شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

بسم الله الرحمن الرحيم

HANAA ALY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

HANAA ALY

شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

HANAA ALY

A Chemometric Approach for the Quality Control of *Trigonella foenum-graecum* (Family Fabaceae) Seeds

A Thesis

Submitted for Partial Fulfillment of the Requirements for the

Master's degree

In Pharmaceutical Sciences

(**Pharmacognosy**)

By

Nariman Hossam El-Din Kandil
Bachelor of Pharmaceutical Sciences, 2012
Faculty of Pharmacy – Misr International University (MIU)

Under the supervision of

Prof. Safaa Ahmed El-Moghazy

Professor of Pharmacognosy
Faculty of Pharmacy
Badr University in Cairo

Prof. Sherweit Hamed El-Ahmady

Professor of Pharmacognosy
Faculty of Pharmacy
Ain Shams University

Assoc. Prof. Iriny Mohsen Mansour Ayoub

Associate Professor of Pharmacognosy,
Faculty of Pharmacy
Ain Shams University

Department of Pharmacognosy
Faculty of Pharmacy
Ain Shams University
Abbassia, Cairo, Egypt

2022

Acknowledgments

First of all, I would like to thank Allah; without his virtue, I would have never been able to achieve this work.

I would like to express my gratitude to my supervisors: ***Prof. Dr. Safaa Ahmed El-Moghazy****, Professor of Pharmacognosy, Head of Pharmacognosy Department, Badr University in Cairo (BUC), for her continuous constructive comments, and the unlimited encouragement and patience in supervising this work.* ***Prof. Dr. Sherweit Hamed El-Ahmady,*** *Professor of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, for suggesting the research point and for the unlimited encouragement, help, and scientific support and guidance throughout this work.* ***Assoc. Prof. Iriny Mohsen Ayoub,*** *Associate Professor at the Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University for the outstanding effort, helpful discussion, and encouragement on every step whenever needed and constructive criticism, guided me immensely throughout the work.*

Finally, I would like to thank you for setting an example of what dedicated professors, scientists, and advisors should be, that I wish one day I would be. No words can express how thankful I am to all of you. It is a great honor to work under your supervision.

My deep thanks to ***Prof. Dr. Mohamed Ahmed El-Shanawany,*** *Professor of Pharmacognosy, the founder of Faculty of Pharmacy, Badr University in Cairo (BUC), for his tremendous support and motivation.*

I would like to express my deepest thanks to members, colleagues, and professors of the Department of Pharmacognosy, Badr University in Cairo (BUC), and Ain Shams University for their help and support.

My family and my greatest support, love, and endless help, without which I would have never been able to accomplish this work. I would like to thank my role model in life, my mother ***Dr. Fatma Kandil****, and my father* ***Dr. Hossam Kandil****. They have always been a true helping hand throughout my life, for the endless support, love, guidance in every moment, for teaching me the persistence to succeed in life may God bless them. I would like to thank my dear husband* ***Eng. Hassan Soliman*** *for being understanding and extremely supportive.*

I would like to dedicate this work to the two blessings in my life Taleen and Yasseen.

Nariman Hossam

List of Contents

List of Figures

List of Tables

List of Abbreviations

Abbreviation	Full-term
4-HIL	4-Hydroxyisoleucine
APTT	Activated Partial Thromboplastin Time
ATR	Attenuated Total Reflectance
CA	Cluster Analysis
DNA	Deoxyribonucleic Acid
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
F-GAL	Fenugreek Galactomannan
FT-IR	Fourier-Transformed Infrared
GAE	Gallic Acid Equivalent
GC/MS	Gas Chromatography coupled to Mass Spectrometry
HCA	Hierarchical Cluster Analysis
HPLC	High Performance Liquid Chromatography
HPLC- MS/MS	High-Performance Liquid Chromatography-tandem Mass Spectrometry
HPLC/UV-vis	High Performance Liquid Chromatography with Ultraviolet visible detector
HRI	Horticulture Research Institute
IC_{50}	Half Maximal Inhibitory Concentration
LC/MS	Liquid Chromatography/Mass spectrometry
NCBI	National Center for Biotechnology Information
NMR	Nuclear Magnetic Resonance
PCA	Principal Component Analysis
PLS	Partial Least Squares
PLS-DA	Partial Least Squares-Discriminate Analysis
PPh	Polyphenol Content
RAPD	Random Amplification of Polymorphic DNA
R_f	Retention Factor
SIMCA	Soft Independent Modelling of Class Analogy
TE	Trolox Equivalents
TF	Total Flavonoids
TRG	Trigonelline
UPLC-QTOF-MS	Ultra-High Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry
UV	Ultra-violet
UV-DAD	Ultra-violet Diode Array Detector
λ	Wavelength

Introduction

Family Fabaceae is the third-largest angiosperm family, comprising approximately 9.4% of flowering plants with about 19,325 species, primarily distributed in tropical areas **(Mabberley, 1997, Yahara *et al.*, 2013)**. This family is divided into three sub-families: Papilionoideae, Caesalpinioideae, and Mimosoideae. *Trigonella* is one of the largest genera of the tribe Trifolieae. Fenugreek is an annual herb that belongs to the family Fabaceae **(Petropoulos, 2002)**. It exhibits various medicinal uses, which include hypoglycemic **(Gad *et al.*, 2006),** hypocholesterolemic **(Al- Habori and Raman, 1998)**, anti-inflammatory **(Bhanger *et al.*, 2008)**, anticancer **(Alsemari *et al.*, 2014)** as well as antioxidant activity **(Mashkor, 2014)**. Also, due to its outstanding effects on digestion, fenugreek plays a beneficial role in the food industry due to its high fiber, protein, and gum content. It is used as an emulsifying agent, adhesive, and food stabilizer **(Wani and Kumar, 2016)**. The seeds exhibit pungent aromatic properties **(Max, 1992)**, which explains why it is used as a spice in many cuisines besides in the preparation of curry **(Parry, 1945).**

Historically, fenugreek (*Trigonella foenum-graecum* Linnaeus) is considered one of the oldest used medicinal plants. The desiccated seeds were found in the tomb of Tutankhamun, dating to ca. 1323 B.C. **(Hepper, 2009)**. Fenugreek was known to the Ancient Egyptians in pre-dynastic times, about 3000 B.C. **(Evans, 2009)**. In ancient Egypt, fenugreek was used to ease childbirth, and recently it is used to relieve menstrual cramps **(Wani and Kumar, 2016)**. Non-medicinal applications of fenugreek by ancient Egyptians included burning and fuming with incense in religious rites and mummifying bodies **(Yoshikawa *et al.*, 1997)**. While fenugreek is a widely grown spice, India is considered the primary producer, and it is also cultivated mainly in Egypt, Ethiopia, and Morocco **(Parthasarathy, 2008, Vidyashankar, 2014, Balodi, 1991).**

Moreover, fenugreek is a rich source of diosgenin which serves as a substrate for synthesizing more than two hundred kinds of steroidal drugs **(Van Wyk and Wink, 2018, Zhou *et al.*, 2019)**. Fenugreek extract is widely used in many consumer goods **(Waqas *et al.*, 2010)**, such as foods **(Ahmad *et al.*, 2016, Burdock, 2019, Wankhede *et al.*, 2016)**. Fenugreek is a forage crop representing 68,000 tons per year globally, of which more than

50% is consumed in India **(Petropoulos, 2002)**. Bibliometric data indicates that the number of publications and clinical trials about fenugreek has steadily increased in the era of re-emergence of natural products in drug development **(El Bairi *et al.*, 2017)**.

Traditional medicine is considered among the hot topics in the scientific field worldwide **(Van Wyk and Wink, 2018)**. According to the Food and Agriculture Organization's (FAO) report, it is estimated that 70–80 % of the world's population, especially in developing countries, depend on herbal medicine to prevent and cure diseases **(Ekor, 2014)**. Moreover, it is estimated that around 25 % of the synthesized drugs are produced initially from medicinal plants **(Pan *et al.*, 2013)**. Hence, quality control of herbal products has become mandatory. The WHO (World Health Organization) estimated that as many as 30 % of medicines sold in some Asian areas are adulterated **(Newton *et al.*, 2010, Shanmughanandhan *et al.*, 2016, Wilczyński *et al.*, 2016)**.

Identifying different species of Fabaceae is difficult when based solely on morphological characteristics **(Steven G and Subramanyam, 2009, Xin *et al.*, 2007)**; additionally, some limitations in traditional taxonomy prevent this technique from meeting the complicated demands of species recognition **(Maddison *et al.*, 2007)**. In some cases, a morphological examination is used as a tool for determining the quality of herbal drugs, but it is considered time-consuming, requiring specialized personnel, and does not reflect the number of chemical constituents and adulterants **(British Retail Consortium, Kucharska-Ambrożej and Karpinska, 2019)**. As such, a method for the simple and accurate authentication of Fabaceae is indispensable. Recently, metabolic fingerprinting and standardization methods using internal or external standards are conducted for quality control **(Bansal *et al.*, 2014, Liang *et al.*, 2010)**. Thus, chromatography plays a unique role in the quality control and standardization of phytotherapeutics. Various chromatographic and spectroscopic techniques are widely used to seek herbal quality control. UV spectrophotometry is widely applied as a primary spectroscopic tool for the analysis and quality control of herbal extracts and chemicals **(Marques *et al.*, 2013)**. It is a simple, available, and low-cost technique compared to other analytical instruments; however, it is not explicitly quantitative for chemical constituents **(Gad *et al.*, 2013c)**. HPLC fingerprint analysis of natural products is performed to ensure quality and authenticity **(Gad and**

Bouzabata, 2017). HPLC is extensively used to assess the quality of many closely related herbs by chemical identification **(Gad *et al.*, 2013a)**. According to WHO, medicinal plants' quality control using various methods, including HPTLC, is certified by the regulatory authority **(World Health Organization, 2011)**.

Based on the complex composition of natural products and the need for indirect measurements, the term "Chemometrics" has taken place. Chemometrics is the science of relating measurements made on a chemical system or process to the state of the system *via* the application of mathematical or statistical methods **(Hibbert, 2016)**. A large dataset is increasingly common and is often difficult to interpret by performing calculations on measurements of chemical data. However, chemometry regroups several related data results leading to extract 'relevant' information and reduce 'irrelevant' information.

Chemometrics can be classified into Pattern Recognition (unsupervised and supervised) and Multiple Calibrations. Pattern recognition methods are used for the qualitative purpose in which it aims to determine similarities and consistencies in the data according to a certain measurement made on the samples. It includes Exploratory Data Analysis (EDA) that is performed initially to analyse data sets to identify underlying structures and extract important variables which in turn embraces Principal Component Analysis (PCA) and Factor Analysis (FA). PCA can be considered the starting point of multivariate data analyses, an orthogonal transformation of multivariate data first formulated by Pearson **(Pearson, 1901)**. Both are applied as the main tools for EDA to reduce the dimensionality of the data. Unsupervised Pattern Recognition aims to group samples based on chemical measurements. Its most common methods include Hierarchical Cluster Analysis that represents correlation among samples in the form of a dendrogram and *K*-means method. Supervised Pattern Recognition aims to identify unknown samples as a member of predefined classes, in which training sets are used to build a classification model. Among the most popular techniques for supervised pattern recognition are Linear Discriminate Analysis (LDA), *k*-Nearest Neighbours (*k*NN), Partial Least Squares Discriminate Analysis (PLS-DA), Artificial Neural Networks (ANN); and Soft Independent Modelling of Class Analogy (SIMCA). While in Multiple Calibration a quantitative model is obtained by combining several independent variables to predict the dependent variable. The commonly applied methods are Partial Least Square (PLS) and