



DEDICATED TO
MY PARENTS

DECLARATION BY THE CANDIDATE

The thesis entitled “**Supercritical Fluid Extraction and Ultrasound Assisted Extraction of Phytochemicals from Underutilized Bhimkol (*Musa balbisiana*) Banana Blossom, its Antidiabetic Property and Application**” is being submitted to the School of Engineering, Tezpur University in the partial fulfillment for the award of the degree of Doctor of Philosophy in the Department of the Food Engineering and Technology is a record of bonafide research work accomplished by me under the supervision of **Prof. Sankar Chandra Deka**.

All the helps from various sources have been duly acknowledged.

No part of this thesis has been submitted elsewhere for the award of any degree.

Date:

Sangita Muchahary

Sangita Muchahary

Place:

Reg. No. TZ189881 of 2018

Department of the Food Engineering and Technology

School of Engineering, Tezpur University

Assam- 784028, India



TEZPUR UNIVERSITY

(A Central University)

NAPAAM, TEZPUR-784028, ASSAM
DISTRICT: SONITPUR, ASSAM, INDIA

Prof. Sankar Chandra Deka, FRSC, FRSB
Department of Food Engineering & Technology

CERTIFICATE OF THE SUPERVISOR

This is to certify that the thesis entitled “**Supercritical Fluid Extraction and Ultrasound Assisted Extraction of Phytochemicals from Underutilized Bhimkol (*Musa balbisiana*) Banana Blossom, its Antidiabetic Property and Application**” submitted to the School of Engineering, Tezpur University in the partial fulfillment for the award of the degree of Doctor of Philosophy in the Department of the Food Engineering and Technology is a record of research work carried out by **Ms. Sangita Muchahary** under my supervision and guidance.

All the helps received by her and from various sources have been duly acknowledged. No part of this thesis has been submitted elsewhere for the award of any degree.

Date: *May 16, 2023*

Place: Tezpur

Prof. S.C. Deka (Supervisor)

Department of Food Engineering and Technology
School of Engineering, Tezpur University
Assam- 784028, India

ACKNOWLEDGEMENTS

First and the foremost, I would like to express my gratitude to Almighty for his blessing during my course of Ph.D work till the end. The dream of Ph.D would have never come without His blessings.

I express my sincere thanks to my supervisor Prof. S.C Deka, Department of Food Engineering & Technology, Tezpur University for his immeasurable and unwavering guidance, insightful suggestion with constructive critics throughout the course of investigation and preparation of thesis.

I express my sincere gratitude to esteemed members of my Doctoral Committee Dr. N. Sit, Department of Food Engineering & Technology and. Dr. M.V. Satish Kumar, Department of Molecular Biology & Biotechnology, Tezpur University and Prof. S. K. Dolui, Department of Chemical Sciences for their valuable suggestion and encouragement at various stage of the investigation.

I wish to express my gratitude to my Departmental Research Committee and research scholars for their valuable suggestion and encouragement.

Solemn regards and thanks goes to Prof. Vinod Kumar Jain, Vice Chancellor, Tezpur University, Prof. P. P. Sahu, Dean, School of Engineering and Dr. L. S. Badwaik, Head, Department of Food Engineering & Technology for providing necessary facilities to carry out the research work.

I sincerely acknowledge Dr. P. Chattopadhyay and Dr. Y. D. Bhutia, Defence Research Laboratory (DRL), Tezpur, Assam for providing me lab facilities with full guidance in performing in vivo study in DRL, Tezpur. I also sincerely thank the entire DRL team Mr. Amartya Banaejee, Mr. P. Subramanyam, Ms. Pakter Niri, Ms. Sanghita Das and Mr. Ajay Kakati, DRL, Tezpur for their perspicacious technical help to complete my in vivo studies in DRL, Tezpur. I express my sincere gratitude to SAIC and Food quality control laboratory, library Tezpur University, Assam, IIFPT (NIFTEM), Thanjavur, Tamil Nadu for assisting for technical assistance during the work.

I sincerely thank Dr. S. Dasgupta and Dr. R. Mukhopadhyay for permitting me to access their laboratory facilities to conduct some of my research works and I also express my sincere acknowledge to Mr. Manoj Sharma, Ms. Archana Singha, Mr. Dipanjan

Banerjee, Department of Molecular Biology & Biotechnology, Tezpur University for their immense technical contributions in some analyses.

I express my heartfelt thanks to the faculty and staff of Department of Food Engineering & Technology for their assistance and cooperation from time to time throughout the period of research. I also thank Dr. Nickhil C. for his valuable technical suggestion and assistance to complete some of my research analyses.

I sincerely acknowledge the Ministry of Tribal affairs (MoTA), New Delhi for funding financial support in the form of fellowship under National Fellowship and Scholarship for Higher Education of ST Students (No. 201718-NFST-ASS-01198) for the period of five years (2017-2022).

Special thanks to my seniors, friends, batchmates, labmates Dr. A. J. Das, Mr. Bhaskar Jyoti Das, Dr. Khurshida, Ms. Noon Haokip, Dr. Duyi Samyor, Dr. Yesmin ara Begum, Mr. Manas Jyoti Das, Dr. Manas Sharma, Urbashi Neog, Maibam Baby, Payel Dhar, Tapasya Kumar for supporting me technically and emotionally.

I convey my acknowledgement to all FET PhD alma mater, friends, and juniors, Tezpur University whose name is not included here for directly and indirectly help and moral support during my entire PhD work.

I would like to thank my mother Mrs. Padumi Muchahary, father Dr. Gambaru Muchahary and my siblings for their strong love, support and motivation throughout my PhD journey.

Date:

(Sangita Muchahary)

Place: Tezpur

List of Tables

Table No.	Title	Page No.
1.1	Nutritional compositions of banana blossom	1.4
1.2	Phytochemical compositions of <i>Musa paradisiaca</i> bract	1.5
1.3	Health beneficial properties of banana blossom	1.9
2.1	Coded response surface methodology-central composite design (RSM-CCD) design for ultrasonic assisted extraction (UAE) method	2.4
2.2	Coded response surface methodology-central composite design (RSM-CCD) design for supercritical fluid extraction (SCFE) method	2.6
2.3	ANOVA table (Quadratic model) of response 1 (TPC) for ultrasound assisted extraction (UAE)	2.13
2.4	ANOVA table (Quadratic model) of response 2 (antioxidant activity) for ultrasound assisted extraction (UAE)	2.14
2.5	ANOVA table (Quadratic model) of response 1 (TPC) for supercritical fluid extraction (SCFE)	2.15
2.6	ANOVA (Quadratic model) of response 2 (Antioxidant activity) for supercritical fluid extraction (SCFE)	2.16
2.7	Observed and the predicted value of responses TPC and antioxidant activities for ultrasound assisted extraction (UAE) and supercritical fluid extraction (SCFE) method in response surface methodology (RSM)	2.21
2.8	Phytochemical evaluation of major parts of bhimkol blossom using a conventional method of determination	2.23
2.9	Retention time of phytochemical standards in RP-HPLC at 254 nm	2.24
2.10	Phytochemical contents detected by HPLC in bhimkol blossom extracts from supercritical fluid extraction (SCFE), ultrasound assisted extraction (UAE) and conventional extraction method	2.26
3.1	Experimental design by OMD for formulation of nachos	3.6

3.2	Fat and water-soluble vitamins in bhimkol blossom	3.12
3.3	Volatile phytochemicals profiling in bhimkol blossom	3.14
3.4	GC-MS fatty acids profiling in bhimkol blossom	3.16
5.1	Experimental design by response surface methodology (Box-Behnken Design)	5.10
5.2	Bioavailability of quercetin from SIF	5.13
5.3	Binding affinities of encapsulating materials and quercetin	5.17
6.1	Body weight and weight of organs of control and rats after 14 days dose of BBE	6.8
6.2	Biochemical parameters of body organs of rats after 14 days dose of BBE	6.8
6.3	Hematological study of body organs of rats after 14 days dose of BBE	6.9
6.4	Rotated component matrix of sensory parameters of control RTC-SM soup	6.14
6.5	Rotated component matrix of sensory parameters of BBP RTC-SM soup	6.15
6.6	Rotated component matrix of sensory parameters of BBQM BBP RTC-SM soup	6.16

List of Figures

Figure No.	Title	Page No.
1.1	Global production of banana	1.2
1.2	Bhimkol (<i>Musa balbisianana</i>) banana blossom	1.3
2.1	Various parts of Bhimkol blossom (<i>Musa balbisiana</i>): (a) Bhimkol blossom (inflorescence), (b) Bract, (c) Male flowers, and (d) Spadix	2.2
2.2	Response surface methodology (RSM) graphs for ultrasound assisted extraction (UAE); (a) Amplitude-temperature (AB) for TPC, (b) Temperature-time (AC) for TPC, (c) Amplitude-time (BC) for TPC, (d) Amplitude-temperature (AB) for Antioxidant activity (e) Temperature-time (AC) for Antioxidant activity (f) Amplitude-temperature (AB) for Antioxidant activity	2.17
2.3	Fig. 2.3 Response surface methodology (RSM) graphs for supercritical fluid extraction(SCFE); (a) Temperature-pressure (AB) for TPC, (b) Temperature-time (AC) for TPC, (c) Temperature-carbon dioxide(AD) for TPC, (d) Pressure-time (BC) for TPC, (e) Pressure-Carbon dioxide (BD) for TPC, (f) Time-carbon dioxide (CD) for TPC, (g) Temperature-pressure (AB) for antioxidant activity, (h) Temperature-time (AC) for antioxidant activity, (i) Temperature-carbon dioxide(AD) antioxidant activity, (j) Pressure-time (BC) for antioxidant activity, (k) Pressure-Carbon dioxide (BD) for antioxidant activity, and (l) Time-carbon dioxide (CD) for antioxidant activity	2.18
2.4	Comparative bar diagram of conventional extraction, ultrasound assisted extraction (UAE), and supercritical fluid extraction (SCFE) with respect to extraction yield, TPC, antioxidant activity and extraction time. Error bar with different letters are significant different values ($p < 0.05$)	2.22
2.5	RP-HPLC Chromatograms of phytochemical compounds	2.25

	detected of whole bhimkol blossom extracts from conventional extraction, ultrasound assisted extraction (UAE), and supercritical fluid extraction (SCFE) at 254 nm. (2-syringic acid, 3- tannic acid, 5-ferulic acid, 6-caffeic acid, 7-rutin, 8- quercetin, 10-gallic acid, 12-coumarin)	
2.6	Antioxidant activities of major phytochemicals detected in HPLC. Error bar with different letters are significant different values ($p<0.05$)	2.27
3.1	Formulated nachos prepared from bhimkol blossom	3.8
3.2	HPLC chromatograms of vitamins in bhimkol blossom: vitamin E (1F), vitamin A (2F), thiamine (1W), and pyridoxine (2W)	3.13
3.3	GC chromatographs of volatile phytochemicals and fatty acids profiling; (a) volatile phytochemicals profiling in bhimkol blossom: (1) l-Gala-l-ido-octose; (2) d-Gala-l-ido-octonic amide; (3) β -D-Glucopyranose, 4-O- β -D-galactopyranosyl-; (4) 2H-Oxecin-2-one, 3,4,7,8,9,10-hexahydro-4-hydroxy-10-methyl-, [4S-(4R*,5E,10S*)]-; (5) 4a,8a-Butano[1,4]dioxino[2,3-b]-1,4-dioxin, tetrahydro-; (6) Thiazole, 2-amino-4-(p-aminophenyl)-; (7) n-Hexadecanoic acid; (8) 17-Octadecynoic acid; (9) Oleic Acid; (11) Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate; (14) Methoxyacetic acid, 2-tridecyl ester; (16) Cholestan-3-ol, 2-methylene-, (3 β ,5 α)-; (17) 12-Methyl-E,E-2,13-octadecadien-1-ol; (18) Ethanol, 2-(octadecyloxy)-; (19) Ethanol, 2-(9-octadecenyloxy)-, (Z)-; (20) Octadecane, 3-ethyl-5-(2-ethylbutyl)- (b) fatty acids profiling in bhimkol blossom: (2) Oleic Acid, 9,12-Octadecadienoic acid (Z,Z)-; (3) 9,12-Octadecadienoic acid (Z,Z)-; (4) cis-13-Eicosenoic acid; (5) 7-Methyl-Z-tetradecen-1-ol acetate; (6) 8,11,14-Eicosatrienoic acid, (Z,Z,Z)-; (7) 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-; (8) Linoleic acid ethyl ester; (9) Z-8-Methyl-9-tetradecenoic acid; (10) Erucic acid; (11) 9-	3.15

	Octadecenoic acid (Z)-, phenylmethyl ester and (12) 10-Undecenoic acid, octyl ester	
3.4	Antioxidant activities and antibacterial activity of bhimkol blossom extract; (a) Antioxidant activities by various parts of bhimkol blossom: Br; bract, Mf; male flower, Sp; spadics WB; whole blossom. Error bar with different letters are significant different values ($p<0.05$), and (b) Antibacterial activity of bhimkol blossom extract and kanamycin	3.18
3.5	Radar chart and graph of sensory evaluation for experimental run nachos; (a) sensory evaluation and (b) sensory attributes	3.20
3.6	Performance of ANN-PSO for training, validation, dataset and all data	3.22
3.7	Product component analysis (PCA) of sensory evaluation of nachos: (a) commercial nachos (b) control nachos (c) bhimkol blossom nachos	3.24
4.1	Flowchart of collection of preparative from bhimkol blossom extract (BBE)	3.3
4.2	HPLC chromatogram of standards and preparative fraction; (a) quercetin standard, (b) catechin standard, and (c) preparative fraction	3.6
4.3	Evaporated quercetin rich fraction (BBQ) collected	3.7
4.4	NMR spectra of quercetin rich fraction (BBQ)	3.8
4.5	FTIR spectra of quercetin standard and quercetin rich fraction (BBQ), (a) quercetin standard, and (b) quercetin rich fraction (BBQ)	3.9
4.6	Major volatile compounds of fraction; (a) GC-MS chromatograph of volatile compounds, (b) mass-to-charge ration at 10.07 min, (c) mass-to-charge ration at 11.38 min, (d) mass-to-charge ration at 12.96 min, and (e) mass-to-charge ration at 14.99 min	3.11
5.1	Optimal artificial neural network structure	5.3
5.2	Dialysis of microbeads containing SQM and BBQM in	5.7

	dialysis tubing	
5.3	Performance of ANN-ACO for training, validation, test and all data	5.11
5.4	Quercetin release in water, simulated intestinal fluid (SIF) and simulated gastric fluid (SGF)	5.12
5.5	NMR spectra, FTIR and XRD graphs of quercetin and microbeads; (a) FTIR graph of Q-quercetin, BBQ, SQM-standard quercetin microbeads and BBQM-bhimkol blossom quercetin microbeads and (b) XRD graph of BBQM	5.14
5.6	Micrographs of microbeads (bhimkol blossom quercetin microbeads, BBQM): (a) SEM micrograph of dried BBQM (b) SEM micrograph of dried BBQM wall and (c) Optical microscopic image of wet BBQM	5.15
5.7	Particle size distribution of microbeads (BBQM- bhimkol blossom quercetin microbeads)	5.16
5.8	Molecular interaction of encapsulating materials (quercetin derivative + sodium alginate + CaCl ₂ + chitosan)	5.18
5.9	Quercetin loss of BBQM in 6 months. Error bar with different letters are significant different values ($p<0.05$)	5.19
6.1	Acclimatized Wister rats (<i>Rattus norvegicus</i>)	6.3
6.2	Incorporation of BBQM in ready to cook soup mixes (RTC-SM)	6.4
6.3	Cell viabilities of BBE treated THP-1 cells	6.6
6.4	Relative organ weights of control and rats after 14 days dose of BBE	6.7
6.5	Histopathology of kidney and liver of control and BBE dosed rats; (a) kidney of BBE dosed rat, (b) kidney of control rat, (c) liver of BBE dosed rat, and (d) liver of control rat	6.10
6.6	Soup prepared from RTC-SM: (1) control, (2) BBP, and (3) BBQM + BBP	6.11
6.7	Sensory analysis of soup prepared from RTC-SM soups; (a) Control, (b) BBP, and (c) BBQM + BBP	6.12

6.8	Significant difference between overall acceptability of BBP and BBQM BBP RTC-SM, different letters indicates significant difference ($p \leq 0.05$).	6.13
6.9	Scree plot and rotated component plot of control RTC-SM soup; (a) Scree plot and (b) Rotated component plot	6.14
6.10	Scree plot and rotated component plot of BBP RTC-SM soup; (a) Scree plot and (b) Rotated component plot	6.15
6.11	Scree plot and rotated component plot of BBQM BBP RTC-SM soup; (a) Scree plot and (b) Rotated component plot	6.16
6.12	DPPH inhibition activity of RTC-SM	6.17
7.1	Inhibition activity of BBE against some diabetes enhancing enzymes; (a) α -amylase, (b) α -glucosidase, and (c) DPP-IV enzyme	7.8
7.2	Glucose uptake intensity of control, insulin, insulin + palmitate and BBE on L6 cells. Error bar with different letters are significant different values ($p < 0.05$)	7.9
7.3	Blood glucose of different treatment groups in A-control, B- diabetic group no treatment, C- BBE treated, and D- Insulin treated	7.10
7.4	Change in blood glucose of rats at different treatment groups, A-control, B- diabetic group no treatment, C- BBE treated, and D- Insulin treated	7.11
7.5	Body weight of different treatment groups in A-control, B- diabetic group no treatment, C- BBE treated, and D- Insulin treated	7.12
7.6	Change in body weights of rats at different treatment groups, A-control, B- diabetic group no treatment, C- BBE treated, and D- Insulin treated	7.12
7.7	Glucose tolerance level and insulin tolerance level of experimental group of rats; (a) Glucose tolerance test and (Insulin tolerance test), A-control, B- diabetic group no treatment, C- BBE treated, and D- Insulin treated	7.14

List of Abbreviations

A_t	Experimental value
F_t	Predicted value
μg	Microgram
μm	Micrometer
μmol	Micromolar
2_NBDG	D-Glucose, 2-deoxy-2-((7-nitro-2,1,3-benzoxadiazol-4-yl)amino)-
3D	Three dimensional
a^*	Redness
ABTS	2,2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid
ABX	Virtual coupling
ACO	Ant colony optimization
A_{control}	Absorbance of control
Al	Aluminum
ANN	Artificial neural networking
ANOVA	Analysis of variance
A_{sample}	Absorbance of sample
ATCC	American type culture collection
A_v	Average
b^*	Yellowness
BB	Bhimkol blossoms
BBE	Bhimkol blossom extract
BBP	Bhimkol blossom powder
BBQ	Quercetin from bhimkol blossom
BBQM	Microbeads of quercetin from bhimkol blossom
BGL	Blood glucose level
BOD	Biological oxygen demand
Br	Bract
BW	Body weight
C	Carbon atom
$\text{C}_{15}\text{H}_{20}\text{O}_7$	Quercetin

$C_2HCl_3O_2$	Trichloroacetic acid
C_6H_5OH	Phenol
Ca^+	Cationic calcium
CCD	Central composite design
Cd	Cadmium
CE	Catechin equivalent
cm	Centimeter
Co	Cobalt
CO_2	Carbon dioxide
Cu	Copper
CV	Coefficient of variation
df	Degree of freedom
DLS	Dynamic light scattering
DM	<i>Diabetes mellitus</i>
DM-1	Type 1 diabetes
DM-2	Type 1 diabetes
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
DPP-IV	Dipeptidyl peptidase-IV
EDTA	Ethylenediamine tetraacetic acid
EE	Encapsulation efficiency
FCR	Folin-Ciocalteu reagent
Fe	Iron
$FeCl_3$	Iron (III) chloride
$FeSO_4$	Iron sulfate or ferrous sulfate
FRAP	Ferric reducing ability of plasma
FTIR	Fourier transform infrared spectroscopy
g	Gram
GA	Genetic algorithm
GAE	Gallic acid equivalent
GC	Gas chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GI	Gastrointestinal

h	Hour
H	Hydrogen atom
H ₂ SO ₄	Sulfuric acid
HCl	Hydrochloric acid
IC ₅₀	Half maximal inhibitory concentration
ID	Internal diameter
IDF	Insoluble dietary fiber
IR	Infrared
K	Potassium
kHz	Kilohertz
kJ	Kilo joule
L*	Lightness
LC	Liquid chromatography
LD	Loading capacity
LPS	Lipopolysaccharide
M	Molarity
m/z	Mass by charge
MAE	Microwave assisted extraction
MAE	Mean absolute error
MAPE	Mean absolute percentage error
MF	Male flowers
MFI	Mean fluorescence intensity
mg	Milligram
mm	Millimeter
Mn	Manganese
MRT	Mean release efficiency
MSE	Mean square error
MTT	3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide)
MUFA	Monounsaturated fatty acid
MWh	Megawatt hour
n	Number
N	Normality
Na	Sodium

Na ₂ CO ₃	Sodium carbonate
Na ₂ WO ₄	Sodium tungstate
ND	Not detected
NH ₄ OH	Ammonium hydroxide
Ni	Nickel
nm	Nanometer
NMR	Nuclear magnetic resonance
O	Oxygen atom
OA	Overall acceptability
OH	Hydroxide
OMD	Optimal mixture design
p	p-value
Pb	Lead
PCA	Product component analysis
PDI	Polydispersity index
PLE	Pressurized liquid extraction
ppm	Parts per million
PSO	Particle swarm optimization
PUFA	Polyunsaturated fatty acid
Q	Quercetin
QE	Quercetin equivalent
R ²	Correlation coefficient
RE	Release efficiency
RMSE	Root mean square error
RP-HPLC	Reversed phase-high performance liquid chromatography
rpm	Rotation per minute
RSM	Response surface methodology
RT	Retention time
RTC	Ready to cook
s	Second
SCFE	Supercritical fluid extraction
SD	Standard deviation
SDF	Soluble dietary fiber

SEM	Scanning electron microscopy
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SM	Soup mix
Sp	Spadics
SQM	Microbeads of quercetin standard
STZ	Streptozotocin
t	Time
TAE	Tannic acid equivalent
TDF	Total dietary fiber
TEM	Transmission electron microscopy
TFC	Total flavonoid content
TLC	Thin layer chromatography
TPC	Total phenolic content
UAE	Ultrasound assisted extraction
UV	Ultraviolet
W	Watt
w/v	Weight by volume
w/w	Weight by weight
WB	Whole blossom
wb	Wet basis
XRD	X-ray diffraction
y	Multiplication of titer value
Y_{TPC}	Computed values of ANN output
Zn	Zinc
α	Alpha
θ	Theta