PHYTOCHEMICAL CONSTITUENTS OF *MUSA X PARADISIACA* ABB FLOWER EXTRACTS AND ITS GALACTAGOGUE EFFECTS ON LACTATING RATS

BY

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ABSTRACT

Banana, which is native to Southeast Asia, is considered as one of the most important contribution to the international fruit industry and being a very important source of income, employment and export earnings in some developing countries. The present research focused on the agricultural by product in which the flower of *Musa x paradisiaca* ABB was selected to study for its phytochemical constituents and galactagogue effects on lactating rats. Initially, varieties of musa species were screened for the presence of secondary metabolites, followed by quantitative analysis and antioxidant activities of the phytochemicals in the selected species. The potential use of the flower in the form of crude and aglycone form of aqueous extract (AFAE) were then extensively study for its effects on galactagogue, growth rate of the suckling pups, mammary glands development and nutritional quality of the milk produced. Chemical compounds that responsible to the galactagogue activities were isolated and characterized for its chemicals structure determination at the end of the study. Result of phytochemicals screening on Musa extracts showed the presence of alkaloids, glycosides, steroids, saponins, tannins, flavanoids and terpenoids. DPPH free radical scavenging assay indicated potential use of the extracts as antioxidant agent. Galactagogue measurement with the crude by weight-suckle-weight method showed that the aqueous extract gave significant milk production compared to petroleum ether and ethanol extracts. Comparable amount of milk was also identified in the rats treated with AFAE (4.65± 1.67 g/pup/day) and the aqueous crude extracts (4.86 ± 2.36 g/pup/day). These values are equivalent to 28.03% and 24.87% higher of milk production in the aqueous and AFAE extracts, respectively, than the control group. AFAE and aqueous extract showed higher weight gain of pups than condensed tannin (CT) and metoclopramide within the experimental period. Pups growth rate was significantly improved for the groups treated with the aqueous and AFAE extract. Analysis on mammary gland showed that AFAE was capable to significantly increase the weight of mammary gland (31.73 ± 0.44 mg/g body weight), protein content of the gland (11.66 ± 0.44 g/100g mammary gland) and size of alveoli (124.49 – 139.91 µm) compared to CT (106.83 – 112.85 µm). Consistency of the results from observations on mammary gland and measurement of milk production *in vivo* confirmed the role of AFAE in promoting galactagogue activities. AFAE was also found to provide more wholesome of milk production because analysis showed it contains higher protein (34.29 ± 0.30%) and lower fat (43.71± 0.90%) than the control group. Spectroscopy data of ^{1}H-NMR, ^{13}C-NMR, IR and MS spectra indicated that bioassay guided isolation of the AFAE afforded four types of compounds; stigmasterol, β-sitosterol, caryophyllene and ophiobolin. In conclusion, the bioactive compounds exist in the flower were identified as the compounds responsible to the increased of milk production in lactating rats. This study provides novel information regarding phytochemical compounds from *Musa x paradisiaca* ABB species which influenced galactagogue activities. The benefits of this work could be extended for pharmacological use of the extract compound for mothers of inadequate milk production and/or could be exploited for commercialization for pets and livestock.

ABSTRAK

Pisang, yang berasal dari Asia Tenggara, adalah salah satu penyumbang utama industri buah global dan menjadi sumber penting pendapatan, pekerjaan dan eksport sesetengah negara membangun. Kajian ini memberi tumpuan kepada hasil sampingan pertanian di mana jantung pisang daripada *Musa x paradisiaca* ABB dipilih untuk dikaji kandungan fitokimia dan kesannya sebagai agen peningkatan susu (*galactagogue*) pada tikus yang menyusu. Peringkat awalnya, pelbagai spesis Musa disaring berdasarkan kehadiran fitokimia sekunder, diikuti analisis kuantitatif dan aktiviti antioksidan fitokimia berkaitan untuk spesis terpilih. Potensi kegunaan jantung pisang dalam bentuk ekstrak kasar dan ekstrak air tanpa glukosida (AFAE) dikaji secara intensif dari aspek kesan *galactagogue*, kadar pertumbuhan anak susuan, perkembangan kelenjar susu dan nilai nutrisi susu. Bahan kimia yang bertanggungjawab terhadap aktiviti *galactagogue* ditentukan di akhir kajian. Keputusan pemeriksaan fitokimia ekstrak *Musa* menunjukkan kehadiran alkaloid, glikosida, steroid, saponin, tanin, flavanoid dan terpenoid. Radikal bebas *scavenging assay* dengan DPPH, menunjukkan ekstrak ini berpontensi sebagai agen anti pengoksidaan. Pengukuran pengeluaran susu menggunakan kaedah timbang-susu-timbang menunjukkan ekstrak air dapat meningkatkan susu secara ketara berbanding ekstrak petroleum eter dan etanol. Kesetaraan jumlah susu dikenalpasti bagi tikus yang dirawat dengan AFAE (4.65± 1.67 g/anak/hari) dan ekstrak air (4.86 ± 2.36 g/anak/hari). Ukuran ini adalah bersamaan dengan peningkatan susu sebanyak 28.03% dan 24.87% lebih tinggi secara relatif bagi kumpulan ekstrak air dan AFAE berbanding kawalan. Ekstrak AFAE dan air menunjukkan peningkatan berat anak yang lebih tinggi berbanding condensed tannin (CT) dan metoclopramide dalam tempoh eksperimen. Kadar pertumbuhan anak meningkat secara ketara bagi kumpulan yang dirawat dengan ekstrak air dan AFAE. Analisis ke atas kelenjar susu menunjukkan bahawa AFAE dapat meningkatkan secara ketara berat kelenjar (31.73 ± 0.44 mg/g berat badan), kandungan protein kelenjar (11.66 ± 0.44 g/100g kelenjar susu) dan saiz alveoli (124.49 – 139.91 µm) berbanding CT (106.83 – 112.85 µm). Keputusan yang konsisten dari pemerhatian ke atas kelenjar susu dan pengukuran pengeluaran susu secara *in vivo* mengesahkan peranan AFAE dalam merangsang aktiviti *galactagogue*. AFAE juga menyumbang kepada pengeluaran susu yang sihat kerana analisis menunjukkan ia mengandungi protein yang lebih tinggi (34.29 ± 0.30%) dan lemak yang lebih rendah (43.71 ± 0.90%) berbanding kumpulan kawalan. Data spektroskopi menggunakan spektrum ^{1}H-NMR , ^{13}C-NMR , IR dan MS menunjukkan pengasingan bioesei berpandu ke atas AFAE menghasilkan empat kompoun; stigmasterol, β-sitosterol, caryophyllene dan ophiobolin. Secara kesimpulannya, sebatian bioaktif yang wujud dalam bunga telah dikenal pasti sebagai sebatian yang bertanggungjawab kepada peningkatan pengeluaran susu bagi tikus yang menyusu. Kajian ini memberikan novel maklumat mengenai sebatian fitokimia dari spesis *Musa x paradisiaca* ABB yang mempengaruhi aktiviti *galactagogue*. Manfaat dari hasil kerja ini boleh dimajukan untuk kegunaan farmakologi bagi kegunaan ibu-ibu yang ketidakcukupan pengeluaran susu dan/atau seterusnya boleh dieksploitasi untuk dikomersialkan bagi kegunaan haiwan peliharaan dan ternakan.

خلاصة البحث

الموز، والتي تعود اصولها الى مناطق جنوب شرق اسيا، وتعتبر من اهم المساهمات لصناعة الفاكهة الدولية ومصدراً مهماً للدخل والعمالة والصادرات في بعض الدول النامية. نمط نمو الموز يؤدي الى وفرة الزهور. تم اختيار موز البرا سيديا ABB لدراسة التركيب الكيميائي والفعالية الحيوية كمدر للحليب عند الفئران. بداية، تم فحص بعض أصناف الموز للبحث عن المستقلبات الثانوية ثم التحليل الكمي والفعالية المضادة للأكسدة للمواد الكيميائية النباتية في الانواع المختارة. تمت دراسة امكانية استخدام خلاصة الازهار وكذلك الشكل اللاسكري للخلاصة المائية (AFAE) كمدر للحليب وكذلك معدل نمو الجراء الرضيعة، وتطور الغدد الثديية والجودة الغذائية للحليب المنتج. تم عزل وتوصيف البنية الكيميائية للمركبات الكيميائية المسؤولة عن ادرار الحليب حيث أظهرت النتائج وجود قلويدات، جليكوسيدات، والمنشطات، الصابونين, العفص، فلافونيدات وتيربينوئيدات. بينت نتائج فحص الجذور الحرة DPPH فعالية عالية للخلاصات كمضادات للأكسدة. استخدمت طريقة الوزن–الرضاعة– الوزن وقد بينت النتائج إنتاج كمية حليب كبيرة مقارنة لدى استخدام الخلاصة المائية مقارنة مع خلاصة الايتر والإيثانول. وقد تم الحصول على كميات معتبرة من الحليب أيضا في الفئران المعالجة ب AFAE(1.67 ±4.65 غ/الجرو/يوم) والخلاصة المائية (4.86± 2.36 ملغ/الجرو /يوم). هذه القيم تعادل 28.03 ٪ و 24.87 ٪ أعلى من مجموعة الشاهد للخلاصة المائية و AFAE، على التوالي. أظهرت AFAE و المستخلص المائي أعلى زيادة وزن في الجراء من مستخلص التانين المكثف (CT) و ميتوكلوبراميد وكذلك معدل نمو الجراء. وأظهر تحليل الغدة الثديية أن AFAE قادرة على زيادة كبيرة في وزن الغدة الثديية (31.73± 0.44 ملغ / غ من وزن الجسم) محتوى البروتين (11.66 ± 0.44 غ/ 100 غ الغدة الثديية) وحجم الحويصلات الهوائية 124.49–139.91ميكرون) مقارنة مع (112.85 – 106.83 ميكرون) لل CT. أكدت نتائج الدراسة على في الانسجة دور AFAE في تعزيز ادرار الحليب وبين تحليل AFAE أنه يحتوي على أعلى نسبة بروتين (34.29 ± 0.30٪) واقل نسبة دهون (43.71 ± 0.90٪) من المجموعة الشاهد. وأظهرت نتائج التحليل الطيفي ^{1}H-NMR, ^{13}C-NMR ، اشعة تحت الحمراء و الكتلة عزل اربعة مركبات من AFAE باسلوب العزل الحيوي وهي ستيغماستيرول، β سيتوستيرول، ، كريوفيلين و اوفيوبولين. في الختام، أثبتت هذه الدراسة وجود مركبات فعالة حيويا كمدرات للحليب في زهور الموز. من الممكن عزل هذه المركبات واستخدامها للأمهات اللواتي يعانين عن عدم كفاية إنتاج الحليب يمكن استغلالها للتسويق وزيادة الحليب لدى الحيوانات الاليفة والمواشي.

APPROVAL PAGE

The thesis of Azizah Binti Mahmood has been approved by the following

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DECLARATION PAGE

I hereby declare that this dissertation is the results of my own investigations, 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.

Azizah Mahmood

Signature ..... Date.....

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PHYTOCHEMICAL CONSTITUENTS OF *MUSA X PARADISIACA* ABB FLOWER EXTRACTS AND ITS GALACTAGOGUE EFFECTS ON LACTATING RATS

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Azizah Mahmood

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LIST OF SYMBOLS AND ABBREVIATIONS

1D	One dimentional
2D	Two dimentional
µL	10^{-6} litre (s) or microlitre (s)
µg	10^{-6} gram (s) or microgram(s)
AA	Amino acid
ABs	Alveolar buds
AFAE	Aglycon form of aqueous extract
ANOVA	Analysis of variance
BST	Bovine growth hormone
CP	Crude protein
cps	Counts per second
CNS	Central nervous system
COSY	Correlated Spectroscopy
CT	Condensed tannins
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	1, 1-diphenyl-2-picryl-hydrazyl
DSHEA	Dietary Supplement Health and Education Act
EAA	Essential amino acids
EGF	Epidermal growth factor
EtOAc	Ethyl acetate
EtOH	Ethanol
FI-O*	aroxyl radical
FI-OH	Alavanoid
FDA	Food and Drug Administration
GC	Gas Chromatography
GH	Growth hormone
HCl	Hydrochloric acid
H&E	Haematoxylin and eosin
HNO_3	Nitric acid
HPLC	High Performance Liquid Chromatography
H_2SO_4	Sulfuric acid
HT	Hyroysable tannins
Hz	Hertz
IACUC	Institutional Animal Care and Use Committee
IC_{50}	Concentration that inhibit a response by 50% relative to positive control
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
L	Liter(s)
LSD	Least square difference
L/min	Liter per minute
λ (nm)	Wavelength (in nanometers)
M	Molar
MAPK	Mitogen activated protein kinase
MeOH	Methanol

MG	Ratio of milk protein (grams per day)
mg	10^{-3} gram (s) or milligram (s)
mg/g	Miligram per gram
ml	10^{-3} liter (s) or milliliter (s)
ml/min	Mililiter per minute
mbar	Milibar
m.p.	Melting point
MΩ cm	Milliohm (definition of purified water quality)
MS	Mass Spectroscopy or Mass Spectrum
m/z	Mass to charge ratio
ND	Not determined
N	Normality
NAN	Non-ammonia nitrogen
NIR	Near Infrared Reflectance
nm	Nanometer
NMR	Nuclear Magnetic Resonance
P	Probability
PEG	Polyethylene glycol
Pet ether	Petroleum ether
PMS	Premenstrual syndrome
ppb	Part per billion
ppm	Parts per million
PRL	Prolactin
r^2	correlation coefficient
R*	Radicals
R_f	Retention factor; migration distance of analyte as a fraction of distance to solvent front in thin-layer chromatography
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rpm	Round per minute
SHBG	Sex hormone binding globulin
STAT	Signal transducers and activators of transcription
Si	Silica
Sp	Species
SPSS	Statistical Package for the Social Sciences
TA	Tannic acid
TDLUs	Terminal ductule lobular units
TEBs	Terminal end buds
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
UV	Ultraviolet
VFI	Voluntary feed intake
W	Watt
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION, STATEMENT OF THE PROBLEM AND LITERATURE REVIEW

1.1 INTRODUCTION AND STATEMENT OF PROBLEM

Bananas and plantains are among the popular and cheapest foods throughout the tropical and sub-tropical regions of the world. Globally in 2009, approximately 4.9 million hectares of the harvested area planted with bananas and plantains, with an annual production of around 97.3 million metric tonnes (Evans & Ballen, 2012). A tremendous increase of banana yield production (49%) was recorded from 2000 to 2009. According to the Food and Agriculture Organization of the United Nations (FAO) Statistics estimation, world total exports of banana accounted for 18.3 million tonnes in 2009. In some developing countries of Latin America, the Caribbean, Southeast Asia and West Africa, banana industry being a very important source of income, employment and export earnings. Although banana is one of the most important commercial crops in the world, it is estimated that 87% of the production is purposely for local consumption (Dzomeku et al., 2007).

The flower of banana is mostly removed from the center of the pseudostem before harvesting to prompt the growth of fruits thus gain higher yield. Most flowers have only been used as organic material and fertilizer in plantations and only little of them were consumed. Although, recently more attention has been focused on the utilization of agricultural by-products, banana flowers have received little attention from the world of science toward favorable utilization of the resources. As reported by

Alisi (2008), banana family has some traditional medicinal value even though it is more of interest for its nutrients than for its medicinal properties.

It is believed that the flowers of *musa sp* have tremendous pharmacological value based on ethnomedicinal surveys around the world supported by limited bioactivities and clinical research. Banana flowers have been used traditionally to alleviate menorrhagia, dysentery, diabetes mellitus, asthma, heart pain, diarrhea and stomach cramps (Imam & Akter, 2011; Sumathy et al., 2011). Sheng et al. (2010) mentioned that "further studies are needed to document the biological effect of banana flower in physiology and healthy benefits of human being. These may include the investigation of its effect of tannin, phytate, total phenolics flavonoid, alkaloid and sterol in human health as daily diet components".

Most of banana flowers are acknowledged remarkably astringent in taste. Because of its astringency, not all flowers of banana species can be eaten and many were recognized to be taboo for post-partum mothers due to its "cooling" properties. As a consequence, the flowers have limited uses in cooking and food products. There is no research reported concerning medicinal uses, isolated substances or pharmacological effects of banana flower means for galactagogue. Therefore, it is meaningful to search for alternative utilizations of the flower to make it more appreciable and valuable. Through the development of standardized extracts, as well as well-controlled clinical trials, it is hope to increase public interest for products of banana flowers. Thus, this research suggests the alternative therapy for its potential efficacy of bioactive compounds in the management of inadequate breast milk supply (hypogalactia) of breastfeeding women. The utilization of banana flower as lactogenic food could provide additional benefits in reducing its waste, and increasing its uses in pharmaceutical and nutraceutical as a therapeutic agent.

1.2 LITERATURE REVIEW

1.2.1 The Family of Musaceae

Bananas and plantains derive from the family of Musaceae. They are classified as perennial, monocotyledonous herbs with 2-9 m (6.6-30 ft) in height that arise from subterranean rhizomes. Musaceae family is composed of the genera *Musa*, *Ensete* and, possibly a third, *Musella*. *Musa* is the largest genus in the family and all edible banana fruits are produced by plants belonging to the genus. The word *Musa,* thought to be derived either from "mouz" (the Arab word for banana), or from Antonia Musa, a Roman physician to Augustus Caesar (Vàlmayor et al., 1991). Historically, *Musa* has been classified into five sections based on their chromosome number and moephological characters i.e., Musa x=11, Rhodochlamys x=11, Australimusa x=10, Callimusa x=10 and Ingentimusa x=7 (x is the number of chromosome in haploid genome) (Daniells et al., 2001).

1.2.1.1 Synonym Used Species of Banana Plants

Scientifically, banana plants can be recognized by many other names as the following; *Ensete glaucum Musa rubra. M. balbisiana. M. basjoo. M. coccinieа. M. uranoscopos.M. feji. M. lasiocarpa. M. nana. M. cavendishii. M. sinensis. M. sapientum. M. troglodytarum. M. wilsonii. Musella lasiocarpa.*

1.2.1.2 Other Common Names of Banana Plants

Banana plants can be easily recognized based on their common or local names such as; cooking banana, dessert banana, plantain [English], *Mai'a* [Hawai'i], *ba jiao, gan jiao, xiang jiao, ba jiao gen* (stump), *kan-lu* (nectar), *jiao yu* (sap) [China]; *guinea* [Spanish], *box haaz. Mopan* [Maya]; *jaina* [Fiji], *kera* [Nepal]; *mei'a* [Tahiti]; *meika* [Cook

Island]; *fa'i. soa'a* [Samoa]; *kadali* [India]; *pisang, jantung pisang* (flower)[Malaysia]; *pisang, getang* [Indonesia].

1.2.2 The Earliest History of Musaceae Family

The earliest agriculture in the Pacific region has been dated at around 8,000 BC, in which the Fe'i bananas were of some importance (Sharrock & Frison, 1998). Banana plants in the earliest cultivation have been described as 'proto-agriculture' and were collected from the wild rather than planted (Price, 1995). The first scientific term given to banana was *Musa paradisiaca* Linn. by Karl Linnaeus, in his book Species Plantarum in 1753. Later *Musa sapientum* Linn. was introduced in 1759 to describe a dessert banana (Valmayor et al., 2000).

1.2.3 Classification of Edible Bananas

Simmonds & Shepherd (1955) developed a classification system to classify all the edible bananas cultivars systematically. They concluded that the edible bananas originated from two wild and seedy species, *Musa acuminata* Colla and *Musa balbisiana* Colla which are native to Southeast Asia. Figure 1.1 shows geographical distribution of both the edible bananas in the region (De Langhe et al., 2009). *Musa sapientum* were the scientific term given to dessert bananas which stand for sweet fruits that were eaten fresh. *Musa paradisiaca*, on the other hand was applied to plantains which require cooking before they are eaten.