

**ANTIOXIDANTS AND ANTIOXIDANT ACTIVITY
IN 7 PUNGENT THAI HERBS
AND THE ANTIOXIDATIVE STRESS EFFECT
IN TRAINING SOLDIERS**

The seal of Mahidol University is a large, circular emblem. It features a central blue circle containing a golden stupa-like structure. This is surrounded by a yellow ring with Thai script. The outermost ring is white with more Thai script. The author's name is printed across the center of the seal.

CAPTAIN JIRAPORN CHAOPOTHONG

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE (NUTRITION)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY**

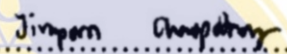
2006

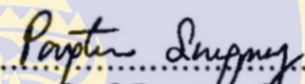
ISBN 974-04-7789-5

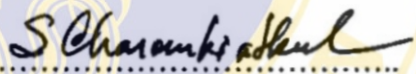
COPYRIGHT OF MAHIDOL UNIVERSITY

Thesis
Entitled

**ANTIOXIDANTS AND ANTIOXIDANT ACTIVITY
IN 7 PUNGENT THAI HERBS
AND THE ANTIOXIDATIVE STRESS EFFECT
IN TRAINING SOLDIERS**

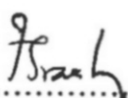

.....
Captain Jiraporn Chaopothong
Candidate

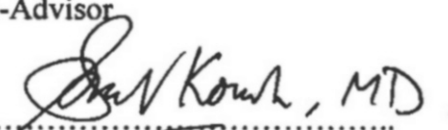

.....
Assoc. Prof. Pongtorn Sungpuag, D.Sc.
Major-Advisor


.....
Assist. Prof. Somsri Charoenkiatkul, D.Sc.
Co-Advisor


.....
Assoc. Prof. Prapaisri Sirichakwal, Ph.D.
Co-Advisor


.....
Maj. Gen. Prasart Laothavorn
M.D.
Co-Advisor


.....
Prof. Dr. M.R. Jisnuson Svasti, Ph.D.
Dean
Faculty of Graduate Studies


.....
Prof. Surat Komindr, M.D., F.R.C.P. (T)
Chair
Master of Science Programme in
Nutrition.
Faculty of Medicine, Ramathibodi Hospital
and Institute of Nutrition

Thesis
Entitled

**ANTIOXIDANTS AND ANTIOXIDANT ACTIVITY
IN 7 PUNGENT THAI HERBS
AND THE ANTIOXIDATIVE STRESS EFFECT
IN TRAINING SOLDIERS**

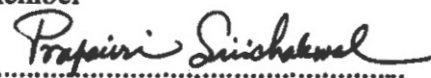
was submitted to the Faculty of Graduate Studies, Mahidol University
For the degree of Master of Science (Nutrition)
on
31 October, 2006

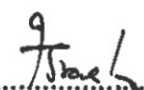

.....
Captain Jiraporn Chaopothong
Candidate

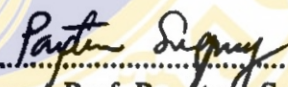

.....
Prof. Surat Komindr, M.D., F.R.C.P. (T)
Chair


.....
Assoc. Prof. Col Chumpol Piamsomboon,
M.D.
Member

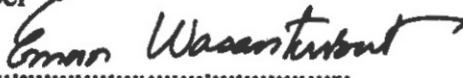

.....
Maj. Gen. Prasart Laothavorn
M.D.
Member

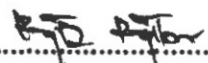

.....
Assoc. Prof. Prapaisri Sirichakwal
Ph.D.
Member


.....
Prof. M.R. Jisnuson Svasti, Ph.D.
Dean
Faculty of Graduate Studies
Mahidol University


.....
Assoc. Prof. Pongtorn Sungpuag,
D.Sc.
Member


.....
Assist. Prof. Somsri Charoenkiatkul,
D.Sc.
Member


.....
Assoc. Prof. Emorn Wasantwisut, Ph.D.
Director
Institute of Nutrition,
Mahidol University


.....
Prof. Rajata Rajatanavin, M.D., F.A.C.E.
Dean
Faculty of Medicine, Ramathibodi Hospital
Mahidol University

ACKNOWLEDGEMENT

The success of this thesis can be attributed to the extensive support and assistance from my major advisor, Assoc. Prof. Pongtorn Sungpuag and all of my co-advisors, Assist. Prof. Somsri Charoenkiatkul, Assoc. Prof. Prapaisri Sirichakwal and Maj. Gen. Prasart Laothavorn, M.D. for their guidance, kindness, encouragement, and valuable advices throughout the period of my research.

I would like to thank Professor Dr. Surat Komindr, M.D., F.R.C.P. (T), who was the Chair of my thesis examination, and Assoc. Prof. Col. Chumpol Piamsomboon, M.D., who was the external examiner of my thesis, for their useful comments, guidance and suggestion.

I would like to thank a Commander – in - Chief of the Infantry Regiment, King's guard 1st, Bangkok for his helpful collaborated in data collecting and many thanks go to all of my volunteers, 50 persons in the first shift training soldiers who were joined in this thesis for their participation.

I would like to thank Mrs. Umaporn Udomsubpayakul for her assistance in the statistical analysis, Mrs. Yupaporn Nakngamanong, Miss Wachiraporn Santawong, and all staffs of INMU for their helpful and kind suggestions.

Special thanks go to Faculty of Science, Mahidol University and all staffs in lyophilized room for their helpful in Kitchen mint preparation and Col. Picha Klaisingha for her help in freeze dried Kitchen mint capsule filling.

Finally, I thank all of my friends for their encouragement in the study and my colleagues for their help in my regular work.

This study was supported in part by Institute of Nutrition, Mahidol University and by the Thesis Grant, Faculty of Graduate Studies, Mahidol University.

Cpt. Jiraporn Chaopothong

ANTIOXIDANTS AND ANTIOXIDANT ACTIVITY IN 7 PUNGENT THAI HERBS AND THE ANTIOXIDATIVE STRESS EFFECT IN TRAINING SOLDIERS

CAPTAIN JIRAPORN CHAOPOTHONG 4536907 RANU/M
M.Sc. (NUTRITION)

THESIS ADVISORS: PONGTORN SUNGPUAG, D. Sc.

SOMSRI CHAROENKIATKUL, D. Sc., PRAPASRI SIRICHAKWAL, Ph.D.

MAJ.GEN. PRASART LAOTHAVORN, M.D.

ABSTRACT

Chronic diseases, caused from the changes in consumption patterns, are a major problem in several countries. In the past, Thailand has had a lower rate of chronic diseases than now. Diet patterns are important because Thai foods have several kinds of herbs. Herbs were used for relieving and curing many symptoms. Therefore, herbs may contain some important substances. This study is an experimental study. In the first phase, quantities of β -carotene, vitamin E, vitamin C, total polyphenol and total antioxidant capacity (TAC) in 7 pungent Thai herbs were analyzed. Holy basil, sweet basil, hairy basil, peppermint, ginger, galangal and lesser ginger were randomly bought from Taladtai market and pooled for analyzed antioxidant vitamins, polyphenol and TAC. Then the highest TAC herb was selected for the second phase.

In the second phase, the antioxidative stress effect of 7 pungent Thai herbs was tested. 50 volunteers, training soldiers from an Infantry Regiment, King's guard 1st Bangkok, were randomly divided into 2 groups to receive dietary supplement of 2,000 mg/day. One group received a placebo and the other group received herb capsules. Blood pressure, pulse rate, body weight, other symptoms, plasma lipid, plasma TAC, plasma hs-CRP and plasma malondialdehyde (MDA) were assessed. General information, 3-day- food record, food frequency questionnaires (FFQ) and 2- day- physical activity were recorded by all volunteers.

Peppermint had the highest TAC and total polyphenol; a freeze dried form was selected for the supplement during the supplementation period. After 8 weeks of the supplement, systolic and diastolic BP, pulse rate in the experimental group and diastolic BP in the placebo group were significantly decreased at P value < 0.05. Plasma cholesterol, plasma triglyceride in the experimental group and plasma triglyceride in the placebo group were significantly increased at P value < 0.05. While, plasma LDL-c and plasma HDL-c in the placebo group were significantly decreased at P value < 0.05. Plasma TAC in experimental group was significantly increased at P value < 0.05. In addition, plasma high sensitivity c-reactive protein (hs-CRP) in both groups was significantly decreased at P value < 0.05. Plasma MDA in both groups was not significantly changed. No volunteers had signs of complication from the supplementation.

In conclusion, the supplementation study in healthy volunteers with freeze - dried peppermint leaves for 8 weeks significantly increased the plasma TAC level of the volunteers in the experimental group. Lipid peroxidation was not significantly affected by this intervention.

Military training activity of the first shift training soldiers in Infantry Regiment, King's guard 1st did not cause high oxidative stress.

KEYWORDS: ANTIOXIDANT VITAMINS/ TOTAL ANTIOXIDANT ACTIVITY/
MALONDIALDEHYDE/ C- REACTIVE PROTEIN/ PUNGENT THAI
HERBS

170 P. ISBN 974-04-7789-5

การศึกษาฤทธิ์ในการเป็นสารต้านอนุมูลอิสระของสมุนไพรไทยรสเผ็ดร้อน 7 ชนิด และฤทธิ์ในการลดภาวะออกซิเดทีฟ
สเตรสในทหารเกณฑ์ (ANTIOXIDANTS AND ANTIOXIDANT ACTIVITY IN 7 PUNGENT THAI
HERBS AND THE ANTIOXIDATIVE STRESS EFFECT IN TRAINING SOLDIERS)

ร้อยเอกหญิง จิราพร เชาวโพธิ์ทอง 4536907 RANU/M

วท.ม. (โภชนศาสตร์)

คณะกรรมการควบคุมวิทยานิพนธ์: พงศธร สังข์เผือก, วท.ค. , สมศรี เจริญเกียรติกุล, วท.ค., ประไพศรี ศิริจักรวาล,
ปร.ค., พลตรีนายแพทย์ประสาท เหล่าถาวร, อายุรแพทย์

บทคัดย่อ

ปัจจุบันโรคเรื้อรังเป็นปัญหาที่สำคัญในทุกประเทศ สาเหตุหนึ่งเกิดจากการเปลี่ยนแปลงด้านการรับประทานอาหาร เพื่อให้เหมาะสมกับสภาพการดำเนินชีวิตในปัจจุบัน ในอดีตจะพบว่าคนไทยมีอัตราการเป็นโรคเรื้อรังน้อยกว่าปัจจุบันมาก รูปแบบการรับประทานอาหารของคนไทยในอดีตน่าจะเป็นส่วนหนึ่งที่มีความสำคัญเพราะในอาหารไทยมีสมุนไพรประกอบอยู่หลายชนิด การรับประทานสมุนไพรไทยได้ถูกนำมาใช้ในการบรรเทาและรักษาอาการต่างมากมาย เพราะอาจจะมีสารสำคัญบางอย่างในสมุนไพร การศึกษานี้เป็นการวิจัยเชิงทดลองทางคลินิก เพื่อศึกษาปริมาณเบตาแคโรทีน วิตามินอี วิตามินซี ปริมาณรวมของโพลีฟีนอล และ ปริมาณรวมของวิตามินที่ต้านออกซิเดชันในสมุนไพรไทยรสเผ็ดร้อน 7 ชนิด ได้แก่กะเพรา โหระพา สะระแหน่ แมงลัก ขิง ข่า และ กระชาย โดยสุ่มซื้อตัวอย่างจากตลาดไท มาเตรียมเพื่อการวิเคราะห์หาวิตามิน ปริมาณรวมของโพลีฟีนอลและปริมาณรวมของวิตามิน ที่ต้านออกซิเดชันในสมุนไพรรสเผ็ดร้อนทั้ง 7 ชนิด และเลือกใบสะระแหน่เพราะมีปริมาณรวมของวิตามินที่ต้านออกซิเดชันมากที่สุด โดยศึกษาถึงผลในการลดภาวะออกซิเดทีฟสเตรสในทหารเกณฑ์ที่อยู่ในช่วงการฝึกจำนวน 50 นาย โดยสุ่มแบ่งเป็น 2 กลุ่มให้ รับประทานเสริมด้วยยาหลอกและสมุนไพรบรรจุแคปซูล วันละ 2,000 มิลลิกรัม โดยประเมินผลจากความดันโลหิต น้ำหนักตัว อาการ ท้องไส้ ระดับของไขมันชนิดต่างๆในพลาสมา ปริมาณรวมของวิตามินที่ต้านออกซิเดชันในพลาสมา ปริมาณสารที่บ่งบอกถึงการ อักเสบที่เกิดขึ้นในร่างกายและปริมาณสารที่เป็นผลจากการออกซิไดส์ของไขมันในพลาสมา โดยมีการสอบถามข้อมูลทั่วไปของ อาสาสมัคร การบันทึกอาหารที่รับประทานทั้งหมดในเวลา 3 วัน มีการบันทึกกิจกรรมและเวลาที่ทำทั้งหมดเป็นเวลา 2 วัน เพื่อนำ ข้อมูลมาวิเคราะห์และดูความแตกต่างระหว่างอาสาสมัครทั้ง 2 กลุ่ม

การศึกษายพบว่า หลังจากอาสาสมัครรับประทานใบสะระแหน่เสริมเป็นระยะเวลา 8 สัปดาห์ มีค่าความดันโลหิตขณะ หัวใจบีบและคลายตัวและอัตราการเต้นของชีพจร ลดลงอย่างมีนัยสำคัญทางสถิติที่ $P < 0.05$ ในขณะที่กลุ่มที่ได้รับยาหลอกมีค่า ความดันโลหิตขณะหัวใจคลายตัวลดลงอย่างมีนัยสำคัญทางสถิติที่ $P < 0.05$ ระดับโคเลสเตอรอล ไตรกลีเซอไรด์ ในกลุ่มที่ได้รับ สะระแหน่ และระดับไตรกลีเซอไรด์ ในกลุ่มที่ได้รับยาหลอกเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ที่ $P < 0.05$ ระดับไขมันแอลดีแอล และ เอชดีแอล ในกลุ่มที่ได้รับยาหลอก ลดลงอย่างมีนัยสำคัญทางสถิติ ที่ $P < 0.05$ ปริมาณรวมของวิตามินที่ต้านออกซิเดชันใน พลาสมา ในกลุ่มที่ได้รับสะระแหน่สูงขึ้นอย่างมีนัยสำคัญทางสถิติ ที่ $P < 0.05$ นอกจากนี้ปริมาณสารที่บ่งบอกถึงการอักเสบที่ เกิดขึ้นในร่างกายในพลาสมาของอาสาสมัครทั้ง 2 กลุ่มลดลงอย่างมีนัยสำคัญทางสถิติที่ $P < 0.05$ ในขณะที่ปริมาณสารที่เป็นผล จากการออกซิไดส์ของไขมันในพลาสมาของอาสาสมัครทั้ง 2 กลุ่มไม่มีความแตกต่างกันมาก่อนและหลังของการเสริม อาสาสมัคร ทุกคนไม่เกิดอาการผิดปกติใดๆหลังจากได้รับการเสริมด้วยสะระแหน่และยาหลอก

จากการศึกษาครั้งนี้สรุปว่าการเสริมด้วยสะระแหน่เป็นเวลา 8 สัปดาห์ในอาสาสมัครที่มีสุขภาพแข็งแรงทำให้ปริมาณ รวมของวิตามินที่ต้านออกซิเดชันในพลาสมาสูงขึ้น แต่ไม่มีผลต่อระดับของไขมันในพลาสมา นอกจากนี้พบว่าการฝึกของทหาร เกณฑ์ในกองพันทหารราบที่ 1 รักษาพระองค์ ไม่ทำให้เกิดภาวะออกซิเดทีฟสเตรส

CONTENTS

	PAGE
ACKNOWLEDGEMENT	iii
ABSTRACT	iv
LIST OF TABLES	ix
LIST OF FIGURES	x
LIST OF ABBREVIATIONS	xi
CHAPTER	
1 INTRODUCTION	1
1.1 Background and importance of the problem	1
1.2 Research questions	5
1.3 Objective	5
2 LITERATURE REVIEWS	6
2.1 Free radicals	6
2.2 Oxidative stress	7
2.3 Dietary antioxidants	9
2.4 Betacarotene	11
2.5 Vitamin E	11
2.6 Vitamin C	14
2.7 Phenolic compound	15
2.8 Total antioxidant capacity	17
2.9 Pungent Thai medicinal plants and herbs, the curing by Thai knowledge and pharmacology effect	18
3 MATERIALS AND METHODS	36
3.1 1 st phase of experiment	36
3.1.1 Materials	36

CONTENTS (cont)

	PAGE
3.2 2 nd phase of experiment	37
3.2.1 Subjects	37
3.2.2 Sample size	38
3.3 Method	38
3.3.1 Ethics approval	38
3.3.2 Experimental design	38
3.3.3 Dietary assessment	40
3.3.4 Physical activity assessment	40
3.4 Supplementation procedure	40
3.5 Biomarkers	41
3.6 Experimental places	42
3.7 Experimental period	42
3.8 Statistical analysis	42
4 RESULTS	44
4.1 Antioxidant vitamins, total polyphenol and total antioxidant activity in 7 pungent Thai herbs	44
4.2 General characteristics of the volunteers	48
4.3 The characteristics of the volunteers from placebo and experimental group	49
4.4 Data collected from food frequency questionnaires (FFQ)	49
4.5 Energy intake from commissary of the volunteers	49
4.6 Anthropometric and physical characteristics of the volunteers	49
4.7 Anthropometric data	50
4.8 Food intake and physical	51
4.9 Biochemical data	53

CONTENTS (cont)

	PAGE
4.10 Physical assessment of all volunteers	58
5 DISCUSSION	60
5.1 Antioxidant activity in peppermint	60
5.2 Plasma total antioxidant activity after supplementation	64
5.3 ORAC and FRAP method	65
5.4 Plasma lipid after supplementation	66
5.5 Exercises induce oxidative stress	68
5.6 MDA: product of lipid peroxidation	68
5.7 C-reactive protein and oxidative stress	71
6 CONCLUSION	73
REFERENCES	75
APPENDIX	88
BIOGRAPHY	170

LIST OF TABLES

TABLE	PAGE
LITERATURE REVIEWS	
2.1 Antioxidant properties of antioxidant vitamins and polyphenol	16
2.2 Antioxidant vitamins in 7 pungent Thai herbs	34
2.3 The carotenoid content in herbs	35
RESULTS	
4.1 The quantity of β -carotene, vitamin E, vitamin C in 7 pungent Thai herbs	45
4.2 The amount of total polyphenol in 7 pungent Thai herbs	46
4.3 The antioxidant capacity of 7 pungent Thai herbs by ORAC, FRAP and percent of water	48
4.4 Characteristics of the volunteers in placebo group and experimental group at baseline	50
4.5 Comparison anthropometric data of the volunteers at 0 and 8 weeks after supplementation in placebo and experimental group both groups at 0 week of supplementation	52
4.6 Comparison plasma level of lipid, TAC, hs- CRP and MDA at 0 and 8 week after supplementation between groups both groups at 8 week of supplementation	53
4.7 Comparison plasma Chol, TG, LDL-c, HDL-c, at 0 week and 8 weeks after supplementation in placebo and experimental group	55
4.8 Comparison plasma ORAC, FRAP, MDA and hs- CRP at 0 week and 8 weeks after supplementation in placebo and experimental group	56
4.9 Comparison plasma MDA in placebo and experimental group at 0, 2 and 8 weeks after supplementation	57

LIST OF FIGURES

FIGURE	PAGE
2.1 Overall mechanism of lipid peroxidation	8
2.2 Lipid radical detoxification cascade	13
2.3 Holy basil	19
2.4 Sweet basil	22
2.5 Hairy basil	23
2.6 Peppermint	25
2.7 Ginger	27
2.8 Galangal	30
2.9 Lesser ginger	32
3.1 Blood collection planning	42
3.2 Peppermint capsule and placebo capsule	43
4.1 Plasma MDA in placebo and experimental group at 0, 2 and 8 weeks after supplementation	58

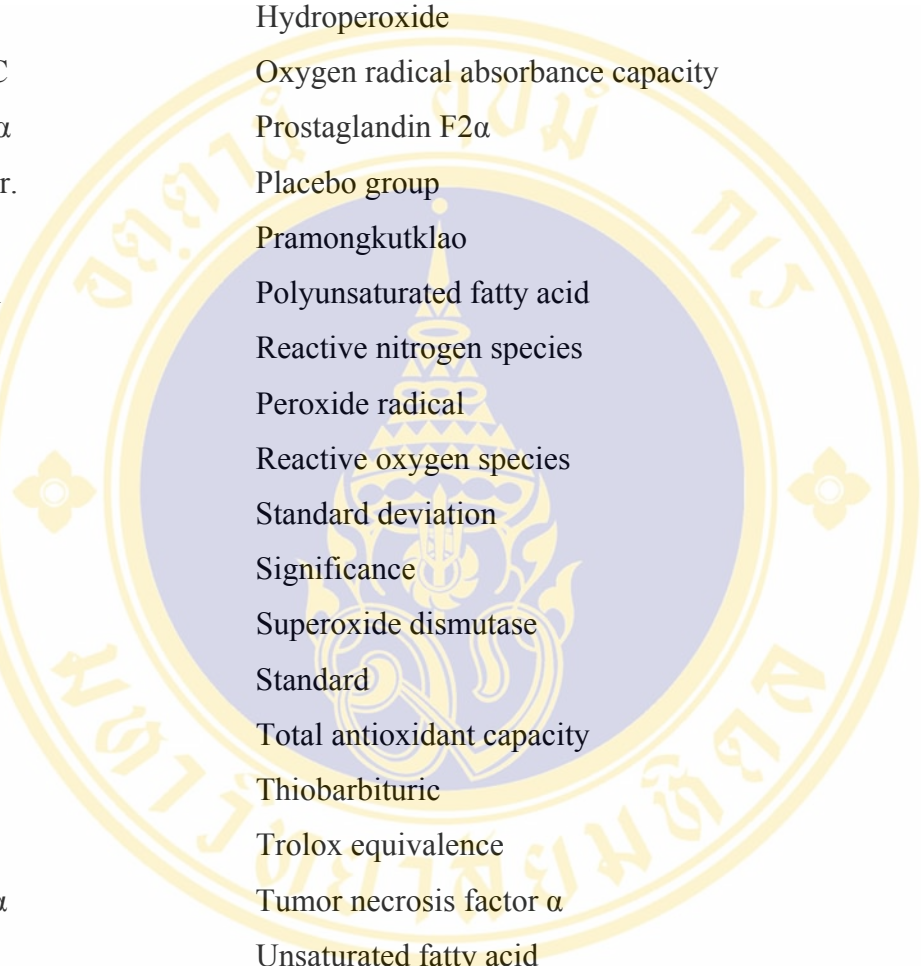
LIST OF ABBREVIATIONS

AOS	Activated oxygen species
AUC	Area under curve
BHT	Butylated hydroxytoluene
BMI	Body mass index
BP	Blood pressure
BW	Body weight
C*	Oxidized vitamin C
cap.	Capsule
CD4	Cluster of differentiation 4
CHCl ₃	Chloroform
cm	Centimeter
dL	Deciliter
DNA	Deoxyribonucleic Acid
DPPH	1, 1- Diphenyl-2- pierylhydrazyl
E*	Oxidized vitamin E
EE	Energy expenditure
EI	Energy intake
Exp. Gr.	Experiment group
Fe ³⁺ - TPTZ	Ferric tripyridyltriazine
Fe ²⁺ - TPTZ	Ferrous tripyridyltriazine
FFQ	Food frequency questionnaires
FRAP	Ferric reducing ability of plasma
g	Gram
GPx	Glutathione peroxidase
GSH	Glutathione
GSSG	Oxidized glutathione

LIST OF ABBREVIATIONS (cont)

H·	Hydrogen atom
HDL-C	High density lipoprotein cholesterol
HIV	Human immune deficiency virus
H ₂ O ₂	Hydrogen peroxide
HPLC	High performance liquid chromatography
hr	Hour
hs- CRP	High sensitivity C- reactive protein
ht	Height
hydro	Hydrophilic
Ig-NH ₂ MDA	Autoantibodies directed to MDA adducts to proteins
IL-6	Interleukin- 6
INMU	Institute of Nutrition, Mahidol University
IU/d	International unit per day
Kcal	Kilocalory
Kg	Kilogram
LDL	Low density lipoprotein
LDL-C	Low density lipoprotein cholesterol
lipo	Lipophilic
m	Meter
MDA	Malondialdehyde
mg	Milligram
µg	Microgram
nm	Nanometer
NADPH	Nicotinamide adenine dinucleotide phosphate
O ₂ ⁻	Superoxide anion
8OHdG	8 –Hydroxy-deoxy-guanosine
OH·	Hydroxyl radical

LIST OF ABBREVIATIONS (cont)



OO·	Peroxyl radical
OOH	Hydroperoxide
ORAC	Oxygen radical absorbance capacity
PGF2 α	Prostaglandin F2 α
Pla. Gr.	Placebo group
PMK	Pramongkutklao
PUFA	Polyunsaturated fatty acid
RNS	Reactive nitrogen species
ROO·	Peroxide radical
ROS	Reactive oxygen species
SD	Standard deviation
Sig.	Significance
SOD	Superoxide dismutase
Std	Standard
TAC	Total antioxidant capacity
TBA	Thiobarbituric
TE	Trolox equivalence
TNF- α	Tumor necrosis factor α
UFA	Unsaturated fatty acid

CHAPTER 1

INTRODUCTION

1.1 Background and importance of the problem

Chronic disease or degenerative disease such as cardiovascular disease, cancer, diabetic mellitus and hypertension are the major public health problems in developed and developing countries. In Thailand, a public health record from 1989-1993 showed that cardiovascular disease was the first cause of death and trend to be higher. [1] From 1995 – 1997, classification of death by sex and cause of deaths according to ICD Mortality Tabulation List 1, 10th Revision, number and rates per 100,000 population Cardiovascular disease was still in the first rank with the death rate from ischemic heart disease was 2,266 per 100,000 population and the death rate from other heart disease was 37,457 per 100,000 population. Furthermore, cancer was in the second rank with the death rate of 29,888 per 100,000 populations. The death rate from diabetic mellitus, hypertension, cerebrovascular disease and atherosclerosis was 4383, 3985, 1463 and 2 per 100,000 population, respectively. [2]

In the future, trend of those diseases will be higher because Thailand developed from agricultural to industrial country. [1]

Thai people have changed their diets a lot over the past decade. Lifestyle and environment change into Western culture. Processed food and fast food are in favorable. Much fat and oil or less vegetable in food and less exercise can be induced chronic diseases. These problems can prevent by changes the eating behavior and a way of life.

Thai foods are composed of a great number of vegetables, herbs and spices. In the past, Thais used herbs and spices for treatment. [3] Herbs and spices have many important ingredients.

Now, the nutritional scientists are alert and pay attention to the local food such as Mediterranean food. The study of Michel de Lorgeril, et al. in 2001 [4] showed that

Mediterranean food consisted of olive oil, vegetables and fruits. Vegetables and fruits contained a lot of vitamins, which had antioxidant property.

In addition, the report of The Lyon Trial, The American Heart Association Science Advisory and Coordinating Committee elucidated that “It would be short-sighted not to recognize the enormous public health benefit that Mediterranean – style diet could confer” and “there is a pressing need to identify unknown risk factors and effective intervention strategies” so the professors from The American Heart Association and The European Society of Cardiology are accept and recommend that eating the Mediterranean food can prevent coronary heart disease. [4]

Thai foods are the useful food. They are composed of many vegetables, spices and herbs. They may have a preventive role and lower the occurrence of many diseases such as coronary heart disease, hypertension, diabetes mellitus, etc.

The study of Charoenkiatkul presented that Thai foods were suitable for health because they were composed of various herbs and spices. In the past, Thai used herbs and spices for treatment. [5]

Thai foods consist of vegetables, herbs and spices. [3] Many studies presented the useful elements in Thai foods. Especially, the substance that are not nutrient but they are the plants derivative - chemical substances. They have many types, such as alkaloids, fragrant oil, glycosides and flavonoids. These compounds are called phytochemicals. They have the important role to prevent and cure some diseases such as cardiovascular disease, hypertension, diabetic mellitus and some types of cancer. [3]

The studies of Thai medicinal and local plants showed the important of food taste because they reflect the properties of that food. The taste of local plants is able to classify into 9 groups: harsh, sweet, bitter, mao-buer, pungent, oily, cool smelling, salty and sour [6], each group has a different benefit.

Thai foods integrate local plants with herbs and spices. Herbs and spices are the important ingredient in Thai foods. Thai people can be used herbs and spices in many kinds of Thai food and they are Thai food identity. Thai people like pungent taste. The local Thai foods are mostly hot and spicy food. [6]

The pungent Thai medicinal plants such as chili, pepper, long pepper, sweet basil, garlic, hairy basil, holy basil, peppermint, ginger, parsley, galangal, lesser ginger, curcuma, piper sarmentosum etc. [6]

Example of pungent Thai herbs, which had ever been studied:

Chili - In the animal experiment showed the effective cure for lower plasma triglyceride in rat and increase the antioxidant enzyme in pleural. [3] Moreover, capsaicin showed the effect on reduces pain and inflammation in rat. [7]

Garlic – From human and animal experiment, garlic could prevent atherosclerosis by reduce plasma cholesterol, inhibit cancer cell, inhibit platelet aggregation. Furthermore, it could fuse fibrin, reduce blood pressure and reduce plasma glucose. It could protect CD4 from HIV, reduce the generation of new virus in infected cell, inhibit bacterial growth, inhibit fungi growth, inhibit excretion of aflatoxin, tapeworm and threadworm excretion in children, relieve bloated, reduce inflammation, inhibit bacterial that cause sore throat. [3, 8]

Pepper – The studies showed that pepper extract had antioxidant effect, increased the use of amino acid. In rat experiment, when feed grind pepper, it could stimulate enzyme in the detoxification system. [9]

The previous studies indicated that antioxidant in many types of Thai medicinal plants can be lowering plasma lipid. In this study, we are interesting in pungent Thai medicinal herbs that use for seasoning in popular Thai foods. Herbs are cheap and easy to buy or grow.

Furthermore, the main ingredients of Thai foods are composed of variety of herbs and spices in each menu. From this information, pungent Thai herbs and spices that are in the ingredient of popular Thai foods were chosen for this study namely: holy basil, hairy basil, sweet basil, peppermint, ginger, galangal and lesser ginger.

Antioxidants in Thai food are β -carotene from vegetables and fruits, vitamin C from fresh vegetables and fruits, vitamin E from half - milled rice. Scientists discovered that vegetables and fruits had useful nutrient for cell. It was called “nutrition for cell working” or “phytonutrient”. [10] Phytonutrient is not only vitamins and minerals but also carotenoid, flavonoid, catechin, plant sterol, nucleic acid and tocopherol. [10] At present, most peoples believe that these substances protect the body from oxidative stress. [10]

The autoxidation of lipid is initiated with the formation of free radicals. When contact with oxygen, an unsaturated fatty acid gives many free radicals an overbalance between radical – generating and radical-scavenging system occurred, this condition is oxidative stress.

Lipid oxidation is believed to play an important role in coronary heart disease, atherosclerosis, cancer, and the aging process. So, food antioxidant is very important. For example, first, endogenous or added antioxidant may protect the food from oxidative damage during storage or cooking. Second, dietary antioxidant may absorb into the body and give beneficial effects. Third, food – derived antioxidant could exert beneficial effects without being absorbed. Fourth, there is considerable interest in plant extracts for therapeutic use. [11]

Another possible way to minimize free radical damage is to eat foods that contain antioxidant. Vitamin C is present in variety of fruits and vegetables. β -carotene is found in orange, yellow or red and dark-green leafy vegetables. Vitamin E is found in vegetable oils, wheat germ, nuts and green leafy vegetables. [12]

Analyse the quantity of antioxidants such as: β -carotene, vitamin C, E, phenolic compound and total antioxidant capacity of pungent Thai herbs and spices: holy basil, sweet basil, hairy basil, peppermint, ginger, galangal and lesser ginger will perform in this study. The antioxidative stress effect on reducing lipid peroxidation will analyse as a quantity of plasma malondialdehyde. The reduction of inflammation from oxidative DNA damage and lipid peroxidation will analyse from of plasma C-reactive protein level.

The highest level of total antioxidant capacity herbs will be using in the supplementation period for high level of oxidative stress person. The results of this study will encourage further study in Thai herbs and spices. The usefulness in consume Thai foods will support the expansion of Thai food's business in the future.

1.2 Research questions

- 1.2.1** Do the pungent Thai herbs in this study have the antioxidant: β -carotene, vitamin C, E and phenolic compound? And which one has the highest level?
- 1.2.2** What kind of pungent Thai herbs in this study that has the highest level of total antioxidant capacity?
- 1.2.3** Could the highest level of total antioxidant capacity pungent Thai herb in this study reduce the oxidative stress in high level of oxidative stress person: training soldiers?

1.3 Objective

1.3.1 General objective

To study the antioxidant effects in holy basil, sweet basil, hairy basil, peppermint, ginger, galangal and lesser ginger.

1.3.2 Specific objectives

1. To analyse the antioxidant nutrients: β -carotene, vitamin C, E and phenolic compound in 7-pungent Thai herb: holy basil, sweet basil, hairy basil, peppermint, ginger, galangal and lesser ginger.
2. To determine the total antioxidant capacity of 7- pungent Thai herb.
3. To study the ability of selected pungent Thai herbs in lowering lipid peroxidation, in human subjects.

CHAPTER 2

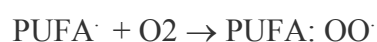
LITERATURE REVIEWS

2.1 Free radicals

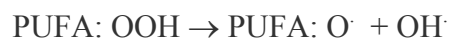
Free radicals are reactive molecules with an unpaired electron; they have the potential to damage a wide range of biomolecules. Forming of free radicals will initiate biological damage that occurs in normal aerobic metabolism. Activated oxygen species are formed during the reduction step of the oxygen into the water and secondary reactions with protons and transition metals such as copper and iron. For example, the superoxide anion (O_2^-) was produced in many cell redox systems such as xanthine oxidase, aldehyde oxidase, membrane-associated NADPH oxidase, and in cytochrome P_{450} oxidase. 1-4% of total oxygen that transport into cell by mitochondria will use to produce O_2^- and 20% of these will take into cell. Stimulated to macrophages and monocytes will release many O_2^- .

While free radicals are in none particularly reactive, they are scattered and lived far from cells. Until they were changed in metal-catalyzed reaction to the more reactive: Hydroxyl radical ($OH^\cdot$). The free radicals that can damage cells may in the forms of pollutant and halogenated anaesthetics. [13]

Polyunsaturated fatty acid is sensitive to the oxidation by free radicals because methylene interrupted double structure in PUFA. Lipid peroxidation will start from the free radicals such as $OH^\cdot$ extracted hydrogen atom from PUFA: H in a PUFA radical (PUFA $^\cdot$) and followed by the change of double bond into conjugated diene. After this, they will assemble with oxygen to be a peroxy radical (PUFA: $OO^\cdot$), that will react with numerous PUFA: H and formed to a hydroperoxide (PUFA: OOH) and the other forms of PUFA. The reaction is now self-propagating [13] :



Moreover, in the presence of iron or copper, PUFA: OOH will lose double bond and one electron will change to free radical including the highly reactive such as OH[·] [13]:



LDL is a fraction of cholesterol. That is composed of PUFA at phospholipid layer. So, they are sensitive to free radical-mediated peroxidation. The oxidation reaction of LDL caused the damage to PUFA and increases the quantity of toxicant such as PUFA: OOH, aldehydes and lysolecithin. [13]

In the experiment, the oxidation reaction changed a chemical substance and physical characteristic of LDL to more atherogenic and the changes from oxidation reaction include [13]:

1. Recognition and preferential uptake of modified LDL by scavenger receptors of macrophages.
2. Enhancement of chemotactic responses with respect to other monocytes/ macrophages, leading to accumulation of macrophages at specific sites.
3. Increased tendency for platelet aggregation.
4. Increased release of adhesion molecules by endothelial cells.

2.2 Oxidative stress

Oxidative stress is defined as the result of an imbalance between pro- oxidants and antioxidants, resulting in cell damage. Modern life leads us to pollution, consumption of alcohol or several drug, prolonged exposure to sunlight and smoking. All of these causes over produce the activated oxygen species (AOS) in our organism, and induce the weakening of our defense mechanism then cell damage occurs. When AOS are produced in excessive amount, they will harm normal cells and activate the change of gene coding.

The examples of the environmental factors that can cause the rising of oxidative stress:

- Prolonged exposure to sunlight
- Smoking

- Excessive physical exercise
- Excessive alcohol consumption
- Pollution
- Intellectual stress, etc.

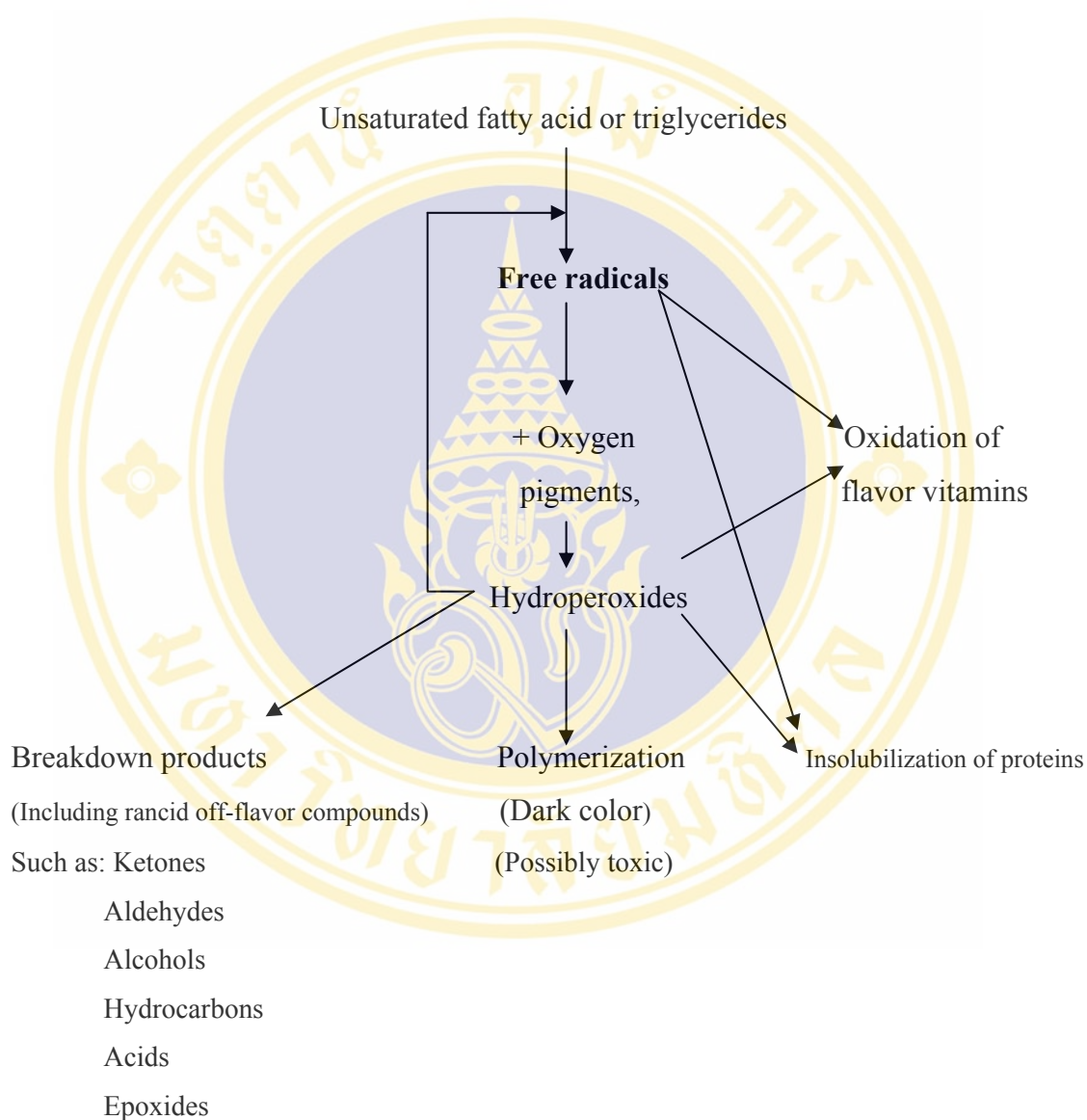


Figure 2.1 Overall mechanism of lipid peroxidation [14]

2.3 Dietary antioxidants: β -carotene, vitamin E, vitamin C, phenolic compound and antioxidant activity and anti oxidative stress effect and the prevention of degenerative diseases

At present, many studies indicated that atherosclerosis and ischemic heart disease occurred from the reaction of free radical formation, lipid peroxidation and oxidative modification of LDL. The end products of free radicals and lipid peroxidation are toxic to cells by form the inflammation and scar at the inner wall of vessels. It stimulated an increased penetration of LDL, platelet aggregation, an abnormal of eicosanoid homeostasis and the collection of inflammatory cells. [15]

Those causes are the important factor of atherosclerosis and other vascular diseases. LDL is the cause of atherosclerosis because UFA was oxidized by free radicals and form many kinds of aldehydes such as malondialdehyde, 4-hydroxynonenal, propanol, hexanal and 2, 4-alkadienals.

MDA is a product of lipid peroxidation. It reacted with other cells and caused a cross-link and polymerization of cell membrane components are the important signs of cell damage. MDA levels have been increasing when exercise and found in biological sample.

When the substances hold with amino acid from apolipoprotein-B and E for forming new compounds that LDL receptor is unfamiliar with them. When plasma LDL is increasing, phagocytic cells or scavenger cells such as macrophage and monocyte will come to eliminate LDL. LDL is lipoprotein contain with high cholesterol. When phagocytic cells or scarvenger cells eat a lot of LDL, cell will packed with cholesterol and cholesteryl ester and became to foam cells, an important composition of atherosclerotic plaques or streaks. By moved to the inner wall of vessel and infiltrated in the intima of vessel. [15]

As stated, lipid peroxidation was toxic and made inflammation to cells. C-reactive protein (CRP) is a reactant that produced when the body is in acute injury, infection or has inflammatory stimuli. C- reactive protein, a marker of inflammation, was associated with significant elevations in both biomarkers of lipid peroxidation in bivariate analyses and with MDA in the multivariate model.

Because atheromatous plaques contained inflammatory cells, when ruptured, they lead into the mechanism for myocardial infarction and acute coronary syndrome.

So, c- reactive protein has been shown to be a powerful predictor of cardiovascular disease risk. [16]

Chiayu Chen, M.D., F.A.C.C., Riverside cardiology associated medical group suggested that c-reactive protein may be a good marker of cardiovascular risk in addition to lipid level. [17]

Normally, the body have the auto-defense mechanism from the invasion of free radicals in 2 forms [18]:

1. The body set up antioxidants such as superoxide dismutase (SOD), glutathione peroxidase.
2. Taken from food: dietary antioxidants.

Example of dietary antioxidants [18]

- 2.1 Minerals: zinc, copper, manganese, selenium
- 2.2 Vitamins: vitamin A, E, C, β -carotene, B complex
- 2.3 Coenzyme Q₁₀

Several studies showed the usefulness of antioxidant and cardiovascular disease. Daniel Steinberg [19, 20] collected the data about the large- scale prospective studies in 40,000 men and 80,000 women. He found that supplementation with high dose natural vitamin E have statistically reduced risk of cardiovascular disease. And the study about beta-carotene in smoking men showed that consumption of carotene could reduce risk of cardiovascular disease. [21]

In vitro studies, presented that vitamin C and E could reduce lipid peroxidation. Vitamin C protected cell wall from peroxidative damage in aqueous phase, vitamin E protected in lipid soluble and chain breaking micronutrient antioxidant. Furthermore, in vivo studies presented that vitamin C could synergist with vitamin E by decrease of tocopheroxyl radicals. [22]

Moreover, Margareta Kristenson and co-worker [23] found that lower serum level of beta-carotene from lower intake β -carotene food lead to increase cell damage.

In this study, we are interested in dietary antioxidant such as β -carotene, vitamin E, vitamin C, total phenolic compound and total antioxidant capacity.

2.4 β -Carotene: 536.85 MW, $C_{40}H_{56}$ [24]

β -carotene is a pro-vitamin A, in carotenoid group that have more than 500 types but only 32 types can react as vitamin A such as α - carotene, δ - carotene, β -cryptoxanthine and β -carotene-5, 6- epoxide but they can react as half of β -carotene. [25, 26]

Substances in carotenoid group found in different colors of fruits and vegetables for example: yellow, red, orange and green. [18, 25]

The important substance in this group is beta-carotene, has two identical beta-ionone rings connect together with carbon bond. When the central bond was oxidized by enzyme from small intestine and liver the result are 2 molecules of retinol. As for α - carotene, δ - carotene and β -cryptoxanthine give a molecule of retinol because they have one β -ionone ring. [27]

The studies presented that vitamin A was absorbed and dissolved in lipid, but it is in only animal food source. Food source of vitamin A are liver, milk, butter and yolk.[28] The later studies presented plants had the substances reacted like vitamin A. Finally, Moore detected that plants β -carotene could convert to vitamin A in the body. In 1930, Karrer discovered a chemical formula of vitamin A and β -carotene. [27]

Food sources of β -carotene are carrot, gourd, chilli, holy basil, garlic leaf, piper sarmentosum. [27] β -carotene from food can covert to vitamin A only one part of 6. [27]

The studies presented vitamin A had many important functions in the body especially in antioxidant defense system, protected membrane from free radicals by reduced the formation of methyl linoleate hydroperoxides. [27]

2.5 Vitamin E: 430.69 MW, $C_{40}H_{56}$ [24]

Vitamin E is a lipid soluble vitamin found by Evans and Bishop in 1992 and in 1936 namely as Alpha-tocopherol. In 1938, Fernhoiz found the chemical formula and Karrer could synthesize alpha-tocopherol. [27]

Tocopherol is an antioxidant of enzymes and proteins. Normally, vitamin E has an important function against lipid peroxidation of cell wall, prevention of cell lysis. [27]

Vitamin E is the first choice of preventing lipid peroxidation and is the natural vitamin that has the highest capacity in preventing cell membrane from free radicals.

Food sources of Vitamin E. Vitamin E has found in both plants and animals. The higher in plants, vegetables oil that has vitamin E are safflower oil, rice bran, cottonseed, corn and soybean. Green leaf vegetables also had but in the minimal amount. [27]

Cell membrane consisted of polyunsaturated fatty acid and had double bond in many position. So, free radicals can damage both of structure and function of cell membrane, caused of degenerative diseases.

Vitamin E will increase body immune system, controlled mechanism of platelet aggregation by inhibited prostaglandin production; moreover it has many important functions in the body.

2.5.1 Vitamin E and cardiovascular disease

Cholesterol come from LDL-c and the rising of LDL-c increase the risk to atherosclerosis because the collection of foam cell in the inner wall was the part of LDL-c that will oxidize and forming to foam cell which plenty of cholesterol and was an important composition of fatty streak lesion.

LDL-c was the important target of free radicals and oxidizing process. In animal studies found that when LDL-c was oxidized, it will change macrophage into foam cell and antioxidants will protect LDL-c from oxidizing. Vitamin E is an antioxidant vitamin that important for LDL oxidation protection, but the mechanism of vitamin E in the body is unclear; it will need further study. Several studies found that consumption of vitamin E will reduce risk to cardiovascular disease.

The studies of Gay KF, Brubacher GB and Stahelin HB showed that the person with serum vitamin E level more than 10.8 mg/dL ($>25 \mu\text{mol/L}$) had the lower rate of ischemic heart disease than the person with serum vitamin E level less than 10.8 mg/dL. Because vitamin E had the ability to eliminate free radicals that damage inner vessels. The studied by Steinberg D and Lewis A showed that consumption lipid-

soluble antioxidant vitamin especially vitamin E decreased atherosclerosis in animal experiment.

Furthermore, Eric B., Meir J. Stampfer and et al. [19, 20] studied in 40,000 men and more than 80,000 women and followed their eating for 4-8 years, found that risk to cardiovascular disease decreased in the person that continuously consumed vitamin E more than 2 years especially natural vitamin E such as cereal grains, vegetables oil and nut but consumed vitamin E supplementation did not decrease risks. An amount of vitamin E that suitable and give good results for consumer will need further studies.

To confirm with Prabhat Jha, Marcus Flather and their coworker [29] in their review prospective epidemiologic study found that consumption vitamin E in natural form or continuously supplementation at least 2 year with the dose of 100 IU/d gave the advantage to reduced cardiovascular risk factors.

Moreover, Peter L Zock and Martijn B Katan [30] found that consumed lipid-soluble antioxidants including vitamin E will be able to reduce atherosclerosis in animal experimental and taken vitamin E by consumed vegetables oil will protect LDL oxidation.

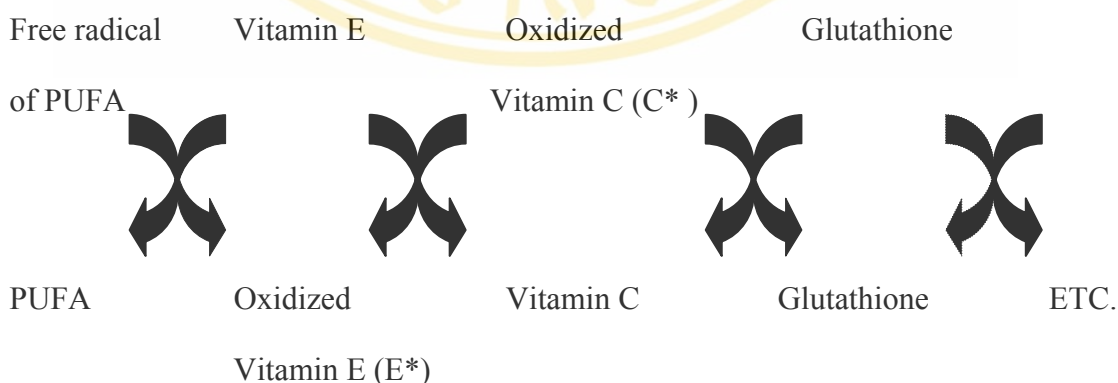


Figure 2.2 Lipid radical detoxification cascade [14]

2.6 Vitamin C: 176.12 MW, $C_6H_8O_6$ [24]

Vitamin C is a water-soluble vitamin. In 1928, Szent-Gyorgyi was able to separate this vitamin from adrenal gland and oranges and namely hexuronic acid. In 1932, C. Glenn King was separated from lemon and found that hexuronic acid was alike vitamin C, could prevent and cure scurvy guinea pigs then namely vitamin C. In 1933, Haworth and Reichstein known the chemical formula and synthesize them. [27]

Vitamin C is easily oxidized even by oxygen in an air and will change to dehydro ascorbic acid and was hydrolyzed to neutral or alkaline phase. Moreover, vitamin C will oxidize by plant enzyme: ascorbic acid oxidase. So, in vitamin C analyses should add meta-phosphoric acid for removed enzyme and oxidized ascorbic acid. [27]

Food sources of vitamin C.

Fresh fruits and vegetables: orange, lemon, *Phyllanthus emblica*, tomato, sweet bell pepper and green leaf vegetables. [27]

Presently, many studies indicate about atherosclerotic plaque and the advantages of vitamin C in decreased atherosclerosis because vitamin C had the important function in collagen synthesis, that make healthy vascular system. If the body received adequate vitamin C, collagen synthesis will complete. The insertion of LDL to form foam cell is difficult because the strengthen vessels and the occurrence of atherosclerosis is less. [31]

In addition, vitamin C has a role to work in cooperation with Alpha-tocopheroxyl radical to change alpha-tocopherol that hold free radicals in lipid phase to be in aqueous phase and prevented alpha-tocopherol from oxidation in lipoprotein. Many studies express that plasma vitamin C can prevent lipid hydroperoxide and the formation of F2-isoprostane from the induction of aqueous peroxy radicals, peroxynitrite, smoking and activated neutrophils [18].

The studies in smoking presented 33% reduction of a quantity of urine F2-isoprostane and 8-epi PGF2 α , the product of lipid peroxidation, if intake vitamin C 2000 mg/d in only 5 days. Moreover, the studies in nonsmoking showed the reduction of plasma malondialdehyde in vitamin C consumer. [18]

Furthermore, Dr. Plotnick and co-worker found that intake vitamin C before eating high cholesterol food. The function of inner cell wall was stable as eating low cholesterol food. [31]

Many studies indicated that when vitamin C worked with proline and lysine, the inhibitory effect to atherogenesis occurred with 3 reasons [31]:

1. Protected inner cell wall damage from inflammatory that caused lipoprotein aggregation.
2. Plaque fusion when these substances hold with lipoprotein aggregation and excrete.
3. Complete collagen formation made the vessels more flexibility and consistent normality.

2.7 Phenolic compound

Phenol or phenolic compound gather a wide range of substances that have an aromatic carbon ring with an OH group or hydroxyl substituent, including their functional derivatives. Usually, phenolic compound have several kinds such as flavonoids and their relatives. Phenolic compound was found in many kinds of fruits and vegetable other than their antioxidant vitamins. Hence, the total number of phenols in a given sample of food is referred to as its "phenolic content".

Flavonoid is a subgroup of glycoside, one of an important substance in plants. Flavonol glycosides or flavonoids consist of aglycone and the structure is $C_6-C_3-C_6$.

Flavonoids are classified in 12 subgroups [32]:

1. Flavones
2. Isoflavones
3. Flavonols
4. Flavonones
5. Flavanonols
6. Leukoanthocyanins
7. Anthocyanins
8. Catechins
9. Chalcones

10. Dihydrochalcones

11. Aurones

12. Xanthones

Table 2.1 Antioxidant properties of antioxidant vitamins and polyphenols [33]

Antioxidant vitamins.	Effect.
β -carotene	- Decrease deoxyribonucleic acid strand breaks - Lower copper-induced oxidation of low density lipoprotein - Decrease lipid peroxidation - trapping peroxy free radical at low pO₂
Vitamin E	- Chain-breaking antioxidant that prevent the propagation of free radicals reactions - A peroxy radical scavenger and specially protects PUFAs within membrane phospholipids and in plasma lipoproteins
Vitamin C	- Scavenge reactive oxidants in activated leukocytes, lung and gastric mucosa - Protect against lipid peroxidation - Interaction with vitamin E - Metal chelating
Polyphenols	- Free radical scavenging in initiation phase - Metal chelating in initiation phase - Chain-breaking antioxidants in propagation phase

2.8 Total antioxidant capacity (TAC)

TAC is the power of substances that can reduce pro-oxidants or scavenge free radical molecules from the body including antioxidant vitamins, polyphenols, flavonoids, and etc. that can act as antioxidant and give the advantages to the body.

The previous studies showed many antioxidants, although the body was auto set up and received from other sources: Enzymatic antioxidants and non-enzymatic antioxidants. [34, 35]

Many studies indicated that increasing intake of dietary antioxidants had a benefit to the body. Although, the body had lower level of antioxidants, can prevent and delay the changes of pro-oxidant-initiated oxidation of the substance. Examples of pro-oxidants include reactive oxygen and nitrogen species. (ROS, RNS) [34]

The method of TAC evaluation was studied. Including the ferric reducing ability of plasma (FRAP assay) by Benzie et al. and the oxygen-radical absorbance capacity (ORAC assay) by Cao G. [36]

Total antioxidant capacity assay methodology.

The method that is used for evaluating antioxidant capacity of a biological sample refers to oxidants or oxidizing agents that endure electrons from the reductants, which are treated as the antioxidants being measured. Therefore, the methods are divided into two groups [34]:

1. Methods using oxidants that are not necessarily pro-oxidants. Including FRAP assay that was defined as:
 - Ferric-reducing ability of plasma.
 - Depend on the reduction of ferric tripyridyltriazine (Fe^{3+} -TPTZ) complex to the ferrous tri-pyridyltriazine (Fe^{2+} -TPTZ) by a reductant at low pH.
 - Fe^{2+} -TPTZ has a dark blue color and can detect at 593 nm.

This method is measure in ability of substance to reduce Fe^{3+} that is the oxidant in the assay to produce Fe^{2+} .

2. Methods using oxidants that are pro-oxidants including ORAC assay. The total antioxidant capacity assays using pro-oxidants beside use an oxidizable substrate. The pro-oxidants induce oxidative damage, and will inhibit with antioxidants. The inhibition is measured and related to antioxidant capacity of the antioxidants.

ORAC assay is based on the method of Glazer et al. It is the method that use free radical action to completion and use an area under curve (AUC) for quantitation and combines both percentage of inhibition and the length of inhibition time of free radical action by antioxidants into a single quantity.

2.9 Pungent Thai medicinal plants and herbs, the curing by Thai knowledge and pharmacology effects

The study of Chavasit V. et al. reported the data of the national culture and Datum group information center about top ten of Thai food popular menu from 5 - Thai restaurant in 5 continents. [37] The top ten- menu are Thai style soup with shrimp (Tom-yam-koong), Green curry (Kang- keow- wan), Thai noodle stir-fry (Phad-Thai), Phad kaprao, Red curry with roast duck (Kang phed ped yang), Galangal soup, Meat spicy salad (Yam neur), Pork satay, Stir-fry Chicken with cashew nut, and Dry curry cooked in a pan (Pa-nang). [3]

Furthermore, Thai foods such as: kang liang, sour-peppery soup (Kang som), Kang khua, Kang Pa, Spicy Muslim curry, Curry, Labb and Spicy salad. The main ingredients of Thai foods are composed of variety of herbs and spices in each menu.

Kang liang: pepper, onion [3], hairy basil leaf [38]. (Some formula add lesser ginger)

Kang khua: dried chili, garlic, onion, galangal, lemon glass [3], [38].

Sour-peppery soup (Kang som): dried chilli, garlic, onion, galangal [3].

Kang pa: dried chilli, garlic, onion, galangal, lemon glass, holy basil leaf [3]. (Some formula add lesser ginger)

Kang phed, Pa-nang: dried chili, garlic, onion, coriander root, galangal, lemon lass, kaffir lime peel, pepper, cumin [3]. (Add sweet basil leaf in Pa-nang)

Green pepper curry (Kang- keow- wan): green chili, garlic, onion, galangal, lemon glass, kaffir lime peel, coriander root, pepper, cumin [3]. (Some formula add sweet basil leaf)

Curry: dried chili, garlic, onion, galangal, ginger, lemon glass, cumin [3].

Spicy Muslim curry: dried chili, onion, galangal, lemon glass, cumin, cloves, pepper [3]

Tom yam, Tom Kha: chili, lemon grass, kaffir lime leaf, galangal [3]. (Some formula add holy basil leaf in Tom yam)

Phad kaprao: chili, garlic, holy basil leaf. (Some formula add onion and pepper)

Meat spicy salad: chili, onion, peppermint leaf [38]. (Some formula add ginger)

Thai noodle stir-fry (Phad-Thai): garlic, dried chilli, onion.

The pharmacology effect data from the study of the researcher of medicinal plant research center, Pharmacy faculty, Mahidol University, Institute of Thai traditional medicine, Department of Medical Services, Ministry of Public Health, Forestry Department and Institute of Nutrition, Mahidol University [3, 10, 39-45] could be summarized as follow.

2.9.1 Holy basil



Figure 2.3 Holy basil

In Thailand have 3 types: Red, White and Combined Holy basil. [45]
Holy basil is an annual plant, stem and leaves have soft hair, fragrant leaf and a bunch of flower. Leaves and branches of Red Holy basil have a green and purple color. [45]
Leaves and branches of White Holy basil have a pale green color. [46]

Growing: Using seed or a scion grafting, easy to grow in loose ground. Red Holy basil is more popular than White. [45, 46]

A taste and Thai medical properties: Pungent. Relief stomachache, bloated, colicky and used for flavour. [45]

Botanical information [45, 46]

Scientific name *Ocimum tenuiflorum* Linn., *Ocimum sanctum* Linn.

Family name Labiatae

English name Holy basil, Sacred basil

Common Thai name Ka-prao

Other local names กอมก้อดง, กอมก้อ, กะเพราขาว, กะเพราแดง, ห่อแก้วชู, ห่อตูปลู, อ่อมลิ้มหล้า

Scientific evidences

Anti-bloating effect: From the fragrant oil of Holy basil. [46]

Anti-peptic ulcer effect: In animal experiment, feeding 100 mg/Kg.BW. Holy basil extract with 70% Alcohol via gastric tube to white rat, resulting in curing peptic ulcer from Aspirin by the method of Shay-induced ulcers or Specific repeated cold stress animals. [46]

Anti-peristalsis effect: Leaf extract with 50% Alcohol, resulting in relieve smooth muscle of a guinea pig and rabbit intestine when stimulate with histamine, acetylcholine and carbachol. [46]

Bile secretory effect: From eugenol in Holy basil. [46]

Anti- inflammatory effect: From eugenol in Holy basil, inhibit the synthesis of prostaglandin. The important substances that inhibit the secretory of histamine from mast cell is ursolic acid. [46]

Anti- hepatitis effect: Holy basil extract with 70% Alcohol injected to abdominal cavity of a mouse at the dose of 200 mg/Kg. BW. , found that it can prevent hepatitis from carbon tetrachloride. [46]

Toxicity testing

1. LD₅₀ of Holy basil extract with 70% Alcohol, feeding dose to mouse is 4.5 g/Kg. BW. And abdominal cavity injection dose is 3.24 g/Kg. BW. [46]
2. Inject leaves extract with 50% Alcohol in abdominal cavity of mouse in the dose of 1 g/Kg. BW., non toxic and subcutaneous inject with 10 g/Kg. BW. is non toxic. [46]
3. No mutation effect: fresh leaves extract with hot water in the dose of 0.5 cc./Disc, no mutation effect in *Bacillus subtilis* , H-17 (REC+) and M-45 (REC-). [46]
4. Fetal toxicity: the water extract in the dose of 100 and 200 mg/Kg. BW. is toxic and withstand the implantation of fetal in white rat. The benzene extract in the dose of 200 mg is toxic to fetal and the study in white rat, showed the petroleum ether extract and the water extract in the dose of 100 mg/Kg and the alcohol extract (1:1) in the dose of 150 mg/Kg are non toxic to the fetal. [46]

2.9.2 Sweet basil



Figure 2.4 Sweet basil

Sweet basil is an annual plant, several years old, 0.3-0.9 m. high. With the specific odor, corner stem young branch have a red- purple and green color. Single oval leaves opposite arrange 2-3 cm. width and 4-6 cm. long. The leaf edge like saw-tooth, a bunch of flower give at the top, the white petal have 2 notches, the lower notch has a red- purple color, the ornament leaf has a green- purple color, one dried fruit have 4 small fruits. [47]

Growing: The methods are cultivated seedlings and plants and peking. Loosen ground and good draining. [48]

A taste and Thai medical properties: Pungent. Fresh leaves relieve bloating. The fragrant oil increased the peristalsis of intestine in the experimental animal, good for release air from the intestine. The soaking fruits will swell and used for a laxative. [47]

Botanical information [47]

Scientific name *Ocimum basilicum* Linn.

Family name Labiatae

English name Sweet basil

Common Thai name Ho – ra- pa

2.9.3 Hairy basil



Figure 2.5 Hairy basil

Hairy basil is an annual plant, 65 cm. high. Fragrant Leaf with a soft hair, a bunch of flower gives at the top. White petal gives around the stem of the flower, easy falling. [45]

Growing: The methods are cultivated seedlings and plants and Peking in clay mixed with sand and cow manure. [45]

Botanical information [45, 49]

Scientific name	<i>Ocimum basilicum</i> Linn. var. citratum Back.
Family name	Labiatae
English name	Hairy basil
Common Thai name	Mang- luk
Other local names	ก้อมก้อขาว (ภาคเหนือ), มังลัก (ภาคกลาง)

Scientific evidences

Lubricate effect: Seed help for excretion because it can swell to 45 times without digestion, increase waste and lubricant, easy for excretion. [45, 49]

Clinical experiment: relieve constipation. [49]

Used 2 teaspoonfuls of Hairy basil seed mixed with 240 cc. of water or one glass. [45] Give the result as 2 teaspoonfuls of psyllium in normal people, and the interesting results such as [45] :

- Increase the excretion time.
- Increase the stool amount.
- Soften stool.

The experiment in older patients with intake the medicine in the dose of 1/2, 1 and 1 1/2 teaspoonful in 240 cc. of water three times per day, found 80.6% of the patients increased times to excrete. [49]

Toxicity testing

Acute toxicity: male and female mouse eat the seed in the dose of 0.255 g/Kg.BW. for 7 days, non toxic.

Semi-acute toxicity: eat in the dose of 1 g/Kg. BW. for 10 days, no effect with mouse, rabbit and cat. [49]

Chronic toxicity: mouse eats in the dose of 0.25, 0.5, 1 and 2 g/Kg.BW. for 12 weeks, not found the changing of liver, small intestine and large intestine. [49]

Precaution

Hairy basil seed that not fully swell will absorb water from intestine, may cause dehydration and intestinal obstruction or hard stool [45], especially in the powder form. [49]

2.9.4 Peppermint



Figure 2.6 Peppermint

Peppermint is an annual plant. The stem split into a branch and spread along the ground. Shortsoft hair covers all the plant, fragrant. Oval leaves opposite arrange, the leaf edge like saw-tooth, the nib of a leaf is short. [37]

Growing: loose soil, grow in low lying, moist and soaked area. Breed by divide the branches.

A taste and Thai medical properties: Pungent. Releasing air and sweat, relief bloating by release break wind, a drink from leaves for digest assistant. [37]

Botanical information [37]

Scientific name	<i>Mentha cordifolia</i> Opiz.
Family name	Labiatae
English name	Peppermint [47]
Common Thai name	Sa-ra-nae
Other local names	สะระแหน่สวน (กลาง), มักเงาะ, สะแน (ใต้), หอมด่วน (เหนือ)

A taste and Thai medical properties: Pungent and cool. Release the heat by sweat; release the remaining blood in the body. A drink from leaves used for relief cold by sweat. [37]

2.9.5 Ginger



Figure 2.7 Ginger

Ginger is an annual plant. Long life, underground stem call “root”, root has a white color. A part grow over-ground is 0.5 m. high from root. Single and tapering leaf, a tip of leaf is pointed; a rim is smooth and 1-3 cm. width, 10-25 cm. long. A bunch of flower give from root, the stem of flower is 10-20 cm. long, a yellow color flower with purple-red at the end of a flower petal, has a green-yellow color fold coat. [45, 50]

Growing: used aging root, growing in clay mixed with sand and good in drain water with moderately sunshine. [45, 50]

Botanical information [45, 50]

Scientific name	<i>Zingiber officinale</i> Roscoe.
Family name	Zingiberaceae
English name	Ginger
Common Thai name	Khing
Other local names	ขิงแกลง, ขิงแดง, ขิงเผือก, สะเอ

Scientific evidences

Anti-emetic effect: injected ginger extract with 50% methanol in the dose of 10 g/Kg.BW. in emetic frog from copper sulfate, found anti-emetic effect. Furthermore, ginger extract with acetone or 50% ethanol has the anti-emetic effect from cisplatin, but the water extract has no effect. Ginger extract has no anti-emetic effect with apomorphine. The important substances in the anti-emetic effect are [6]-, [8]-, [10]-shogaol and 10-gingerol. [50]

Clinical experiment in anti-emetic effect [50]

Ginger extract with 50% methanol in the dose of 10g/Kg.in 18-20 years old, 36 volunteers are 18 men and women, showed anti-emetic effect at 940 mg.

Relief dizziness: used ginger powder in the patient with dizziness, showed the better result than dimenhydrinate.

In ovarian surgery patient: by taking ginger powder capsule before surgery, found lesser nausea and vomiting in consumed patient.

Relieve bloating effect: Ginger is good in relief colicky symptom [45], because it has a volatile oil that have anti-bloating effect. The effective substances are menthol and cineole. Ginger extract with acetone (75 mg/Kg), shogaol and gingerol have the effect of propulsive movement, relieve bloating symptom. [50]

Bile secretory effect: Ginger extract with acetone and ginger powder have a bile secretory effect. The important substances that have the bile secretory effect are borneol, fenchone, 6-gingerol and 10-gingerol. [50]

Reduction of peristalsis effect: Volatile oil from root has the anti-peristalsis effect for small intestine, 12% of papaverine. Ginger extract with water found, in the

low dose has the stimulate effect to separated small intestine of white rat. The intra abdominal injection, in the dose of 0.005-0.020 g/Kg relieved the propulsive movement of small intestine and stimulated the peristalsis of small intestine in the dog. The effect pass through the auto-nervous system, the effect is not looked like histamine. So the anti colicky symptom is from the secretion of 5-HT or in the ginger has 5-HT, that has the anti-colicky symptom. The important substances are 6-gingerol and 6-shogaol. [50]

Reduction of peptic ulcer effect: White rat eating ginger extract with acetone in the dose of 1 g/Kg, found 97.5% of the anti-peptic ulcer effect and the important substance is furanogermerone, when stressing rat with reduced flow of acid. Feeding zingiberine and 6-gingerol to the rat at the dose of 100 mg/Kg, showed the reduction of peptic ulcer of ethanol-acid peptic ulcer white rat, the reduction rate are 97.5, 53.6 and 54.5%. 6-gingesulfonic acid, ginger glycolipid A, B and C have the anti-peptic ulcer effect. [50]

Reduction of inflammatory effect: Pungent substance in ginger that has this effect is 6-shogaol found in dry more than fresh ginger, by protected cyclooxygenase activity effect. Gingerol and diarylheptanoid are anti-inflammatory substance and can inhibit arachidonate-5-lipoxygenase enzyme. [50]

Reduction of acid secretion effect: Ginger extract with 50% alcohol reduced gastric acid secretion. [50]

Anti-bacterial effect: Ginger extract have the bactericidal effect to E. coli. and pus formation bacteria. [45]

Cough inhibitory effect: 6-Shogaol has a cough inhibitory effect [50], and reduction of phlegm. [45]

Increase secretory effect in throat: From 6-shogaol. [50]

Mutagenic effect [50]:

Ginger extract with hot water is a mutagen for B 291I cell and TA 100 - Salmonella typhimorium in the dose of 12.5 mg/disc, but not for TA 98 - Salmonella typhimurium in the dose of 50 mg/disc.

Ginger extract with alcohol is a mutagen for TA 510 and 4 - Salmonella typhimorium.

Gingerol and shogaol are the mutagen for TA 100 and 1535 but no effect with TA 98; zingerone is not a mutagen and can inhibit the mutagenic effect of gingerol and shogaol.

3 hrs of 135°C and UV light can reduce the mutagenic effect of ginger extract with ethanol.

2.9.6 Galangal



Figure 2.8 Galangal

Galangal is an annual plant. A white stem is in underground call “root”. The segment root is obvious seen. The stem of a leaf and leaf are on overground, 1-2 m. high. Single and dark green leaf, a leaf sheath coat over the stem, oval leaf with pointed tip, smooth rim. A bunch of flower give from the tip, long stem of a flower, each flower is small and pink color. [45]

Growing: Using root to grow, loose soil with suitable soaked, good in rainy season. [45]

Botanical information [45, 51]

Scientific name *Alpinia galanga* (Linn.) Swartz.
Languas galanga (Linn.) Stuntz.

Family name Zingiberaceae

English name Galangal [52]

Common Thai name Kha

Other local names กุฎกโรหิณี, ข่าหยวก, ข่าหลวง, สะเอเซช, สะเออเคย

Scientific evidences

Reduction of colicky effect: Found the important substances are Cineole, camphor and Eugenol. [51]

Bile secretory effect: By Eugenol, helping for digestion. [51]

Relief bloating effect: From the volatile oil. [51]

Reduction of inflammatory effect: From 1'-acetoxychavicol acetate, 1'-acetoxy eugenol acetate and Eugenol, relieve colicky symptom from peptic ulcer. [51]
a bactericidal effect

Bactericidal effect: The cause of colicky symptom may be from intestinal bacteria. Galangal has a intestinal bactericidal effect. [51] The important substance is Eugenol. [51]

Fungicidal effect: Galangal extract with alcohol, chloroform and petroleum ether have a good result for fungi that are the cause of a ringworm disease; *Microsporum gypseum* and *Trichophyton rubrum* [53] The water extract give lesser result than alcohol extract. The important substances for fungicidal effect are 1'-acetoxychavicol acetate and 1'-acetoxyeugenol acetate. [51]

Clinical experiment in ringworm and Tinea disease: Compared with Tolnaftate, the result was good.

Anti-mutagenic effect: From Eugenol, used 0.4% mixed in the food of male mouse. [51]

Toxicity testing

Galangal extract with 50% alcohol at the dose of 10 g/Kg in the mouse by feeding and subcutaneous injection had no toxic at the dose of 250 times of reference in textbook. [51]

2.9.7 Lesser ginger



Figure 2.9 Lesser ginger

Lesser ginger is an annual plant, several years old. Underground spindle root with yellow color and specific fragrance. Single oval leaf 4.5-10 cm width, the leaf coat has a pale red color. The inside of the stem of a leaf has a crack, a bunch of white and pink color flower give between shells of leaf at a base of a stem. [45, 54]

Growing: Using root in loose soil mixed with sand, good draining, growing in both of under the tree or clear area, in a beginning of rainy season. [45, 54]

A taste and Thai medical properties: Pungent and bitter. Relieve stomachache, bloating and nourishment. [45]

Botanical information [55]

Scientific name	<i>Boesenbergia rotunda</i> (Linn.) Mansf., <i>Boesenbergia pandurata</i> (Roxb.) Schltr., <i>Gastrochilus panduratus</i> Ridl.
Family name	Zingiberaceae
English name	Wild ginger [52], lesser ginger
Common Thai name	Kra-chai
Other local names	กะแอน, จิงทราย, จี่, ชี่, เป้าชะเอม, เป้าลิ, ระแอน, ว่านพระอาทิตย์

Scientific evidences

The important substance in the root of lesser ginger is essential oil but found a little. 0.08% of essential oil composed of many chemical substances: 1.8 - Cineol, Boesenbergin A, dl – Pinostrobin and Camphor. The essential oil has the anti-bloating effect, helps the movement of bowels and is an appetizing agent. Department of Medical Sciences studied and reported no acute toxicity in mouse. [54]

Anti-bloating effect: by the fragrant oil. [55]

Reduction of peristalsis effect: Lesser ginger can reduce peristalsis of segmented intestine in rat by 4 stimulator substances: Acetylcholine, Histamine, Barium chloride and Dimethyl-4-phenyl-piperaziniumiodide (DMPP). [55] Cineole is an important substance in lesser ginger, has the reduction of peristalsis effect. [55]

Bactericidal effect: Lesser ginger extract has a bactericidal effect to *E. coli*, the cause of colicky symptom. [55] The experiment found root substances could reduce the growth of microorganism, better in bacteria: *Bacillus subtilis*, intestinal bacteria and pus formation bacteria than fungi. [54]

Reduction of inflammatory effect: From 5, 7-dimethoxyflavone by inhibit the analysis of Prostaglandin. [55]

Toxicity testing

Hippocratic screening, using 5, 7-dimethoxy flavones in the dose of 3 g/Kg (10 times of inflammation curing), showed small toxin and non-toxic to mouse. In 7 days trial, showed the dose at 1.26 g/Kg could reduce respiration and temperature from the pressure on central nervous system. [55]

Mutagenic effect: Lesser ginger extract with hot water and water in the dose of 0.5 cc/ disc, no mutagenic effect to both breed of B. Subtilis: H-17 (Rec+) and M-45 (Rec-). And lesser ginger squeeze has no mutagenic effect. [55]

Table 2.2 Antioxidant vitamins in 7 pungent Thai herbs. [52, 56]

Name of medicinal herbs	Nutrient composition per 100 g. edible portion		
	Beta carotene (Microgram)	Vitamin E (Milligram)	Vitamin C (Milligram)
Holy Basil*	5501-7875	-	24-25
Sweet Basil*	2713	-	21
Hairy Basil*	3348	-	3-18
Kitchen Mint*	3163	-	88
Ginger**	11	-	10
Galangal***	2-14	-	12-22
Lesser Ginger**	0	-	2

* Leaves

** Young rhizome

*** Young tuber

Table 2.3 The carotenoid content in herbs. (μg / 100 g. edible portion, fresh weight) [57]

Herbs	Edible portion	β -carotene	Retinol equivalent
Holy basil leaves	38	6803	1134
Sweet basil leaves	72	3543	591
Kitchen mint leaves	63	3230	538

CHAPTER 3

MATERIALS AND METHODS

3.1 The first phase of experiment

To determine the quantities of beta-carotene, vitamin C, E, phenolic compounds and total antioxidant capacity per 100 gram wet weight and per one cup of 7- pungent Thai herbs: holy basil, sweet basil, hairy basil, peppermint, ginger, galangal and lesser ginger. All herbs were bought from 3 shops of Taladtai market, Rangsit boundary, Bangkok.

3.1.1 Materials

3.1.1.1 Medicinal herbs

Simple random sampling: bought 7 popular Thai medicinal herbs: holy basil, sweet basil, hairy basil, peppermint, ginger, galangal and lesser ginger from randomized 3 shops of Taladtai market in Rangsit boundary, Bangkok.

3.1.1.2 Characteristics of medicinal herbs

A part of medicinal pungent Thai herbs, which were used in this study:

1. Holy basil – leaf
2. Sweet basil - leaf
3. Hairy basil – leaf
4. Peppermint - leaf
5. Ginger – young root
6. Galangal – young root
7. Lesser ginger – young root

The edible portion of herb from the first, second and third shops of Taladtai market were pooled. Each pool sample was analyzed for β -carotene, vitamin C, vitamin E, total polyphenol and total antioxidant capacity.

3.2 The second phase of experiment

Supplementation to volunteers with placebo or herb capsule then comparison plasma level of cholesterol, triglyceride, LDL-c and HDL-c, malondialdehyde and C - reactive protein of training soldiers before and after daily intake of the highest level of total antioxidant capacity of pungent Thai herb at 2 and 8 weeks.

3.2.1 Subjects

50 volunteer training soldiers were invited from the first shift training soldier of Infantry Regiment, King's guard 1st Bangkok and divided into two groups.

3.2.1.1 Inclusion criteria

The following volunteers were eligible for the study:

- Male with age between 18 and 25 years.
- Healthy from any illness during the past 4 weeks and free from serious medical illness.
- Neither taking any medication nor vitamin supplement.
- No smoking.
- Willing to follow with the study protocol and stay on the study for 8 weeks.
- Able to consume Thai medicinal herbs.

3.2.1.2 Exclusion criteria

The volunteers were not participated if they had the following:

- Regular intake of antioxidants, any other drug with antioxidant properties.
- Smoking.
- Severe obesity (BMI > 30 kg/m²)
- Diabetic mellitus, cataracts, uncontrolled hypertension (sitting diastolic BP > 105 mmHg), cardiovascular disease, cerebrovascular disease, cancer or other disease or condition worsening the adherence to the measurement or treatment.
- Any conditions limiting volunteers follow the study protocol.
- Medical compliance < 50%

3.2.2 Sample size

Calculated by:

$$n_0 = \frac{Z_{\alpha}^2 PQ}{d^2}$$

Setting: Confidential interval (CI) at 95% and an error at 20%

n_0 = Sample size

Z = Z-Table at Probability $\alpha/2 = 1.96$ (CI = 95%)

P = Rate of reducing oxidative stress in smoking after supplementation with antioxidants = 0.35 [58]

Q = $1-P = 0.65$

d = an error in probability of population and sample = 0.2

Calculation:

$$\begin{aligned} n_0 &= \frac{(1.96)^2 (0.35) (0.65)}{(0.2)^2} \\ &= 21.8 \approx 22 \end{aligned}$$

3.3 Methods

3.3.1 Ethics approval

The objectives and details of the study were explained, and a written informed consent was obtained from all volunteers. The study was approved by the ethics committees of Mahidol University and performed in accordance with the principles of the declaration of Helsinki.

3.3.2 Experimental design

In this study, the experiment was divided into 2 phase. In the first phase, 7 pungent Thai medicinal herbs were tested for their antioxidant activity and antioxidant vitamins. Then the highest herb was selected in the second phase experiment.

3.3.2.1 In the first phase experiment

- Total phenolic compound analyze by Folin Ciocalteu's reagent [59] by Spectrophotometer.
- β -carotene, vitamin E and vitamin C analyses by HPLC-method.[60-62]
- Total antioxidant capacity analyses by ORAC or FRAP – method [63-65] by Spectro-fluorometer.

β -carotene, vitamin E and vitamin C were analysed in the laboratory at Institution of Nutrition, Mahidol University followed the laboratory protocol. The analytical method for total polyphenol, β -carotene, vitamin C, vitamin E, ORAC and FRAP are shown in the appendix.

The highest TAC herb was prepared by freeze-dried at Faculty of Science and Institution of Nutrition, Mahidol University. Herb and corn starch, which used as supplement in the experimental and the placebo group, were encapsulated by the pharmacist of Pramongkutklao Hospital.

3.3.2.2 In the second phase experiment

Subject assessment:

- General information by the questionnaires.
- Anthropometric data by physical examination.
- Dietary assessment by 3 days record form and analyzed by INMUCAL.
- Physical activity by physical activity record form.
- Blood sample at 0, 2 and 8 week of supplementation for Lipid profile (Cholesterol, HDL-c, LDL-c, Triglycerides) analyzed by Spectrophotometer. (PMK Hospital Lab. center)
- Total antioxidant capacity in plasma analyzed by ORAC or FRAP – method [63-65] by Spectro-fluorometer or Spectrophotometer.
- Plasma malondialdehyde analyzed as TBA assay by HPLC-method.[66]
- C-reactive protein in plasma analyzed by Radial immunodiffusion assay. (PMK Hospital Lab. Center)

In the morning, after 10 hrs overnight fasted. Blood samples were drawn through an indwelling catheter from an antecubital vein in the fasting state. They were analyzed for lipid profile and hs- CRP at PMK Hospital Lab. Center in 1 hr after drawing, Reserve plasma were kept in -80° C until the analysing day for plasma TAC and MDA. The analytical method for MDA was shown in the appendix.

3.3.3 Dietary assessment

The volunteer were orientated before recording in the food record form. They were recorded the whole food that they eat in 2 working days and 1 weekend day. The 24 hrs food record will analyze by program “INMUCAL”.

3.3.4 Physical activity assessment

The volunteers were orientated before using the physical activity recorded form. They were recorded their all activities and time in 1 working day and 1 weekend day. The information was calculated and averaged total energy expenditure per day.

3.4 Supplementation procedure

All volunteers were randomly divided into 2 groups: experimental group and placebo group. The volunteers in each group were treated as following procedure.

3.4.1 Experimental group

3.4.1.1 All volunteers in experimental group intake the highest-level TAC herb in the form of capsule totally 2,000 mg/day.

3.4.1.2 All volunteers in experimental group intake the herb capsule two times a day. The diets were the same because they stayed in an Infantry Regiment, King's guard 1st military training center.

3.4.1.3 All volunteers in experimental group completely intake the same amount of a high level of TAC medicinal herb in the form of capsule, each capsule contain 500 mg of freeze dried herb. They were intake 2 capsules after breakfast and 2 capsules after dinner, totally 2,000 mg/day.

3.4.1.4 In experimental period (8 weeks), all volunteers in experimental group intake the herb capsule everyday.

3.4.2 Placebo group

3.4.2.1 The food intake of placebo group was the same as experimental group because their food was come from commissary.

3.4.2.2 All volunteers in placebo group daily intake the placebo capsule two times a day. The whole foods are the same because they stayed in Infantry Regiment, King's guard 1st military training center.

3.4.2.3 All volunteers in placebo group completely intake the same amount of corn starch in the form of capsule, each capsule contain 500 mg of corn starch. They must intake 2 capsules after breakfast and 2 capsules after dinner, totally 2,000 mg/day.

3.4.2.4 In experimental period (8 weeks), all volunteers in placebo group intake the placebo capsule everyday.

3.5 Biomarkers

All volunteers had blood drawn at 0, 2 and 8 week of supplementation for analyzed: plasma lipid, plasma MDA, plasma C-reactive protein and plasma TAC.

3.5.1 Plasma lipid (Cholesterol, HDL-c, LDL-c, and Triglyceride) analyses by Spectrophotometer. (Pramongkutklao Hospital Lab. Center)

3.5.2 Plasma malondialdehyde analyze by HPLC- method.

3.5.3 Plasma hs C-reactive protein analyze by Radial immunodiffusion assay (Pramongkutklao Hospital Lab. center)

3.5.4 Plasma total antioxidant capacity determined by ORAC and FRAP method

Blood sample of all volunteers were collected at 0 and 8 week after supplementation. They were sent to Pramongkutklao Hospital laboratory center for determining plasma lipid and hs-CRP on the same day and 1 hour after blood drawing.

Plasma ORAC, FRAP and MDA level were determined in laboratory at Institution of Nutrition, Mahidol University. The plasma of 0, 2 and 8 weeks after supplement were kept in – 80°C freezer until examination.

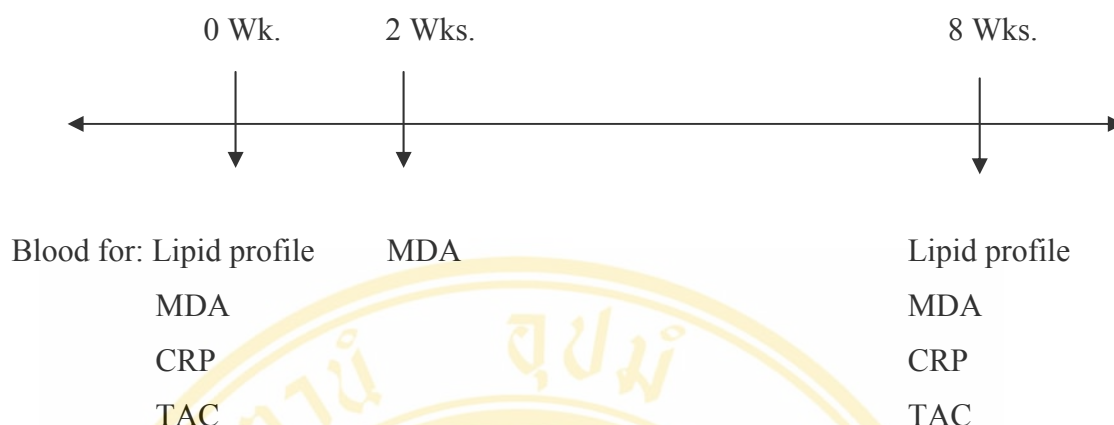


Figure 3.1 Blood collection planning

3.6 Experimental places

Institute of Nutrition, Mahidol University

An Infantry Regiment, King's guard 1st. Bangkok

3.7 Experimental period

Throughout the military training period: 8 weeks

3.8 Statistical analysis

Statistical analysis was performed using the computerized statistical program (SPSS 11.5 for Windows Inc., 2002 Chicago, Illinois, USA).

Normal distribution was tested by Shapiro - Wilk test. The results of anthropometric data were reported as mean $\pm$ SD.

The differences in values at pre and post supplementation were determined by related 2- sample Paired t-test for parametric data and Wilcoxon Signed Ranks test for non parametric data.

2- Independent sample T- Test and Mann- Whitney U Test were used for calculating the differences in parametric and nonparametric data between groups.

Pearson and Spearman's rho correlation were used for determining the relationship of parametric and nonparametric data, respectively.

Percent change was used for determine the change of data, which were different at baseline, from baseline to after supplementation time.

P-value of less than 0.05 was considered significant.



Placebo - Corn starch capsule

Peppermint Capsule

Figure 3.2 Peppermint capsule and placebo capsule

CHAPTER 4

RESULTS

4.1 Antioxidant vitamins, total polyphenol and total antioxidant activity in 7 pungent Thai herbs

β -carotene, vitamin E and vitamin C were determined in 7 pungent Thai herbs together with total polyphenol and total antioxidant activity.

4.1.1 β -carotene in 7 pungent Thai herbs

β -carotene is a lipid soluble compound which is called pro-vitamin A because it can be converting to vitamin A in the body. It found in fruits and vegetables. In this study, β -carotene was determined in 7 pungent Thai herbs by HPLC; the data are in Table 4.1

In this study, the amount of β -carotene is found in holy basil, sweet basil, hairy basil and peppermint. Ginger, galangal and lesser ginger have not detected.

4.1.2 Vitamin E in 7 pungent Thai herbs

Vitamin E is a lipid soluble vitamin that prevents lipid peroxidation. It can be found in 8 different forms. Alpha-tocopherol is the most active form in human body. In this study, alpha-tocopherol was determine in 7 pungent Thai herbs as vitamin E level by HPLC, the data are in Table 4.1

In this study, the amount of vitamin E is found in sweet basil, hairy basil, peppermint, holy basil and lesser ginger. Vitamin E was not detected in ginger and galangal.

4.1.3 Vitamin C in 7 pungent Thai herbs

Vitamin C is a water soluble vitamin; it can oxidize even by air. Vitamin C was detected in 7 pungent Thai herbs by HPLC, the data are in Table 4.1

In this study, the amount of vitamin C is found in holy basil, hairy basil, sweet basil, peppermint. Vitamin C have found in ginger, galangal and lesser ginger about 0.1 mg/100g sample.

Table 4.1 The quantity of β -carotene, vitamin E and vitamin C in 7 pungent Thai herbs

Name of pungent Thai herb	Betacarotene		Vitamin E		Vitamin C	
	mg/100 g edible portion	mg / 1 cup (g)	mg/100 g edible portion	mg / 1 cup (g)	mg/100 g edible portion	mg / 1 cup (g)
Holy basil (<i>Ocimum sanctum</i> Linn.)	3.01	0.4515	0.72	0.429	0.68	0.102
Sweet basil (<i>Ocimum basilicum</i> Linn.)	2.43	0.3645	2.05	0.3075	0.58	0.087
Hairy basil (<i>Ocimum canum</i> Sims.)	2.33	0.5126	1.95	0.429	0.6	0.132
Peppermint (<i>Mentha cordifolia</i> Opiz.)	1.29	0.1935	1.08	0.162	0.33	0.0495
Ginger (<i>Zingiber officinale</i> Roscoe.)	0	0	0	0	0.1	0.126
Galangal { <i>Alpinia nigra</i> (Gaertn.) B. L. Burtt}	0	0	0	0	0.1	0.126
Lesser ginger { <i>Boesenbergia pandurata</i> (Roxb.) Schltr.}	0	0	0.48	0.6048	0.1	0.126

4.1.4 Total polyphenol in 7 pungent Thai herbs

Phenolic compound was found in many kinds of fruits and vegetable. The total number of phenols in food is referred to "phenolic content".

In this study, total polyphenol in 7 pungent Thai herbs was detected by react with Folin Ciocalteu's reagent and read with Spectrophotometer by modify method of Botanical center for age and related diseases. [59] The data shown in Table 4.2

The polyphenol was highest in peppermint then holy basil, sweet basil, lesser ginger, hairy basil, ginger and galangal, respectively.

Table 4.2 The amount of total polyphenol in 7 pungent Thai herbs.

Name of pungent Thai herb	Total polyphenol	
	mg /100 g edible portion	mg / 1 cup (g)
Peppermint (<i>Mentha cordifolia</i> Opiz.)	124.56	18.684
Holy basil (<i>Ocimum sanctum</i> Linn.)	118.48	17.772
Sweet basil (<i>Ocimum basilicum</i> Linn.)	80.13	12.02
Lesser ginger { <i>Boesenbergia pandurata</i> (Roxb.) Schltr.}	74.94	94.424
Hairy basil (<i>Ocimum canum</i> Sims.)	72.39	15.926
Ginger (<i>Zingiber officinale</i> Roscoe.)	63.31	79.77
Galangal { <i>Alpinia nigra</i> (Gaertn.) B. L. Burtt}	57.86	72.904

4.1.5 Total antioxidant capacity in 7 pungent Thai herbs

In this study, ORAC and FRAP are selected for determine total antioxidant capacity.

4.1.5.1 ORAC assay

Oxygen radical absorbance capacity (ORAC) method is specific for measuring total antioxidant capacity in various samples including all herbs in this study. The method was followed the standard operating protocol of Brunswick Laboratories.[63, 67] The data are shown in Table 4.3

From the table, ORAC was found highest in peppermint then holy basil, ginger, hairy basil, lesser ginger, sweet basil and galangal respectively.

4.1.5.2 FRAP assay

The ferric reducing ability of plasma (FRAP) method was used for evaluating antioxidant capacity in various samples followed the method of Iris F. Benzie and J. J. Strain.[65] The data are shown in Table 4.3

From the table, galangal has highest FRAP follow by peppermint, sweet basil, holy basil, ginger or lesser ginger and hairy basil.

4.1.6 Water content in 7 pungent Thai herbs

The data showed that ginger had 96% water, followed by galangal, hairy basil, peppermint, sweet basil, lesser ginger and holy basil.

Interesting that peppermint had highest quantity of total polyphenol and ORAC then it was chosen for study in 2nd Phase.

Table 4.3 The antioxidant activity of 7 pungent Thai herbs by ORAC, FRAP and water content

Name of pungent Thai herb	ORAC		FRAP		% Moisture
	$\mu\text{mol TE}$ /100 g edible portion	$\mu\text{mol TE}$ /1 cup (g)	$\mu\text{mol TE}$ /100 g edible portion	$\mu\text{mol TE}$ /1 cup (g)	
Peppermint (<i>Mentha cordifolia</i> Opiz.)	1839	276	64	9.6	90.54
Holy basil (<i>Ocimum sanctum</i> Linn.)	1570	236	55	8.25	86.65
Ginger (<i>Zingiber officinale</i> Roscoe.)	1280	1612	47	59.22	96.19
Hairy basil (<i>Ocimum canum</i> Linn.)	1120	246	42	9.24	90.69
Lesser ginger { <i>Boesenbergia pandurata</i> (Roxb.) Schltr.}	962	1212	47	59.22	88.89
Sweet basil (<i>Ocimum basilicum</i> Linn.)	527	79	60	9	89.37
Galangal { <i>Alpinia nigra</i> (Gaertn.) B. L. Burtt}	525	662	71	89.46	93.03

4.2 General characteristics of the volunteers

All of the volunteers are the first shift training soldier of Infantry Regiment, King's guard 1st. In the first 2 months, their training schedule was taken approximately 8-10 hrs per day from Monday to Friday and 4-6 hrs per day in Saturday. The activities were recorded in their recording booklet for physical activity assessment. Their food intake was also recorded in 3 day-record form for calculating total energy intake. All volunteers consumed the regular diet from their commissary.

4.3 The characteristic of the volunteers from placebo and experimental group

The personal information of the volunteers' are home address, education level, ailment and health condition, history of using medicine, alcohol consumption, smoking habit, drinking habit in coffee and tea, exercise habit. The data are not significantly different between groups, except, type of alcohol is significantly different at P value < 0.05 . The data are shown in appendix table A2.

4.4 Data collected from food frequency questionnaires (FFQ)

The information of volunteers' in habitual diet was collected. Most of the data are not significantly different, except, the frequency of intake in Kang lieng and Kang pa are significantly different at P value < 0.05 . The data are shown in appendix table A23-46.

4.5 Energy intake from the commissary of the volunteers

Average energy intake of the commissary of Infantry Regiment, King's guard 1st is 1992 Kcal/person/day. Which was got from interviewing and observing while the diet preparation by the cooker. Everyday, all of the volunteers have eaten food from the commissary, their foods are the same, except on the weekend and the leisure time, and some of them may eat different food. The diets from commissary were recorded for calculating the overall energy intake of all soldiers at Infantry Regiment, King's guard 1st. The data are shown in the appendix table A 48.

4.6 Anthropometric and physical characteristics of the volunteers

All volunteers were divided into 2 groups, each of 25 volunteers. The anthropometric and physical characteristics of the volunteers are shown in table 4.4.

After determining all of the inclusion criteria and medication compliance, the placebo group has 19 volunteers and the experimental group has 14 volunteers. Their age, body weight, BMI, waist-hip circumference, blood pressure, pulse rate, total energy intake and energy expenditure per day are not significantly different while body height is significantly different. (P value < 0.05)

Table 4.4 Characteristics of the volunteers in placebo and experimental group at baseline. The data are presented as mean $\pm$ SD.

Parameter	Placebo group (n=19)	Experimental group (n=14)	p-value
Age (years)*	21.21 $\pm$ 0.63	21.71 $\pm$ 2.2	0.419
Body weight (kgs) ^a	56.13 $\pm$ 6.34	56.64 $\pm$ 7.32	0.884
Body height (cms) ^a	163.45 $\pm$ 2.48	167.61 $\pm$ 6.09	0.010
BMI (kg/m ²) ^a	21.0 $\pm$ 2.32	20.17 $\pm$ 2.53	0.172
Waist circumference (cms) ^a	72.74 $\pm$ 5.29	72.85 $\pm$ 4.93	0.927
Hip circumference (cms) ^a	88.47 $\pm$ 4.1	89.43 $\pm$ 5.63	0.608
Systolic blood pressure (mmHg) ^a	120.74 $\pm$ 13.51	120.00 $\pm$ 11.42	0.675
Diastolic blood pressure (mmHg) ^a	69.47 $\pm$ 9.28	68.35 $\pm$ 8.56	0.869
Pulse rate (beat/min) ^a	75.58 $\pm$ 13.23	85.07 $\pm$ 13.64	0.068
Total energy intake per day (kcal) ^a	2038.03 $\pm$ 332.34	2025.45 $\pm$ 291.21	0.970
Total energy expenditure per day (kcal) ^a	3420.12 $\pm$ 409.61	3570.1 $\pm$ 523.27	0.407

^a Test by Mann-Whitney Test, P value = 0.05.

* Test by 2-independent sample t-test, P value = 0.05.

The data in bold text are significantly different at P value < 0.05.

4.7 Anthropometric data

In placebo group, body weight, BMI, waist-hip circumference, systolic blood pressure and pulse rate at 0 and 8 week are not significantly different. Diastolic blood pressure at 0 and 8 week are significantly decreased.

In experimental group, body weight and BMI are significantly increased at 8 week. However, systolic blood pressure, diastolic blood pressure and pulse rate are significantly decreased at 8 weeks after supplementation.

All antropometric data did not correlate with medication compliance when tested with Pearson correlation and Spearman's rho at P value < 0.05.

The result showed that antioxidant supplementation could cause significantly reduced in pulse rate and systolic blood pressure eventhough not significantly

different. It might be illustrate that daily intake of pungent Thai herb had a beneficial effect. The data are shown in Table 4.5.

4.8 Food intake and physical activity

From table 4.4, 18 volunteers in placebo group have completed 3-day- record form. Average total energy intake per day of placebo group is 2,038 Kcal. The number of volunteers who completed physical activity record form is 15. Average total energy expenditure per day of this group is 3,420 Kcal. Whereas, in experimental group, 14 volunteers have completed 3-day- record form. Average total calories intake of this group is 2,025 Kcal/day. The number of volunteers who completed physical activity record form is 12; average total energy expenditure of this group is 3,570 Kcal/day. The ratio of energy intake and energy expenditure (EI: EE) in both groups is 0.55. Both groups are not significantly different in total energy intake per day and total energy expenditure per day.

From table 4.4, the data of BMI and body weight in experimental group were significantly increased at 8 weeks after supplementation; whereas in placebo group the data were not significantly different.

Table 4.5 Comparison anthropometric data of the volunteers at 0 week and 8 weeks after supplementation in placebo and experimental group

Parameter	Placebo group			Experimental group		
	Time		p-value	Time		p-value
	(Week after supplement)			(Week after supplement)		
	0 Week	8 Week		0 Week	8 Week	
Body weight (kg)	56.13±6.34	56.74±3.52	0.555	56.6±7.3	59.2±5.64	0.031
BMI (kg/m ²)	21.0±2.31	21.24±1.34	0.556	20.17±2.53	21.07±1.7	0.034
Waist circumference (cm)	72.74±5.29	72.13±2.65	0.981	72.85±4.93	73.71±2.46	0.171
Hip circumference (cm)	88.47±4.1	89.26±2.49	0.347	89.43±5.62	90.14±3.88	0.227
Systolic blood pressure (mmHg)	120.74±13.51	116.84±10.68	0.295	120.0±11.42	114.64±10.4	0.116
Diastolic blood pressure (mmHg)	69.47±9.28	63.94±6.79	0.051	68.36±8.56	65.78±7.16	0.248
Pulse rate (beat/min)	75.58±13.23	74.05±9.38	0.672	85.07±13.63	72.71±8.09	0.002

Tested by Wilcoxon Signed Ranks test, P value = 0.05.

The data in bold text are significantly different at P value < 0.05.

4.9 Biochemical data

4.9.1 Plasma levels of cholesterol, triglyceride, LDL-c, HDL-c, ORAC, FRAP, MDA and hs – CRP.

Table 4.6 Comparison plasma levels of lipid, TAC, hs-CRP and MDA at 0 and 8 week after supplementation

Parameter	Time					
	0 Week			8 Week		
	Pla. Gr.	Exp. Gr.	p-value	Pla. Gr.	Exp. Gr.	p-value
Cholesterol ^a (mg/dL)	155.16±32.73	157.2±33.2	0.913	150.74±25.4	172.35±33.3	0.105
Triglyceride ^a (mg/dL)	58.16±14.49	59.21±17.8	0.870	134.05±77.9	194.36±95.9	0.016
LDL-c ^a (mg/dL)	98.26±31.52	97.64±27.46	0.757	86.74±22.1	93.5±21.99	0.423
HDL-c ^a (mg/dL)	50.26±9.22	53.78±11.9	0.316	45.63±8.05	51.5±8.23	0.037
ORAC ^a (μmol TE/100 g sample)	576.12±121.44	494.1±162.9	0.126	624.02±245.2	1507.75±604.8	0.000
FRAP ^a (μmol TE/100 g sample)	5.6±0.77	12.74±2.24	0.000	5.34±0.51	14.37±3.64	0.000
hs-CRP* (mg/L)	2.75±4.24	2.57±3.05	0.896	1.25±2.22	0.8±1.34	0.507
MDA* (μmol/L)	0.127±0.04	0.072±0.037	0.001	0.131±0.04	0.073±0.035	0.000

^a Tested by Wilcoxon Signed Ranks test, P value = 0.05.

* Tested by related 2-sample Paired t-test, P value = 0.05.

The data in bold text are significantly different at P value = 0.05.

As showed in table 4.6 and 4.7, the biochemical data showed not significantly different between experimental and placebo groups in level of cholesterol, LDL-c and hs-CRP at 0 and 8 week of supplementation. In addition, plasma triglyceride, HDL-c and ORAC at baseline are also not significantly different, however at 8 week they are significantly different. Furthermore, the change of FRAP and MDA values are significantly different, after supplement for 8 weeks. The data was shown in table 4.8.

In placebo group, plasma cholesterol, ORAC, FRAP and MDA were not significantly different between 0 and 8 weeks. Plasma triglyceride, LDL-c, HDL-c and hs-CRP were significantly decreased at 8 weeks after supplement.

In experimental group, after 8 weeks of supplementation, plasma cholesterol, triglyceride and ORAC were increased. Plasma LDL-c, HDL-c, FRAP and MDA were not significantly different between 0 and 8 weeks. However, hs-CRP was significantly decreased at 8 week.

Although, plasma hs-CRP level of volunteers from experimental group was significantly decreased at 8 weeks after supplement and military training period. In table 4.8 the plasma hs-CRP level in experimental group was reduced more than placebo group. In spite of, plasma hs-CRP level at 0 week of experimental group was higher than placebo group.

The data showed benefit of pungent Thai herb in increasing plasma level of total antioxidant capacity (ORAC), maintained level of HDL-c and may have preventive effect on chronic diseases by reducing the level of hs-CRP.

Plasma lipid, ORAC, FRAP and hs-CRP at 0 and 8 weeks of supplementation did not correlate with medication compliance when tested with Pearson correlation and Spearman's rho at P value < 0.05.

In table 4.8, FRAP level at 8 weeks after supplement in placebo group was decreased 4.6% from baseline whereas in experimental group was increased 12.8% from baseline. The data showed the important of peppermint supplement can increase level of total antioxidant capacity, reduce the inflammatory process and control lipid peroxidation by stabilizing the level of plasma MDA

Table 4.7 Comparisons of plasma cholesterol, triglyceride, LDL-c, HDL-c, at 0 and 8 weeks after supplementation in placebo and experimental group. The data are presented as mean $\pm$ SD.

Parameter	Placebo group			Experimental group		
	Time (Week after supplement)		p-value	Time (Week after supplement)		p-value
	0 Week	8 Week		0 Week	8 Week	
Cholesterol (mg/dL)	155.16 $\pm$ 32.73	150.74 $\pm$ 25.4	0.376	157.2 $\pm$ 33.2	172.35 $\pm$ 33.3	0.013
Triglyceride (mg/dL)	58.16 $\pm$ 14.49	134.05 $\pm$ 77.98	0.000	59.21 $\pm$ 17.8	194.36 $\pm$ 95.88	0.001
LDL-c (mg/dL)	98.26 $\pm$ 31.52	86.74 $\pm$ 22.1	0.056	97.64 $\pm$ 27.46	93.5 $\pm$ 21.99	0.379
HDL-c (mg/dL)	50.26 $\pm$ 9.22	45.63 $\pm$ 8.05	0.008	53.78 $\pm$ 11.9	51.5 $\pm$ 8.23	0.248

Tested by Wilcoxon Signed Ranks test, P value = 0.05.

The data in bold text are significantly different at P value = 0.05.

Table 4.8 Comparisons of plasma ORAC, FRAP, MDA and hs - CRP at 0 week and 8 weeks after supplementation in placebo and experimental group. The data are presented as mean $\pm$ SD.

Parameter	Placebo group			Experimental group		
	Time (Week after supplement)		p-value	Time (Week after supplement)		p-value
	0 Week	8 Week		0 Week	8 Week	
ORAC ^a (μ mol TE/100 g sample)	576.12 $\pm$ 121.44	624.02 $\pm$ 245.19	0.376	494.1 $\pm$ 162.98	1507.75 $\pm$ 604.77	0.001
FRAP ^a (μ mol TE/100 g sample)	5.6 $\pm$ 0.77	5.34 $\pm$ 0.51	0.147	12.74 $\pm$ 2.24	14.37 $\pm$ 3.64	0.140
hs-CRP* (mg/L)	2.75 $\pm$ 4.24	1.25 $\pm$ 2.22	0.181	2.57 $\pm$ 3.05	0.8 $\pm$ 1.34	0.011
MDA* (μ mol/L)	0.127 $\pm$ 0.04	0.131 $\pm$ 0.04	0.302	0.072 $\pm$ 0.037	0.073 $\pm$ 0.035	0.829

^a Tested by Wilcoxon Signed Ranks test, P value = 0.05.

* Tested by related 2-sample Paired t-test, P value = 0.05.

The data in bold text are significantly different at P value = 0.05.

4.9.2 Plasma MDA level of all volunteers

From table 4.9, plasma MDA of volunteers in placebo group was slightly increased at every period of time during supplementation 0.79% at 2 week and 2.34% between 2 and 8 week, but there is not significantly different. Whereas, plasma MDA of volunteers in experimental group were significantly increased 2.53% at 2 weeks after supplement and slightly decreased 2.47% from 2 to 8 weeks after supplement, but it was not significantly different. In conclusion, plasma MDA in placebo group was increased 3.15% from 0 to 8 weeks, whereas, in experimental group was not changed from 0 to 8 weeks.

Although, there are not significant difference in plasma MDA level after supplement. The trend of rising in plasma MDA level may happen in placebo group at a longer period of military training and it may stable in experimental group under the same condition as showed in Figure 4.1

Table 4.9 Comparison of plasma MDA in placebo and experimental group at 0, 2 and 8 weeks after supplement. The data are presented as mean $\pm$ SD.

Treatment condition	Plasma MDA (μmol/L)		
	0 week	2 week	8 week
Placebo group	0.127±0.04	0.128±0.04	0.131±0.04
% Change at 0-2 week	0.79%		
% Change at 2-8 week		2.34%	
% Change at 0-8 week	3.15%		
Experimental group	0.079±0.036	0.081±0.035	0.079±0.03
% Change at 0-2 week	2.53%		
% Change at 2-8 week		-2.47%	
% Change at 0-8 week	0%		

The statistical differences tested by related 2 sample paired t-test at P value = 0.05 in plasma MDA levels of placebo and experimental group at 0 and 2 weeks after supplement were 0.225 and 0.143 respectively, at 0 and 8 weeks after supplement were 0.302 and 0.829 respectively and at 2 and 8 weeks after supplement were 0.428 and 0.267 respectively.

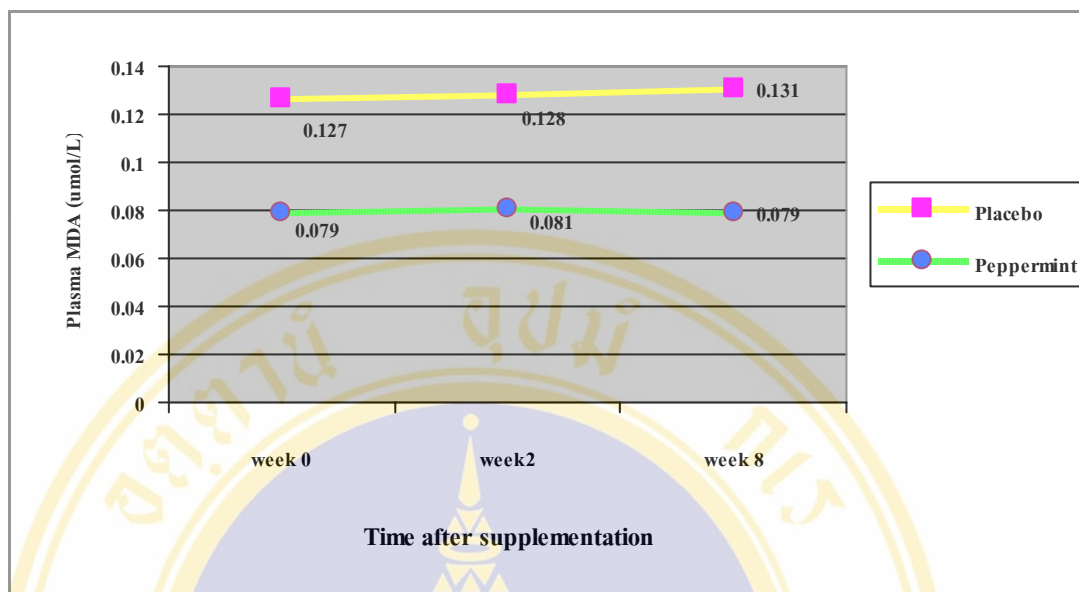


Fig 4.1 Plasma MDA in placebo and experimental group at 0, 2 and 8 weeks after supplement

Plasma MDA at 0, 2 and 8 weeks of supplementation did not correlate with medication compliance when tested with Spearman's rho at P value < 0.05.

4.10 Physical assessment of all volunteers

All volunteers in placebo and experimental group had physical examination by a doctor and nurses from Pramongkutklao Hospital every 2 weeks on the medication dispensing time at 0, 2 and 8 weeks after supplementation. Every 2 weeks, the nurse checked their blood pressure, pulse rate, performed physical examination and interviewed their symptoms; the doctor was consulted if volunteer had abnormal signs or symptoms.

At 0 week, 4 volunteers from placebo group and 2 volunteers from experimental group got sick from cold. One of volunteers from experimental group had Tinea corporis. All of volunteers' conjunctivas were not pale and no jaundice. Their lymph node and liver were not palpable when checked by fumble. Their thyroid size was normal. All extremities were not edema.

At 2nd week, 2 volunteers from placebo group and 2 volunteers from experimental group got cough from cold. The volunteer that had Tinea corporis at 0

week was recovered from Tinea corporis at 2nd week after used medicine. 3 volunteers from placebo group complained about their calf and leg pain. All of volunteers' conjunctivas were not pale and no jaundice. Their lymph node and liver were not palpable when checked by fumble. Their thyroid size was normal. Nobody complained about stomachache, diarrhea or allergy. All extremities are no edema.

At 4th week, 2 volunteers from placebo group and 3 volunteers from experimental group had cough. 2 volunteers from placebo group and 3 volunteers from experimental group had headache. All of volunteers' conjunctivas were not pale and no jaundice. Their lymph node and liver were not palpable when checked by fumble. Their thyroid size was normal. Nobody complained about stomachache, diarrhea or allergy. All extremities were not edema.

At 6th and 8th week, all of volunteers' conjunctivas were not pale and no jaundice. Their lymph node and liver were not palpable when checked by fumble. Their thyroid size was normal. Nobody complained about stomachache, diarrhea or allergy. All extremities were not edema.

In conclusion, all volunteers had no complication or any side effect from corn starch in the dose of 2,000 mg/day in placebo group or freeze dried peppermint powder in the dose of 2,000 mg/day in experimental group for the period of 8 weeks supplementation.

CHAPTER 5

DISCUSSION

In this study, the quantities of antioxidant vitamins and TAC in 7 pungent Thai herbs were analysed. Peppermint had the highest TAC, then this herb was chosen to use as supplement in 2nd phase of experiment. Most of results from 2nd phase of experiment were not different between placebo and experimental group.

5.1 Antioxidant activity in peppermint.

There are several methods for determining antioxidant activity. The methods can define by their types such as enzymatic antioxidant and non-enzymatic antioxidant or lipophilic antioxidant and hydrophilic antioxidant.

The specific method for determining the antioxidant vitamins and antioxidant activity in samples is very important. The method of storage and extract sample is also important. The reliable values of antioxidant vitamins or antioxidant activity depend on suitable solution in the storage or extraction step. [68]

Joseph O. Kuti and Hima B. Konuru [69] found the amount of polyphenol and total antioxidant activity in fresh leaf extract more than cooked leaf extract.

ORAC (Oxygen Radical Antioxidant Activity) is the method for determining total antioxidant activity by measuring the duration of lag phase [70]. At present, ORAC is the method for determining lipophilic antioxidant activity and hydrophilic antioxidant activity in samples by the specific procedure that is different in some chemicals but has the same principle.

Joseph M. Awika et al. [71] studied and compared the antioxidant activity of sorghum and sorghum products by using ORAC and FRAP. They found ORAC was a good method for total antioxidant activity in vegetables and better than FRAP. Their studied showed that ORAC_{Lipo} had low quantity in vegetables while ORAC_{Hydro} was closely ORAC_{Total}, so the ORAC_{Hydro} can be use for predicting ORAC_{Total} in other vegetables.

In this study, the total antioxidant activity of 7 pungent Thai medicinal plants were determined by ORAC and FRAP. The result shown peppermint was the highest total antioxidant activity by ORAC method.

Moreover, total polyphenol content in 7 pungent Thai medicinal plants was determined by Folin Ciocaltau's method as the previous studies. [68, 71-74] The highest total polyphenol content in these medicinal plant was peppermint, that are the same as the study of Joseph M. Awika et al. [71] They reported that total polyphenol content was related to the total antioxidant activity of that plant. Joseph O. Kuti and Hima B. Konuru [69] studied antioxidant activity and total polyphenol content in leaf extract of tree spinach and reported the significant correlation of quantity between total polyphenol content and ORAC. In addition, J. Javanmardi et al. [73] found the relationship between total antioxidant activity and total polyphenol content in Iranian basils. 71% of antioxidant activity in Iranian basil was resulted from the contribution of phenolic compounds. In this study, peppermint has the highest TAC and total polyphenol content. It might be concluded that antioxidant activity of plant extracts come from phenolic compounds and other antioxidant 2° metabolites such as volatiles oil, carotenoids and vitamins in that plants. [73]

Mi-Hyun Ka et al. [75] found that peppermint leaves had several volatiles phenolic compounds. For example, eugenol and thymol had antioxidant activity. Furthermore, volatile extract of peppermint leaves had the inhibitory activity against hexane oxidation or H_2O_2 that induced cytotoxicity, but not affected to MDA level and concluded that antioxidant activity of volatiles extract from medicinal plants may be from the consociation of many phenolic compounds. H.R. Juliani and J.E.simon [76] reported that the main antioxidant activity from plants was not from their essential oil but come from phenolic compounds such as flavonoids in green basil and anthrocyanin in purple basil. King James Medical laboratory, Inc. reported that antioxidant system can protect cells from the attack of free radicals such as H_2O_2 , RO° , ROO° and $O_2^{\cdot 2}$ radical. Antioxidant system is consisting of 3 main groups of antioxidant: primary antioxidants work by preventing the formation of new free radical species such as SOD, GPx, and metal binding proteins such as ferritin or ceruloplasmin. Secondary antioxidants trap free radical by preventing chain reactions such as vitamin E, vitamin C, β -carotene, uric acid, bilirubin and albumin. Tertiary

antioxidant repair biomolecules damaged by free radicals such as DNA repair enzymes.

This study showed peppermint was a source of antioxidant. It contained the highest TAC when analyzed by ORAC method. R.Paul Chadhury et al [77] found mint extract with diethyl ether showed 100% activity of DPPH free radical scavenging at 40 µg/L, they concluded that mint was a strong antioxidant plant. H.J. Damien Dorman et al. [78] studied in DPPH· radical scavenging assay concluded that the mentha extract was capable of scavenging free radicals in solution at pH 7.4 and may be able to protect the initiation of free radical-mediated chain reactions by preventing the abstraction of H⁺ from susceptible PUFAs.

Moreover, Mi-Hyun-Ka et al. [75] indicated that peppermint leaves had phenolic compounds such as eugenol and thymol. Miss Anchana Chanwitheesuk [79] studied antioxidant from local vegetables and Thai herbs concluded that peppermint was a strong antioxidant species. The same as other researchers, Chamila Jayasinghe et al.[72] reported the potency of *Occimum basilicum* as a natural antioxidant due to high prevalence of phenolic compound.

Many studies presented the antioxidant properties of peppermint. [75, 77-80] Mint extract with diethyl ether not only showed 100 % activity of DPPH· free radical scavenging [77], but menthol, that was a volatile oil which found in peppermint, inhibited the movement of calcium across the cell membrane and produced the choleretic and antifoaming effects. [80] Total phenolic content has related with DPPH· radical activity [78] in volatile extract of peppermint leaves. It has inhibitory activity against hexanal oxidation or H₂O₂ induced cytotoxicity but not effected to MDA level [75]. Some of free phenolic compound in the extracts of *Mentha cordifolia* Opiz were identified as gallic acid, which has important roles for antioxidant substances [79] The polyphenol had a power to reduce oxidation because it was an inhibitor of lipid peroxidation. [81]

Several previous studies showed a great number of the benefit of peppermint, but few information about the toxicity of peppermint. The collected data of Nair B. [82] in final report on the safety assessment of *Mentha piperita* oil, leaf extract, *Mentha piperita* leaf and *Mentha piperita* leaf water. He indicated that peppermint oil was minimal toxic in acute oral studies if peppermint oil which consisting of pulegone

in the dose that has menthone $> 200 \mu\text{g/Kg/day}$. Pulegone is recognized as hepatotoxin. Because of the toxicity of pulegone, the safe concentration of this constituent was limited to $\leq 1\%$. In addition, when repeated inject intradermal dose of peppermint oil, moderate and severe reactions in rabbits were happened because peppermint oil can rapidly dermal absorption due to the power of abundant menthol in peppermint oil.

In conclusion, *Mentha piperita* oil; *Mentha piperita* extract, *Mentha piperita* leaves, and *Mentha piperita* leaves water were safe as used in cosmetics formulations.

In this study, healthy men were supplemented with *Mentha cordifolia* Opiz laves for 8 weeks. The safe assessment of *Mentha cordifolia* Opiz performed by volunteers' physical examination and volunteers complaint. Since they were first supplemented, we found that all supplemented volunteers were not suffering from freeze dried peppermint at the dose of 2,000 mg/day and medication compliance was 40-82%. The average of medication compliance of volunteers in placebo group (76.16%) is more than experiment group. (61.46%)

Unextracted and unpurified leaves of *Mentha cordifolia* Opiz. were made in tablet form for using in clinical trial phase 1, 2 and 3. It is antigenotoxic to dimethylnitrosamine, N-nitrosopyrrolidine and tetracycline. Moreover, it did not show DNA damaging and chromosome- breaking effect before and after metabolic activation. [83]

Peppermint was used in various purposes a long time. For example, peppermint and caraway were used for dyspepsia curing [84]. In previous study, the researchers could separate analgesic constituents from peppermint leaves. It can increase the tolerance to pain 157-300%, while mefenamic acid, analgesic substance, can increase 171%. That is a reason of the use of *Mentha cordifolia* Opiz. in tablet form for relieving pain in children and adults. [83]

In addition, β -sitosterol in peppermint had antihelminthic and antimutagenic activities with tetracycline by 65.3% at a dose of 0.5 mg/Kg mouse. At this dose had no chromosome breaking activity. [85] The chloroform extract of *Mentha cordifolia* Opiz leaves was an antiteratogenic at the dose of 2.5 mg/ 20 mg mouse and anticarcinogenic potential when applied at the dose of 0.05mg/ 0.2 mL acetone after croton oil in mouse. [86] The essential oil of many Thai lamiaceous plants including

peppermint has antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis*. [87] The CHCl_3 extract of *Mentha cordifolia* Opiz. had an antimutagenicity effect against tetracycline 68.7% at 0.01mg/ 20 g mouse. [83]

In conclusion, peppermint is a strong antioxidants herb because it has many volatile phenolic compounds which can measure by ORAC and the Folin Ciocaltau's assay. In addition, peppermint has a protective effect. It can protect cells from other substances such as teratogen, carcinogen or microbacterial. Moreover, peppermint was used for curing remedy a long time with no losses; therefore peppermint should be promoted for high antioxidant herb.

5.2 Plasma total antioxidant activity after supplementation

The plasma total antioxidant activity of volunteers in experiment group was significantly increased after supplementation for 8 weeks, while in placebo group was slightly increased. These may effect from the daily food intake from their commissary.

In this study, we stored the plasma of all volunteers at -80°C till analysed that is around 3 months after the first drawing blood at 0 week of supplementation. The collected plasma was thaw for total antioxidant activity analyzed by ORAC and FRAP. After that, they were stored at -80°C for other analyses. In the ORAC and FRAP method, plasma samples were thaw only one time.

Marilena Kampa et al. [88] reported that collected plasma sample which passed freeze thawing cycle showed the same result even it passed 1-3 cycle. The level of antioxidant in plasma decreased or disappeared after it passed the fourth cycle of thawing. They concluded that plasma sample must be analysed either immediately or after a maximum of two freeze thawing cycles. It might be concluded that the freeze thawing has no effected in this study because the plasma samples were passed only 2 cycles of thawing.

Furthermore, plasma total antioxidant activity was tested by ORAC method higher in experimental group than placebo group when compared with FRAP method. That is the same as reported in the study of Guohua Cao. and Ronald L Prior. [89] FRAP method is very simple and not expensive but it can not detect SH-group antioxidant while ORAC is a high specific method and response to many antioxidants.

Moreover, ORAC method can analyze by using serum, it can detect antioxidants that contain with low molecular weight SH-group such as lipoic acid and some amino acid. For this reason, the different result between ORAC and FRAP occurred.

5.3 ORAC and FRAP

In resting period, the body is consisting of non enzymatic and enzymatic antioxidants, reserved for protecting the attack from ROS. The fine physiological balance between oxidative reactions and antioxidant capacity may be perturbed by physical activity. Antioxidant defense system preserves homeostasis for normal cell function at rest, and during mild oxidative stress. [90] Guohua Cao. and Ronald L. Prior [89] found antioxidant capacity of human serum measured as either ORAC or FRAP was stable over an 8 weeks. Plasma total antioxidant activity is part of a tightly regulated homeostatic mechanism. Furthermore, the level of plasma antioxidant in normal people may affect from several factors except nutrients from food for example the absorption rate, other nutrients intake and homeostatic regulation. [91]

Helle Bruunsgaard et al. [92] concluded that long term combined supplement with α - tocopherol and vitamin C, in suitable dose, was not affected to the systemic anti-inflammatory effects in healthy people and no signs of inflammation. CRP level, TNF- α and IL-6 at baseline and after 3 years of supplementation were not difference.

In this study, we supplemented freeze dried peppermint leaves at dose of 2,000 mg/day, the result was closed to the study of Helle Bruunsgaard et al [92] which supplement with other antioxidants such as 91 mg of α - tocopherol and 250 mg of vitamin C two times a day for 3 years period. CRP level before and after supplementation was not difference. [92] In addition, supplement with vitamin C 515 mg/day could reduce plasma F₂-isoprostane in smokers, while supplement with vitamin C and vitamin E were not significantly affected to plasma F₂-isoprostane after supplementation. [93]

Supplement with vitamin C 515 mg/day for 2 months reduced plasma level of CRP about 24%. However, supplement with vitamin C 515 mg/day, mixed with tocotrienols 252 mg/day and α -lipoic acid 95 mg/day can reduce plasma level of CRP

but not statistical significance [94]. This finding was the same as plasma CRP in this study.

H. Sung et al [95] found the significantly increased in plasma TAC when drawing at the first and second hour after green tea drinking at the dose of 300 or 450 mL. Moreover, A.R. Leeds et al. [96] studied in dehydrated fruit capsule (containing vitamin E 30 mg, vitamin C 150 mg and β -carotene 6 mg, 850 mg/cap) and dehydrated vegetable capsule (containing vitamin E 30 mg, vitamin C 150 mg, and β -carotene 9 mg, 750 mg/cap) 2 caps/day for 7 days reported plasma β - carotene and vitamin C were significant higher in both groups. Plasma vitamin E was higher after supplement with dehydrated fruit capsule.

Cao G. and Cutler RG. [97] indicated that the oxygen radical defense increased after supplement with plenty of antioxidants. They did not show the suitable concentration because other oxygen radicals, formed in the body, and antioxidant may not relate with each other. They could not control the oxidant such as vitamin C and vitamin E for reducing NOO-.

Furthermore, plasma vitamins C, α - carotene and β - carotene were increased if consumption more fruits and vegetables. [98] Consumption of high carotenoids fruits or vegetables by healthy and non smoking people either small or large amount, were not affected to the markers of immune function.

The consumption of fruits and vegetables 8 servings/day could reduce inflammatory process by the reduction of plasma CRP level. [28] Other study reported that even consumed high antioxidants diet such as Mediterranean diet, plasma antioxidant and urine MDA were not changed. [91]

Plasma TAC depended on the dose of antioxidant. [95] Bary Halliwell [99] found vegetables and fruits consumption reduced free radical attacked to DNA. In supplementation with ascorbate, vitamin E or β - carotene did not reduce DNA damage. Guohua Cao et al. [100] found the consumption of diet with high phenolic compound increased plasma TAC in human. These were the same as reporting in this study.

5.4 Plasma lipid after supplementation

In this study, supplement with peppermint, which had the highest TAC and total polyphenol, can not reduce plasma cholesterol, plasma triglyceride, plasma HDL-

c and not increase plasma LDL-c in 2 months. Linda Hagfors et al. [100] studied the relationship between plasma lipid and tocopherols from baseline to 12th week of subjects who consumed the different diet. They did not find any relation. Moreover, D. Palli et al. [101] found plasma α -tocopherol and retinol were correlated with plasma level of cholesterol. Jacqueline J.M. Castinmiller et al. [102] found plasma triacylglycerol and cholesterol concentration did not change over the experimental period of carotenoids supplement to healthy and no smoking volunteer for 3 months.

Furthermore, BMI and body weight were significantly increased in experiment group whereas not changed in placebo group. It may be concluded that the data from 3-day-record form that collected at baseline of military training of volunteers in both groups could not represent all of the diet that they had eaten in 8 weeks of military training. Because they can eat other food on weekend and it could be differ from the others. The volunteers in the experimental group may eat high calory diet more than placebo group, so their BMI and body weight were significantly increased together with their cholesterol, triglyceride and LDL-c.

In this study, plasma cholesterol, plasma triglyceride and plasma LDL-c of all volunteers were higher than baseline while plasma HDL-c was lower; even they exercised over 8 weeks of the experiment. The information from physical activity and 3-day record may be errored from malpractice. This is the same as Linda Hagfors et al. [91] in the study about the reliability of the physical activity and dietary data. They concluded that prediction of reliable dietary data should compare with energy expenditure of volunteers, if the ratio of energy intake and energy expenditure (EI: EE) is 0.79 – 1.21, it was reliable data. If the ratio less than 0.79, it was under estimated. If the ratio more than 1.21, it was over estimated. The ratio of EI and EE from both groups of volunteers in this study is 0.55, and then the data of energy intake and energy expenditure was under estimated.

Furthermore, physical activity and dietary assessment was the methods that have more errors due to true random variation in the behavior or errors in translation of frequency and information of diet and energy expenditure values. Recording of physical activity by each volunteer often recorded more active than actually or over reported. [103] Moreover, Black A.E. et al. [104] concluded that individual volunteers were identified as under report if EI: EE < 0.79.

5.5 Exercises induce oxidative stress

In this study, the volunteers, who expected as high level of oxidative stress, are training soldiers. They had an outdoor training everyday for endurance and strong body to be qualified as soldier. Moreover, the change of environment and stress increased the oxidative stress. The mechanism that link acute aerobic exercise and oxidative stress are the increasing of pro - oxidant activity during exercise more than resting period by 10-15 times. The lack of antioxidant activity related with pro – oxidant. Exhaustive aerobic exercise attacked the balance between antioxidants and pro – oxidant activity, so the oxidative stress from exercise is increasing. [105]

Furthermore, plasma lactate level was significantly increased after exercise and muscle TBARS level increased after sprint exercise then return to baseline level at 24 hours post exercise. [90] Alessio H.M. [106] reported an unclear of exercise in free radical process and found an increasing of oxygen consumption and free radical activity in cells during exercise. Although, duration and severity of exercise are affected biomarkers of free radical activity but exercise with moderate intensity can tolerate with the production of free radical activity.

Plasma MDA after supplementation period is not significantly different from baseline because all volunteers passed the military training and received the supplement only 8 weeks. Alessio H.M. and Goldfarb A.H. [107] studied in rats. They found endurance training resulted in lowering MDA during moderate intensity exercise. It possible that catalase enzyme was stimulated and respiratory capacity was increased; those are the important factors for controlling lipid peroxidation during exercises.

5.6 Malondialdehyde: product of lipid peroxidation

MDA is a product of lipid peroxidation; there are several methods for measuring MDA. In this study HPLC was used for measuring MDA.

The thiobarbituric acid test for MDA is a popular method for determining lipid peroxidation and free radical activity. [91, 108-111] The HPLC method is very sensitive, it can be detected less than 0.05 mmol/L. [111]

Several studies indicated that the TBA test is nonspecific to MDA because other aldehydes can react with TBA [95, 110, 112] and other pigments can absorb at

532 nm and made over estimation of MDA concentration. [110] Then plasma MDA concentration from TBA test may limit in specificity [109, 113] and sensitivity. [108] Therefore many researchers improve TBA test by using HPLC for separating MDA (TBA)₂ adduct from other interfering compounds by C18 reverse – phase. [96, 109, 110, 113] After the deproteinization step, in the TBA incubation step, it could be improved the column life and gave better sensitivity. [110]

In this study, after supplement with peppermint in experimental group, plasma MDA at baseline and after supplement was not significantly different. A.R.Leeds et al. [63] studied the supplement with fruits and vegetables capsule to healthy subjects for 7 days. They found plasma MDA was significantly decreased at the end of study in both groups. They concluded that supplement with fruits or vegetables could reduce lipid peroxidation.

Plasma MDA from the supplement with peppermint at 0 and 8 weeks was not significantly different. It is noteworthy that plasma MDA in experimental group tends to be stable in the stress condition, while plasma MDA in placebo group is slightly increased and this could be resulted from the antioxidant activity of peppermint.

Mi Hyun ka et al. [75] found peppermint leaves had phenolic compounds such as uegenol and thymol, an antioxidant activity in volatile extract from peppermint leaves. It had inhibitory activity against hexanal oxidation or H₂O₂ induced cytotoxicity but not affected to MDA level. Antioxidant activity of volatile extract from medicinal plants may come from various phenolic compounds. Paul Preziosi et al. [114] supplemented healthy subjects with multivitamins for 6 months reported plasma MDA was not changed, although plasma glutathione peroxidase and red cell glutathione peroxidase were increased.

Druso D. perez et al. [115] studied the effect of different diets plus wine supplementation on antioxidant defense and oxidative damage. They found volunteers in red wine group had lower level of 8OHdG. Moreover, plasma polyphenol was increased and had protective effect to oxidative damage. In this study, plasma polyphenol and vitamins were not measured; the result of plasma TAC could be a representative for other antioxidant vitamins.

Eun Ju Cho et al. [81] studied antioxidant activity of 12 medicinal plants and inhibitory effects on lipid peroxidation. They found anti-lipid peroxidation compounds

such as 3, 4- dihydroxylbenzoic acid, quercetin, quercetin glycosides, catechin, gallic acid, methyl gallate and rosmarinic acid. They showed that polyphenols can protect lipids from peroxidation and inhibitory effect of polyphenols on lipid peroxidation resulted from scavenging radicals and / or inhibition of a wide range of enzymes involved in oxidation system. [81]

J. Voces et al. [116] studied in rats found acute exercise increased MDA level and lipid peroxidation based on the concentration of GSH in tissues and the reducing capacity of GSH- GSSG cycle. Lipid peroxidation, measured by MDA value, will increase in all muscles after acute exercise. They concluded that ginseng can reduce the formation of free radicals in skeleton muscles after exhaustive exercise.

Linda Hagfors et al. [91] studied in rheumatoid arthritis patients consumed antioxidant rich foods and received multivitamin in high dose for 3 month was not affected to urine MDA level or plasma antioxidant but plasma total cholesterol was significantly decreased in antioxidant group. Moreover, MDA level may change from the storage method. Jill Templar et al. [110] studied by added BHT in plasma sample before TBA was incubation for reduction of lipid peroxidation in heating stage. MDA value was come from the oxidation only in the laboratory. They concluded that the stability of sample was better when BHT added in sample before storage at -70 °C. If storage sample for 14 days, it would not affect to MDA level, the precision of untreated plasma was less than BHT treated plasma.

In this study, plasma was kept at -80 °C but not added BHT, so plasma MDA of all volunteers may be slightly higher. Jill Templar et al. [110] found the slight reduction in oxidation when samples were kept at -70 °C for 60 days but MDA level was increased 9% for treated sample and 17% for untreated sample. Kharb S. Ghalaut Veena singh [117] indicated that lipid peroxidation will increase with aging effect therefore it is very important that volunteers' age should not different between groups.

Moreover, Lecomte E. et al. [118] found MDA and Ig-NH₂MDA concentration in alcoholics was higher than men who consumed low or moderate amount of alcohol but it will reduce in withdrawal period. So alcohol drinking habit of volunteers is very important for MDA measurement.

5.7 C- Reactive protein and oxidative stress

C- reactive protein (CRP) is a marker of inflammation; it is recognized as a predictor of cardiovascular disease and related with other metabolic syndromes such as diabetes mellitus. [119] Several studies indicated that CRP is presented in atherosclerotic plaques and activates the complement system. CRP is produced by hepatocytes in response to IL-6, IL-1, TNF α and other cytokines. CRP is presented in the vessel wall, where it induces expression of the adhesion molecules vascular adhesion molecules -1 (VAM-1) and intercellular adhesion molecule-1 (ICAM-1) by endothelial cells and serves as a chemoattractant for monocytes. [120]

It is possible that increasing of CRP levels is the inflammatory activity of atherosclerotic lesions throughout the arterial system [121] and significantly worse metabolic pattern. [122] Endothelial dysfunction and inflammation are related with most forms of cardiovascular disease such as essential hypertension and coronary artery disease or even chronic renal failure. There are several evidences that oxidative stress induce hypertension and arterogenesis. Santina Cottone et al. [120] found significantly increased in essential hypertension when compared with healthy control subjects, moreover, ICAM-1 and VCAM-1 were significantly increased even in the subjects with lower CRP. They concluded that CRP and TNF- α were increased related to oxidative stress and endothelial dysfunction.

In this study, hs- CRP used for CRP because of its high sensitivity, with a minimum detectable concentration of 0.004 mg/L. [123] The result showed plasma hs-CRP of volunteers from peppermint group was significantly decreased at the end of study period and this is an effect of peppermint supplementation. Yunshing Ma et al. [119] studied the relation between dietary fiber and CRP level found that dietary fiber had a protective against high CRP. Gladys Block et al. [94] found that supplement in smokers with vitamin C 515 mg/day could reduce 24% of plasma CRP but supplement with mixed antioxidant and placebo had the same result due to their slightly decreased of plasma CRP.

Furthermore, Marian Dietrich et al. [93] found daily supplementation for 2 months with vitamin C alone was significantly decreased plasma F2- Isoprostanes in smokers. Esther Lopez- Garcia et al [124] found highly intake of fruits, vegetables, legumes, fish and whole grain had lower plasma CRP. Bernhard Watzl et al [28] found

high intake of carotenoid- rich fruits and vegetables in healthy non smoked not affected to the marker of immune function but can reduce inflammatory process. The reduction of CRP was used as detector. CRP was significant decreased at week 8 in subject who consumed fruit and vegetable in the quantity of 8 servings/ day when compared with those who consumed 2 servings/ day.

Furio Brighenti et al. [123] found TAC was significantly higher in subjects with low plasma hs-CRP. That is the same as our study. Moreover, Gunilla Nerdin Fredrikson et al. [121] found that there is no associated between CRP level and alcohol consumption or physical activity. CRP level was significantly associated with high BMI. So, alcohol drinking habit and energy expenditure in each volunteer do not affect to plasma hs- CRP level in this study.

CHAPTER 6

CONCLUSION

This study showed that the 7 pungent Thai herbs contained many antioxidant vitamins in different quantities. Peppermint had a great quantity of TAC. It showed the highest amount of total polyphenol and total antioxidant capacity by Folin Ciocaltau's reagent method and ORAC assay.

The supplementation study in healthy volunteers with freeze - dry peppermint leaves for 8 weeks significantly increased the plasma TAC level of the volunteers in experimental group. Lipid peroxidation was not significantly affected by this intervention.

Military training of the first shift training soldiers in Infantry Regiment, King's guard 1st is not the activity that causes high oxidative stress.

The results from this study will help for finding the other methods of supplement or suitable range of time for supplement in healthy subjects.

6.1 The usefulness of study

- 6.1.1 The information of a quantity of antioxidants and total antioxidant capacity in pungent Thai herbs will encourage growing of Thai medicinal plants and their products to the high quality of functional food.
- 6.1.2 To support Thai food's business with a quantity of antioxidants and total antioxidant capacity in pungent Thai herbs.
- 6.1.3 To encourage the alteration of western style eating behavior to Thai style due to the benefit information about Thai medicinal plants.
- 6.1.4 To stimulate further studies, with the antioxidative stress effect and lower plasma lipid of pungent Thai herbs.

- 6.1.5** To generate the information for people, especially in high-risk people group to select foods for reduces risks of chronic diseases, particularly cardiovascular disease.

6.2 The suggestion for future study

6.2.1 Subject should be truly high level of oxidative stress such as smokers or laborers for determine the occurring changes.

6.2.2 Expand the time of supplement for the clearing effects of herb supplement.

6.2.3 Increasing dose of supplement/ day for the other researches in vitamins supplement to get the effects from the supplement.

6.3.4 Further study should select a suitable product of lipid peroxidation in healthy volunteers, who have experienced with oxidative stress. And the other indicators of lipid peroxidation such as

: 8-OHdG, which is a product of DNA damage, used as a biomarker for oxidative stress.

: 8-epi-PGF2a, which is detected in plasma and urine as a result of oxidative stress. It is a specific product of lipid peroxidation, which is very stable.

: F2- isoprostane, which is a reliable index of lipid peroxidation in vivo, detected in every biological fluid and tissues.

6.3.5 Standard toxicity testing should be done for the safe results from herbs supplement.

6.3.6 3-day record diet and 2- day record of physical activity should perform in every week of supplement for the accurate data of energy intake.

REFERENCES

1. สุนีย์ เอี่ยมศิริกุล. ปัจจัยที่เกี่ยวข้องกับการมารับรักษาของผู้ป่วยกลุ่มอาการหัวใจขาดเลือดเฉียบพลัน [วิทยานิพนธ์ปริญญาวิทยาศาสตรมหาบัณฑิต สาขาวิชาพยาบาลศาสตร์]. กรุงเทพฯ: บัณฑิตวิทยาลัย มหาวิทยาลัยมหิดล; 2540.
2. กระทรวงสาธารณสุข. สถิติสาธารณสุข พ.ศ. 2541. กรุงเทพฯ: 2541.
3. วิสิฐ จະวะสิต และคณะ. การวิเคราะห์หาโอกาสและศักยภาพของการพัฒนาอาหารไทยสู่ตลาดโลก. นครปฐม: 2545.
4. Michel LD, et al. Mediterranean diet and French paradox: two distinct biographic concepts for one consolidated scientific theory on the role of nutrition in coronary heart disease. Cardiovascular research 2002; 54: 503-15.
5. Somsri Charoenkiatkul. Nutrition value of Thai food for health. Available from: URL: http://www.nu.mahidol.ac.th/new_02 [Accessed 2002 Mar 20].
6. สถาบันการแพทย์แผนไทย กรมการแพทย์ กระทรวงสาธารณสุข สำนักงานคณะกรรมการการสาธารณสุขมูลฐาน. ผักพื้นบ้าน: ความหมายและภูมิปัญญาของสามัญชนไทย. กรุงเทพฯ: โรงพิมพ์องค์การสงเคราะห์ทหารผ่านศึก; 2538.
7. วิโรจน์ วิทยาเวโรจน์. ผลของสารเผ็ดแคปไซซินที่ฉีดผ่านแม่หนูที่ตั้งท้องต่อระดับความเจ็บปวด และการรั่วของน้ำเลือด(พลาสมา) ในลูกหนู [วิทยานิพนธ์ปริญญาวิทยาศาสตรมหาบัณฑิต]. กรุงเทพฯ: บัณฑิตวิทยาลัย มหาวิทยาลัยมหิดล; 2531.
8. กรมการแพทย์ กระทรวงสาธารณสุข. ปรรณนา สถิติชีวภาพ และคณะ, บรรณาธิการ. ผลของกระเทียมสกัดชนิดเม็ดต่อระดับไขมันในผู้ที่มิโคเลสเฮอร์อลสูง. กรุงเทพฯ; 2545.
9. LS, S VK, and SM. Turmerin: a water soluble antioxidant peptide from turmeric (Cureuma longa). Archives of Biochemistry and Biophysics. 1992; 292(2): 617-23.

10. กระทรวงสาธารณสุข สถาบันการแพทย์แผนไทย. รวมบทความการประชุมวิชาการ ผักพื้นบ้านและอาหารพื้นบ้าน 4 ภาค. กรุงเทพฯ: โรงพิมพ์องค์การสงเคราะห์ทหารผ่านศึก; 2542.
11. Halliwell B. Food derived antioxidants: How to evaluate their importance in food and in vivo. In: E. Cadanas and L. Packer, editors. Handbook of antioxidants. Los angeles, California: Marcel Dekker; 2002. p. 1-45.
12. Saari JT. Vitamin and minerals, free radicals and aging. Grand Forks Human Nutrition Research center; 2002. p. 1-2.
13. Duthie GG and KM Brown. Reducing the risk of cardiovascular disease in Functional foods. In: I Goldberg. Editor. Designer foods, pharmafoods, nutraceuticals. Chapman& Hall; 1994. p. 19-38.
14. SJ Jadhav, et al. Lipid oxidation in biological and food system. In: DL Madhavi, SS Deshpandie and DK Salunkhe, editors. Food antioxidants. Technological. Toxicological and health perspectives. New York: Marcell Dekker; 1995. p. 5-63.
15. อธิป ลิขิตลิลิต และคณะ. The potential role of vitamin A, C, E and betacarotene on lowering the plasma and lipoprotein lipids and on scavenging enzyme activities in hypercholesterolemic humans. ภาควิชาชีวเคมี และภาควิชาเวชศาสตร์ป้องกันและสังคม คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล: กรุงเทพฯ; 2537.
16. Block G, et al. Factor associated with oxidative stress in human populations. Am J Epid 2002; 156(3): p 274-85.
17. Chen C. C-reactive protein. 1999. Available from URL: <http://www.pubmed.com> [Accessed 2003 Mar 20]
18. C. CA and F. Blaz. Vitamin C and cardiovascular disease. In: C Enrique and P Lester, editors. Handbook of antioxidants. New York: Marcel Dekker; 2002.
19. Stampher MJ, Ch Hennekens, et al. Vitamin E consumption and the risk of coronary heart disease in women. The New England journal of medicine 2001; 328: 1444-9.

20. Rimm EB, MJ Stanpher, et al. Vitamin E consumption and the risk of coronary heart disease in men. The New England journal of medicine 1993; 328: 1450-6.
21. Daniel S. Antioxidant vitamins and coronary heart disease. The New England journal of medicine 1993; 328: 1487-9.
22. Huang HY, et al. Effects of vitamin C and vitamin E in vivo lipid peroxidation: results of a randomized controlled trial. Am J Clin Nutr 2002; 76: 549-55.
23. Margareta Kritenson, et al. Lower serum levels of betacarotene in Lithuanian men are accompanied by higher urinary excretion of the oxidative DNA adduct, 8-hydroxydeoxyguanosine: the LiVicia study. Nutrition 2003; 19: 11-5.
24. Network AB. Vitamin C/ betacarotene/ vitamin E/ flavonoids/ selenium/ superoxide dismutase. Available from URL: <http://www.pubmed.com> [Accessed 2003 Mar 20]
25. Berry OP. The technology of vitamins in food. 1st ed. Cornwell. England: Hartnolls; 1993.
26. Lifestyle, T. Available from URL: <http://www.tiscali.co.uk/lifestyle/healthfitness/menhealth/part3> 019-1.html. [Accessed 2003 Mar 20]
27. คารณี ชุมชุมศิริวัฒน์ และสมทรง เลขะกุล. ชีวเคมี เล่มที่ 2. พิมพ์ครั้งที่ 4. คณะแพทยศาสตร์ศิริราชพยาบาล. กรุงเทพฯ: พรประเสริฐ พรินดิง 2536.
28. Bernhard Watzl, SE. K, Jatta Moseneder, Stephan W Barth and Achim Bub. A 4 – wk intervention with high intake of carotenoid-rich vegetables and fruits reduces plasma C-reactive protein in healthy, non smoking men. Am J Clin Nutr 2005; 82: 1052- 8.
29. Jha P, M Flather, et al. The antioxidant vitamins and cardiovascular disease. A critical review of epidemiologic and clinical trial data. Annals of internal medicine 1995; 123: 860-72.
30. Zock PL and MB Kartan. Diet, LDL oxidation and coronary artery disease. Am J Clin Nutr 1998; 68: 759-60.

31. Michael L. Vitamin C therapy in cardiovascular disease. Available from URL: <http://www.pubmed.com> [Accessed 2003 May 02]
32. วันดี กฤษณพันธ์. Introduction to phytochemistry. ใน: วิชา อัจฉริยาคุณ, บรรณาธิการ. ยาและผลิตภัณฑ์ธรรมชาติ. ภาควิชาเภสัชวินิจัย คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล. กรุงเทพฯ: 2534. หน้า 25-72.
33. Medicine, F.a.n.B.I.o. DRI, Dietary reference intakes for vitamin C, vitamin E, selenium and carotenoids. 1st ed. Washington DC: National academy Press; 2000. p. 509.
34. Cao G and RL Prior. Measurement of total antioxidant capacity in nutritional and clinical studies. In: E Cadenas and L Packer, editors. Handbook of antioxidants. Los Angeles, California: Marcel Dekker; 2002. p. 47-55.
35. Laboratories, B. oxidative stress services. 2003. Available from URL: <http://www.pubmed.com> [Accessed 2004 Jun 07]
36. McAnalley S, et al. In vitro methods for testing antioxidant potential. Glycoscience. Available from URL: <http://www.pubmed.com> [Accessed 2003 May 02]
37. สาระแทน. Available from URL: <http://www.kph.go.th/departmt/social/Hearb9.html>. [Accessed 2003 May 02]
38. ประไพศรี สิริจักรวาล และ สิริพร โกสุม. โภชนาการเพื่อสุขภาพดี ดำรับอาหารพื้นบ้านภาคกลาง. พิมพ์ครั้งที่ 1. สำนักงานปลัดกระทรวงสาธารณสุข. กรุงเทพฯ: Siam M&B Publishing; 2545.
39. Medicinal Plant in Thailand. Department of Pharmaceutical botany, Faculty of Pharmacy, Mahidol University. Bangkok: Amarin printing and publishing public; 1996.
40. รุ่งรัตน์ เหลืองนทีเทพ. พืชเครื่องเทศและสมุนไพร: ตำรา-เอกสารวิชาการ ภาคพัฒนาตำราและเอกสารวิชาการ, ฉบับที่ 59. หน่วยศึกษานิเทศก์ กรมการฝึกหัดครู. กรุงเทพฯ: โรงพิมพ์การศาสนา กรมศาสนา; 2535.

- 41.มหาวิทยาลัยมหิดล, ศูนย์ข้อมูลสมุนไพร และกรมป่าไม้. โครงการสมุนไพรเพื่อการพึ่งตนเอง. ก้าวไปกับสมุนไพร. กรุงเทพฯ: ชรรคมถการพิมพ์; 2527.
- 42.เพาว์ เหมือนวงษ์ญาติ. สมุนไพรก้าวใหม่. พิมพ์ครั้งที่ 2. กรุงเทพฯ: ที พี พรินตัง; 2537.
- 43.รุ่งระวี เต็มศิริฤกษ์กุล. สมุนไพรรักษาโรคเรื้อรังบางชนิด. พิมพ์ครั้งที่ 1. ภาควิชาเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล. กรุงเทพฯ; 2536.
- 44.นันทวัน บุญประภัสร์ และอรนุช โชคชัยเจริญพร. สมุนไพรไม้พุ่มบ้าน. พิมพ์ครั้งที่ 1. กรุงเทพฯ: โรงพิมพ์ประชาชน จำกัด; 2539.
- 45.ปัจจุบัน เหมหงษา. สมุนไพรในงานสาธารณสุขมูลฐาน. พิมพ์ครั้งที่ 1. สำนักงานการสาธารณสุขมูลฐาน กระทรวงสาธารณสุข. กรุงเทพฯ: โรงพิมพ์สงเคราะห์ทหารผ่านศึก; 2541.
- 46.กะเพรา. Available from URL:
<http://www.mahidol.ac.th/mahidol/py/mpcenter/html/ocimten.html>.
[Accessed 2003 May 02]
- 47.พร้อมจิตร ศรีลัมพ์ และคณะ. สมุนไพรสวนสิริรุกษชาติ. พิมพ์ครั้งที่ 1. กรุงเทพฯ: บริษัท อมรินทร์ พรินตังก์ จำกัด; 2535.
- 48.โหระพา. Available from URL:
<http://www.doae.go.th/library/html/detail/horapa/#33> [Accessed 2003 May 02]
- 49.แมงลัก. Available from URL:
<http://www.mahidol.ac.th/mahidol/py/mpcenter/html/ocimbas.html>.
[Accessed 2003 May 02]
- 50.ขิง. Available from URL:
<http://www.mahidol.ac.th/mahidol/py/mpcenter/html/zinoff.html>.
[Accessed 2003 May 02]
- 51.ข่า. Available from URL:
<http://www.mahidol.ac.th/mahidol/py/mpcenter/html/alpinia.html>.
[Accessed 2003 May 02]

52. สุริย์พันธ์ บุญวิสุทธิ์. ตารางแสดงคุณค่าทางโภชนาการของอาหารไทย. พิมพ์ครั้งที่ 1. กลุ่มงานวิเคราะห์อาหารและโภชนาการ กรมอนามัย กระทรวงสาธารณสุข. นนทบุรี: โรงพิมพ์องค์การสงเคราะห์ทหารผ่านศึก; 2544.
53. สมณีย์ สุขรุ่งเรือง. การพัฒนาข้าวเพื่อใช้เป็นยารักษาโรคกลาก ภาค 1. การทดสอบฤทธิ์การต้านเชื้อราและฤทธิ์ของข้าวสาคัด. ใน คณะกรรมการโครงการพัฒนาการใช้สมุนไพร และยาไทยทางคลินิกของมหาวิทยาลัยมหิดล, บรรณาธิการ. หนังสือรวบรวมงานวิจัย โครงการใช้สมุนไพรและยาไทยทางคลินิกปี 2525 -2536.
54. กระชาย. Available from URL:
<http://www.kph.go.th/departmt/social/hearb12.html>. [Accessed 2003 May 02]
55. กระชาย. Available from URL:
<http://www.mahidol.ac.th/mahidol/py/mpcenter/html/boesen.html>. [Accessed 2003 May 02]
56. Puwastein P, et al. Thai food composition tables. Institute of nutrition, Mahidol University: Nakorn Pathom; 1999.
57. E WC. and PE. J. The carotenoid content of foods with special reference to developing countries. 1993.
58. Dillion SA, et al. Dietary supplementation with aged garlic extract reduces plasma and urine concentrations of 8-isoprostaglandin F2-alpha in smoking and non smoking men and women. American Society for nutrition sciences 2001: 168-170.
59. Standard operating protocol (SOP). Total phenolic contents in green tea, grape products, echinacea product by UV spectrometric method using gallic acid as standard 2001. Project No. Core B (SOP No. CB0101): 18-20.
60. AOAC. AOAC official method 984.26. Vitamin C (total) in food semi-automated fluorometric method. First Action 1984, final action 1985. , AOAC official methods of analysis (2000): p. 18-20.

61. AOAC. AOAC official method 971.30. Alpha-tocopherol and alpha- tocopheryl acetate in foods and feeds. Colorimetric method. First action 1971, final action 1972. , AOAC official method of analysis (2000): p.31-34.
62. Kangsadalampai K and Sungpuag P. Vitamin A and beta-carotene. Laboratory manual for food analysis 1984. Mahidol University: Bangkok, Thailand: p. 46-50.
63. B. Laboratories. Standard operating procedure. Hydro-ORAC-platereader. 22-09-2003. Review#5: p. 1-17.
64. University. T.R.G., S.O.P.11 FRAP assay on plasma& faecal extracts, in standard operating procedure for FRAP assay on plasma & faecal extracts, T.R.G.U. The school of life sciences. Editor: Aberdene. Available from URL: [http:// www.pubmed.com](http://www.pubmed.com) [Accessed 2003 Jun 13]
65. Benzie IF and JJ strain. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Annal Biochem* 1996; 239 (1): 70-6.
66. Lykkesfeldt J. Determination of malondialdehyde as dithiobarbituric acid adduct in biological samples by HPLC with fluorescene detection: comparison with ultraviolet-visible spectrophotometry. *Clin Chem* 2001; 47: 1725-7.
67. T. Bulletin. ORAC lipo-free radical absorption activity of oil soluble antioxidants. *J Agric Food Chem* 2001: 1-11.
68. Prakash A. Antioxidant activity. *Agric Food Chem* 2001; 44: p.701-5.
69. Fabio Chinici AG., Nadia Natali, Claudio Riponi and Sergio Galassi. Improbed HPLC determination of phenolic compounds in CV. Golden delicious apples using a monolithic column. *J Agric Food Chem* 2004; 52: 3-7.
70. Niki E. Antioxidant activity: Are we measuring it correctly? *Nutrition* 2002; 18: 524-5.
71. Joseph M. Awika, LW R., Xianli Wu, Ronald L. Prior and Luis Cisneros Zevallos. Screening methods to measure antioxidant activity of Sorghum (*Sorghum bicolor*) and Soghum products. *Agric Food Chem* 2003; 51: 6657-62.
72. Chamila Jayasinghe, N. G, Tomoro Aoki and Shun Wada. Phenolics composition and antioxidant activity of sweet basil (*Ocimum basilicum* L.). *J Agric Food Chem*, 2003. 51: 4442-9.

73. J. Javanmardi, C.S., E. Locke and J.M. Vivanco. Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. *Food Chem* 2003; 83: 547-50.
74. Susan gahler, K.OA. V.B. Alterations of vitamin C, total phenolics and antioxidant capacity as affected by processing tomatoes to different products. *J Agric Food Chem* 2003; 51 (27): 7962-8.
75. Mi-Hyun ka, E.H.C., Hang-Sok Chun and Kwang-Geun lee. Antioxidative activity of volatile extracts isolated from *Angelica tenuissimae* roots, peppermint leaves, pine needles and sweet flag leaves. *J Agric Food Chem* 2005; 53: 4124-9.
76. Simon HR.J and J.E. Antioxidant activity of basil. Alexandria, VA. ASHS Press. 2002: p.575-9.
77. R. Paul Houdhury, AK, AN. Garg. Analysis of Indian mint (*Mentha spicata*) for essential, trace and toxic elements and its antioxidant behavior. *Journal of Pharmacoceutical and biomedical analysis* 2006; 41: 825-32.
78. HJ. Damien Dorman, M.K., Kirsti Lahlos, Yvonne Holm and Raimo Hiltunen. Antioxidant properties and composition of aqueous extracts from *Mentha* species, hybrids, varieties and cultivars. *J Agric Food Chem* 2003; 51: 4563-9.
79. Chanwitheesuk A. Screening and identification of antioxidants from local vegetables and Thai herbs [M.S. Thesis in Biotechnology]. Faculty of Graduate Studies, Chiangmai University.
80. Grigoleit HG, G.P. Pharmacology and preclinical pharmacokinetics of peppermint oil. *Phytomedicine* 2005; 12 (8): 612-6.
81. Eun Ju Cho, T.Y., Dong Young Rhyu, Hyun Young Kim and Naotoshi Shibahara. The inhibitory effects of 12 medicinal plants and their component compounds on lipid peroxidation. *American Journal of Chinese Medicine* 2003; 31 (6): 907-17.
82. B.N. Final report on the safety assessment of *Mentha piperita* (peppermint) oil, *Mentha piperita* (peppermint) leaf extract, *Mentha piperita* (peppermint) leaf and *Mentha piperita* (peppermint) leaf water. *Int J Toxicol* 2001; 20 (Suppl 3): 61-73.

83. Irene M. Villasenor, D.E.E. and J.S.A. A new antimutagen from *Mentha cordifolia* Opiz. *Mutation Research* 2002; 515: 141-6.
84. Ernst JT.C and E. Systemic review: herbal medicinal products for non- ulcer dyspepsia. *Aliment Pharmacol Ther* 2002; 16: 1689-99.
85. Irene M. Villasenor, J.A., Arlyn P. Canlas, Deborah Echegoyen. Bioactivity studies on beta-sitosterol and its glycoside. In office of research coordination and natural science research institute. University of the Philippines, Diliman, Quezon City, Philippines; 2001.
86. Irene M. Villasenor, DP.SA and JS. A. Antimutagenicity and antiteratogenicity potential of the antimutagenic chloroform leaf extract from *Mentha cordifolia* Opiz. *PJS* 1997; 126 (3).
87. Daojan Choochoat, Wanchai De-Eknamkul and Nijisiri Ryangrunsi. Antimicrobial activities of the essential oils from Thai Lamiaceous plants. Faculty of Pharmacy, Rangsit University, Department of Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University. Rangsit University and Chulalongkorn University: Pathumthani and Bangkok; 2543.
88. Marilena Kampa, AN, Vassilios Tsaousis, Niki Maliaraki. A new automated method for the determination of the total antioxidant capacity (TAC) of human plasma, based on the crocin bleaching assay. *BMC Clinical Pathology* 2002; 2 (3).
89. Prior GC. And R.L. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clin Chem* 1998; 44 (6): 1309-15.
90. Berkant Muammer Kayatekin, S.G., Osman Ackgoz, Nazan Uysal, Ayfer Dayi. Effects of sprint exercise on oxidative stress in skeletal muscle and liver. *Eur J Appl Physiol* 2002; 87: 141-4.
91. Linda Hagfors PL., Lar Skolstam, Jan Anderson and Gunnar Johansson. Antioxidant intake, plasma antioxidants and oxidative stress in a randomized, control parallel, Mediterranean dietary intervention study on patients with rheumatoid arthritis. *Nutrition* 2003; 2(5).
92. Helle Bruunsgaard, H.E.P., Bente K Pederson, Kristiina Nyysönen, Jari Kaikkonen and Jukka T Salonen. Long term combined supplementations

- with alpha-tocopherol and vitamin C have no detectable anti-inflammatory effects in healthy men. *American society for nutrition science* 2003; 1170-3.
93. Marion Dietrich, G.B., Mark Hudes, Jason D Morrow. Edward P. Norkus, Marer G Traber, Carroll E Cross and Lester Packer. Antioxidant supplementation decrease lipid peroxidation biomarker F2-isoprostanes in plasma of smokers. *Cancer epidemiology, biomarkers& prevention* 2001; 7-13.
94. Gladys Block, C.J., Marion Dietrich, Edward P. Norkus, Mark Hudes and Lester Packer. Plasma C-reactive protein concentrations in active and passive smokers: Influence of antioxidant supplementation. *J Am Coll Nutr* 2004; 23 (2): 141-147.
95. H Sung, J.N., S Chun, H Park, S E Yang and W K Min. In vivo antioxidant effect of green tea. *Eur J Clin Nutr* 2000; 54 (7): 527-9.
96. A.R. Leeds, EA. E.F., J. Staley, R. Atesh and F. Ross, Availability of micronutrients from dried, encapsulated fruit and vegetable preparations: a study in healthy volunteers. *Hum Nutr Dietet* 2000; 13: 21-7.
97. Cao G and C.R., High concentrations of antioxidants may not improve defense against oxidative stress. *Arch Gerontol Geriatr* 1993; 17 (3): 189-201.
98. Sarah Zino, M.S., Sheila Williams, Jim Mann. Randomized controlled trial of effect of fruit and vegetable consumption on plasma concentrations of lipids and antioxidants. *BMJ* 1997; 314.
99. Halliwell B. The antioxidant paradox. *Lancet* 2000; 355: 1179-80.
100. Guahua Cao, R.M.R., Neal Lischner and Ronald L Prior. Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women. *J Nutr* 1998; 128: 2383-90.
101. D. Palli, A.D., A.Russo, F. Cipriani, A. Giacosa, D. Anadori, R. Salkeld, S. Salvini, E. Buiatti. Plasma levels of antioxidant vitamins and cholesterol in a large population sample in Central-Northern Italy. *Eur J Nutr* 1999; 38: 90-8.
102. Susana Liesuy. P.E, Ana Maria Campoa and Eduardo. Methodologies for evaluation of total antioxidant activities in complex mixtures. A critical review. *Biological Research* 2001; 34 (2).

103. Charles E. Matthews, P.S.F. James R. Hebert, Edward J. Stanek III, Phillip A. Merriam and Ira S. Ockene. Comparing physical activity assessment methods in the seasonal variation of blood cholesterol study. *Medicine & science in Sports & Exercise* 1999: 976-84.
104. Black A.E., B.S., Johanson G, Coward WA. Validation of dietary intakes of protein and energy against 24 hour urinary N and DLW energy expenditure in middle-aged women, retired men and post-obese subjects: comparisons with validation against presumed energy requirements. *Eur J Clin Nutr* 1997; 51 (6): 405-13.
105. Helaine M. Alessio, A.E.H., Methany K. Fulkerson, Jessica Ambrose, Robyn E. Rice and Ronald L. Wiley. Generation of reactive oxygen species after exhaustive aerobic and isometric exercise. *Medicine & Science in Sport & Exercise* 1999: 1576-81.
106. HM. A. Exercise-induced oxidative stress. *Medicine & Science in Sport & exercise* 1993; 25 (2): 218-24.
107. Alessio H.M.G.A. Lipid peroxidation and scavenger enzymes during exercise: adaptive response to training. *J Appl Physiol* 1998; 64 (4): 1333-6.
108. Northwest-Life science specialties. L., Premier products for superior life science research. 2004: Vancuever.
109. Ah Siew Sim, C.S., Daya Naidoo, David Emil Leon Wilcken. Improved method for plasma malondialdehyde measurement high-performance liquid chromatography using methyl malondialdehyde as an internal standard. *Journal of chromatography B* 2003; 785: 337-44.
110. Jill Templar, S.P.K., Tom P. Milligan, David Newman and Martin J. Raftery. Increased plasma malondialdehyde levels in glomerular disease as determined by a fully validated HPLC method. Department of renal medicine and transplantation and department of clinical biochemistry, Royal London Hospital; 1999.
111. Young IS and T.E. Measurement of malondialdehyde in plasma by high performance liquid chromatography with fluorometric detection. *Ann Clin Biochem* 1991; 28 (Pt 5): p. 504-8.

112. DR.J. Malondialdehyde and thiobarbituric acid- reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radic Biol Med* 1990; 9 (6): 515-40.
113. D. Erba, P. Riso, F. Criscuoli and G. Testolin. Malondialdehyde production in Jurkat T cells subjected to oxidative stress. *Nutrition* 2003; 19: 545-8.
114. Paul Preziosi, P.G., Bernard Herbeth, Poerre Valeix et al. Effects of supplementation with a combination of antioxidant vitamins and trace elements at Nutritional doses on biochemical indicators and markers of the antioxidant system in adult subjects. *Journal of the American College on Nutrition* 1998; 17 (3): 244-9.
115. Druso D. Perez, P.S., Rocio Foncea, Soledad Diez, Luis Vasquez et al. Wine , diet antioxidant defenses and oxidative damage. *Annals of the New York Academy of sciences* 2002; 957: 136-45.
116. J. Voces, A.C.C.d.O, I.G. Preto, L. Vila et al. Ginseng administration protects skeletal muscle from oxidative stress induced by exercise in rats. *Braz J Med Biol Res* 2004; 37 (12): 1863-71.
117. Khab S. G.V.S. Plasma lipoperoxides: a preliminary reference range. *Indian Journal of Medical Sciences* 2003; 57 (3): 105-7.
118. Lecomte E, H.B., Pirollet P, Chancerelle Y et al. Effect of alcohol consumption on blood antioxidant nutrients and oxidative stress indicators. *Am J Clin Nutr* 1994; 60 (2): 255-61.
119. Yunsheng Ma, J.A.G., Lisa Chasan_Tuber, Barbara C Olendzki et al. Association between dietary fiber and serum C-reactive protein. *Am J Clin Nutr* 2006; 83: 760-6.
120. Santina Cottonem Giuseppe Mule, E.N., Anna Valada, Marco Guarneri et al. Relation of C- reactive protein to oxidative stress and to endothelial activation in essential hypertension. *AJH* 2006; 19: 313-8.
121. Gunilla Nordin Fredrikson, B.H., Jan-Ake Nilsson, Ragnar Alm, Gortran Berglund and Jan Nilsson. Association between diet, lifestyle, metabolic cardiovascular risk factors and plasma C –reactive protein levels. *Metabolism* 2004; 53 (11): 1436-42.

122. S. Bo., R.G., B. Uberti, M.P. Mangiameli, G Colosso, E. Repetti, L. Gentile, M. Cassader and G.F. Pagano. Does C- reactive protein identify a subclinical metabolic disease in healthy subjects? *Eur J Clin Investigation* 2005; 35: 265-70.
123. Furio Brighenti, S.V., Nicoletta Pellegrini, Diego Ardigo, Daniele Del Rio, Sara Salvatore, PterMarco Poatti, Mauro Serafini and Ivana Zavaroni. Total antioxidant capacity of the diet is inversely and independently related to plasma concentration of high –sensitivity C-reactive protein in adult Italian subjects. *British Journal of Nutrition* 2005; 93: 619-25.
124. Esther Lopez-Garcia, M.B.S., Teresa T Fung, James B Meigs, Nader Rifai, JoAnn E Manson and Frank B Hu. Major dietary patterns are related to plasma concentrations of markers of inflammation and endothelial dysfunction. *Am J Clin Nutr* 2004; 80: 1029-35.
125. อรพินธ์ บรรจง, ธรา วิริยะพานิช และ อุไรพร จิตต์แจ้ง. คู่มือการประเมินปริมาณอาหาร. พิมพ์ครั้งที่ 1. ฝ่ายโภชนาการชุมชน สถาบันวิจัยโภชนาการ มหาวิทยาลัยมหิดล. นครปฐม: 2538.
126. Re'ka Szollosi and I.S. Varga. Total antioxidant power in some species of Labiatae (Adaptation of FRAP method). *Acta Biol Szeged* 2002; 46 (3-4): 125-7.
127. AJ. S., T. CR and S.J. Determination of betacarotene content and vitamin A activity of vegetables by high performance liquid chromatography and spectrophotometry. *Food Chemistry* 1986; 19: 65-74.
128. AR.B., W. DC. And I. HE. Determination of total vitamin C in Fruit juices and related products by liquid chromatography: Interlaboratory study. *J AOAC Inter* 2003; 86(2): 367-74.
129. Jeampiriyakul N. Effect of vitamin C and E supplementation on lipid peroxidation in non smokers and smokers. [M.S. Thesis in Nutrition]. Faculty of Graduate Studies, Mahidol University, 2003.
130. William D. Mcardle, F.I.K.V.L.K. Sports and Exercise nutrition. Philadelphia, PA: Lippincott Williams & Wilkins; 2004.
131. Tritachler K. Practical measurement and assessment. Philadelphia, Pennsylvania: Lippincott Williams & Wilkins; 2000.



1. Data from laboratory experiment

Table A1 Weight (g) per 1 cup of 7 pungent Thai herbs [125]

Name of pungent Thai herb	Weight (g) / 1 cup
Ginger (<i>Zingiber officinale</i> Roscoe.)	126
Galangal { <i>Alpinia nigra</i> (Gaertn.) B. L. Burtt}	126
Hairy basil (<i>Ocimum canum</i> Sims.)	22
Peppermint (<i>Mentha cordifolia</i> Opiz.)	15
Sweet basil (<i>Ocimum basilicum</i> Linn.)	15
Lesser ginger { <i>Boesenbergia pandurata</i> (Roxb.) Schltr.}	126
Holy basil (<i>Ocimum sanctum</i> Linn.)	15

Biochemical data**Table A2** Total energy intakes and total energy expenditure per day of the volunteers in both groups

Number of volunteers	Total energy intake/day (Kcal.)		Total energy expenditure/day (Kcal.)	
	Placebo group (n=19)	Experimental group (n=20)	Placebo group (n=19)	Experimental group (n=20)
1	2182	-	-	-
2	1857	1579	-	3492
3	2550	1496	3141	3472
4	1710	2083	3675	4208
5	2182	1999	-	4131
6	2158	2587	3119	3161
7	1690	1485	3343	2701
8	1499	1850	3909	-
9	1820	1853	3388	2973
10	2386	1610	3293	3363
11	1797	1918	4265	3287
12	2752	1795	3728	-
13	2119	1878	2849	4453
14	2354	1936	3193	3030
15	1712	2085	-	2950
16	-	1991	3340	3178
17	1995	2001	3994	3442
18	2109	1803	2908	4142
19	1811	2419	3157	3445
20		2446		-
Average	2038*	1938*	3420**	3464**

- Data not completed.

*, ** No significance different between groups. Test by 2-independent sample t-test, P value = 0.05.

Table A3 Plasma lipids of volunteers in both groups at baseline of supplementation

Number of volunteers	Cholesterol		Triglyceride		HDL-c		LDL-c	
	P group (n=19)	E group (n=20)	P group (n=19)	E group (n=20)	P group (n=19)	E group (n=20)	P group (n=19)	E group (n=20)
1	109.00	116.00	56.00	68.00	44.00	45.00	60.00	62.00
2	123.00	164.00	52.00	44.00	48.00	51.00	68.00	103.00
3	138.00	139.00	47.00	59.00	68.00	50.00	67.00	86.00
4	177.00	205.00	93.00	96.00	47.00	64.00	116.00	131.00
5	109.00	218.00	56.00	47.00	44.00	71.00	60.00	154.00
6	136.00	172.00	38.00	56.00	57.00	59.00	78.00	103.00
7	161.00	119.00	53.00	55.00	59.00	49.00	94.00	60.00
8	209.00	125.00	88.00	59.00	43.00	54.00	156.00	68.00
9	176.00	125.00	64.00	34.00	67.00	66.00	105.00	58.00
10	180.00	156.00	68.00	56.00	53.00	46.00	119.00	105.00
11	168.00	128.00	45.00	41.00	52.00	40.00	111.00	83.00
12	164.00	148.00	63.00	76.00	43.00	51.00	111.00	88.00
13	92.00	152.00	52.00	70.00	36.00	51.00	48.00	91.00
14	209.00	95.00	42.00	40.00	48.00	38.00	157.00	48.00
15	145.00	152.00	54.00	57.00	36.00	42.00	97.00	99.00
16	193.00	131.00	73.00	54.00	42.00	52.00	142.00	77.00
17	146.00	127.00	44.00	68.00	57.00	29.00	86.00	82.00
18	149.00	127.00	57.00	47.00	52.00	49.00	88.00	70.00
19	164.00	163.00	60.00	40.00	59.00	60.00	104.00	103.00
20		214.00		84.00		69.00		142.00
Average	155.16	150.74	58.16	57.55	50.26	51.8	98.26	90.65
P-value.	0.551*		0.944 ^a		0.634*		0.432*	

^a Tested by Mann- Whitney U Test, P value = 0.05.

* Tested by related 2-Independent sample T-test, P value = 0.05.

Table A4 Plasma lipid levels of volunteers in both groups at 8 weeks of supplementation.

Number of volunteers	Cholesterol		Triglyceride		HDL-c		LDL-c	
	P group (n=19)	E group (n=20)	P group (n=19)	E group (n=20)	P group (n=19)	E group (n=20)	P group (n=19)	E group (n=20)
1	116.00	160.00	68.00	148.00	37.00	39.00	69.00	103.00
2	125.00	169.00	105.00	119.00	51.00	51.00	58.00	101.00
3	143.00	151.00	83.00	182.00	65.00	48.00	71.00	79.00
4	173.00	239.00	95.00	317.00	40.00	61.00	109.00	129.00
5	116.00	215.00	68.00	136.00	37.00	63.00	69.00	131.00
6	149.00	170.00	71.00	221.00	51.00	53.00	92.00	85.00
7	131.00	135.00	111.00	182.00	44.00	41.00	65.00	76.00
8	187.00	136.00	114.00	100.00	44.00	51.00	126.00	65.00
9	159.00	143.00	137.00	230.00	52.00	54.00	85.00	59.00
10	172.00	152.00	222.00	189.00	39.00	41.00	99.00	81.00
11	135.00	136.00	86.00	102.00	49.00	38.00	72.00	80.00
12	128.00	200.00	229.00	163.00	36.00	53.00	61.00	118.00
13	109.00	136.00	89.00	153.00	38.00	37.00	59.00	67.00
14	193.00	93.00	206.00	110.00	49.00	31.00	119.00	40.00
15	159.00	158.00	126.00	113.00	44.00	55.00	98.00	89.00
16	165.00	154.00	87.00	204.00	37.00	52.00	113.00	80.00
17	181.00	146.00	105.00	96.00	56.00	39.00	116.00	91.00
18	166.00	160.00	380.00	116.00	43.00	54.00	86.00	86.00
19	157.00	192.00	165.00	243.00	55.00	59.00	81.00	109.00
20		213.00		445.00		55.00		106.00
Average	150.74	162.9	134.05	178.45	45.63	48.75	86.74	88.75
P-value.	0.214*		0.020 ^a		0.259*		0.782*	

^a Tested by Mann- Whitney U Test, P value = 0.05.

* Tested by related 2-Independent sample T-test, P value = 0.05.

Table A5 Plasma ORAC, FRAP and hs-CRP levels of volunteers in both groups at baseline of supplementation

Number of volunteers	ORAC		FRAP		hs-CRP	
	P group (n=19)	E group (n=20)	P group (n=19)	E group (n=20)	P group (n=19)	E group (n=20)
1	671.9400	436.9934	5.8517	8.4094	5.1680	21.8440
2	879.3900	403.0926	5.4034	11.6850	2.5990	0.9840
3	559.5571	378.9494	5.7724	10.5197	2.6960	0.5860
4	609.6830	728.6935	5.3345	11.7323	2.2220	0.5390
5	671.9400	419.2434	5.8517	13.5118	5.1680	0.4480
6	656.1876	646.2134	5.6759	15.2126	0.8190	10.2220
7	417.3140	522.8611	6.2345	12.2992	1.5380	3.9970
8	550.4602	518.0541	5.3862	13.7795	0.3850	0.5210
9	578.0734	572.5095	7.5883	13.3228	1.6940	0.4490
10	491.6692	704.1605	3.8886	14.3150	0.9050	1.4660
11	517.3545	621.4860	4.8664	12.8819	0.7390	0.6010
12	364.8904	759.4171	5.7800	11.7323	0.2970	2.6750
13	477.7154	649.3524	5.6365	14.8661	2.2950	2.0990
14	467.4407	756.6826	6.4029	7.6507	0.4620	1.3760
15	659.6171	294.4894	5.9612	6.3416	19.0670	7.8030
16	754.0154	319.8555	5.6629	14.4235	0.8060	0.7040
17	502.0408	415.2421	5.3609	12.8714	0.5430	4.6560
18	557.9347	445.3775	4.3031	13.7963	4.1260	3.0420
19	559.0426	312.7455	5.3346	13.8277	0.6560	0.3360
20		353.5770		13.3887		1.8290
Average	576.12	512.95	5.59	12.32	2.746	3.309
p-value	0.166*		0.000 ^a		0.955 ^a	

^a Tested by Mann- Whitney U Test, P value = 0.05.

* Tested by related 2-Independent sample T-test, P value = 0.05.

Table A6 Plasma ORAC, FRAP and hs-CRP level of volunteers in both groups at 8 weeks of supplementation

Number of volunteers	ORAC		FRAP		hs-CRP	
	P group (n=19)	E group (n=20)	P group (n=19)	E group (n=20)	P group (n=19)	E group (n=20)
1	782.7700	1719.885	4.9517	11.2756	0.7480	0.3390
2	1065.980	1970.168	4.8172	15.3701	0.4330	1.7030
3	975.2303	3077.338	4.8207	16.9449	1.1090	0.0710
4	870.8077	1899.472	4.8793	12.0000	10.0980	0.6700
5	782.7700	1708.621	4.9517	13.1654	0.7480	0.2230
6	982.2379	1724.518	4.7138	18.9449	0.4850	5.3440
7	246.3585	1613.960	5.8586	10.5197	1.6610	0.5580
8	712.7448	1902.115	5.2000	13.1339	0.2250	1.3160
9	676.0500	1644.192	5.5459	21.6693	0.3740	0.1350
10	723.0290	1560.268	5.4515	13.0079	2.0380	1.3370
11	407.7773	1792.127	5.3081	15.4646	1.5940	0.2660
12	284.7972	2271.341	5.7686	15.6693	0.1110	0.2280
13	314.1095	1990.229	5.5497	13.6378	0.6450	0.9320
14	457.5337	1942.194	5.9838	6.2554	0.2280	1.2230
15	539.4052	808.2101	5.0589	6.3024	1.3090	0.5660
16	607.4932	836.3625	5.6592	14.7527	0.151	0.9560
17	492.8937	821.0952	6.3463	13.0438	0.3010	0.5280
18	440.3038	1110.302	4.5512	11.1625	0.9860	0.8090
19	494.0885	800.5909	6.0630	12.8714	0.4260	0.1790
20		624.0978		15.5836		0.2480
Average	624.02	1590.85	5.34	13.54	1.246	0.882
p-value	0.000*		0.000 ^a		0.643 ^a	

^a Tested by Mann-Whitney U Test, P value = 0.05.

* Tested by related 2-Independent sample T-test, P value = 0.05.

Table A7 Plasma MDA ($\mu\text{mol/L}$) of volunteers in placebo and experiment group at 0, 2 and 8 weeks after supplementation

Number of volunteers	0 week plasma MDA		2 week plasma MDA		8 week plasma MDA	
	P group (n=19)	E group (n=20)	P group (n=19)	E group (n=20)	P group (n=19)	E group (n=20)
1	0.0935	0.0365	0.0954	0.0350	0.0951	0.0304
2	0.0980	0.0312	0.0952	0.0312	0.0963	0.0335
3	0.0966	0.0319	0.0964	0.0319	0.0947	0.0427
4	0.0954	0.0327	0.0989	0.0365	0.1008	0.0358
5	0.0935	0.0335	0.0954	0.0358	0.0951	0.0319
6	0.0986	0.0273	0.1010	0.0319	0.0982	0.0319
7	0.0999	0.0319	0.0997	0.0335	0.0982	0.0350
8	0.0987	0.0358	0.0945	0.0442	0.1078	0.0412
9	0.0991	0.1269	0.1029	0.1264	0.1002	0.1196
10	0.0981	0.1219	0.1025	0.1287	0.0988	0.1250
11	0.0943	0.1200	0.1064	0.1191	0.1022	0.1196
12	0.0939	0.1232	0.0971	0.1219	0.1749	0.1237
13	0.1812	0.1232	0.1784	0.1214	0.1816	0.1196
14	0.1820	0.0836	0.1828	0.0836	0.1773	0.0828
15	0.1784	0.0810	0.1765	0.0844	0.1737	0.0802
16	0.1816	0.0758	0.1741	0.0945	0.1733	0.0767
17	0.1765	0.0774	0.1788	0.0794	0.1745	0.0824
18	0.1713	0.0856	0.1753	0.0824	0.1765	0.0788
19	0.1761	0.0840	0.1780	0.0860	0.1757	0.0840
20		0.0806		0.0824		0.0803
Average	0.1267	0.0722	0.1278	0.0745	0.1313	0.0727
p-value *	0.000		0.000		0.000	

*Tested by Mann-Whitney U Test, P value = 0.05.

2. Data from general information questionnaires

Table A8 General Characteristics of the volunteers: placebo group and experiment group

Parameter	p-value
1. Home address	0.741
2. Education	0.318
3. Disease	0.955
4. Health condition	0.137
5. Used to medication	0.549
6. Alcohol habit	0.083
7. Type of alcohol	0.047*
8. Smoking habit	0.491
9. Coffee/tea habit	0.391
10. Type of exercise	0.16
11. Frequency on exercise	0.744
12. Range of time on exercise	0.583

Tested by Mann Whitney test, P value = 0.05.

* Significantly different between groups.

Table A9 Home address of the volunteers.

Home address	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Bangkok	6	31.6	5	25.0
North	0	0	1	5.0
Northeast	11	57.9	11	55.0
East	0	0	1	5.0
Central	2	10.5	2	10.0

The volunteers in placebo group are coming from Northeast region more than Bangkok, and central region. The volunteers in experimental group are coming from Northeast region more than Bangkok and central region as same as the placebo group. Also home addresses of both groups are not significantly different.

Table A10 Education levels of the volunteers

Education	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Elementary	2	10.5	2	10.0
Junior high school	4	21.1	6	30.0
Senior high school	3	15.8	6	30.0
Vocational	1	5.3	0	0
Higher Diploma	7	36.8	5	25.0
Studying	2	10.5	0	0
Bachelor's degree	0	0	1	5

The volunteers in placebo group had got higher diploma more than junior high school, senior high school, elementary and vocational school. The volunteers in experimental group had got junior high school and senior high school more than higher diploma, elementary and Bachelor's degree. The educations in both groups are not significantly different.

Table A11 Diseases of the volunteers

Disease	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Allergy	1	5.3	2	10.0
None	16	84.2	17	85.0
Other	0	0	1	5.0

Almost all volunteers in both groups are healthy. Some of them had allergy. Both groups are not significantly difference.

Table A12 Health condition of the volunteers.

Health Condition	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Previous month	2	10.5	6	30.0
Healthy	17	89.5	14	70.0

Most of volunteers in both groups are healthy. Some of them in placebo group (10.5%) and experimental group (30%) were sick in one month before study period. Both groups are not significantly different.

Table A13 History of medicine using

Medication	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Relieve pain drugs	8	42.1	12	60.0
Relieve cold drugs	1	5.3	1	5.0
Supplement	2	10.5	0	0
None	7	36.8	6	30.0
Other	0	0	1	5.0
Missing	1	5.3	0	0

The volunteers from placebo group were used to intake relieve pain medicine more than did not intake any medicine, supplement and relieve cold medicine. In experimental group, the volunteers were used to intake relieve pain medicine like placebo and used to intake it more than did not intake any medicine and relieve cold medicine. Nevertheless, both groups are not significantly different.

Table A14 Alcohol drinking of the volunteers.

Alcohol Habit	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Drink	8	42.1	14	70.0
No Drink	11	57.9	6	30.0

42.1 % of volunteers from placebo group are drinker and 70% of volunteers in experimental group are drinker. Both groups are not significantly different in alcohol drinking habit.

Table A15 Types of alcohol of the volunteers

Type of alcohol	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Spirits	2	10.5	6	30.0
Local Wine	0	0	1	5.0
Beer	6	31.6	7	35.0
Not Drink	11	57.9	6	30.0

The data was shown 57.9% of volunteers in placebo group and 30% of volunteers in experimental group are not drink. 31.6% in placebo group and 35% in experimental group are beer drinkers. 10.5% in placebo group and 30% in experimental group are spirits drinkers. Furthermore, 5% in experimental group are local wine drinker. However, both groups are not significantly different.

Table A16 Smoking habit of the volunteers

Smoking Habit	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Cease to Smoke	2	10.5	1	5.0
Not Smoke	16	84.2	19	95.0
Missing	1	5.3	0	0

In both groups the volunteers are not smoke. In placebo group, there are 10.5% of volunteers that used to smoke but now they are cease to smoke. In experimental group, there are 5% of volunteers that used to smoke and cease to smoke. Both groups are not significantly different.

Table A17 Nature of coffee and tea drinking of the volunteers.

Coffee/Tea drinking habit	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Drink both of them	7	36.8	4	20.0
Only coffee	2	10.5	2	10.0
Only tea	1	5.3	3	15.0
Drink none of them	9	47.4	11	55.0

The most of volunteers from placebo (47.4%) and experimental group (55%) were not drunk both tea and coffee. 36.8% in placebo group and 20% in experimental group were drunk both of them. 10.5% in placebo group and 10% in experimental group were drunk only coffee. 5.3% in placebo group and 15% in experimental group were drunk only tea. However, both groups are not significantly different in coffee/tea drinking habit.

Table A18 Nature of exercise of the volunteers.

Exercise Habit	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Yes	16	84.2	18	90.0
No	3	15.8	2	10.0

Most of the volunteers had exercised before recruitment. Some of them (15.8% in placebo group and 10% in experimental group) did not exercise. However, both groups are the same.

Table A19 Types of exercise of the volunteers

Type of Exercise	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Running	11	57.9	9	45.0
Football	4	21.1	4	20.0
Playing sports	1	5.3	3	15.0
Unspecified	0	0	2	10.0
No Exercise	3	15.8	2	10.0

57.9% of volunteers in placebo group and 45% of volunteers in experimental group had exercised by running. 21.1% in placebo and 20% in experimental group had exercised by playing football. 5.3% in placebo and 15% in experimental group had exercised by playing sports. Both groups are not significantly different.

Table A20 Frequency on exercise of all volunteers

Time on Exercise	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
1-2 times/week	5	26.3	7	35.0
3-4 times/week	7	36.8	3	15.0
Everyday	3	15.8	7	35.0
Leisure Time	1	5.3	1	5.0
No Exercise	3	15.8	2	10.0

The data was shown that 36.8% of volunteers in placebo group had exercised 3-4 times per week. 26.3% of them had exercised 1-2 times per week. 15.8% had exercised everyday and 5.3% had exercised in leisure time. In experimental group, 35% of them had exercised everyday or 1-2 times per week. 15% of them had done 3-

4 times per week and 5% of them had done in leisure time. Both groups are not significantly different.

Table A21 Duration of exercise of all volunteers

Range of Time on Exercise	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
≤ 30 min	3	15.8	6	30.0
31-60 min	7	36.8	2	10.0
61-120 min	3	15.8	4	20.0
> 120 min	2	10.5	1	5.0
No answer	1	5.3	5	25.0
No Exercise	3	15.8	2	10.0

In Placebo group, 36.8% of volunteers had 31-60 min. on each exercise. 15.8% of them had ≤ 30 min or 61-120 min. on each exercise. 10.5% of them had > 120 min. on each exercise. In experimental group, 30% of volunteers had ≤ 30 min on each exercise. 20% of them had 61-120 min. on each exercise. 10% of them had 31-60 min. on each exercise. 5% of them had > 120 min. on each exercise. Both groups are not significantly different.

3. Data from food frequency questionnaires

Table A22 Statistical data of the difference in vegetables, fruits and food intake of the volunteers from placebo and experimental group

Types of food	p-value
Green leave vegetable	0.681
Red, orange and yellow vegetable	0.839
White leave vegetable	0.14
Herb – root	0.856
Herb – leaf and fruit	0.694
Other vegetable	0.576
Fruit – sweet	0.323
Fruit – general	0.292
Phad kaprou	0.915
Phad khing	0.322
Phad phed	0.486
Kang lieng	0.002*
Kang pa	0.025*
Kang som	0.353
Tom yum and tom kha	0.74
Kang phed and Choo chi	0.545
Other Yum	0.109
Phla, Labb and Koy	0.341
Mieng	0.481
Thai vermicelli	0.608
Nam prik with vegetable	0.384
Food – prefer and often eat	0.84
Vegetable– prefer and often eat	0.149
Fruit– prefer and often eat	0.622

Tested by Mann Whitney test, P value = 0.05.

* Significantly different between groups

Table A23 Frequency of green leaf vegetable intake of all volunteers.

Green leaf vegetable	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
1-2 times/Mo	3	15.8	3	12.0
1-2 times/Wk	10	52.6	9	48.0
eat everyday	5	26.3	7	32.0
Missing	1	5.3	1	4.0
Total	19	100.0	20	100.0

52.6% of volunteers from placebo group and 48% of volunteers from experimental group had eaten green leaf vegetable 1-2 times/wk. 26.3% from placebo group and 32% from experimental group had eaten everyday. 15.8% from placebo group and 12% from experimental group had eaten 1-2 times/mo. However, both groups are not significantly different.

Table A24 Frequency of red, yellow or orange vegetable intake of all volunteers

Red-yellow vegetable	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Don't eat	1	5.3	2	10.0
1-2 times/Mo	5	26.3	4	20.0
1-2 times/Wk	11	57.9	11	55.0
eat everyday	2	10.5	2	10.0
Missing	0	0	1	5.0
Total	19	100.0	20	100.0

57.9% of volunteers from placebo group and 55% from experimental group had eaten red-yellow-orange vegetables 1-2 times/wk. 26.3% from placebo and 20% from experimental group had eaten red-yellow-orange vegetables 1-2 times/mo. 10.5%

from placebo group and 10% from experimental group had eaten everyday. Some of them (5.3% from placebo and 10% from experimental group) had not eaten red-yellow-orange vegetables. Both groups are not significantly different.

Table A25 Frequency of White leaf vegetable intake of all volunteers

White leaf vegetable	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
1-2 times/Mo	2	10.5	9	45.0
1-2 times/Wk	10	52.6	10	50.0
eat everyday	7	36.8	1	5.0
Total	19	100.0	20	100.0

Mostly volunteers from placebo (52.6%) and from experimental group (50%) had eaten white leaf vegetables 1-2 times/wk. 36.8% of volunteers from placebo group and 5% of volunteers from experimental group had eaten everyday. 10.5% from placebo group and 45% from experimental group had eaten 1-2 times/Mo. Both groups are not significantly different.

Table A26 Frequency of herb's root intake of all volunteers

Root herb	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Don't eat	1	5.3	2	10.0
1-2 times/Mo	3	15.8	2	10.0
1-2 times/Wk	9	47.4	10	50.0
eat everyday	5	26.3	6	30.0
Missing	1	5.3	0	0
Total	19	100.0	20	100.0

In placebo group, 47.4% of volunteers had eaten herb's root 1-2 times/wk, 26.3% of them had eaten everyday, 15.8% of them had eaten 1-2 times/mo, and 5.3% of them had not eaten. In experimental group, 50% of them had eaten 1-2 times/wk, 30% of them had eaten everyday, 10% of them had eaten 1-2 times/Mo or had not eaten. However, they are not significantly different between groups.

Table A27 Frequency of herb's leaf or fruit intake of all volunteers

Leaf and fruit herb	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Don't eat	0	0	1	5.0
1-2 times/Mo	2	10.5	2	10.0
1-2 times/Wk	11	57.9	11	55.0
eat everyday	5	26.3	6	30.0
Missing	1	5.3	0	0
Total	19	100.0	20	100.0

In placebo group, 57.0% of them had eaten herb's leaf or fruit 1-2 times/wk, 26.3% of them had eaten everyday, 10.5% of them had eaten 1-2 times/mo. In

experimental group, 55% of them had eaten 1-2 times/wk, 30% of them had eaten everyday, 10% of them had eaten 1-2 times/mo and 5% of them had not eaten. Both groups are not significantly different.

Table A28 Frequency of other vegetables intake of all volunteers

Other vegetable	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Don't eat	0	0	3	15.0
1-2 times/Mo	1	5.3	1	5.0
1-2 times/Wk	14	73.7	11	55.0
eat everyday	3	15.8	4	20.0
Missing	1	5.3	1	5.0
Total	19	100.0	20	100.0

Mostly volunteers in placebo (73.7%) and experimental group (55%) had eaten other vegetables 1-2 times/wk, 15.8% in placebo and 29% in experimental group had eaten everyday, 5.3% in placebo and 5% in experimental group had eaten 1-2 times/mo and 15% of volunteers from experimental group had not eaten. However, there is not significantly different between groups.

Table A29 Frequency of sweet fruit intake of all volunteers

Sweet fruit	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Don't eat	1	5.3	0	0
1-2 times/Mo	4	21.1	9	45.0
1-2times/Wk	11	57.9	9	45.0
eat everyday	3	15.8	1	5.0
Missing	0	0	1	5.0
Total	19	100.0	20	100.0

In placebo group, 57.9% of them had eaten sweet fruit 1-2 times/wk, 21.1% of them had eaten 1-2 times/mo, 15.8% of them had eaten everyday and 5.3% of them had not eaten. In experimental group, 45% of them had eaten 1-2 times/wk or 1-2 times/mo and 5% of them had eaten everyday. Both groups are not significantly different.

Table A30 Frequency of general fruit intake of all volunteers

General fruit	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Don't eat	2	10.5	0	0
1-2 times/Mo	4	21.1	8	40.0
1-2 times/Wk	7	36.8	11	55.0
eat everyday	5	26.3	0	0
Missing	1	5.3	1	5.0
Total	19	100.0	20	100.0

36.8% of volunteers from placebo group had eaten general fruit 1-2 times/wk, 26.3% of them had eaten everyday, 21.1% of them had eaten 1-2 times/mo and 10.5%

of them had not eaten. Whereas, 55% of volunteers from experimental group had eaten 1-2 times/ wk and 40% of them had eaten 1-2 times/ mo. There is not significantly different between groups.

Table A31 Frequency of Phad kaprou intake of all volunteers

Phad kaprou	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Don't eat	3	15.8	2	10.0
1-2 times/Mo	2	10.5	7	35.0
1-2 times/Wk	13	68.4	7	35.0
eat everyday	1	5.3	2	10.0
Missing	0	0	2	10.0
Total	19	100.0	20	100.0

Mostly volunteers from placebo group (68.4%) had eaten phad kaprou 1-2 times/wk, 10.5% of them had eaten 1-2 times/mo, 5.3% of them had eaten everyday but 15.8% of them had not eaten. In experimental group, 35% of them had eaten 1-2 times/wk or 1-2 times/mo and 10% of them had eaten everyday. There is not significantly different between groups.

Table A32 Frequency of Phad khing intake of all volunteers

Phad khing	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Don't eat	5	26.3	9	45.0
1-2 times/Mo	8	42.1	6	30.0
1-2 times/Wk	3	15.8	2	10.0
Eat everyday	0	0	1	5.0
Missing	3	15.8	2	10.0
Total	19	100.0	20	100.0

In placebo group, 42.1% of them had eaten Phad khing 1-2 times/mo, 15.8% of them had eaten everyday or 1-2 times/wk and 26.3% of them had not eaten. In experimental group, 45% of them had not eaten, 30% of them had eaten 1-2 times/mo, 10% of them had eaten 1-2 times/wk and 5% of them had eaten everyday. There is not significantly different between groups.

Table A33 Frequency of Phad phed intake of all volunteers

Phad phed	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Don't eat	2	10.5	1	5.0
1-2 times/Mo	3	15.8	8	40.0
1-2 times/Wk	11	57.9	8	40.0
eat everyday	3	15.8	2	10.0
Missing	0	0	1	5.0
Total	19	100.0	20	100.0

In placebo group, 57.9% of them had eaten Phad phed 1-2 times/wk, 15.8% of them had eaten everyday or 1-2 times/mo and 10.5% of them had not eaten. In

experimental group, 40% of them had eaten 1-2 times/mo or 1-2 times/wk and 10% of them had eaten everyday and 5% of them had not eaten. There is not significantly different between groups.

Table A34 Frequency of Kang lieng intake of all volunteers

Kang lieng	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Don't eat	2	10.5	13	65.0
1-2 times/Mo	9	47.4	4	20.0
1-2 times/Wk	7	36.8	2	10.0
Missing	1	5.3	1	5.0
Total	19	100.0	20	100.0

In placebo group, 47.4% of them had eaten Kang lieng 1-2 times/mo, 36.8% of them had eaten 1-2 times/wk and 10.5% of them had not eaten. In experimental group, 20% of them had eaten 1-2 times/mo, 10% of them had eaten 1-2 times/wk and 65% of them had not eaten. There is significantly different between groups.

Table A35 Frequency of Kang pa intake of all volunteers

Kang pa	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Don't eat	3	15.8	9	45.0
1-2 times/Mo	8	42.1	8	40.0
1-2 times/Wk	5	26.3	2	10.0
eat everyday	1	5.3	0	0
Missing	2	10.5	1	5.0
Total	19	100.0	20	100.0

In placebo group, 42.1% of them had eaten Kang pa 1-2 times/mo, 26.3% of them had eaten 1-2 times/wk 5.3% of them had eaten everyday and 15.8% of them had not eaten. In experimental group, 40% of them had eaten 1-2 times/mo, 10% of them had eaten 1-2 times/wk and 45% of them had not eaten. There is significantly different between groups.

Table A36 Frequency of Kang som intake of all volunteers

Kang som	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Don't eat	1	5.3	3	15.0
1-2 times/Mo	5	26.3	6	30.0
1-2 times/Wk	12	63.2	10	50.0
Eat everyday	0	0	1	5.0
Missing	1	5.3	0	0
Total	19	100.0	20	100.0

In placebo group, 63.2% of them had eaten Kang som 1-2 times/wk. 26.3% of them had eaten 1-2 times/Mo and 5.3% of them had not eaten. In experimental group,

50% of them had eaten 1-2 times/wk, 30% of them had eaten 1-2 times/mo and 5% of them had eaten everyday and 15% of them had not eaten. There is not significantly different between groups.

Table A37 Frequency of Tom yum or Tom kha intake of all volunteers

Tom yum Tom kha	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Don't eat	1	5.3	3	15.0
1-2 times/Mo	10	52.6	7	35.0
1-2 times/Wk	7	36.8	7	35.0
eat everyday	1	5.3	2	10.0
Missing	0	0	1	5.0
Total	19	100.0	20	100.0

Mostly volunteers from placebo group (52.6%) had eaten Tom yum or Tom kha 1-2 times/mo, 36.8% of them had eaten 1-2 times/wk and 5.3% of them had eaten everyday or had not eaten. In experimental group, 35% of them had eaten 1-2 times/wk or 1-2 times/mo and 10% of them had eaten everyday and 15% of them had not eaten. There is not significantly different between groups.

Table A38 Frequency of Kang phed or Choo chi intake of all volunteers

Kang phed Choo chi	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Don't eat	4	21.1	7	35.0
1-2 times/Mo	9	47.4	7	35.0
1-2 times/Wk	3	15.8	3	15.0
eat everyday	1	5.3	1	5.0
Missing	2	10.5	2	10.0
Total	19	100.0	20	100.0

Mostly volunteers in Placebo (47.4%) and experimental group (35%) had eaten Kang phed or Choo chi 1-2 times/mo, 15.8% in placebo and 15% in experimental group had eaten 1-2 times/wk, 5.3% in placebo and 5% in experimental group had eaten everyday and 21.1% in placebo and 35% of volunteers from experimental group had not eaten. However, there is not significantly different between groups.

Table A39 Frequency of other Yum intake of all volunteers

Other Yum	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Don't eat	2	10.5	6	30.0
1-2 times/Mo	7	36.8	9	45.0
1-2 times/Wk	6	31.6	1	5.0
eat everyday	3	15.8	3	15.0
Missing	1	5.3	1	5.0
Total	19	100.0	20	100.0

In placebo group, 36.8% of them had eaten other Yum 1-2 times/mo, 31.6% of them had eaten 1-2 times/wk, 15.8% of them had eaten everyday and 10.5% of them

had not eaten. In experimental group, 45% of them had eaten 1-2 times/mo, 5% of them had eaten 1-2 times/wk and 15% of them had eaten everyday and 30% of them had not eaten. There is not significantly different between groups.

Table A40 Frequency of Phla, Labb or Koy intake of all volunteers

Phla, Labb, Koy	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Don't eat	6	31.6	7	35.0
1-2 times/Mo	4	21.1	8	40.0
1-2 times/Wk	6	31.6	3	15.0
Eat everyday	0	0	1	5.0
Missing	3	15.8	1	5.0
Total	19	100.0	20	100.0

In placebo group, 31.6% of them had eaten Phla, Labb or Koy 1-2 times/wk. 21.1% of them had eaten 1-2 times/mo and 31.6% of them had not eaten. In experimental group, 40% of them had eaten 1-2 times/mo, 15% of them had eaten 1-2 times/wk and 5% of them had eaten everyday and 35% of them had not eaten. There is not significantly different between groups.

Table A41 Frequency of Mieng intake of all volunteers

Mieng	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Don't eat	10	52.6	12	60.0
1-2 times/Mo	4	21.1	5	25.0
1-2 times/Wk	2	10.5	2	10.0
Missing	3	15.8	1	5.0
Total	19	100.0	20	100.0

In placebo group, 21.1% of them had eaten Mieng 1-2 times/mo, 10.5% of them had eaten 1-2 times/wk and 52.6% of them had not eaten. In experimental group, 25% of them had eaten 1-2 times/mo, 10% of them had eaten 1-2 times/wk and 60% of them had not eaten. There is not significantly different between groups.

Table A42 Frequency of Thai vermicelli intake of all volunteers

Thai vermicelli	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
don't eat	3	15.8	7	35.0
1-2 times/Mo	8	42.1	4	20.0
1-2 times/Wk	6	31.6	7	35.0
Missing	2	10.5	2	10.0
Total	19	100.0	20	100.0

In placebo group, 42.1% of them had eaten Thai vermicelli 1-2 times/mo 31.6% of them had eaten 1-2 times/wk, and 15.8% of them had not eaten. In experimental group, 35% of them had eaten 1-2 times/wk, 20% of them had eaten 1-2 times/mo and 35% of them had not eaten. There is not significantly different between groups.

Table A43 Frequency of Nam prik with vegetable intake of all volunteers

Nam prik with vegetable	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Don't eat	2	10.5	1	5.0
1-2 times/Mo	4	21.1	4	20.0
1-2 times/Wk	12	63.2	12	60.0
eat everyday	1	5.3	2	10.0
Missing	0	0	1	5.0
Total	19	100.0	20	100.0

In placebo group, 63.2% of them had eaten Nam prik with vegetable 1-2 times/wk, 21.1% of them had eaten 1-2 times/mo, 5.3% of them had eaten everyday and 10.5% of them had not eaten. In experimental group, 60% of them had eaten 1-2 times/wk, 20% of them had eaten 1-2 times/mo, 10% of them had eaten everyday and 5% of them had not eaten. There is not significantly different between groups.

Table A44 Types of prefer food of volunteers

Prefer food or often eat	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
Tom yum	4	21.1	7	35.0
Phadped	6	31.6	4	20.0
Nam prik	1	5.3	1	5.0
Fried vegetable	1	5.3	1	5.0
Other	6	31.6	3	15.0
Missing	1	5.3	4	20.0
Total	19	100.0	20	100.0

In placebo group, 31.6% of them had preferred Phadphed or other food, 21.1% of them had preferred tom yum and 5.3% of them had preferred Nam prik or fried vegetable. In experimental group, 35% of them had preferred Tom yum, 20% of them had preferred Phadphed, 15% of them had preferred other food and 5% of them had preferred Nam prik or fried vegetable. There is not significantly different between groups.

Table A45 Types of prefer vegetable of volunteers

Prefer vegetable or often eat	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
green leaf	6	31.6	7	35.0
yellow-red-	6	31.6	2	10.0
orange	2	10.5	10	50.0
white leaf	4	21.1	0	0
other vegetable	1	5.3	1	5.0
Missing				
Total	19	100.0	20	100.0

In placebo group, 31.6% of them had preferred green leaf vegetable or white leaf vegetable, 21.1% of them had preferred other vegetable and 10.5% of them had preferred white leaf vegetable. In experimental group, 50% of them had preferred white leaf vegetable, 35% of them had preferred green leaf vegetable and 10% of them had preferred red or yellow or orange vegetable. There is not significantly different between groups.

Table A46 Types of prefer fruit of volunteers

Prefer fruit or often eat	Placebo group (n=19)		Experimental group (n=20)	
	Number of volunteers	Percent	Number of volunteers	Percent
sweetest	10	52.6	8	40.0
watery	6	31.6	10	50.0
other	1	5.3	0	0
Missing	2	10.5	2	10.0
Total	19	100.0	20	100.0

In placebo group, 52.6% of them had preferred sweetest fruit, 31.6% of them had preferred watery fruit and 5.3% of them had preferred other fruit. In experimental group, 50% of them had preferred watery fruit and 40% of them had preferred sweetest fruit. There is not significantly different between groups.

Table A 47 Medication Compliance of All Volunteers. The data was shown as percent.

No. of volunteers	Compliance in Placebo group (%)	Compliance in Experimental group (%)
1	85.91*	48.64
2	62.27	49.55
3	55.00	82.27
4	66.82	81.82
5	72.73	61.36
6	85.91*	53.64*
7	62.27	66.36
8	83.64	50.00
9	88.18	49.55
10	89.55	70.00
11	60.00	45.00
12	64.09	65.00*
13	90.00	59.55
14	80.45	60.45
15	72.73*	53.64
16	81.82	40.45
17	82.27	64.55*
18	69.09	74.55*
19	60.00	53.64*
20	78.18	52.27
21	79.55*	65.45
22	81.82*	66.82
23	78.64	80.45
24	79.55*	78.64
25	93.64	62.73

* The volunteers who excluded from study.

The data of volunteers whose compliance are more than 50% were used in the study.

4. Analytical Methods

1. Analyses of malondialdehyde (MDA) in plasma by high pressure liquid chromatography (HPLC) [66]

Principle

The assay is based on the reaction of MDA with thiobarbituric acid forming an MDA-TBA₂ adduct that absorbs strongly at 532 nm. BHT is added to the sample and reaction mixture to minimize oxidation of lipids that contribute artifactually during sample processing and the TBA reaction. The temperature of the reaction mixture has been reduced to minimize the decomposition of lipid peroxides. MDA is protein bound, mostly as a Schiff base, the pH of the reaction has been optimized to facilitate hydrolysis of the MDA. The sample is heated with TBA under acidic conditions and amount of pink-coloured MDA-TBA₂ adduct produced is measured with fluorescence detector (Excitation 515 nm, emission 553 nm).

Chemicals

1. H₂SO₄
2. Phosphotungstic acid
3. BHT
4. Ethanol
5. TBA reagent 6.7 g/L TBA in H₂O diluted 1: 1 with glacial acetic acid
6. n-butanol
7. Phosphate-buffer saline
8. NaOH
9. Butanol-pyridine
10. Tetramethoxypropane
11. Methanol
12. Potassium dihydrogen phosphate buffer
13. Sodium azide

Procedure

1. Pipette 10 µL of plasma in a microcentrifuge tube
2. Add 500 µL of 42 mmol/L H₂SO₄ and mixed gently

3. Add 125 μL of 100 g /L phosphotungstic acid and vortex
 4. 5 min at room temperature, the mixture was centrifuged 3 min at 16000 g
 5. The supernatant was discarded, and the precipitate was resuspended in 300 μL of 42 mmol/L H_2SO_4 and 45 μL of 100 g /L phosphotungstic acid
 6. The sample was centrifuged 3 min at 16000 g
 7. The supernatant was again discarded, and the pellet was resuspended in 350 μL of H_2O , 50 μL of 0.7 mmol /L BHT in 200 mL /L ethanol, and 100 μL of TBA reagent (consisting of 6.7 g/L TBA in H_2O diluted 1:1 with glacial acetic acid)
 8. The mixture was heated immediately for 60 min at 95°C and cooled on ice
 9. The MDA (TBA) ₂ adduct was then extracted with 500 μL of n-butanol
 10. The layers were separated by centrifugation 3 min at 16000 g
 11. Calibration curves were constructed using tetramethoxypropane 0.1-5.0 $\mu\text{mol/L}$
 12. Butanolic extracts were analyzed on an automated HPLC gradient system (Agilent Model 1100 units: Gradient pump) Vacuum degasser, thermostated autosample (4°C), thermostated column compartment operated at 30°C, and Fluorescence detector (excitation, 515 nm; emission, 553 nm)
 13. Chromatographic separation was achieved on a Zorbax Eclipse C₈ column (particle size 5 μm ; column size 150 x 4.6 mm) fitted with a Security guard C₈ guard column
- Mobile phase A** consisted of 300 mL /L methanol in 50 mmol /L potassium dihydrogen phosphase buffer, pH 7.0
- Mobile phase B** consisted of 800 mL /L methanol in the same buffer

Both mobile phases contained 0.2 g /L sodium azide to prevent bacterial growth.

The gradient (percentage of solvent B) and flow rates were as follows: 0 min, 0% B, 1 mL /min;

2.5 min, 0% B, 1 ml /min;

1.5 min, 100% B, 2 ml /min;

8.5 min, 100% B, 2 mL /min;

9.5 min, 0%B, 2 ml /min;

10 min, 0% B, 2 mL /min;

11 min, 0% B, 1 mL /min.

The injection volume was 5 μ L, and the MDA (TBA) ₂ adduct eluted at 3.75 min

2. Standard Protocol for ORAC[63]

Principle

The ORAC assay measures the capacity of antioxidants to protect the fluorescent protein from damage by free radicals. In this assay, AAPH is used as the source for the peroxy radical, which is generated as a result of the spontaneous decomposition of AAPH at 37°C. Fluorescein is the chosen target protein, whose loss of fluorescence is an indication of the extent of damage from its reaction with the peroxy radical. The protective effect of the antioxidants is measured by assessing the longer fluorescence time/ intensity are area under the curve of the sample compared to the blank in which no antioxidant compounds are present. Trolox is used as the calibration standard. The ORAC assay provides a unique assessment, as the reaction goes to completion both the inhibition time and inhibition degrees are measured.

Sample preparation

1. Spices were cut into small pieces
2. Weigh 0.5 g. of dried sample or 2-3 g of fresh sample into Erlenmeyer flask with cap 250 ml
3. All samples should be extract with 20 mL of Acetone/H₂O (50:50 v/v)

4. Shaken on an orbital shaker at 400 rpm at room temp for 1 hr
5. All samples should be centrifuge at 14,000 rpm for 15 min
6. Supernatant was diluted with 75 mM Phosphate buffer solution before analyze

Reagent preparation

1. ORAC Buffer stock solution

- Weigh 102.07 g of KH_2PO_4 in volumetric flask 1L
- Dilute with DI water to the volume, this will yield 1 L of 0.75M stock solution of KH_2PO_4 store in refrigerator
- weigh 130.64 g of K_2HPO_4 in polystyrene bottle 1L
- Dilute with DI water to the volume, then stir for 1 hr and filter, this is 1L of 0.75M stock solution of K_2HPO_4 .
- Take 603 ml of 0.75M K_2HPO_4 stock solution and add 351 ml of 0.75M KH_2PO_4 stock solution, this will yield 954 ml of ORAC stock solution.

2. ORAC Buffer Working solution

- Take 100 ml of ORAC buffer stock solution into volumetric flask 1L
- Add DI water to the volume and mix well
- pH should be 7.2, use NaOH concentrated solution drop by drop to bring pH up if it is below 7.2

3. AAPH 153 mM

- Weigh 0.414 g of AAPH into volumetric flask 10 mL
- Dilute with Phosphate buffer (ORAC working solution) to the volume
- Store in an ice bath before use

4. Fluorescein Concentrated solution.

- Weigh 0.045 g of Fluorescein into 100 mL volumetric flask
- Dilute with Phosphate buffer (ORAC working solution) to the volume this will yield 100 ml of 8.37×10^{-4} mM Fluorescein Concentrated solution.

5. Fluorescein Stock solution

- Taking 20 ml of ORAC buffer working solution and removing 100 μ L. Next, take 100 μ L of Fluorescein concentrated solution and add to the ORAC buffer working solution. This will yield 20 ml of 4.19 μ M FL Stock solution.

6. Fluorescein working solution (Store 1 Mo.)

- Pipette 1.95 ml of Fluorescein Stock solution into 100 ml volumetric flask
- Dilute with Phosphate buffer (ORAC working solution) to the volume

7. 1000 μ M Trolox stock solution (Store 1 Mo.)

- Weigh 0.025 g of Trolox into 100 ml volumetric flask
- Dilute with Phosphate buffer (ORAC working solution) to the volume

8. 100 μ M Trolox working solution (for serial dilution)

- Pipette 1 ml of 1000 μ M Trolox stock solution into test tube 15 ml
- Add 9 ml of Phosphate buffer (ORAC working solution)
- Make serial dilution to 50, 25, 12.5, 6.25, 3.125 μ M Trolox standard

Measurement

1. Blank: pipette 3 ml of Fluorescein working solution and 0.5 ml of Phosphate buffer (ORAC working solution) and mixed well
2. Sample: pipette 3 ml of Fluorescein working solution and 0.5 ml of extract sample or standard Trolox and mixed well
3. Before measurement, warm sample in water bath at 37 $^{\circ}$ C then add 0.5 ml of AAPH and vortex, measure at Excitation wavelength 493 nm and Emission wavelength 515 nm in 37 $^{\circ}$ C every 1 min.

3. Analyses total antioxidant capacity by ferric reducing antioxidant power (FRAP) assay [64, 126]

Principle

FRAP assay depends upon the reduction of ferric tripyridyltriazine complex to the ferrous tripyridyltriazine by a reductant at low pH. TPTZ has an intensive blue color and can be monitor at 593 nm. The relative activities of samples were assessed by comparing their activities with that of trolox.

Chemicals

1. 2, 4, 6-tripyridyl-s-triazine (TPTZ)
2. Sodium acetate
3. Glacial acetic acid
4. Distilled water
5. 11M conc. HCl
6. 20 mM Ferric chloride
7. Standard Trolox

Chemicals preparation

1. 300 mM Acetate buffer, pH 3.6
 - 3.1 g of Sodium acetate.3H₂O
 - 16 ml glacial acetic acid
 - Distilled water to 1 litre
 - Check pH, store at 4°C
2. 40 mM Dilute HCl
 - 8.33 ml conc. HCl (11 M)
 - Distilled water to 100 ml
 - Store at room temperature
3. 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine)
 - 0.31 g TPTZ in 100 ml of 40 mM HCl, dissolve at 50°C in water bath
 - make fresh on day of assay in a new Corning tube.

4. 20 mM Ferric chloride
 - 0.54 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$
 - dissolve in 100 ml distilled water
 - make fresh on day of assay in a new Corning tube.

5. Standards Trolox
 - The method as std. For ORAC

Sample preparation [126]

1. One g. of Herbals were cut into small pieces and mashed with a cool mortar and pestle using quartz sand and 9 ml cool 0.1 M phosphate buffer (pH 7.6, containing 0.1 mM EDTA).
2. This mixture was filtered through a filter paper.
3. Centrifuged at 15000 rpm for 10 min.
4. The supernatant was used for the measurements.

Measurement procedure (Adapted from Benzie & Strain, 1996, Analytical Biochemistry, 239, 70-76) [64]

1. Switch water baths & spectrophotometer on
2. Defrost samples & standards
3. Prepare FRAP reagent:
 - 20 ml acetate buffer
 - 2 ml TPTZ solution
 - 2 ml FeCl_3 solution

Keep in a dedicated plastic bottle. The solution should be straw coloured, if it is tinged with blue discard and prepare fresh solution after rinsing bottle thoroughly with demineralised water. Mix; keep in water bath at 37°C.

4. Run a set of blanks first. Place a cuvettes in the spectrophotometer. Add 30 μl distilled water to each cuvette (let the water run down the side of cuvette). Add 1 mL of FRAP reagent vigorously to each

cuvette (using a Gilson pipette) so that the contents mix thoroughly (there is no need to mix the cuvettes further).

5. Set the spectrophotometer at 593 nm, the temperature strip should read 37 °C. Set timers for 4 minutes.
6. After 4 minutes, zero the blank and press run to record the absorbance of the samples. Take written records of the absorbances then reset the spectrophotometer and prepare the next set of samples.
7. Discard recorded standards/samples down drain with plenty of water.
8. Repeat 4-6 with 3 sets of standards, the first of which should always be a blank.
9. Then repeat 4-6 with samples, always using a blank in cuvette 1. All samples must be run at least in triplicate.
10. **Analysis [64]:**
 - Subtract the water blank from the sample/standard values.
 - Construct a linear regression for the standards. (absorbance against concentration)
 - Use the regression equation to calculate the FRAP values (units, mM Fe (II) per liter) of the Samples.

4. Analyses β -carotene and vitamin E by high performance liquid chromatography (HPLC) [127]

Chemicals

1. 95% Ethanol
2. 2N Potassium hydroxide
3. 10% Ascorbic acid
4. Petroleum ether –hexane (2:1)
5. 5% Potassium hydroxide
6. 10% Sodium chloride
7. Absoluted methanol
8. 0.5% Tetrahydrofuran in methanol

9. Standard β -carotene, vitamin E

10. Reverse osmosis water

Appliances

1. Water bath
2. Condenser
3. Amber round bottom flask 250 ml
4. Analytical balance
5. Water flow hood
6. Cylinder 100 ml
7. Beaker 1 L
8. Serological pipette 10 ml
9. Amber separating funnel 250 ml
10. Rotation evaporator
11. Nitrogen gas
12. Filter paper No. 1
13. HPLC with UV-Vis detector

Sample preparation

1. Herbals were cut into small pieces and blended with blender
2. Keep the sample into thick bottle, Freeze at -20°C until analysis
3. Thawn the sample in water bath at room temperature, mix thoroughly.
4. Weight 0.5-3 g of the sample

Saponification

1. Add RO water in Warm water bath at $T = 100^{\circ}\text{C}$.
2. Weight 0.5-3 g of the sample in Amber-round bottom volumetric flask 250 ml. in duplicate.
3. Prepared 10% Ascorbic acid: Ascorbic acid 1 g. + RO. Water make volume to 10 ml. in Erlenmeyer flask 250 ml and gently mixed. (Prepare daily , this volume for 1 flask.)
4. Prepared 2N alcoholic potassium hydroxide: weight KOH 11.2 g +RO. water make volume to 10 ml., then added 95%

ethanol 90 ml. to be 2N alcoholic potassium hydroxide in Hood (Prepare daily) Add 50 ml. KOH in amber-round bottom volumetric flask 250 ml. that contained sample. (This volume for 2 flasks.)

5. Warm in water bath, used condenser on amber-round bottom volumetric flask.

6. Measure time 30 min after boiled and gently mixed flasks in an interval of time.

7. Switch off water bath then keep them cool.

Extraction method [62]

1. Prepared amber separating funnel 250 ml

2. Added 70 mL of petroleum-hexane (2:1) in amber-round bottom flask 250 ml with sample and shake for 2 min. Let it separated in to 2 layers.

3. Add 50 mL of 5% KOH in extraction flask and repeated saponification

4. Mix the clear solution from petroleum-hexane in amber separating funnel with 5% KOH

5. Add 35 mL of petroleum-hexane (2:1) in amber-round bottom flask 250 ml. 2 times gently shake and mixed the clear solution in amber separating funnel

6. Gently shake amber separating funnel and release gas, carefully discard the lower phase

7. Add 80-100 ml of 10% NaCl in amber separating funnel, 3 times shake and release gas, carefully discard the lower phase

8. Add 80-100 ml of RO water shake and release gas, carefully discard the lower phase

9. Add a few pieces of filter paper No. 1 in amber separating funnel, pour the residue solution in a new amber-round bottom flask 250 ml for Evaporation

10. Dissolve the dried matter residue with Chloroform : Methanol (1:4) before measurement by HPLC

HPLC condition:

1. column : Reverse phase C₁₈ CAT* 201TP54 (Vitamin E = Resolve 5 μ Spherical C₁₈)
2. mobile phase: 0.5% THF in CH₃OH (Vitamin E = Absolute Methanol)
3. flow rate: 0.6 ml / min, detector: UV- Vis at 450 nm (Vitamin E = 295 nm)

5. **Analyze vitamin C by high performance liquid chromatography. (HPLC) [128]**

Chemicals

1. 2% Metaphosphoric acid
2. Nanopure water
3. Dithiothreitol acid
4. L-ascorbic acid (Standard)
5. Tang- orange juice powder (Control sample)

Appliances and glasswares

1. Volumetric flask
2. Beaker
3. Funnel
4. Analytical balance
5. Autopipette and tip
6. Volumetric pipette
7. Vortex
8. Whatman filter paper No. 1
9. Centrifuge
10. Blender
11. Reverse phase C₁₈ column

Sample preparation

1. Samples were homogenized with blender.

2. The homogenate sample (1-5 g) was added to 20 mL of 2% metaphosphoric acid and vortexed, diluted to 50 mL volumetric flask
3. Extracts were centrifuged at 1640g for 15 min at 20 °C
4. 10 mL of supernatant adjust pH to 5-5.2 with NaOH
5. Add 10 mg of dithiothreitol, shake gently for a few second
6. Keep sample in the dark for one hour
7. Dilute to 25 mL volumetric flask with 2% metaphosphoric acid, shake

HPLC method

1. Mobile phase was KH_2PO_4 (2.5g/ 450mL water) brought to pH 2.5 with K_3PO_4 .
2. The flow rate was 0.8 mL/min. and the detection wavelength was 254 nm.
3. Samples were filtered through a 0.45 μm poly-tetrafluoroethylene filter prior to injection; the injection volume was 20 μL . All samples were run in triplicate.
4. Prepare standard 1, 2, 4, 6, 8, 10 $\mu\text{g/mL}$ and inject to HPLC for drawing calibration curve
5. Read concentration of vitamin C from calibration curve

6. Standard operating protocol for total phenolic content by UV spectrometric method using gallic acid as standard.[59]

Principle

The total phenolic compounds of each fraction were determined and expressed as milligrams of gallic acid equivalents per gram of ethanol extract. Each fraction was dissolved in deionized water. Folin ciocaltau's phenol reagent was added to the sample then 10% aqueous sodium carbonate was added and allowed to stand for reaction at room temperature. Absorbance of the developed dark blue color was measured by spectrophotometer at 530 nm. Deionized water was used as blank sample.

Materials

1. Ultrasonic cleaner (Sonicate)
2. Balance, accurate to 0.0001 gram.
3. Ethanol, HPLC grade (ST Baker)
4. Water, HPLC grade
5. Sodium carbonate HPLC grade.
6. Folin-Ciocalteu's Phenol reagent (SigmaBrand P/N F-9252)
7. Gallic acid standard
8. 25 mL, 50 mL and 100 mL of volumetric flasks class A
9. 1 mL Reusable Class B volumetric pipettes
10. Auto pipette and tip
11. Microplate reader
12. Microplate 96 wells

Preparation of sample

1. Weigh 5-25 mg of sample into 25 mL volumetric flask.
2. 15 mL of 40% Ethanol is added. Mixed and then sonicate for about 30 min and shake about 10 min. Allow the extracts cool down to room temperature and fill to 25 mL with 40% Ethanol. Mix well.
3. Filter the extracts with whatman paper No. 1 and get at least 10 mL of clear solution for UV test.
4. From clear extraction solution prepared from sample preparation,
 - Transfer 1 mL to 100 mL volumetric flask with 60-70 mL deionized water. Swirl contents to mix.
 - Add 5 mL of Folin ciocalteu's phenol reagent and mix again.
 - After 1 min and before 8 min, add 15 mL of 20% Sodium carbonate, record the time as zero, and mix again.

- Make the volume to 100 mL with deionized water, stopper the flask and mix thoroughly by inverting it several times.
- After 2 hrs. (to with in 1-2 min) record the UV absorption and maximum absorbance 760 nm.
- Use the same solution without the extraction solution as blank solution

Preparation of standard

1. Accurately weigh 20 mg of gallic acid into 25 mL volumetric flask and record the weigh for standard.
2. Added 15 mL of 40% Ethanol into 25 mL volumetric flask and sonicate until no solid was present in the flask.
3. Allow the solution cool down to room temperature and fill to full volume with 40% Ethanol.
4. Mix well and make the serial dilution to 1:2, 1:4, 1:8, 1:16 and 1:32.

Method of Folin ciocalteu's reagent for phenolic content**Extraction**

5-25 mg of sample (or 20 mg of gallic acid for standard) + 15 mL Of 40% Ethanol

Mix, sonicate 30 min and shake 10 min

Make the volume to 25 mL with 40% Ethanol

Mix

Filtrate by Whatman No. 1

Preparation for determination phenol content in sample or standard

1mL of filtrate solution (or standard solution) + 60-70 mL Deionized water

Mix

Add 5 mL of Folin- Ciocalteu's phenol reagent

Mix 1-8 min

Add 15 mL of 20% Sodium carbonate

Mix

Make the volume to 100 mL with deionized water

Mix 2 hrs.

Measure at maximum absorbance at 760 nm

5. ปริมาณสระระแทนต่อนึ่งแคปซูล

สมุนไพรสระระแทน 1 กิโลกรัม Freeze Dried แล้วได้ผงหนักประมาณ 25 กรัม (24.585 g) สมุนไพรสระระแทน 1 แคปซูลมีผง Freeze Dried 500 mg

สมุนไพรสระระแทน 1 กิโลกรัมหรือ 25 g จะได้สระระแทน 50 แคปซูล

อาสาสมัครรับประทานสมุนไพรวันละ 4 แคปซูล (2 g) เท่ากับรับประทานสมุนไพรสระระแทนสดวันละ 80 g



6. Documents used in 2nd phase experiment

คำชี้แจงสำหรับผู้เข้าร่วมวิจัยและการพิทักษ์สิทธิของผู้เข้าร่วมวิจัย

เรียน ผู้เข้าร่วมวิจัยทุกท่าน

ดิฉัน ร.อ.หญิง จิราพร เชาวน์โพธิ์ทอง นักศึกษาปริญญาโท หลักสูตรโภชนาการ คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล ขณะนี้กำลังทำการศึกษาวิจัยเรื่อง “การศึกษาฤทธิ์ในการเป็นสารต้านอนุมูลอิสระของสมุนไพรไทยรสเผ็ดร้อน และฤทธิ์ในการลดภาวะออกซิเดทีฟสเตรสใน: ทหารเกณฑ์” เพื่อนำผลการศึกษาวิจัยที่ได้เป็นแนวทางในการวางแผนพัฒนา เปลี่ยนพฤติกรรมกรรมการบริโภคที่เลียนแบบชาติตะวันตกให้กลับมาเป็นแบบไทย และรณรงค์ให้มีการบริโภคอาหารไทย และสมุนไพรไทยเพิ่มมากขึ้น ตลอดจนขยายตลาดธุรกิจอาหารไทยให้เป็นที่แพร่หลาย เนื่องจากคุณประโยชน์ของสมุนไพรต่างๆ ที่เป็นส่วนประกอบของอาหารไทยหลากหลายชนิด ดังนั้นจึงใคร่ขอความกรุณาจากท่านช่วยตอบแบบสอบถาม และบันทึกอาหารที่ท่านรับประทานในแบบฟอร์มที่แจกให้ท่าน ตามความเป็นจริงที่เกี่ยวกับตัวท่าน ซึ่งข้อมูลทั้งหมดจะถูกเก็บเป็นความลับนำมาใช้ตามวัตถุประสงค์เท่านั้น

ท่านผู้เข้าร่วมการศึกษาวิจัยครั้งนี้จะได้รับการเจาะเลือด ชั่งน้ำหนักและวัดความดันโลหิตทั้งหมด 3 ครั้ง ในสัปดาห์แรก สัปดาห์ที่ 2 และสัปดาห์ที่ 8 ภายหลังจากการศึกษาเพื่อตรวจหาระดับของสารที่เกิดจากภาวะออกซิเดทีฟสเตรส ระดับไขมันในเลือดและระดับของสารต้านอนุมูลอิสระในเลือด

ท่านมีสิทธิที่จะตอบรับหรือปฏิเสธการเข้าร่วมวิจัยครั้งนี้ และเมื่อเข้าร่วมการวิจัยแล้วในระหว่างดำเนินการศึกษาวิจัย ท่านสามารถยกเลิกการเข้าร่วมวิจัยได้ตลอดเวลาตามที่ท่านต้องการ โดยไม่มีผลใดๆ ต่อหน้าที่การทำงานของท่าน หากท่านมีข้อสงสัยประการใดเกี่ยวกับงานศึกษาวิจัยครั้งนี้ ผู้วิจัยยินดีตอบคำถามของท่านให้เข้าใจตลอดเวลา

ขอขอบคุณในความร่วมมือของท่าน

ร.อ.หญิง

(จิราพร เชาวน์โพธิ์ทอง)

นักศึกษาระดับปริญญาโท

หนังสือแสดงเจตนายินยอมเข้าร่วมการวิจัย
(Consent form)

การวิจัยเรื่อง “การศึกษาฤทธิ์ในการเป็นสารต้านอนุมูลอิสระของสมุนไพรไทยรสเผ็ดร้อน และฤทธิ์ในการลด
ภาวะออกซิเดทีฟสเตรสในทหารที่กำลังฝึก”

วันให้คำยินยอม วันที่.....เดือน.....พ.ศ.....

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึงวัตถุประสงค์ของการ
วิจัย วิธีการวิจัย อันตราย หรืออาการที่เกิดขึ้นจากการวิจัย หรือจากสมุนไพรที่ใช้ รวมทั้งประโยชน์ที่จะเกิดขึ้นจาก
การวิจัยอย่างละเอียด และมีความเข้าใจแล้ว

ผู้วิจัยรับรองว่าจะตอบคำถามต่างๆที่ข้าพเจ้าสงสัยด้วยความเต็มใจ ไม่ปิดบังซ่อนเร้น จนข้าพเจ้าพอใจ

ข้าพเจ้ามีสิทธิที่จะบอกเลิกการเข้าร่วมโครงการวิจัยนี้เมื่อใดก็ได้ และเข้าร่วมโครงการวิจัยนี้โดยสมัคร
ใจ และการบอกเลิกการเข้าร่วมวิจัยไม่มีผลต่อหน้าที่การทำงานหรือการฝึกของข้าพเจ้า

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะที่เกี่ยวกับตัวของข้าพเจ้าเป็นความลับและจะเปิดเผยได้เฉพาะในรูปที่
สรุปผลการวิจัย การเปิดเผยข้อมูลของข้าพเจ้าต่อหน่วยงานต่างๆที่เกี่ยวข้อง กระทำได้ในเฉพาะกรณีจำเป็นด้วย
เหตุผลทางวิชาการเท่านั้น

ผู้วิจัยรับรองว่าหากเกิดอันตรายใดจากการวิจัยดังกล่าว ข้าพเจ้าจะได้รับการรักษาพยาบาลโดยไม่คิด
มูลค่าตามมาตรฐานการรักษาพยาบาลทั่วไป

ผู้วิจัยรับรองว่าหากมีข้อมูลเพิ่มเติมที่ส่งผลกระทบต่อการศึกษา ข้าพเจ้าจะได้รับการแจ้งให้ทราบโดยไม่
ปิดบังซ่อนเร้น

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ และได้ลงนามในใบยินยอมนี้ด้วยความ
เต็มใจ

ลงนาม พลทหาร..... ผู้ยินยอม

ลงนาม..... พยาน

ลงนาม..... ผู้วิจัย

Study No.....

แบบสอบถามข้อมูลทั่วไปของอาสาสมัคร

ชื่อพลทหาร.....นามสกุล.....อายุ..... ปี

ข้อมูลทุกอย่างที่ได้รับจากท่านจะถูกปิดเป็นความลับ ไม่เปิดเผยต่อบุคคลอื่นที่ไม่เกี่ยวข้องกับการศึกษา
นี้ ข้อมูลที่ได้จากท่านจะนำมาแปลผลและเผยแพร่ในรูปของผลการทดลองเท่านั้น

ข้อแนะนำในการตอบแบบสอบถาม

1. ตอบคำถามทุกข้อที่มีในแบบสอบถามโดยการเขียนตอบ หรือ ทำเครื่องหมาย ✓ ในช่อง ☐ ที่ตรงกับคำตอบของท่าน
2. ตอบคำถามตามความเป็นจริง โดยคำตอบที่ได้จะไม่มีผลใดๆต่อท่าน และถูกปกปิดเป็นความลับ
3. หากคำตอบที่มีไม่ตรงกับของท่าน สามารถเขียนระบุข้อมูลของท่านในช่องอื่นๆ ระบุ..... ได้

ข้อมูลทั่วไป

1. น้ำหนัก.....กิโลกรัม ส่วนสูง.....เซนติเมตร
2. ภูมิลำเนาเดิม จังหวัด.....
3. ท่านจบการศึกษาระดับใด

☐ ประถมศึกษา ป.1-ป.6	☐ มัธยมศึกษาปีที่ 1-3
☐ มัธยมศึกษาปีที่ 4-6	☐ ปวช.
☐ ปวส.	☐ ปริญญาตรี
☐ กำลังศึกษาในระดับชั้น.....	
☐ อื่นๆ ระบุ.....	
4. โรคประจำตัวของท่านคือ

☐ ลมชัก	☐ ไต
☐ เบาหวาน	☐ หัวใจ
☐ ความดันโลหิตสูง	☐ ภูมิแพ้
☐ อื่นๆ ระบุ.....	☐ ไม่มีโรคประจำตัว

5. ยาที่ท่านใช้เป็นประจำ คือ

- | |
|--|
| ☐ ยาแก้ปวด ลดไข้ เช่น พาราเซตามอล, แอสไพริน |
| ☐ ยาบรรเทาอาการหวัด คัดจมูก น้ำมูกไหล เช่น แอ็กติเฟด, ซีพีเอ็ม (คลอเฟนิรามีน) |
| ☐ ยาบำรุงเลือด ได้แก่..... |
| ☐ ยาบำรุงกำลัง ได้แก่..... |
| ☐ วิตามินต่างๆ ได้แก่..... |

- ☐ สมุนไพร หรือยาหม้อ ได้แก่.....
- ☐ ยารักษาโรคประจำตัว ได้แก่.....
- ☐ อาหารเสริม ได้แก่.....
- ☐ ยาอื่นๆ ระบุ.....
6. ท่านสูบบุหรี่หรือไม่
- ☐ ไม่สูบ
- ☐ เคยสูบแต่ปัจจุบันเลิกสูบแล้ว ระยะเวลาที่เลิกสูบคือ.....
- ☐ สูบ
- จำนวนที่สูบคือ ☐ 1-10 มวนต่อวัน
- ☐ 10-20 มวนต่อวัน
- ☐ มากกว่า 20 มวนต่อวัน
- ☐ อื่นๆ ระบุ.....
7. ท่านดื่มเครื่องดื่มที่มีแอลกอฮอล์ (เหล้า เบียร์ ไวน์) หรือไม่
- ☐ ไม่ดื่ม ☐ ดื่ม
8. ชนิดของเครื่องดื่มแอลกอฮอล์ที่ท่านดื่มเป็นประจำ
- ☐ สุรา ☐ สุราพื้นบ้านทำเอง เช่น เหล้าขาว, กระจ่าง
- ☐ ไวน์ ☐ ไวน์พื้นบ้านทำเอง
- ☐ เบียร์ ☐ เหล้าโรง, เชียงขุน
- ☐ อื่นๆ ระบุ.....
- จำนวนครั้งที่ดื่มต่อสัปดาห์คือ
- ☐ 1-2 ครั้ง ☐ 3-4 ครั้ง
- ☐ ทุกวัน ☐ อื่นๆ ระบุ.....
- ปริมาณที่ดื่มในแต่ละครั้ง
- ☐ 1-2 แก้ว ☐ 3-4 แก้ว
- ☐ มากกว่า 4 แก้ว ☐ อื่นๆ ระบุ.....
9. ท่านมีอาการป่วยครั้งสุดท้ายเมื่อใด
- ☐ กำลังป่วยอยู่ ☐ สัปดาห์ที่ผ่านมา
- ☐ เดือนที่ผ่านมา ☐ แข็งแรงดีตลอด
10. หากท่านเคยป่วย (จากคำถามข้อ 9) อาการป่วยของท่านคือ.....
- ท่านต้องเข้ารับการรักษาในโรงพยาบาล (นอนที่โรงพยาบาล) หรือไม่
- ☐ รักษาตัวในโรงพยาบาล
- ☐ ไม่ต้องรักษาตัวในโรงพยาบาล

11. ก่อนที่ท่านจะมาเกณฑ์ทหาร ท่านเคยออกกำลังกายหรือไม่

☐ เคย โดยวิธีการ.....

จำนวนครั้งที่ท่านออกกำลังกายต่อสัปดาห์

☐ 1-2 ครั้งต่อสัปดาห์

☐ 3-4 ครั้งต่อสัปดาห์

☐ ทุกวัน

☐ อื่นๆ ระบุ.....

☐ ไม่เคย



Example of Food Record [129]

Study No.

ตัวอย่างแบบบันทึกรายการอาหารที่รับประทาน

ชื่อพลทหาร.....กล้าหาญ.....นามสกุล.....เชี่ยวชาญรบ.....วันที่..... 16 ตุลาคม 2547.....

ข้อเสนอแนะในการบันทึก

1. บันทึกอาหารทุกมื้อ ทุกชนิด รวมทั้งขนมและเครื่องดื่มที่ท่านรับประทานตลอดวันตั้งแต่ท่านตื่นนอนจนเข้านอน (เฉพาะที่ท่านรับประทานเท่านั้น)
2. อาหารหรือ ขนมหวานต่างๆ ให้บันทึกอาหารที่รับประทานทั้งที่บ้านและนอกบ้านถ้าเป็นมื้อพิเศษ ให้ระบุด้วย เช่น งานเลี้ยงแต่งงาน เป็นต้น
3. ข้อความต่อไปนี้เป็นสิ่งจำเป็นในการบันทึก
 - 3.1 ระบุเครื่องประกอบของอาหารแต่ละชนิด พร้อมทั้งปริมาณ โดยของแข็งให้ระบุเป็นช้อนตวงหรือทัพพี ส่วนของเหลวระบุเป็นช้อนหรือระบุตามที่ตวงขวด ที่ใช้อยู่ในบ้าน ถ้าไม่สามารถประมาณปริมาณได้ให้พยายามบันทึกในรูปขนาด เช่น ขนาดเล็ก กลาง ใหญ่ หรือ ขนาดกว้างยาวของอาหาร เช่น รับประทานผัดเปรี้ยวหวาน ต้องระบุว่ารับประทานมากกว่า 4 ช้อนโต๊ะ มะเขือเทศ 2 ช้อนโต๊ะ เนื้อหมู 2 ช้อนโต๊ะ หรือระบุว่ารับประทานมากกว่าประมาณ ครึ่งลูกใหญ่ มะเขือเทศ 1 ลูกเล็ก เนื้อหมู 5 ช้อน ขนาดชิ้นละ 1 x 2 เซนติเมตร เครื่องดื่มควรระบุเป็นปริมาตร หรือขนาด เช่น โคล่า 1 ขวดกลาง หรือ 290 ซีซี เป็นต้น
 - 3.2 อาหารที่รับประทานปรุงอย่างไร เช่น ปลาทอด ไก่ย่าง เป็นต้น
 - 3.3 การเติมน้ำตาล น้ำเชื่อม เกลือ หรือน้ำปลา ลงในเครื่องดื่ม ให้ระบุปริมาณด้วย เช่น น้ำตาล 2 ช้อนชา ในกาแฟ 1 แก้ว
 - 3.4 ระบุปริมาณน้ำดื่ม และปริมาณน้ำจากอาหารที่รับประทานทุกมื้อ

ตัวอย่างการบันทึกอาหาร

16 ตุลาคม 2547

วันที่.....

มื้ออาหารและ สถานที่	เวลา	ประเภท	อาหาร	ปริมาณ
เช้า, ที่บ้าน	7.00 น.	ข้าวต้มกุ้ง	ข้าวต้ม (เนื้อข้าว)	2 ทัพพี
			กุ้งน้ำจืด	2 ช้อนโต๊ะ (5 ตัว)
			น้ำข้าว	½ ถ้วยตวง (120 ซีซี)
			น้ำตาล	1 ช้อนชา
		ปลาทูทอด	ปลาทู	1 ตัวกลาง
			น้ำมันพืช (กุก)	1 ช้อนโต๊ะ
		กุนเชียงทอด	กุนเชียง	2 ช้อนโต๊ะ
			น้ำมันพืช (กุก)	1 ช้อนโต๊ะ
		กาแฟ	กาแฟ	1 ช้อนชา
			น้ำตาลทราย	2 ช้อนชา
			นมสด (ตราหมี)	2 ช้อนโต๊ะ
			น้ำ	150 ซีซี
อาหารว่าง, ที่ ทำงาน	10.00 น.	สาเกูปะยก	สาเก	½ ถ้วยตวง
			เผือก	1 ช้อนโต๊ะ
			น้ำตาล	1 ช้อนโต๊ะ
กลางวัน, ที่ทำงาน	12.00 น.	ก๋วยเตี๋ยวเนื้อ	เส้นใหญ่	1 ทัพพี
			ถั่วงอก	2 ช้อนโต๊ะ
			ลูกชิ้นเนื้อ	5 ลูก
			น้ำมันกระเทียมเจียว	2 ช้อนชา
			น้ำก๋วยเตี๋ยว	½ ถ้วยตวง (120 ซีซี)
			น้ำตาลทราย (ปรุงรส)	2 ช้อนชา
		ปอเปี๊ยะสด	แผ่นแป้งปอเปี๊ยะ	3 แผ่น
			กุนเชียง	2 ช้อนชา
			เต้าหู้	2 ช้อนโต๊ะ
			ถั่วงอก	2 ช้อนโต๊ะ
			เนื้อปู	1 ช้อนโต๊ะ

มื้ออาหารและ สถานที่	เวลา	ประเภท	อาหาร	ปริมาณ
			น้ำราด (รสค่อนข้าง หวาน)	1 ช้อนโต๊ะ
		สับปะรด	สับปะรดขนาด 2 x 3 นิ้ว	1 ชิ้น
			น้ำตาลทราย	1 ช้อนชา
		โคล่า	โคล่า ขวดกลาง	290 ซีซี
อาหารว่าง, ที่ ทำงาน	15.00 น.	กล้วยบวชชี	กล้วยน้ำว้า (1 ลูกผ่า 4)	4 ชิ้น
			น้ำกะทิ	4 ช้อนโต๊ะ
			น้ำตาลทราย	2 ช้อนโต๊ะ
		น้ำเปล่า	น้ำ	½ แก้ว (120 ซีซี)
อาหารเย็น, ที่บ้าน	18.00 น.	ข้าวสวย	ข้าว	2 ทัพพี
		ต้มยำไก่ใส่เห็ด	เนื้อไก่	2 ช้อนโต๊ะ
			เห็ดฟาง	2 ช้อนโต๊ะ
			น้ำต้มยำ	1/4 ถ้วยตวง (60 ซีซี)
			มะนาว	1 ช้อนโต๊ะ
			น้ำตาล	2 ช้อนชา
		ผัดผักคะน้าหมู กรอบ	ผัดคะน้า	1 ถ้วยตวง
			หมูกรอบ	2 ช้อนโต๊ะ
			น้ำมันพืช (กูก)	1 ช้อนโต๊ะ
		ไข่เจียวหมูสับ	ไข่ไก่	1 ฟอง
			หมูสับ	1 ช้อนโต๊ะ
			น้ำมันพืช (กูก)	2 ช้อนโต๊ะ
อาหารว่าง, ที่บ้าน	23.00 น.	ส้มเขียวหวาน	ส้มเขียวหวาน ขนาด เส้นผ่าศูนย์กลาง 2 ½ นิ้ว	2 ลูก
ก่อนนอน, ที่บ้าน		นมสด	นมสด หนองโพ ชนิด หวาน	1 กล่อง (240 ซีซี)

FOOD RECORD FORM

Study No.

แบบบันทึกรายการอาหารที่รับประทาน

ชื่อพลทหาร.....นามสกุล.....วันที่.....

ข้อแนะนำในการบันทึก

1. บันทึกอาหารทุกมื้อ ทุกชนิด รวมทั้งขนมและเครื่องดื่มที่ท่านรับประทานตลอดวันตั้งแต่ท่านตื่นนอนจนเข้านอน (เฉพาะที่ท่านรับประทานเท่านั้น)
2. อาหารหรือ ขนมหวานต่างๆ ให้บันทึกอาหารที่รับประทานทั้งที่บ้านและนอกบ้านถ้าเป็นมือพิเศษ ให้ระบุด้วย เช่น งานเลี้ยงแต่งงาน เป็นต้น
3. ข้อความต่อไปนี้เป็นสิ่งจำเป็นในการบันทึก
 - 3.1 ระบุเครื่องประกอบของอาหารแต่ละชนิด พร้อมทั้งปริมาณ โดยของแข็งให้ระบุเป็นช้อนตวงหรือทัพพี ส่วนของเหลวระบุเป็นช้อนชี่หรือระบุตามที่ตวงขวด ที่ใช้อยู่ในบ้าน ถ้าไม่สามารถประมาณปริมาณได้ให้พยายามบันทึกในรูปขนาด เช่น ขนาดเล็ก กลาง ใหญ่ หรือขนาดกว้างยาวของอาหาร เช่น รับประทานผัดเปรี้ยวหวาน ต้องระบุว่ารับประทานมากกว่า 4 ช้อนโต๊ะ มะเขือเทศ 2 ช้อนโต๊ะ เนื้อหมู 2 ช้อนโต๊ะ หรือระบุว่ารับประทานมากกว่าประมาณ ครึ่งลูกใหญ่ มะเขือเทศ 1 ลูกเล็ก เนื้อหมู 5 ชั้น ขนาดชิ้นละ 1 x 2 เซนติเมตร เครื่องดื่มควรระบุเป็นปริมาตร หรือขนาด เช่น โคล่า 1 ขวดกลาง หรือ 290 ซีซี เป็นต้น
 - 3.2 อาหารที่รับประทานปรุงอย่างไร เช่น ปลาทอด ไก่ย่าง เป็นต้น
 - 3.3 การเติมน้ำตาล น้ำเชื่อม เกลือ หรือ น้ำปลา ลงในเครื่องดื่ม ให้ระบุปริมาณด้วย เช่น น้ำตาล 2 ช้อนชา ในกาแฟ 1 แก้ว
 - 3.4 ระบุปริมาณน้ำดื่ม และปริมาณน้ำจากอาหารที่รับประทานทุกมื้อ

วันที่.....

[illegible]

Study No.....

แบบสอบถามเกี่ยวกับความถี่ในการรับประทานอาหารในช่วง 6 เดือนที่ผ่านมาของอาสาสมัคร

ข้อมูลทุกอย่างที่ได้รับจากท่านจะถูกปิดเป็นความลับ ไม่เปิดเผยต่อบุคคลอื่นที่ไม่เกี่ยวข้องกับการศึกษา
นี้ ข้อมูลที่ได้จากท่านจะนำมาแปลผลและเผยแพร่ในรูปของผลการทดลองเท่านั้น

ขอแนะนำในการตอบแบบสอบถาม

1. ตอบคำถามทุกข้อที่มีในแบบสอบถามโดยการเขียนตอบ หรือ ทำเครื่องหมาย ✓ ในช่องว่าง ที่ตรงกับคำตอบของท่าน
2. ตอบคำถามตามความเป็นจริง โดยคำตอบที่ได้จะไม่มีผลใดๆต่อท่าน และถูกปิดเป็นความลับ
3. หากคำตอบที่มีไม่ตรงกับของท่าน สามารถเขียนระบุข้อมูลของท่านในช่องว่างได้

ตัวอย่างการบันทึกความถี่ในการรับประทานอาหารในช่วง 6 เดือนที่ผ่านมาของอาสาสมัคร

กส้าหาญ เชี่ยวชาญรบ
ชื่อพลทหาร.....นามสกุล.....

ประเภทของอาหาร	ความถี่ในการรับประทาน			
	ไม่ รับประทาน	เดือนละ 1-2 ครั้ง	สัปดาห์ละ 1-2 ครั้ง	รับประทาน ทุกวัน
ประเภทผัก				
ผักใบเขียว ได้แก่ คะน้า, กวางตุ้ง ผักหวาน, ตำลึง, ผักบุ้ง ฯลฯ			✓ คะน้า	
ผักที่มีสีเหลือง ส้ม แดง ได้แก่ แครอท, ฟักทอง, มะเขือเทศ ฯลฯ		✓ ฟักทอง		
ผักสมุนไพรรับประทานใบและผล ได้แก่ กะเพรา , โหระพา, พริก, สะระแหน่, ใบแมงลัก, ใบ มะกรูด, ผักชี, ต้นหอม ฯลฯ				✓ พริก
ประเภทผลไม้				
ผลไม้รสหวานจัด ได้แก่ ทุเรียน, ขนุน, มะขาม หวาน, เงาะ, ทุเรียน, ลำไย, มะม่วงสุก, ลิ้นจี่, น้อยหน่า, ละมุด ฯลฯ		✓ เงาะ, ทุเรียน		
ชนิดของอาหาร				
ผัดกะเพรา			✓	
น้ำพริกประเภทต่างๆ+ผักจิ้ม			✓	

แบบสอบถามเกี่ยวกับความถี่ในการรับประทานอาหารในช่วง 6 เดือนที่ผ่านมาของอาสาสมัคร

Study No..... ชื่อ พลทหาร.....นามสกุล.....

ประเภทของอาหาร	ความถี่ในการรับประทาน			
	ไม่รับประทาน	เดือนละ 1-2 ครั้ง	สัปดาห์ละ 1-2 ครั้ง	รับประทาน ทุกวัน
ประเภทผัก				
ผักใบเขียว ได้แก่ คะน้า, กวางตุ้ง ผักหวาน, ตำลึง, ผักบุ้ง ฯลฯ				
ผักที่มีสีเหลือง ส้ม แดง ได้แก่ แครอท, ฟักทอง, มะเขือเทศ ฯลฯ				
ผักใบกบ ได้แก่ กะหล่ำปลี, กะหล่ำดอก, ผักกาดขาว, ผักกาดเขียว ฯลฯ				
ผักสมุนไพรรับประทานราก ต้น หรือหัว ได้แก่ จิง, ข่า, กระชาย, ตะไคร้, หอมแดง , หอมหัวใหญ่, กระเทียม ฯลฯ				
ผักสมุนไพรรับประทานใบและผล ได้แก่ กะเพรา, โหระพา, พริก, สะระแหน่, ใบ แมงลัก, ใบมะกรูด, ผักชี, ต้นหอม ฯลฯ				
ผักอื่นๆ ได้แก่ แตงกวา, มะเขือ, ถั่วต่างๆ, บวบ, ฟักเขียว, ข้าวโพดอ่อน ฯลฯ				
ประเภทผลไม้				
ผลไม้รสหวานจัด ได้แก่ ทุเรียน, ขนุน, มะขามหวาน, เงาะ, ทุเรียน, ลำไย, มะม่วง สุก, ลิ้นจี่, น้อยหน้า, ละมุด ฯลฯ				
ผลไม้รสไม่หวานจัด ได้แก่ ส้ม, พุทรา, แตงโม, แคนตาลูป, แอปเปิ้ล, สับปะรด, สาลี่, มังคุด, ชมพู, ฝรั่ง, มะพร้าวอ่อน, มะละกอ, กล้วยต่างๆ ฯลฯ				

ประเภทของอาหาร	ความถี่ในการรับประทาน			
	ไม่ รับประทาน	เดือนละ 1-2 ครั้ง	สัปดาห์ละ 1-2 ครั้ง	รับประทาน ทุกวัน
ชนิดของอาหาร				
ผักกะเพรา				
ผัดขิง				
ผัดเผ็ด				
แกงเลียง				
แกงป่า				
แกงส้ม				
ต้มยำ, ต้มข่า				
แกงเผ็ด, ญี่เอ่				
ยำต่างๆ				
พล่า ลาบ ก้อย				
เมี่ยงต่างๆ เช่น เมี่ยงคำ				
ขนมจีน + น้ำพริกหรือน้ำยาต่างๆ				
น้ำพริกประเภทต่างๆ+ผักจิ้ม				

อาหารที่ท่านชอบและรับประทานบ่อยๆ คือ

.....

.....

ผักที่ท่านชอบและรับประทานบ่อยๆ คือ

.....

.....

ผลไม้ที่ท่านชอบและรับประทานบ่อยๆ คือ

.....

.....

แบบบันทึกการทำกิจกรรมต่างๆในแต่ละวันของทหารที่กำลังฝึก

คำแนะนำในการบันทึก

ทำการบันทึกเวลาและกิจกรรมทุกอย่างที่ทำตั้งแต่ตื่นเช้า จนเข้านอน โดยบันทึกทั้งหมด 2 วัน คือ วันที่มีการฝึกทหาร 1 วัน และวันหยุด 1 วัน

ตัวอย่างการบันทึก

วันที่ 16 ตุลาคม 2547 (วันธรรมดา / วันหยุด) ✓

เวลา	กิจกรรม
05.00	ตื่นนอน
05.30 – 05.10	ทำเตียง
05.10 – 05.40	วิ่งลงบันได 15 ชั้น รวมแถว, ฝึกกระเบียด
05.40 – 06.30	วิ่งเหยาะๆออกกำลังพร้อมกับร้องเพลงฝึก
06.30 – 07.30	อาบน้ำแปรงฟัน เดินขึ้นบันไดแต่งชุดฝึก
07.30 – 08.00	วิ่งลงบันไดรวมแถว เดิน 500 เมตร ไปตั้งแถว เตรียมเคารพธงชาติ, ร้องเพลงชาติ
08.00 – 08.10	เดินแถวประมาณ 500 เมตร
08.10 – 08.50	นั่งรับประทานอาหารเช้า
08.50 – 09.00	เข้าห้องน้ำ ปัสสาวะ
09.00 – 09.30	วิ่งรวมแถว ขึ้นกลางแดดฟังตารางการฝึกในวันนี้
09.30 – 10.00	เดินฝึกกระเบียดแถวกลางแดด
10.00 – 10.15	นั่งพักใต้ต้นไม้ ดื่มน้ำ คุยกับเพื่อน
10.15 – 10.30	วิ่งรวมแถว
10.30 – 11.30	เดินฝึกท่ามือเปล่ากลางแดด
11.30 – 11.45	วิ่งรวมแถว
11.45 – 12.00	เดินแถวกลางแดดไปโรงอาหารประมาณ 500 เมตร
12.00 – 12.30	นั่งรับประทานอาหารเช้าในโรงอาหาร
12.30 – 13.00	เข้าห้องน้ำ ปัสสาวะ และนั่งพักในร่ม
13.00 – 13.15	วิ่งรวมแถวใต้ต้นไม้
13.15 – 14.00	เดินฝึกท่ามือเปล่า ในโรงฝึกในร่ม
14.00 – 14.30	วิ่งฝึกกระเบียดแถว ในโรงฝึก
14.30 – 14.45	นั่งพักในโรงฝึก ดื่มน้ำ คุยกับเพื่อน

✓

วันที่ 16 ตุลาคม 2547 (วันธรรมดา / วันหยุด)

เวลา	กิจกรรม
14.45 – 15.00	วิ่งรวมแถว
15.00 – 16.00	เลิกแถว เดินเก็บใบไม้ กลางแดด
16.00 – 16.30	เดินขึ้นบันได นั่งพักผ่อน คุยกับเพื่อน
16.30 – 17.00	วิ่งลงบันได รวมแถว วิ่งแคดร้าไร รอบกองหิน ประมาณ 2.4 กิโลเมตร
17.00 – 17.10	พักแถว เดินระยะทาง 500 เมตร ได้ร่มไม้
17.10 – 17.30	นั่งรับประทานอาหารเย็น
17.30 – 18.00	เดินกลับที่พัก 500 เมตร เดินขึ้นบันได 15 ชั้นนั่งพักคุยกับเพื่อน
18.00 – 18.30	เดินลงบันได ซักผ้า
18.30 – 18.40	อาบน้ำ เดินขึ้นบันได แต่งตัวชุดลำลอง
18.40 – 19.40	นั่งพักผ่อน ดูโทรทัศน์ คุยกับเพื่อน
19.40 – 19.45	เดินลงบันได รวมแถว
19.45 – 20.00	ขึ้นสวดมนต์
20.00 – 20.15	เข้าห้องน้ำ ปัสสาวะ แปรงฟัน
20.15 – 20.30	เดินขึ้นบันได เข้านอน

แบบบันทึกข้อมูลที่ได้จากการสัมภาษณ์แม่ครัว

ชื่อผู้ให้สัมภาษณ์ จสอ. ประเสริฐ อุทะมะกุล

รายการอาหารที่จัดใน 1 สัปดาห์

อาหารจะถูกจัดเป็นวงรอบ วงรอบละ 10 วัน

ยอดประกอบเลี้ยงมีโต๊ะ 360 คน มีพลทหาร 270 คน

วัน	รายการอาหาร
จันทร์	เช้า - แกงจืดผักกาดดอง กลางวัน - ต้มโคล้งปลากรอบ, ผัดหมี เย็น - แกงจืดผักกาดขาวลูกชิ้นปลา, ผัดกะเพราไก่ใส่ถั่วฝักยาว, ถั่วเขียวต้มน้ำตาล
อังคาร	เช้า - แกงจืดวุ้นเส้น กลางวัน - ต้มกะทิใส่ปลาหมึก, ไข่เจียว เย็น - แกงเผ็ดหน่อไม้ดองไก่, ปลาเค็มทอด, ขนมสาเกูปะขี้ข้าวโพด
พุธ	เช้า - แกงจืดผักกวางตุ้งไก่ กลางวัน - ผัดผักกาดดองไก่, แกงเผ็ดหน่อไม้ต้มมะเขือพวง เย็น - ผัดพริกขิงหัวหมูถั่วฝักยาว, แกงจืดผักกาดดองไก่, ขนมเต้าส่วน
พฤหัสบดี	เช้า - ต้มผักกาดดองเครื่องในหมู กลางวัน - แกงส้มมะละกอปลาชุกทอด, ปลาเค็มทอด เย็น - ต้มโคล้งปลากรอบ, ไข่ต้ม, ขนมเปี๊ยะ
ศุกร์	เช้า - แกงจืดวุ้นเส้นไก่ กลางวัน - ต้มฟักมะนาวดองไก่, ผัดกะเพราหอยแมลงภู่อั่วฝักยาว เย็น - แกงเลียงไก่, ไข่ต้ม, ขนมถั่วเขียวต้มน้ำตาล
เสาร์	เช้า - ต้มผักกาดดองเครื่องในหมู กลางวัน - แกงเผ็ดหน่อไม้ดองไก่, กุนเชียงทอด เย็น - แกงส้มผักกวางตุ้งปลาหมึกสด, ปลาเค็มทอด, ขนมไข่
อาทิตย์	เช้า - แกงจืดผักกาดขาวลูกชิ้นปลา กลางวัน - ต้มโคล้งปลากรอบ, ไข่ต้ม เย็น - แกงจืดผักกวางตุ้ง, ผัดกะเพราหอยแมลงภู่อั่วฝักยาว, ขนมโดนัท

รายละเอียดของเครื่องปรุงในการทำอาหาร

วันที่ / มื้อ	รายการอาหาร	เครื่องปรุง	ปริมาณที่ใช้
1 พ.ค. 48			
เช้า	ข้าวสวย	ข้าวสาร	40 Kg
	แกงจืดผักกวางตุ้งไก่	ผักกวางตุ้ง	15 Kg
		อกไก่	4 Kg
		เกลือป่น	100 g
		น้ำปลา	1050 cc
		ซีอิ๊วขาว	700 cc
		ผงชูรส	70 g
กลางวัน	ข้าวสวย	ข้าวสาร	40 Kg
	แกงส้มมะละกอบลาคูสด	มะละกอดิบ	15 Kg
	ปลาเค็มทอด	ปลาคูสด	5 Kg
		ปลาช่อนทะเลเค็ม	5 Kg
		น้ำพริกแกงส้ม	3 Kg
		มะขามเปียก	3 Kg
		กระชายหัว	1 Kg
		ผงชูรส	70 g
		พริกไทยป่น	40 g
		ซีอิ๊วขาว	700 cc
		เกลือป่น	100 g
		น้ำปลา	650 cc
		น้ำมันพืช	1.6 Lt
เย็น	ข้าวสวย	ข้าวสาร	40 Kg
	แกงป่าไก่	น้ำพริกแกงป่า	4 Kg
	ไข่ไก่ต้ม	มะเขือพวง	10 Kg
	ขนมเปียะ	หน่อไม้ฝอย	10 Kg
		ถั่วฝักยาว	10 Kg
		ใบมะกรูด	100 g
		ใบกะเพรา	1 Kg
		น้ำตาลปีบ	3 Kg
		กระชายหั่นฝอย	1 Kg

วันที่ / มื้อ	รายการอาหาร	เครื่องปรุง	ปริมาณที่ใช้
(1 พ.ค. 48)		น้ำปลาผสมตราโบว์แดง	1.1 Lt
		อกไก่	6 Kg
		ไข่ไก่	360 ฟอง
		ผงชูรส	70 g
		ขนมเปียะ	360 อัน
2 พ.ค.48			
เช้า	ข้าวสวย	ข้าวสาร	40 Kg
	แกงจืดผักกาดดองไก่	ผักกาดดอง	20 Kg
		อกไก่	6 Kg
		ตังถ่าย	2 Kg
		ซีอิ๊วขาว	700 cc
		ซอสถั่วเหลืองภูเขาทอง	700 cc
		รสดี	40 g
		พริกไทยป่น	40 g
		กระเทียมเจียวสำเร็จรูป	500 g
		ผงชูรส	70 g
กลางวัน	ข้าวสวย	ข้าวสาร	40 Kg
	ต้มโคล้งปลากรอบ	ปลาล้าง	10 Kg
	ผัดหมี่	ข้าว	200 g
		ตะไคร้	200 g
		พริกขี้หนูแห้ง	1 Kg
		ใบมะกรูด	50 g
		น้ำมะนาว	2.9 Lt
		ผักชีฝรั่ง	1 Kg
		พริกขี้หนูสด	2 Kg
		เส้นหมี่	5 Kg
		ซอสแดง	500 g
		ถั่วงอก	5 Kg
		ใบกุยช่าย	1 Kg
		เต้าหู้เหลืองแผ่น	10 แผ่น (แผ่นละ 155g)
		กระเทียม	1 Kg
		เต้าเจี้ยว	680 cc
		น้ำส้มสายชู	600 cc

วันที่ / มื้อ	รายการอาหาร	เครื่องปรุง	ปริมาณที่ใช้
(2 พ.ค.48)		น้ำมันพืช	2.4Lt
		หอมใหญ่	1 Kg
		มะเขือเทศ	1 Kg
		ผงชูรส	70 g
		ซีอิ้วขาว	1.4 Lt
		น้ำปลา	1.14 Lt
		ซอสถั่วเหลืองภูเขาทอง	1.4 Lt
		น้ำตาลทราย	354 g
		เกลือป่น	100 g
เย็น	ข้าวสวย	ข้าวสาร	40 Kg
	แกงจืดผักกาดขาวลูกชิ้นปลา	ผักกาดขาว	30 Kg
	ผัดกะเพราไก่ถั่วฝักยาว	ลูกชิ้นปลาเล็ก	7 Kg
	ถั่วเขียวคั่วมน้ำตาล	ถั่วฝักยาว	25 Kg
		อกไก่	5 Kg
		กระเทียม	1 Kg
		พริกชี้หนูแดง	1.5 Kg
		ใบกะเพรา	1 Kg
		น้ำมันพืช	1.6 Lt
		ต้นหอม	1 Kg
		น้ำตาลทราย	350 g
		น้ำตาลทราย (ขนม)	5 Kg
		เกลือป่น	100 g
		ถั่วเขียว	7 Kg
		น้ำปลา	1.14 Lt
		ซีอิ้วขาว	700 cc
		ซอสถั่วเหลืองภูเขาทอง	700 cc
		ผงชูรส	70 g
		รสดี	30 g
3 พ.ค. 48			
เช้า	ข้าวสวย	ข้าวสาร	40 Kg
	แกงจืดวุ้นเส้นไก่	วุ้นเส้น	4 Kg
		อกไก่	7 Kg
		ต้นหอม	1 Kg

วันที่ / มื้อ	รายการอาหาร	เครื่องปรุง	ปริมาณที่ใช้
(3 พ.ค. 48)		ตั้งน้ำ	200 g
		เกลือป่น	100 g
		ซีอิ้วขาว	700 cc
		ซอสถั่วเหลืองภูเขาทอง	700 cc
		ผงชูรส	70 g
กลางวัน	ข้าวสวย	ข้าวสาร	40 Kg
	ต้มกะทิฟักใส่ปลาทุ	ฟักเขียว	35 Kg
	ไข่เจียว	ปลาทุ	8 Kg
		กะทิขาวเกาะ	12 Kg
		ใบมะกรูด	50 g
		น้ำตาลปีบ	2 Kg
		น้ำมันพืช	1.8Lt
		กะปิ	1 Kg
		หอมแดง	1 Kg
		มะขามเปียก	3 Kg
		ไข่ไก่	360 ฟอง
		ผงชูรส	70 g
		ซอสถั่วเหลืองภูเขาทอง	700 cc
		ซีอิ้วขาว	700 cc
		น้ำปลา	1.4 Lt
		เกลือป่น	100 g
3 พ.ค. 48			
เย็น	ข้าวสวย	ข้าวสาร	40 Kg
	แกงเผ็ดหน่อไม้ดองไก่	สาหร่ายเม็ดเล็ก	5 Kg
	ปลาเค็มทอด	หน่อไม้ดอง	30 Kg
	สาหร่ายเปียกข้าวโพด	กะทิขาวเกาะ	22 Kg
		น้ำพริกแกงคั่ว	6 Kg
		อกไก่	10 Kg
		น้ำปลา	1.4 Lt
		น้ำตาลปีบ	4 Kg
		ใบมะกรูด	100 g
		ปลาช่อนทะเลเค็ม	11 Kg
		น้ำมันพืช	3.2 Lt

วันที่ / มือ	รายการอาหาร	เครื่องปรุง	ปริมาณที่ใช้
(3 พ.ค. 48)		ข้าวโพดดิบ	5 Kg
		น้ำตาลทราย	10 Kg
		ผงชูรส	70 g
		ซอสถั่วเหลืองภูเขาทอง	700 cc
		ซีอิ๊วขาว	700 cc
4 พ.ค. 48			
เช้า	ข้าวสวย	ข้าวสาร	40 Kg
	แกงจืดผักกวางตุ้ง	ผักกวางตุ้ง	40 Kg
		อกไก่	10 Kg
		ตังกาย	200 g
		น้ำปลา	1.14 Lt
		เกลือป่น	100 g
		ซีอิ๊วขาว	700 cc
		ผงชูรส	70 g
กลางวัน	ข้าวสวย	ข้าวสาร	40 Kg
	พะแนงน่องไก่มะเขือพวง	น่องไก่	370 น่อง
	ผัดผักกาดดองไก่	กะทิขาวเกาะ	20 Kg
		มะเขือเทศลูกเล็ก	6 Kg
		น้ำพริกแกงเผ็ด	6 Kg
		มะเขือพวง	8 Kg
		ใบโหระพา	2 Kg
		ผักกาดดอง	45 Kg
		น้ำมันพืช	0.8 Lt
		กระเทียม	1 Kg
		ไข่ไก่	180 ฟอง
		ผงชูรส	140 g
		ซีอิ๊วขาว	1.4 Lt
		ซอสถั่วเหลืองตราภูเขาทอง	1.4 Lt
		น้ำปลา	2.25 Lt
		น้ำตาลปีบ	3 Kg
		เกลือป่น	100 g
เย็น	ข้าวสวย	ข้าวสาร	40 Kg
	ผัดพริกขิงหัวหมูใส่ถั่วฝักยาว	ถั่วฝักยาว	30 Kg

วันที่ / มื้อ	รายการอาหาร	เครื่องปรุง	ปริมาณที่ใช้
(4 พ.ค. 48)	แกงจืดผักกาดขาวไก่	น้ำพริกแกงเผ็ด	5 Kg
	เต้าส่วน	หัวหมูต้ม	10 Kg
		ใบมะกรูด	100 g
		ผักกาดขาว	40 kg
		อกไก่	8 Kg
		น้ำปลา	2.27 Lt
		กระเทียมดอง	1 Kg
		แป้งท้าว	5 Kg
		น้ำตาลทราย	350 g
		ถั่วเขียวผ่าซีก	5 Kg
		เกลือป่น	100 g
		น้ำมันพืช	0.84 Lt
		ผงชูรส	140 g
		ซีอิ๊วขาว	1.4 Lt
		ซอสถั่วเหลืองตราภูเขาทอง	1.4 Lt
		น้ำตาลทราย (ขนม)	10 Kg
5 พ.ค. 48			
เช้า	ข้าวสวย	ข้าวสาร	40 Kg
	ต้มผักกาดดองเครื่องในหมู	ผักกาดดอง	30 Kg
		ปอดหมู	15 kg
		อกไก่	1 Kg
		ตังน่าย	200 g
		ผงชูรส	70 g
		ซีอิ๊วขาว	0.7 Lt
		ซอสถั่วเหลืองตราภูเขาทอง	0.7 Lt
		เกลือป่น	100 g
		รสดี	30 g
กลางวัน	ข้าวสวย	ข้าวสาร	40 Kg
	แกงส้มมะละกอปลาดุกทอด	มะละกอดิบ	40 Kg
	ปลาเค็มทอด	น้ำพริกแกงส้ม	6 Kg
		มะขามเปียก	6 Kg
		น้ำตาลปีบ	5 Kg
		ปลาดุกชิ้น	13 kg

วันที่ / มื้อ	รายการอาหาร	เครื่องปรุง	ปริมาณที่ใช้
(5 พ.ค. 48)		กระชายหัว	2 Kg
		น้ำปลา	1.14 Lt
		ปลาช่อนทะเลเค็ม	10 Kg
		น้ำมันพืช	3.2 Lt
		ผงชูรส	70 Kg
		ซอสถั่วเหลืองตราภูเขาทอง	0.7 Kg
		ซีอิ๊วขาว	0.7 Kg
เย็น	ข้าวสวย	ข้าวสาร	40 Kg
	ต้มโคล้งปลากรอบ	ปลากดย่าง	10 Kg
	ไข่เค็ม	มะเขือเทศ	3 Kg
	ขนมเปี๊ยะ	พริกขี้หนูแห้ง	1 Kg
		ผักชีฝรั่ง	2 Kg
		หอมใหญ่	3 Kg
		น้ำมันงาขาว	5.8 Lt
		พริกขี้หนูสด	3 Kg
		ไข่ไก่	350 ฟอง
		ต้นหอม	1 kg
		น้ำปลา	1.4 Lt
		ผงชูรส	70 g
		ซีอิ๊วขาว	0.7 Lt
		ซอสถั่วเหลืองตราภูเขาทอง	0.7 Lt
		ขนมเปี๊ยะ	350 ชิ้นกลาง
6 พ.ค. 48			
เช้า	ข้าวสวย	ข้าวสาร	40 Kg
	แกงจืดวุ้นเส้นไก่	วุ้นเส้น	5 Kg
		อกไก่	9 Kg
		ต้นหอม	1 Kg
		ตังน่าย	200 g
		เกลือป่น	100 g
		รสดี	30 g
		ซีอิ๊วขาว	0.7 Lt
กลางวัน	ข้าวสวย	ข้าวสาร	40 Kg

วันที่ / มื้อ	รายการอาหาร	เครื่องปรุง	ปริมาณที่ใช้
(6 พ.ค. 48)	ต้มฟักมะนาวดองไก่	ฟักเขียว	50 Kg
	ผัดกะเพราหอยแมลงภู่น้ำจืด	อกไก่	8 Kg
		ถั่วฝักยาว	35 Kg
		หอยแมลงภู่น้ำจืด	8 Kg
		มะนาวดอง	30 ลูก
		ต้นหอม	1 Kg
		ใบกะเพรา	1 Kg
		น้ำมันพืช	2.4 Lt
		พริกขี้หนูแดง	2 Kg
		น้ำตาลทราย	350 g
		เกลือป่น	100 g
		น้ำปลา	2.27 Lt
		รสดี	30 g
		ซีอิ๊วขาว	1.4 Lt
		ซอสถั่วเหลืองตราภูเขาทอง	1.4 Lt
		กระเทียม	1 Kg
		ผงชูรส	140 g
เย็น	ข้าวสวย	ข้าวสาร	40 Kg
	แกงเลียงไก่	ฟักทอง	20 Kg
	ไข่ต้ม	บวบเหลี่ยม	20 Kg
	ถั่วเขียวต้มน้ำตาล	กะปิ	2 Kg
		อกไก่	8 Kg
		ถั่วเขียว	7 Kg
		ใบแมงลัก	2 Kg
		มะละกอ	20 Kg
		หอมแดง	2 Kg
		กุ้งแห้ง	1 Kg
		น้ำตาลทราย	5 Kg
		ผงชูรส	70 g
		น้ำปลา	1.4 Lt
		กระชายหัว	2 Kg
		ไข่ไก่	350 ฟอง
		ซีอิ๊วขาว	0.7 Lt

วันที่ / มือ	รายการอาหาร	เครื่องปรุง	ปริมาณที่ใช้
(6 พ.ค. 48)		ซอสถั่วเหลืองตราภูเขาทอง	0.7 Lt
7 พ.ค. 48			
เช้า	ข้าวสวย	ข้าวสาร	40 Kg
	ต้มผักกาดดองเครื่องในหมู	ผักกาดดอง	30 Kg
		ปอดหมู	15 Kg
		อกไก่	1 Kg
		ตังน่าย	200 g
		ผงชูรส	70 g
		ซีอิ๊วขาว	0.7 Lt
		ซอสถั่วเหลืองตราภูเขาทอง	0.7 Lt
		เกลือ	100 g
กลางวัน	ข้าวสวย	ข้าวสาร	40 Kg
	แกงเผ็ดหน่อไม้ดองไก่	หน่อไม้ดอง	30 Kg
	กุนเชียงทอด	สะโพกไก่	10 Kg
		ใบมะกรูด	100 g
		กะทิขาวเกาะ	20 Kg
		น้ำมันพืช	3 Lt
		น้ำพริกแกงเผ็ด	6 Kg
		น้ำปลา	1.4 Lt
		น้ำตาลปีบ	3 Kg
		ใบโหระพา	2 Kg
		กุนเชียง	11 Kg
		ผงชูรส	70 g
เย็น	ข้าวสวย	ข้าวสาร	40 Kg
	แกงส้มผักกวางตุ้งปลาทุสด	ผักกวางตุ้ง	40 Kg
	ปลาเค็มทอด	มะขามเปียก	6 Kg
	ขนมไข่	กระชายหัว	2 Kg
		ปลาจวดเค็ม	11 Kg
		น้ำพริกแกงส้ม	6 Kg
		ปลาทุสด	10 Kg
		น้ำมันพืช	2.4 Lt
		น้ำปลา	1.4 Lt
		ซีอิ๊วขาว	0.7 Lt

วันที่ / มื้อ	รายการอาหาร	เครื่องปรุง	ปริมาณที่ใช้
(7 พ.ค. 48)		ซอสถั่วเหลืองตราภูเขาทอง	0.7 Lt
		ขนมไข่	350 ชิ้น



Table A 48 7 days-nutrients from commissary. (total 360 person)

Nutrient	หน่วย	May-01	May-02	May-03	May-04	May-05	May-06	May-07	Average/ 360 person/day	Average/ 1 person/day
Ash	g	4,419.92	4,726.79	4,967.71	8,560.97	21,603.69	5,784.12	4,616.65	7,811.41	21.698
B-Carotene	ug	425,652.34	1,058,002.42	246,548.70	1,895,105.30	1,237,798.72	300,297.78	1,273,069.86	919,496.45	2554.157
Calcium	mg	92,444.52	88,362.55	71,630.58	146,090.02	365,753.76	97,680.34	145,211.90	143,881.95	399.672
Carbohydrate	g	115,963.86	119,572.04	122,515.36	126,928.34	196,552.54	122,359.78	123,308.05	132,457.14	367.936
Cholesterol	mg	56,425.00	13,266.64	137,032.00	84,963.10	70,702.16	102,279.32	63,655.00	75,474.75	209.652
Copper	mg	221.95	306.83	262.83	292.07	177.99	398.2	140.93	257.26	0.715
Fat	g	5,254.09	6,373.80	10,573.80	16,214.99	16,356.57	7,649.80	15,812.57	11,176.52	31.046
Fiber-Crude	g	165.2	140.16	97	65	1,580.00	112.78	604	394.88	1.097
Fiber-Dietary	g	2,901.30	4,816.43	2,433.73	5,346.00	12,364.06	6,167.31	1,907.75	5,133.80	14.261
Iron	mg	3,876.61	4,610.46	5,408.16	6,507.65	18,280.29	5,913.25	13,359.92	8,279.48	22.999
Iron-Animal	mg	748.4	673.17	1,932.90	909.65	8,278.75	2,635.65	8,188.23	3,338.11	9.273
Iron-Veg	mg	2,914.21	3,937.28	3,475.26	5,290.00	6,773.54	3,277.60	4,799.70	4,352.51	12.090
K Calories	K	578,024.82	604,559.52	663,206.39	749,305.62	1,059,176.62	639,162.24	725,931.72	717,052.42	1991.812
Magnesium	mg	762.3	0	1,334.03	0	0	0	0	299.48	0.832
Niacin	mg	5,216.19	4,286.55	3,867.45	7,587.78	10,165.13	5,534.11	5,550.66	6,029.70	16.749
Phosphorus	mg	251,184.30	222,857.66	268,119.53	323,571.90	516,338.96	285,236.51	278,042.50	306,478.77	851.330
Phytate	mg	45,597.20	59,741.53	50,645.10	41,070.00	40,700.23	55,560.46	39,600.00	47,559.22	132.109

Nutrient	หน่วย	May-01	May-02	May-03	May-04	May-05	May-06	May-07	Average/ 360 person/day	Average/ 1 person/day
Potassium	mg	326,078.80	460,028.06	334,360.15	551,880.90	825,144.77	588,259.94	221,906.90	472,522.79	1312.563
Prot-Animal	g	6,582.32	5,006.97	9,497.56	12,271.54	16,241.49	8,560.31	12,247.18	10,058.20	27.939
Prot-Veg	g	10,470.64	12,713.98	10,992.24	12,846.86	19,804.79	11,843.93	11,202.84	12,839.33	35.665
Protein	g	17,052.96	17,720.94	20,489.80	25,118.40	36,046.28	20,404.24	23,450.02	22,897.52	63.604
Retinol	ug	40,356.32	4,725.76	112,197.36	37,768.00	4,549.36	66,669.04	3,759.68	38,575.07	107.153
Riboflavin	mg	235.42	176.08	291.04	504.8	306.26	298.99	289.81	300.34	0.834
Selenium	ug	0	0	0	0	0	0	0	0.00	0.000
Sodium	mg	903,006.36	1,202,277.95	879,969.57	2,252,373.80	5,690,615.70	1,128,252.27	1,294,511.92	1,907,286.80	5298.019
Sugars	g	464.36	5,810.11	210.85	10,759.77	15,653.85	5,542.54	554.71	5,570.88	15.475
Thiamin	mg	181.85	255.06	256.32	280.18	442	264.21	258.44	276.87	0.769
Vit A	RE	75,828.07	93,592.67	132,743.49	195,493.79	107,700.33	91,692.97	113,874.80	115,846.59	321.796
Vit B12	ug	0	0	0	0	0	0	0	0.00	0.000
Vit B6	mg	0	0	0	0	0	0	0	0.00	0.000
Vit C	mg	16,870.00	18,412.07	16,830.00	41,820.00	109,640.17	36,439.94	23,430.00	37,634.60	104.541
Vit E	mg	0	0	0	0	0	0	0	0.00	0.000
Water	g	98,970.84	131,544.29	120,443.19	246,855.74	469,195.04	203,946.31	163,820.50	204,967.99	569.356
Zinc	mg	1,031.80	1,058.35	1,187.83	1,625.87	824.56	1,511.88	735.13	1,139.35	3.165

Nutrient	หน่วย	May-01	May-02	May-03	May-04	May-05	May-06	May-07	Average/ 1 person/day
Carbohydrate	%	80.06	78.86	73.45	67.33	72.96	76.49	67.63	73.83
Protein	%	11.77	11.69	12.28	13.32	13.38	12.75	12.86	12.58
Fat	%	8.16	9.46	14.26	19.35	13.66	10.76	19.51	13.59

Table A 49 Energy expenditure in household, occupational. Recreational and sports activities [130]

How to use this appendix, refer to the column that comes closest to your present body mass. Multiply the number in this column by the number of minutes you spend in an activity. Suppose that an individual weighing 80.0 Kg (176 lb) spends 45 minutes working out with free weights. To determine the energy cost of participation, multiply the caloric value per minute (6.8 Kcal) by 45 to obtain the 45-minute gross expenditure of 306 Kcal. If the same individual does aerobic dance for 60 minutes, the gross energy expended would be calculated as 7.8 Kcal x 60 minutes, or 468 Kcal.

Activity	Your body weight (Kg)																	
	47	50	53	56	59	62	65	68	71	74	77	80	83	86	89	92	95	98
Carpet sweeping (M)	2.3	2.4	2.5	2.7	2.8	3.0	3.1	3.3	3.4	3.6	3.7	3.8	4.0	4.1	4.3	4.4	4.6	4.7
Circuit resistance training																		
Free-weight	4.0	4.3	4.5	4.8	5.0	5.3	5.5	5.8	6.1	6.3	6.6	6.8	7.1	7.4	7.6	7.9	8.1	8.4
Hydra-Fitness	6.2	6.6	7.0	7.4	7.8	8.2	8.6	9.0	9.4	9.7	10.2	10.5	10.9	11.4	11.7	12.1	12.5	12.9
Cleaning	2.7	2.9	3.1	3.2	3.4	3.6	3.8	3.9	4.1	4.3	4.5	4.6	4.8	5.0	5.2	5.3	5.5	5.7
Eating (sitting)	1.1	1.2	1.2	1.3	1.4	1.4	1.5	1.6	1.6	1.7	1.8	1.8	1.9	2.0	2.0	2.1	2.2	2.3
Housework																		
Mopping floors	2.8	3.1	3.3	3.5	3.7	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2
Laundry	3.1	3.4	3.5	3.7	3.9	4.1	4.3	4.5	4.7	4.9	5.1	5.3	5.5	5.7	5.9	6.1	6.3	6.5
Running, on flat surface	6.3	6.8	7.2	7.6	8.0	8.4	8.8	9.2	9.6	10.0	10.5	10.9	11.3	11.7	12.1	12.5	12.9	13.3
Sitting quietly	1.0	1.1	1.1	1.2	1.2	1.3	1.4	1.4	1.5	1.6	1.6	1.7	1.7	1.8	1.9	1.9	11.0	2.1
Standing quietly	1.3	1.4	1.4	1.5	1.6	1.7	1.8	1.8	1.9	2.0	2.1	2.2	2.2	2.3	2.4	2.5	2.6	2.6

Activity	Your body weight (Kg)																	
	47	50	53	56	59	62	65	68	71	74	77	80	83	86	89	92	95	98
Walking, leisure outdoors																		
Asphalt road	3.8	4.0	4.2	4.5	4.7	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0	7.1	7.3
Grass tract	3.8	4.1	4.3	4.5	4.8	5.0	5.3	5.5	5.7	5.9	6.1	6.3	6.5	6.7	6.9	7.1	7.3	7.5
Walking tread, hill level	2.4	2.6	2.8	3.0	3.1	3.3	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.7	4.9	5.1	5.3	5.4
Writing (sitting)	1.4	1.5	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.7	2.8

Compendium of physical activities [131]

Part I: Standardized MET intensity Levels

Code	MET	Category	Activity
07030	0.9	Inactivity	Sleeping

BIOGRAPHY

NAME	Cpt. Jiraporn Chaopothong
DATE OF BIRTH	10 February 1973
PLACE OF BIRTH	Saraburi, Thailand
INSTITUTIONS ATTENDED	Royal Thai Army Nursing College, 1995: Bachelor of Nursing Mahidol University, 2006: Master of Science (Nutrition)
POSITION & OFFICE	315 Pramongkutklao Hospital, Bangkok, 10400. Thailand Position: Nurse Tel. 02-3547638 E-mail: kob_jiraporn@yahoo.com
HOME ADDRESS	66/77 Moo 1, Adisorn Hill Village, Nhong- Yao , Muang, Saraburi, 18000. Tel. 036- 302284