used for food preparation and production of biodiesel depending on their oil characteristics and composition. Some fatty acids like linoleic acid and arachidonic acid known as polyunsaturated fatty acids that are being used for human nutrition (Carvalho, Miranda & Pereira, 2006) are essential for growth and development. There are innumerable sources of fat or oil available and oily seeds are one of the important sources among them. There is evidence that some oily seeds and nuts have protective effect on human health by reducing cholesterol, blood pressure and diabetes (Moodley, Kindness & Jonnalagadda, 2007). The available and popular wild seed oils are jatropha seed oil, wild carrot seed oil, rosehip seed oil, wild almond seed oil, Kalahari melon seed oil and so on which have been used from biodiesel preparation to food preparation. There are many studies on the seed oil from different wild plants indicating that those wild seeds can be promising oil sources for many purposes like nutritional, medicinal and industrial purposes (Berchmans & Hirata, 2008; Tang, Liu, Ling, Lai, Zhang et al., 2013; Okieimen & Eromosele, 1999).

Processing method is a vital determinant of the final quality of seed oil. When the oils from seeds are inadequately processed, they can easily be deteriorated by principal deteriorative reaction with the formation of hydroperoxides and several secondary products of oxidation such as aldehydes, peroxides and ketones (Nzikou, Mvoula-Tsiéri, Ndangui, Pambou-Tobi, Kimbonguila et al., 2010). These compounds can decrease quality of oils drastically. Extraction of oil from oily seed is considered as the first processing step.

There are several techniques available to extract oil from plant matrix. Solvent extraction is commonly used for oil extraction. It has, however, some inherent drawbacks; (a) heat is essential for the distillation of oil which can deteriorate thermo-labile compounds, (b) the oil contains residual solvents that possess the risk of degenerative disease upon ingestion and (c) rancidity could occurs during the separation process as oil is oxidatively unstable (Scalia, Giuffreda & Pallado, 1999). For the extraction of oil, supercritical fluid extraction (SFE) attracts considerable attention as a promising alternative to conventional organic solvent extraction. Carbon dioxide is commonly used solvent in SFE because of its low critical temperature (31.1°C) and critical pressure (7.28 MPa). The lower critical temperature makes it an ideal solvent for extracting thermally sensitive materials. Supercritical carbon dioxide (scCO_2) has high extraction rate for oil extraction. Furthermore, scCO_2 is non-toxic, non-flammable, environment friendly, non-explosive, cost effective, less laborious, available and easy to remove from the extract thus eliminates post processing steps (Liu, Yang, Zhang, Ji, Hong et al., 2009; Lang & wai, 2001; Herrero, Cifuentes, & Ibanez, 2006). In addition, products obtained by scCO_2 extraction are completely free from solvent residues. On the contrary, the used solvent must be removed from the solvent-extracted products before further processing. The scCO_2 extracted crude oils can also be more easily refined than conventionally extracted oils as they contain fewer impurities (Devittori, Gumy, Kusy, Colarow, Bertoli et al., 2000, Leque de Castro, Cases & Tena, 1994).

The extraction efficiency of $scCO_2$ can be influenced by many factors such as extraction temperature and pressure, particle size and moisture content of the sample, time of extraction and flow rate of CO_2 (Temelli & Guclu-Ustundag, 2005; Ibañez, Herrero, Mendiola & Castro-Puyana, 2012). Like $scCO_2$ extraction, conventional solvent extraction efficiency also depends on the type of solvent, particle size of the sample, extraction temperature, agitation intensity (Azmir, Zaidul, Rahman, Sharif, Mohamed et al., 2013; Atabani, Silitonga, Ong, Mahlia, Masjuki et al., 2013). Other important influential factors of solvent extraction are solvent-to-feed ratio and time of total extraction (Stanisavljević, Lakićević, Veličković, Lazić & Veljković, 2007; Stroescu, Stoica-Guzun, Ghergu, Chira & Jipa, 2013). Optimization of significant influencing factors of $scCO_2$ extraction and conventional solvent extraction have been reported by several researchers for different seed oils (Kostić, Joković, Stamenković, Rajković, Milić et al., 2013; Stroeascu et al., 2013; Sayyar, Abidin, Yunus & Muhammad, 2009; Zahedi & Azarpour, 2011; Azmir, Zaidul, Rahman, Sharif, Sahena et al., 2014; Ivanov, Čolović, Lević & Sredanović, 2012; Khajeh, 2012). Response surface methodology (RSM) is a very effective technique to optimize the significant operating parameters as well as to determine the effect of individual input variable and the interaction of variables for maximizing the benefits (Wang, Liu, Wei & Yan, 2012; Raymond & Montgomery, 2002). Optimum and valid output can be produced with a minimum effort, time and resources by RSM as it requires minimum experimental runs for optimizing multiple independent variables (Sharif, Rahman, Azmir, Mohamed, Jahurul et al., 2014). Second order polynomial model is used to describe and predict the response variable and to optimize the input variables in RSM. For measuring the impact of various input parameters on the extraction of fats or oils, CCD of RSM is often used (Mani, Jaya & Vadivambal, 2007; Shao, Sun & Ying, 2008; Wei, Liao, Zhang, Liu & Jiang, 2009; Sirisompong, Jirapakkul & Klinkesorn, 2011).

The relationship between the input and output of a complex non-linear process like extraction is usually affected by a number of factors that is difficult to perceive using conventional regression methods. On the other hand, artificial neural network (ANN) is very helpful to predict the response for non-linear system as it is inspired biologically on the basis of different characteristics of the function of brain. ANN is a simplified model of the structure of a biological network (Mandal, Sivaprasad, Venugopal & Murthy, 2009) and an artificial neuron is its main processing element. The main advantage of ANN is that it does not need any mathematical modeling (Mandal et al., 2009) like linear and polynomial regressions based modeling. ANN requires sufficient input and output data (Ali & Cinar, 2005) since it learns from examples and recognizes patterns in a series of input and output values without any prior assumptions about their nature and interrelations (Mandal et al., 2009).

1.2 OBJECTIVE OF STUDY

i. To extract oil from *Phaleria macrocarpa* seeds using conventional solvent extraction and supercritical carbon dioxide extraction techniques.
ii. To compare RSM and ANN model for the prediction of oil yield.
iii. To analyze the fatty acids composition in terms of triglycerides and to determine the physicochemical properties of the extracted oil.

CHAPTER TWO

LITERATURE REVIEW

2.1 *PHALERIA MACROCARPA*

2.1.1 Background

Phaleria macrocarpa is traditionally treated as an important medicinal plant since centuries. *Mahkota Dewa* (God's crown) is the local name of this plant which implies that it descends from heaven, as a benediction from divinity to help mankind. God's Crown is an indigenous plant from the island of Papua (*Irian Jaya*) located in the far east of the Indonesian archipelago. This plant is thought to have several anti-diseases and healing properties which make it valuable in folk medicine. From leaves to root, every parts of this plant have been used for disease prevention. Anti-diabetic, anti-hypertensive, anticancer, anti-allergic, anti-uretic, anti- rheumatic properties and so forth are claimed by informal practitioners (Harmanto, 2003).

P. macrocarpa was first described as *Drimyspermum macrocarpum* by Scheffer based on fruiting specimens collected by Teysmann (with the herbarium collection #7786, L, Bo) near Doré, in western New Guinea. Young fruits are ellipsoid or slightly obovoid (15 by 12 mm) and narrowed stipe-like towards the base. This shape is not uncommon and can also be observed in other herbarium collections e.g. bb25746, BW 5468 from Hollandia and NGF 7298 from Morobe distric. The *P. macrocarpa* fruits with seeds are shown in Figure 2.1.

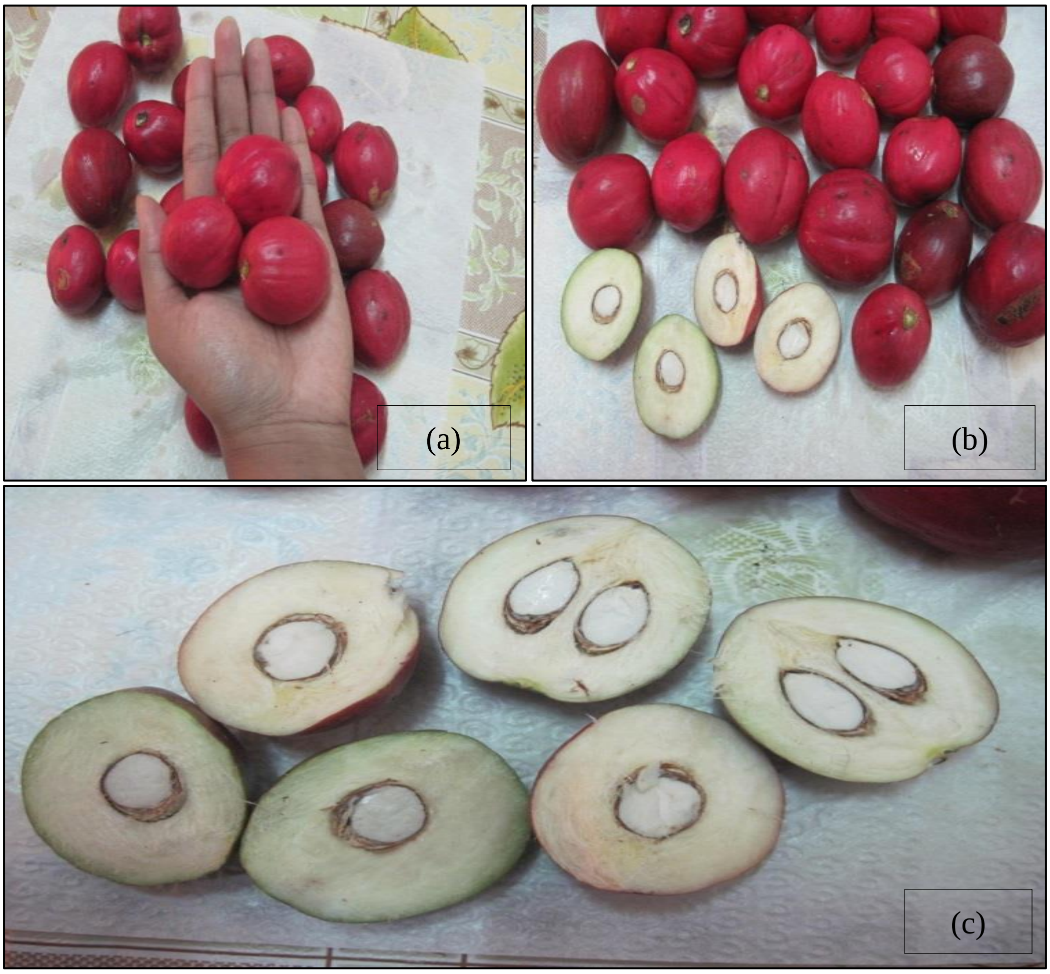

Figure 2.1. The *P. macrocarpa* (a) fruits, (b) fruits with seeds and (c) chopped fruits with seeds.

This plant is found in primary and secondary forests, from the lowland up to 550 m in Malaysia and in western New Guinea. It is a shrub or small tree up to five meters (Harmanto, 2003) but its height could also reach up to 18 meters. It features a many branched crown, a large straight root (one meter long) exuding sap, a brownish green bark and white wood. It has green tapering leaves, 7-10 cm long and 3-5 cm wide. The flowers are little white trumpet-like fragrant flowers and from a compound of 2-4 flowers come out. The fruit can be in various sizes and color, from green to maroon. The pit is round, white and very poisonous. It grows in areas of 10–1,200 m above sea level with productive age between 10-20 years.

2.1.2 Taxonomy of *P. macrocarpa*

Superkingdom	: Eukaryota
Kingdom	: Viridiplantae
Phylum	: Streptophyta
Class	: Eudicotyledons
Subclass	: Eurosids II
Order	: Malvales
Family	: Thymelaeaceae
Genus	: *Phaleria*
Species	: *P. macrocarpa*

2.1.3 Bioactive Compounds of *P. macrocarpa*

Various parts of this plant have been examined for its bioactive contents. Alkaloid, lignin, saponin, terpenoid and poly-phenol are the major types of bioactive compounds containing in this plant. The latex reported to have toluquinone, ethylquinone, octanoic acid, 1-nonene, 1-undecene, 1-pentadecene, 1-heptadene and 6-alkyl1-1-4-naphtoquinone. The plant leaves contain mahkoside A (4,4´-dihydroxy-6-methoxybenzophenone-2-O-β-D- glucoside) and phalerin (4,5-dihydroxy,4´-methoxybenzophenone-3-O-β-D-glucoside) (Zhang et al., 2006). Phalerin was first isolated by Wahyuningsih, Mubarika, Gandjar, Hamann, Rao et al. (2005) but Oshimi, Zaima, Matsuno, Hirasawa, Iizuka et al. (2008) revised it and gave a new structure for it. Additionally, mangiferin, kaempferol-3-O-β-D-glucoside, dodecanoic acid, palmitic acid, ethyl stearate and sucrose have been found from the leaves of *P. macrocarpa* (Zhang et al., 2006). Three compounds namely mahkoside A, mangiferin (xanthone glycosides), kaempferol 3-O-β-D-glucoside have been isolated from seed by Zhang et al. (2006). Winarno (2008) isolated 6,4´-dihyhydroxy-4-methoxybenzophenone-2-O-β-D glucospiranoside from the bark. Icariside C_3 (sesquiterpene glucoside) and benzophenon derivatives were found in chloroform extract and mangiferin (xanthone glycoside) was isolated from methanol extract of fruits (Oshimi et al., 2008). Benzophenon glucoside (6,4´-dihydroxy-4-methoxybenzophenone-2-O-α-D glucospiranoside) and 5-[4 (-methoxy-phenyl-tetrahydrofuro- [3,4-c]furan-1-yl]-benzene 1,2,3-triol, known as cytotoxic lignin have been isolated from ethyl acetate and butanol extracts of fruit pericarp. Bioactive compounds of *P. macrocarpa* are shown in Figure 2.2.

The essential oil content of the flesh of fruit are octadecana tricosan, octacosan, diocthylester and tributyl acetyl citrate, while the seed consists of heptadecana, octadecana, eicosan, tricosan, vinyl laurat, diocthylester.

2.1.4 Pharmacological Effect of *P. macrocarpa*

P. macrocarpa has been used from long time as medicinal plant by local people of Indonesia and Malaysia for curing various diseases such as cancer, diabetic, allergy, hypertension, lowering uric acid etc. Some of these are scientifically proved by various researchers.

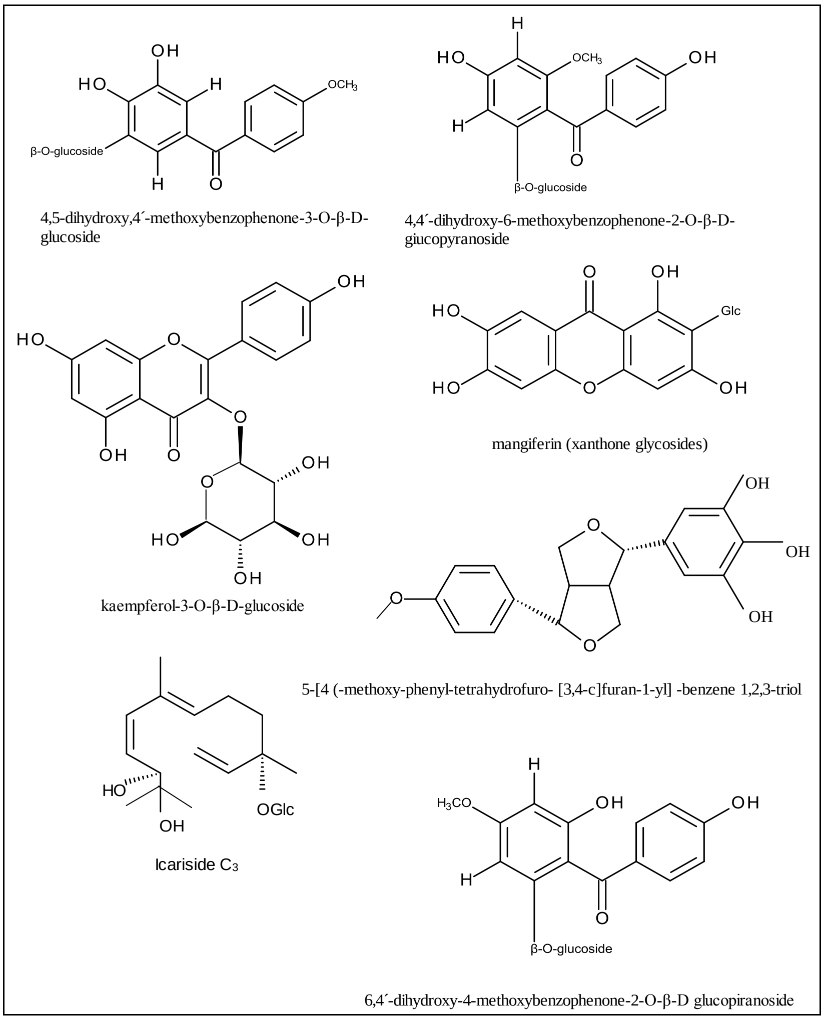

Figure 2.2. Bioactive compounds of *P. macrocarpa*.

2.1.4.1 Anticancer Effect

Use of *P. macrocarpa* extract for cancer treatment is well known. Bakhriansyah (2012) conducted cytotoxicity test of ethanol extract of fruits and found that seeds have toxicity to T47D breast cancer cell line. The ethanol extract of seed and fruit flesh increased the p53 gene expression but no effect on Bcl-2 gene expression and n-hexane extraction of fruits had higher effect of increasing p53 gene expression than fruit flesh but no effect on Bcl-2 gene expression. Astuiti, Raharjo and Eviane (2007) found ethanol extract having no toxicity on human mononuclear cell but having slight toxicity on vero cell line. The fruit parts (pericarp, mesocarp and seed) showed