

# Authentication of Green Tea as Raw Material and in Pharmaceutical Products by Multivariate Analysis

A Thesis presented to
Faculty of Pharmacy, Ain Shams University,
In partial fulfillment for the requirements for the degree of
Master's of Philosophy in Pharmaceutical Sciences
(Pharmacognosy)

By

### Maram Mohammed Mabrouk Aboulwafa

B. Pharm. Sci. (2013)

Department of Pharmacognosy

Faculty of Pharmacy

Ain Shams University

Cairo, Egypt

(2018)

### Under the Supervision of

# **Mohamed Lotfy Ashour**

Associate Professor of Pharmacognosy
Faculty of Pharmacy
Ain Shams University

# **Haidy Abdel Moniem Gad**

Associate Professor of Pharmacognosy
Faculty of Pharmacy
Ain Shams University

#### Fadia Salah Youssef

Lecturer of Pharmacognosy
Faculty of Pharmacy
Ain Shams University



# تقييم الشاي الأخضر كمادة أولية في المستحضرات الصيدلية باستخدام تقنيات التحليل متعدد المتغيرات

رسالة مقدمة الى

كلية الصيدلة - جامعة عين شمس و ذلك الستكمال متطلبات الحصول على درجة الماجيستير في العلوم الصيدلية (عقاقير)

من الصيدلانية مرام محمد مبروك أبوالوفا بكالوريوس في العلوم الصيدلية (2013)

قسم العقاقير
كلية الصيدلة – جامعة عين شمس
القاهرة
جمهورية مصر العربية
(2018)

# تحت اشراف

# أ.م.د. محمد لطفي عاشور

أستاذ مساعد العقاقير

كلية الصيدلة - جامعة عين شمس

# أ.م.د. هايدي عبدالمنعم جاد

أستاذ مساعد العقاقير

كلية الصيدلة - جامعة عين شمس

# د. فادية صلاح يوسف

مدرس العقاقير

كلية الصيدلة - جامعة عين شمس

#### Acknowledgement

First of all, I would like to thank ALLAH for guiding me and giving me the opportunity, health and energy to accomplish this work.

I would like to express my sincere gratitude and appreciation to everyone who supported and gave me the possibility to complete this work.

I have the pleasure to acknowledge my advisory committee: Assoc. Prof. Dr. Mohammed L. Ashour, Associate Professor of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Assoc. Prof. Dr. Haidy A. Gad, Associate Professor of Pharmacognosy, Faculty of Pharmacy, Ain Shams University and Dr. Fadia S. Youssef, Lecturer of Pharmacognosy, Faculty of Pharmacy, Ain Shams University for setting an example to what dedicated scientists and advisors should be, for their encouragement, constructive comments and valuable suggestion throughout the time of research and in writing this thesis.

I have the pleasure to acknowledge my Godfather **Prof. Dr. Mohammed M. Al-Azizi**, Professor of Chemistry of Natural products, Faculty of Pharmacy, Ain Shams University for suggesting the research point and his continuous support and teaching.

I would like to thank my greatest supporter and best friend, my dear mother, **Dr.** Azza El-Bishry, for being the best mother ever, my father, my brother Hamada and my grandmother, Susu for their love and care in all aspects.

Many thanks to my teachers and professors who believed in me and encouraged me throughout my past learning years.

Maram Aboulwafa

# **Table of Content**

Subject	Page
Acknowledgment	i
Table of Content	ii
List of Figures	v
List of Tables	vii
List of Abbreviations	ix
Introduction	1
Literature Review	3
1. Phytoconstituents of Green Tea.	. 5
2. Health Benefits and Biological Activities of Green Tea.	. 9
3. Recent Approaches for Monitoring the Quality of Green Tea.	. 42
4. Authentication of Green Tea.	. 49
Statement of the Problem	. 51
Work Strategy	52
Materials and Methods	53
1. Sample Collection	. 53
2. Solvents, Materials, Reagents and Standards	. 55
3. Sample Preparation for Different Analytical Techniques	. 55
4. Apparatus	56
5. Software	57
6. Methods and Procedures	57
Results	
Chapter I:	
Spectroscopic Analysis as a Chemometric Model for the Identification and	
Authentication of Green Tea	. 60
1. Ultra Violet Spectroscopy as a Chemometric Model for the Identification and	
Authentication of Green Tea	. 60
1.1. Results of Principal Component Analysis (PCA) of Genuine Green tea Samples from	
Different Geographical Origins.	. 61

1.2. Results of Hierarchical Clustering Analysis (HCA) of Genuine Green Tea Samples from
Different Geographical Origins.
1.3. Validation of UV Model of Genuine Green Tea Samples from Different Geographical
Origins.
2. Visible Spectroscopy as a Chemometric Model for the Identification and
Authentication of Green Tea
2.1. Results of Principal Component Analysis (PCA) of Genuine Green Tea Samples from
Different Geographical Origins.
2.2. Results of Hierarchical Clustering Analysis (HCA) of Genuine Green Tea Samples from
Different Geographical Origins.
3. Fourier Transformer Infrared Spectroscopy as a Chemometric Model for the
Identification and Authentication of Green Tea
3.1. Results of Principal Component Analysis (PCA) of Genuine Green Tea Samples from
Different Geographical Origins.
3.2. Results of Hierarchical Clustering Analysis (HCA) of Genuine Green Tea Samples from
Different Geographical Origins.
4. Nuclear Magnetic Resonance Spectrometry as a Chemometric Model for the
Identification and Authentication of Green Tea
Results of Principal Component Analysis (PCA) of Genuine Green Tea Samples from
Different Geographical Origins.
Chapter II:
Chromatographic analysis as a Chemometric Model for the Identification and
Authentication of Green Tea
1. High Performance Thin-Layer Chromatography as a Chemometric Model for the
Identification and Authentication of Green Tea
1.1. Results of Principal Component Analysis (PCA) of Genuine Green Tea Samples from
Different Geographical Origins.
1.2. Results of Hierarchical Clustering Analysis (HCA) of Genuine Green Tea Samples from
Different Geographical Origins

1.3. Validation of HPTLC Model of Genuine Green tea Samples from Different	
Geographical Origins.	91
2. High Performance Liquid Chromatography as a Chemometric Model for the	
Identification and Authentication of Green Tea	94
2.1. Results of Principal Component Analysis (PCA) of Green tea Samples from Different	
Geographical Origins.	94
2.2. Results of Hierarchical Clustering Analysis (HCA) of Green Tea Samples from	
Different Geographical Origins.	94
2.3. Validation of Developed HPLC Method used for the Analysis of Green Tea Samples	
from Different Geographical Origins.	96
Chapter III:	
Biological Assessment of Different Green Tea (Camellia sinensis) Samples from	
Different Geographical Origins	100
The Antioxidant Capacity of Genuine Green Tea Samples collected from Different	
Geographical Origins.	101
Conclusion	104
Summary	106
Recommendations	110
References	111
Appendix	143
Arabic Summary	

# **List of Figures**

Fig	igure Pa	
1.	Photo of green tea plant.	
2.	Chemical structures of phytoconstituents isolated from green tea.	
3.	(A) PCA score plot of the UV data matrix (200-400 nm) of the methanolic extracts of	
	the thirty-one genuine green tea samples collected from different geographical origins.  (B) PCA leverage plot of the UV data matrix (200-400 nm) of the methanolic extracts	
	of the thirty-one genuine green tea samples collected from different geographical origins.	
	(C) PCA score plot of the UV data matrix (200-400 nm) of the methanolic extracts of the genuine green tea samples collected from different geographical origins after	
	removing the outlier SF sample.  (D) PCA score plot of the UV data matrix (200-400 nm) of the methanolic extracts of	
	the genuine green tea samples collected from different geographical origins after removing the outlier SF sample & Kenyan samples.	
4.	Large detailed political map of Asia with the genuine green tea samples from Asian countries distributed on it.	
5.	PCA score plot of the UV data matrix (200-400 nm) of the methanolic extracts of the three replicates of the five genuine Kenyan green tea samples.	
6.	Map of Kenya with the genuine Kenyan green tea samples distributed on it	
7.	Clustering dendogram of the UV data matrix (200-400 nm) of the methanolic extracts of the thirty-one genuine green tea samples collected from different geographical origins.	
8.	PCA score plot of the UV data matrix (200-400 nm) of the methanolic extracts of the	
	twelve commercial green tea samples using PCA model of genuine green tea samples collected from different geographical origins.	
9.	Cooman's plot showing Kenyan & Ceylon genuine green tea samples-to-China and	
	India models distance.	

10.	Cooman's plot showing Kenyan & Ceylon genuine green tea samples along with	
	commerical samples-to-China and India models distance.	72
11.	(A) PCA score plot of the visible data matrix (400-800 nm) of the methanolic extracts	
	of the thirty-one genuine green tea samples collected from different geographical	
	origins.	75
	(B) PCA score plot of the visible data matrix (400-800 nm) of the methanolic extracts	
	of the genuine green tea samples collected from different geographical origins after	
	removing the outlier SF sample.	75
	(C) PCA score plot of the visible data matrix (400-800 nm) of the methanolic extracts	
	of the genuine green tea samples collected from different geographical origins after	
	removing the outlier SF sample & Kenyan samples.	76
12.	PCA score plot of the visible data matrix (400-800 nm) of the methanolic extracts of	
	the three replicates of the five Kenyan green tea samples.	76
13.	(A) Clustering dendogram of the visible data matrix (400-800 nm) of the methanolic	
	extracts of the thirty-one genuine green tea samples collected from different	
	geographical origins.	77
	(B) Clustering dendogram of the visible data matrix (400-800 nm) of the methanolic	
	extracts of the genuine green tea samples collected from different geographical origins	
	after removing Kenyan samples.	77
14.	PCA score plot of the FTIR raw data matrix (4000-400 cm <sup>-1</sup> ) of the thirty-one genuine	
	green tea samples collected from different geographical origins.	81
15.	PCA score plot of the FTIR data matrix (4000-400 cm <sup>-1</sup> ) of the thirty-one genuine	
	green tea samples collected from different geographical origins after Standard Normal	
	Variate (SNV) transformation method.	81
16.	PCA score plot of the FTIR data matrix (4000-400 cm <sup>-1</sup> ) of genuine green tea	
	samples collected from different geographical origins after Standard Normal Variate	
	(SNV) transformation method after removing Kenyan samples.	82
17.	PCA score plot of the FTIR raw data matrix (4000-400 cm <sup>-1</sup> ) of the three replicates of	
	the five genuine Kenyan green tea samples.	82

18.	(A) Clustering dendogram of the FTIR data matrix (4000-400 cm <sup>-1</sup> ) of the thirty-one	
	genuine green tea samples collected from different geographical origins after Standard	83
	Normal Variate (SNV) transformation method.	
	(B) Clustering dendogram of the FTIR data matrix (4000-400 cm <sup>-1</sup> ) of the genuine	
	green tea samples collected from different geographical origins after Standard Normal	83
	Variate (SNV) transformation method after removing the Kenyan samples	
19.	PCA score plot of the chemical shifts of the thirty-one genuine green tea samples	
	collected from different geographical origins.	86
20.	PCA score plot of peak area% of HPTLC chromatograms of the thirty-one genuine	
	green tea samples collected from different geographical origins scanned at 450 nm	90
21.	Clustering dendogram of the peak area% of HPTLC chromatograms of the thirty one	
	genuine green tea samples collected from different geographical origins scanned at 450	
	nm	90
22.	PCA score plot of the peak area% of HPTLC chromatograms of the twelve commercial	
	green tea samples using PCA model of genuine green tea samples collected from	
	different geographical origins.	93
23.	Cooman's plot showing Kenyan & Ceylon genuine green tea samples along with	
	commerical samples-to-China and India models distance (HPTLC).	93
24.	PCA score plot of the chromatograms data matrix of the methanolic extracts of the	
	thirty-one genuine green tea samples collected from different geographical origins	95
25.	Clustering dendogram of the chromatograms data matrix of the methanolic extracts of	
	the thirty-one genuine green tea samples collected from different geographical origins.	95
26.	Calibration curve for standard caffeine showing the relationship between concentration	
	and area under the curve as determined by HPLC.	99
27.	Caffeine content (mg/1 gm DW) of genuine green tea samples collected from different	
	geographical origins.	99
28.	The half maximal inhibitory concentration (IC <sub>50</sub> ) of genuine green tea samples	
	collected from different geographical origins.	101

# **List of Tables**

Γable		Page	
1.	The major biological activities and mechanism of action of different parts, extracts as well as compounds isolated from green tea.		
2.	Collected green tea samples from different geographical origins and commercial ones, codes and sources.	. 53	
3.	Classification table of genuine green tea samples collected from different geographical origins with respect to "East Asia" and "South Asia & Kenya" models using SIMCA	. 70	

#### **List of Abbreviations**

ALT Alanine Aminotransferase

AST Aspartate Aminoransferase

BHA Butylated Hydroxyanisole

BHT Butylated Hydroxytoluene

BP Blood Pressure

BPH Benign Prostate Hyperplasia

CA Cluster Analysis

CAD Coronary Artery Disease

cAMP Cyclic Adenosine Monophosphate

CG Catechin Gallate

COX Cyclooxygenase

CV Cardiovascular

CYP Cytochrome P450

DPPH 2,2-Diphenyl-1-Picrylhydrazyl

EC (-)-Epicatechin

ECG (-)-Epicatechin-3-Gallate

EGC (-)-Epigallocatechin

EGCG (-)-Epigallocatechin-3-Gallate

E-nose Electronic Nose

ES Ensemble Strategy

ESR Electron Spin Resonance

E-tongue Electronic Tongue

FAS Fatty-acid Synthase

FT-IR Fourier Transformer-Infrared

GA Gallic Acid

GABA γ-Aminobutyric Acid

GC Gas Chromatography

GSH Glutathione

GSHpx Glutathione Peroxidase

GST Glutathione S-Transferase

GTPs Green Tea Polyphenols

HCA Hierarchical Clustering Analysis

HIV Human Immunodeficiency Virus

HPLC High Performance Liquid Chromatography

IC<sub>50</sub> The Half Maximal Inhibitory Concentration

ICH International Conference on Harmonization

ICP-MS Inductively Coupled Plasma-Mass Spectrometry

IgE Immunoglobulin E

LDA Linear Discriminant Analysis

LDL Low-Density Lipoprotein

LOD Limit of Detection

LOQ Limit of Quantification

LOX Lipoxygenase

MALDI-TOF- Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight-Mass

MS Spectrometry

MAPK Mitogen-Activated Protein Kinase

MI Myocardial Infarction

MMPs Matrix Metalloproteinases

MS Mass Spectrometry

NIR Near-Infrared
NK Natural Killer

NOS Nitric Oxide Synthase  $O_2^-$  Oxygen-Free Radicals

P-60 Polyphenone 60

PCA Principal Component Analysis

PLS Partial Least Square

PMNs Polymorphonuclear Neutrophils

PSA Prostate-Specific Antigen

PVN Paraventricular Nucleus

QuEChERS Quick, Easy, Cheap, Effective, Rugged and Safe

ROS Reactive Oxygen Species

RR Relative Risk

R.S.D Relative Standard Derivation

Rx Reaction

SIMCA Soft Independent Modeling of Class Analogy

SNV Standard Normal Variate

SOD Superoxide Dismutase

Stdev Standard Deviation

TBHQ Tert-Butylhydroquinone

TEAC Trolox Equivalent Antioxidant Capacity

TGs Triglycerides

TH Tyrosine Hydroxylase

TNF-α Tumor Necrosis Factor-Alpha

UDP Uridine 5'-Diphosphate

UPLC Ultra-High Performance Liquid Chromatography

UV Ultraviolet

VEGF Vascular Endothelial Growth Factor