UNIVERSITI PUTRA MALAYSIA

EVALUATION OF ANTIOXIDANT ACTIVITY AND IDENTIFICATION OF BIOACTIVE COMPOUNDS OF Senna siamea (Lam.) LEAF EXTRACT USING SOLVENT AND ULTRASOUND-ASSISTED EXTRACTIONS

ANUTHIDA PHAIPHAN

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By

ANUTHIDA PHAIPHAN

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EVALUATION OF ANTIOXIDANT ACTIVITY AND IDENTIFICATION OF BIOACTIVE COMPOUNDS OF *Senna siamea* (Lam.) LEAF EXTRACT USING SOLVENT AND ULTRASOUND-ASSISTED EXTRACTIONS

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Chairman : Associate Professor Badlishah Sham Baharin, PhD
Faculty : Food Science and Technology

This research was conducted to evaluate antioxidant activity and identification of bioactive compounds of *S. siamea* leaf extracted using solvent and ultrasound-assisted extractions (UAE). A randomized complete block design with three replicates and central composite design (CCD) as a response surface methodology (RSM) were used to design and optimize the experiments. Effects of different solvents and locations on the bioactive compounds and antioxidant activities of *S. siamea* leaf were investigated. The results showed that the various solvent extractions had different amount of antioxidant compounds and activities. Methanol extract presented the highest efficiency values of yield, TPC, tannin content, DPPH, FRAP, and TEAC values, followed by extracts of water, ethanol, and ethyl acetate, respectively. Water extracts had the highest concentration of ascorbic acid, varying from 6.59-9.43 mg/g dw. Meanwhile, Beta-carotene content of ethyl acetate extracts was the highest (28.20 mg/g dw) with significant (p<0.05) differences among locations. The correlation coefficients of antioxidant compounds with three of the antioxidant activity assays (%DPPH, FRAP, and TEAC) revealed that TPC, tannin content, and ascorbic acid of the extracts correlated strongly with antioxidant activities. The average of R^2 of TPC, tannin content, and ascorbic acid was 0.976, 0.955, and 0.833, respectively. The optimization of solvent extraction and UAE of antioxidant compounds and antioxidant activities from *S. siamea* leaf was investigated. RSM and CCD were used to design and optimize experimental conditions. The result showed that the optimum conditions for solvent extraction were 49% of ethanol concentration, temperature at 51ºC, and 22.5 min extraction time. Ethanol concentration, temperature, and time were the most important variables that had effect on the responses. For UAE, the optimum conditions were 40%ethanol concentration, temperature at 37ºC, and time of 7 min. Ethanol concentration was the most important variable that had an effect on the responses, followed by time and temperature. The UAE extracts exhibited higher efficiency, yield, TPC, %DPPH, and FRAP values than solvent extracts. The yield, TPC, %DPPH, and FRAP values increased 10.6%, 20.7%, 9.4%, and 29.6%, respectively from the solvent extraction. These results from the RSM clearly revealed that UAE has potential and is efficient to be used to extract TPC and antioxidant activities in

higher yields compared with solvent extractions. However, more identification studies are required to identify the bioactive compounds in the most desirable extract from *S. siamea* leaf. The individual phenolic acids and flavonoids were carried out using high performance liquid chromatography. The results showed that bioactive compounds in soluble extracts are significantly (p<0.05) higher than cell wall-bound extracts. It was further revealed that soluble phenolic acids are the major phenolic acids in the leaf. Caffeic acid was found as the most abundant phenolic acid in the leaf extracts, followed by gallic acid, and protocatechuic acid at 42.12, 25.27, and 21.65 mg/g dw, respectively, mainly in its soluble form. On the other hand, protocatechuic acid was the main phenolic acid found in cell wall-bound extracts at 19.13 mg/g dw. The flavonoid contents were in the range of 0.38-137.14 mg/g dw in the extracts. Epicatechin and catechin contents were the main flavonoids present in soluble extracts, which were 137.14 and 27.02 mg/g dw and also were the only flavonoids detected in cell wall-bound extracts, which were 10.19 and 16 mg/g dw, respectively. The results exhibited that the main portions of bioactive compounds in the leaf were present in soluble extracts. It indicated that *S. siamea* leaf may be regarded as a valuable source of antioxidant-rich nutraceuticals. The potential applications of *S. siamea* leaf antioxidants could be a value-added to the cosmetic or processed foods industries.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENILAIAN AKTIVITI ANTIOKSIDAN DAN IDENTIFIKASI SEBATIAN BIOAKTIF DAUN *Senna siamea* (Lam.) DARIPADA PENGEKSTRAKAN PELARUT DAN PENGEKSTRAKAN ULTRASONIK-BERBANTU

Oleh

ANUTHIDA PHAIPHAN

November 2016

Pengerusi : Profesor Madya Badlishah Sham Baharin, PhD
Fakulti : Sains dan Teknologi Makanan

Kajian ini telah dijalankan untuk menilai aktiviti antioksidan dan mengidentifikasi sebatian bioaktif daun *S. siamea* yang diekstrak daripada pengekstrakan pelarut dan pengekstrakan ultrasonik-berbantu (UAE). Reka bentuk blok rawak lengkap dengan tiga ulangan dan reka bentuk komposit pusat (CCD) sebagai kaedah tindak balas permukaan (RSM) telah digunakan untuk merancang dan mengoptimumkan eksperimen. Kesan pelarut dan lokasi yang berbeza terhadap sebatian bioaktif dan aktiviti antioksidan daun *S. siamea* telah diselidik. Hasil kajian menunjukkan kepelbagaian pelarut pengekstrakan mempunyai perbezaan jumlah sebatian dan aktiviti antioksidan. Ekstrak metanol menunjukkan hasil, TPC, kandungan tanin, DPPH, FRAP dan TEAC yang paling efisien, diikuti oleh ekstrak air, etanol dan etil asetat. Ekstrak air mempunyai kepekatan asid askorbik tertinggi, dalam anggaran 6.59-9.43 mg/g bk. Sementara itu, kandungan Beta-karotena daripada ekstrak etil asetat adalah yang tertinggi (28.20 mg/g bk) dengan signifikan (p<0.05) di antara lokasi. Pekali korelasi sebatian antioksidan daripada tiga esei aktiviti antioksidan (%DPPH, FRAP, dan TEAC) mendedahkan bahawa TPC, kandungan tanin, dan asid askorbik di dalam ekstrak berhubungkait dengan kuat terhadap aktiviti antioksidan. Purata nilai R^2 untuk TPC, kandungan tanin, dan asid askorbik ialah 0.976, 0.955, dan 0.833, masing-masing. Pengoptimuman pengekstrakan pelarut dan pengekstrakan ultrasonik-berbantu (UAE) terhadap sebatian antioksidan dan aktiviti antioksidan daripada daun *S. siamea* telah dikaji. RSM dan CCD telah digunakan untuk merancang dan mengoptimumkan kondisi eksperimen. Hasil kajian menunjukkan bahawa kondisi optimum untuk pengekstrakan pelarut ialah 49% kepekatan etanol, suhu 51^{o}C, dan masa pengekstrakan 22.5 min. Kepekatan etanol, suhu, dan masa merupakan pembolehubah yang sangat mempengaruhi respons. Untuk UAE, kondisi optimum ialah 40% kepekatan etanol, suhu 37^{o}C, dan masa pengekstrakan 7.0 min. Kepekatan etanol adalah pembolehubah yang paling mempengaruhi respons, diikuti oleh masa pengekstrakan dan suhu. Ekstrak UAE mempamerkan nilai efisiensi, hasil, TPC, %DPPH dan FRAP yang lebih tinggi berbanding pengekstrakan pelarut. Nilai hasil, TPC, %DPPH dan FRAP masing-masing telah meningkat 10.6%, 20.7%, 9.4%, dan 29.6% daripada pengekstrakan pelarut. Hasil kajian daripada RSM ini jelas

mendedahkan bahawa UAE berpotensi dan efisien digunakan untuk mengekstrak TPC dan aktiviti antioksidan dengan hasil yang lebih tinggi berbanding pengekstrakan pelarut. Walau bagaimanapun, lebih banyak kajian identifikasi diperlukan untuk mengidentifikasi sebatian bioaktif yang paling dikehendaki daripada daun *S. siamea*. Pemencilan individu asid fenolik dan flavonoid telah dijalankan menggunakan kromatografi cecair berprestasi tinggi. Hasil kajian menunjukkan bahawa sebatian bioaktif di dalam ekstrak larut adalah lebih tinggi secara signifikan (p<0.05) berbanding ekstrak terikat-dinding sel. Kajian lanjut turut mendedahkan bahawa asid fenolik larut adalah asid fenolik major di dalam daun. Asid kafeik ditemuikan sebagai asid fenolik yang paling banyak di ekstrak daun, diikuti oleh asid galik dan asid protokatekuik, masing-masing pada 42.12, 25.27, dan 21.65 mg/g bk, terutamanya dalam bentuk larut. Sebaliknya, asid protokatekuik ialah asid fenolik utama yang dijumpai di dalam ekstrak terikat-dinding sel pada 19.13 mg/g bk. Kandungan flavonoid merangkumi 0.38-137.14 mg/g bk di dalam ekstrak. Epikatekin dan katekin ialah flavonoid utama yang hadir di dalam ekstrak larut, iaitu 137.14 dan 27.02 mg/g bk dan merupakan satu-satunya flavonoid yang dikesan di dalam ekstrak terikat-dinding sel, iaitu masing-masing 10.19 and 16 mg/g bk. Hasil kajian mempamerkan bahawa bahagian utama sebatian bioaktif di dalam daun hadir dalam bentuk ekstrak larut. Ini menunjukkan bahawa daun *S. siamea* boleh dianggap sebagai sumber bernilai untuk nutraseutikal kaya-antioksidan. Potensi pengaplikasian antioksidan daun *S. siamea* mampu menjadi nilai tambah kepada industri kosmetik atau pemprosesan makanan.

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I certify that a Thesis Examination Committee has met on 14 November 2016 to conduct the final examination of Anuthida Phaiphan on her thesis entitled "Evaluation of Antioxidant Activity and Identification of Bioactive Compounds of *Senna siamea* (Lam.) Leaf Extract using Solvent and Ultrasound-Assisted Extractions" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Seyed Hamed Mirhosseini, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Jamilah binti Bakar, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal Examiner)

Azizah binti Abdul Hamid, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal Examiner)

T. Parimelazhagan, PhD
Professor
Bharathiar University
India
(External Examiner)

NOR AINI AB. SHUKOR, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 28 February 2017

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Badlishah Sham Baharin, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Tan Chin Ping, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Russly Abdul Rahman, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
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School of Graduate Studies
Universiti Putra Malaysia

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This is to confirm that;

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Signature: _____
Name of Chairman of Supervisory Committee: Associate Professor Dr. Badlishah Sham Baharin

Signature: _____
Name of Member of Supervisory Committee: Professor Dr.Tan Chin Ping

Signature: _____
Name of Member of Supervisory Committee: Professor Dr.Russly Abdul Rahman

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

µL	Microliter
µg/mL	Micrograms per milliliter
µM	Micromolar
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt
ABAP or AAPH	2,2'-azo-bis-(2-amidino-propane) hydrochloride
AH	Biological antioxidants
$AlCl_3.6H_2O$	Aluminium chloride
ANOVA	Analysis of variance
ArOH	Phenolic compounds
ASE	Accelerated solvent extraction
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
^oC	Degree celsius
S. siamea	*Senna siamea*
$C_{13}H_{10}O_3$	Anhydrobarakol
$C_{13}H_{12}O_4$	Barakol
C_3	Three carbons
CA	*p*-Coumaric acid
CAT	Catalase
CCD	Central composite design
CCl_3•	Trichloromethyl
CFA	Caffeic acid
ChA	Chlorogenic acid
cm	Centimeter
DI	Deionized water
DNA	Nucleic acid
DPPH	2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay
DPPH●	Free radical
DPPH-H	Non free radical
EDTA	Ethylene diamine tetraacetic acid
FA	Ferulic acid

Fe^{3+}	Ferric ion
Fe^{2+}	Ferrous ion
$FeCl_3.6H_2O$	Ferric chloride hexahydrate
$FeSO_4.7H_2O$	Ferrous sulfate solution
FL-H	Beta-phycoerythrin or fluorescein
FRAP	Ferric reducing antioxidant power
g	Gram
GA	Gallic acid
GAE	Gallic acid equivalent
GHz	Gigahertz
GPx	Peroxidase
GPX	Glutathione peroxidase
GSH	Glutamylcysteinylglycine or glutathione
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
HOCl	Hypochlorous acid
HPLC	High performance liquid chromatography
I%	Inhibition percentage
IC_{50}	The half maximal inhibition concentration
K	Rate constants
K	Temperature (Kelvin)
KOH	Potassium hydroxide
LD_{50}	Lethal dose
m	Meter
M	Molar
MAE	Microwave-assisted solvent extraction
MAP	Microwave-assisted process
mg	Milligram
$MgCO_3$	Magnesium carbonate
MIC	Minimum inhibitory concentration
min	Minute
mL	Milliliter
mm	Millimeter

mM	Millimolar
MPa	Megapascal
N	Normal
$NaNO_2$	Sodium nitrite
Na_2SO_4	Sodium sulfate
Nd	Not detected
nm	Nanometer
NO•	Nitric oxide radical
$O_2•^-$	Superoxide anion
O_3	Ozone
OH	Hydroxyl group
OH•	Hydroxyl radical
ORAC	Oxygen radical absorbance capacity
p-OH	*p*-Hydroxy benzoic acid
PCCA	Protocatechuic acid
PG	Propyl gallate
PHGPX	Phospholipid hydroperoxide glutathione peroxidase
ppm	Parts per million
PSE	Pressurized solvent extraction
QE	Quercetin equivalent
R^2	Correlation coefficient
R^2	Regression coefficient
R-PE	R-phycoerythrin
R-S-R′	Sulphides
R-SH	Alkyl thiols
RH	Lipid molecules
ROO• and R•	Scavenging radical products
ROOH	Lipid hydroperoxide species
ROS	Reactive oxygen species
RP-HPLC	Reversed phase high performance liquid chromatography
RS•	Thiyl radical
RSM	Response surface methodology
rpm	Rounds per minute

SD	Standard deviation
SCF	Supercritical fluid
SFE	Supercritical fluid extraction
SNA	Sinapic acid
SOD	Superoxide dismutase
SyA	Syringic acid
t	Time (min)
T	Temperature (^{o}C)
TAC	Total antioxidant capacity
TBHQ	Tertiary-butyl-hydroquinone
TC	Tannin content
TE	Trolox equivalent
TEAC	Trolox equivalent antioxidant capacity
TFC	Total flavonoid content
TPC	Total phenolic content
TRAP	Total peroxyl radical trapping parameter
TSE	Traditional solvent extraction
UAE	Ultrasound-assisted extraction
v:v	Volume by volume
VA	Vanilic acid
vitamin A	Carotenoid
vitamin C	Ascorbic acid
vitamin E	Tocopherol
w/w	Weight by weight
w/v	Weight by volume

CHAPTER 1

INTRODUCTION

Antioxidants are more in demand for ready-to-eat food products to gain the purposes of long term storage, odor, color, and flavor preservations (Pokorny, 2007). Food products, especially those consisting of lipids frequently change due to the rancidity produced (Gordon, 2001). The oxidative rancidities such as odor and flavor spoilages created in food products not only affect the quality of food, but also cause the loss of some nutrients, such as vitamins and fatty acids (Velasco *et al.*, 2010). Hence, researchers have become considerably interested in finding new natural antioxidants that are safe for customers and also have nutritional properties (Pokorny, 2007; Botterweck *et al.*, 2000). In fact, many medicinal plants and herbs such as rosemary, oregano, and sage have been studied, and it has been found that they have high amount of polyphenols and potent antioxidant activities (Surveswaran *et al.*, 2007) which can be applied in food products and others (Miliauskas *et al.*, 2004).

Senna siamea is widely used in Thailand as an anxiolytic drug and its fresh young leaves, fruits, and flowers have been used in curries and as vegetables in Thailand, Myanmar, and India for a long time (Otimenyin *et al.*, 2010; Kiepe, 2001; Kaur *et al.*, 2006). *S. siamea* is an evergreen tree that is fast growing in the open and can reach up to 30 feet in height. The Senna leaves are yellowish green in color, are pinnate, and are made up of elliptic leaflets, while the flowers are yellow in color and blossom from spring to summer. The leaves have been used as a laxative in traditional medicine (Deachapunya *et al.*, 2005). The whole plant has various medicinal functions. In addition, the leaves, stem and bark have various bioactive properties. The extract of the bark has been used to treat diabetes, the roots are used as antipyretic, and the leaves are for insomnia, hypertension, and constipation (Kaur *et al.*, 2006). Pharmacological studies have reported that barakol possesses anxiolytic, hypotensive and sedative effects (Wongwitdecha *et al.*, 2009). Chanwitheesuk *et al.* (2001) also studied antioxidants from Thai local vegetables and herbs. The results showed that they have strong antioxidants and the extract of total phenolic compounds show an important role of antioxidant substance.

Traditional extraction methods have been used for a long time, but they have limitations. Modern solvent extraction methods, which are more effective, have been developed in recent years for the extraction of bioactive compounds from plants. Nowadays, ultrasound-assisted extraction has been used for extractions of valuable compounds such as proteins, essential oil, lipids, phenolic compounds, and antioxidants. Cravotto *et al.* (2004) found that ultrasound-assisted extraction can increase extraction kinetics and still improve the quality of extracts. Moreover, some reports showed that ultrasound-assisted extraction can increase the extracted yield of bioactive compounds (Vilkhu *et al.* 2008), increase the extraction rate, and decrease the extraction temperature to allow for the extraction of thermally unstable compounds (Vilkhu *et al.*, 2008). Sun *et al.* (2011) have found that the yield of ethanol extract using the ultrasound-assisted extraction is more efficient than the traditional extraction

due to its exhaustive extraction and the heating effect of the ultrasound-assisted extraction. Similarly, Toma *et al*. (2001) found that ultrasound-assisted extraction considerably exhibits the highest antioxidant activity extraction and increases the potent antioxidant compounds' recovery. Although the extraction of bioactive components from plants by ultrasound-assisted extraction is popular and some studies have reported on the extraction of antioxidants from *S. siamea* using conventional solvent extractions, not many studies have looked into its bioactive compounds and antioxidant activities (Koyama *et al*. 2000). Specific studies pertaining to the antioxidant potential of *S. siamea* leaf, on the other hand, are still scarce. In addition, investigations related to ultrasound-assisted extraction of bioactive compounds and antioxidant activities from *S. siamea* have not been found to be reported yet in present literature.

Therefore, the main objectives of this study are to evaluate the antioxidant activity and identification of bioactive compounds of *S. siamea* (Lam.) leaf extract using solvent and ultrasound-assisted extractions. The specific objectives are:

(1) To determine the effects of different solvents and locations on the bioactive compounds and antioxidant activities of *S. siamea* leaf.
(2) To optimize the solvent and ultrasound-assisted extraction conditions for phenolic content and antioxidant activities from *S. siamea* leaf using response surface methodology.
(3) To identify the bioactive compounds in the most desirable extract from *S. siamea* leaf.

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