



شبكة المعلومات الجامعية
التوثيق الإلكتروني والميكرو فيلم

بسم الله الرحمن الرحيم



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شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلم



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جامعة عين شمس التوثيق الإلكتروني والميكروفيلم

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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)

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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	v
List of Tables	viii
List of Abbreviations	ix
Introduction	1
Aim of Work	5
Literature Review	6
1. Phytochemistry of Fenugreek Seeds:	6
2. Traditional Uses of Fenugreek Seeds in Folk Medicine	21
3. Biological activities reported for Fenugreek:	21
4. Quality Control of Fenugreek	32
Taxonomy	52
Material, Methods, and Apparatus	55
A. Materials	55
1. Sample collection and preparation	55
2. Reagents, Solvents and Apparatus	58
2.1. Extract's preparation	58
2.2. Total polyphenol's assay.....	58
2.3. Total flavonoid's assay	58
2.4. High Performance Liquid Chromatography HPLC analysis	58
2.5. High Performance Thin Layer Chromatography HPTLC analysis.....	59
Part 2: <i>In vitro</i> α -amylase inhibition assay for fenugreek seeds	59
3. Software	59
B. Methods.....	59
Extract's preparation	59
Part 1: Phytochemical analysis.....	60
Part 2: <i>In vitro</i> α -amylase inhibition assay for fenugreek seeds	65

Results and Discussion.....	66
Part 1: Phytochemical Analysis	67
Chapter I: Ultraviolet Spectroscopic Analysis of Fenugreek Seeds	72
Chapter II: Determination of Total Polyphenol and Total Flavonoid Content in Fenugreek Seeds	79
Chapter III: High Performance Liquid Chromatography (HPLC) Analysis of Fenugreek Seeds	86
Chapter IV: High Performance Thin Layer Chromatography (HPTLC) Analysis of Fenugreek Seeds	92
Part 2: <i>In vitro</i> α -amylase inhibition assay for fenugreek seeds	100
Conclusions.....	116
Recommendations.....	117
Summary.....	118
References.....	122
Appendix.....	148
Arabic Summary.....	1

List of Figures

Figure 1: Fenugreek (a) seeds, (b) leaves and flower and (c) a diagram for its different developmental stages	54
Figure 2: Map of the world showing the distribution of fenugreek crop among native countries in green and introduced countries in purple.....	54
Figure 3: Fenugreek pharmaceutical products used in the study.....	57
Figure 4: Extraction yield % (Average of 4 replicates) of fenugreek seeds collected from different geographical origins.....	68
Figure 5: UV absorption spectra of 27 fenugreek extracts over the range 200-400 nm (Three replicates).....	73
Figure 6: PCA score plot based on the spectroscopic data (200-400 nm) for 27 fenugreek seeds (average of three replicates).....	75
Figure 7: PCA score plot based on the spectroscopic data (200-400 nm) for 22 fenugreek seeds (average of three replicates).....	75
Figure 8: Loading plot for fenugreek seeds based on their UV scan (average of three replicates)	76
Figure 9: HCA Dendrogram of fenugreek samples of UV scan data matrix (average of three replicates).....	77
Figure 10: Calibration curve of gallic acid standard used for the assay of total polyphenols in fenugreek seeds.....	80
Figure 11: Calibration curve of quercetin standard used for the assay of total flavonoids in fenugreek seeds.....	82
Figure 12: Total polyphenols and flavonoids for fenugreek seeds.....	83
Figure 13: Calibration curve of standard trigonelline measured at wavelength 265 nm as determined by HPLC.	87
Figure 14: HPLC chromatogram of Trigonelline standard recorded at 265 nm.....	88
Figure 15: HPLC chromatogram of a Egyptian sample (EE1) recorded at 265 nm.	88
Figure 16: Trigonelline content in mg/g powdered fenugreek of samples collected from different geographical origins.....	89

Figure 17: PCA score plot of relative peak areas of HPLC after performing alignment for peaks detected at 230, 265, 330, and 347 nm.	91
Figure 18: Loadings plots for HPLC analysis after performing alignment for peaks detected at 230, 265, 330, and 347 nm.....	91
Figure 19: Matrix plot for the serial dilutions prepared to construct trigonelline calibration curve measured at 265 nm	93
Figure 20: HPTLC Calibration curve of trigonelline measured at wavelength 265nm with R ² value.....	93
Figure 21: HPTLC Calibration curve of 4-hydroxyisoleucine measured at wavelength 395 nm after derivatization with ninhydrin with R ² value.....	94
Figure 22: Matrix plot for the serial dilutions prepared to construct 4-hydroxyisoleucine calibration curve measured at 395 nm after derivatization with ninhydrin.	94
Figure 23: Overlaid three-dimensional (3D) chromatograms for HPTLC analysis performed on fenugreek samples recorded at (a) 265 nm and (b) 395 nm after derivatization with ninhydrin..	95
Figure 24: PCA score plot of relative peak areas (three replicates) of HPTLC after performing alignment for peaks recorded at 265 nm and 395 nm after derivatization with ninhydrin reagen	99
Figure 25: Loading plot of R _f recorded in HPTLC analysis for peaks recorded at 265 nm and 395 nm after derivatization with ninhydrin reagent.....	99
Figure 26: The half-maximal inhibitory concentration (IC ₅₀) of fenugreek samples collected from different geographical origins.	101
Figure 27: Trigonelline and 4-hydroxyisoleucine contents in mg/g dried residue from fenugreek seeds collected from different geographical origins calculated from HPTLC analysis.....	103
Figure 28: PCA score plot (A) and loading plot (B) based on UV spectroscopy, total polyphenols and flavonoids contents, TRG & 4-HIL contents and IC ₅₀ for α -amylase assay for 22 fenugreek samples.....	106
Figure 29: HCA dendrogram based on UV spectroscopy, total polyphenols and flavonoids contents, TRG & 4-HIL contents and IC ₅₀ for α -amylase assay for 22 fenugreek samples.....	108
Figure 30: PCA score plot (A) and loading plot (B), based on UV spectroscopy, total polyphenols and flavonoids contents, TRG & 4-HIL contents and IC ₅₀ for α -amylase assay for 22 fenugreek samples.....	108

Figure 31: HCA dendrogram based on HPLC analysis, total polyphenols and flavonoids contents, TRG & 4-HIL contents and IC₅₀ for α -amylase assay for 27 fenugreek samples 111

Figure 32: PCA score plot (A) and loading plot (B), based on HPTLC analysis, total polyphenols and flavonoids contents, TRG & 4-HIL contents and IC₅₀ for α -amylase assay for 27 fenugreek samples..... 113

Figure 33: HCA dendrogram based on HPTLC analysis, total polyphenols and flavonoids contents, TRG & 4-HIL contents and IC₅₀ for α -amylase assay for 27 fenugreek samples 114

List of Tables

Table 1: Reported nitrogenous compounds in fenugreek.	12
Table 2: Reported saponins/sapogenins in fenugreek.....	12
Table 3: Reported amino acids in fenugreek.	13
Table 4: Flavonoids reported in fenugreek	14
Table 5: Coumarins reported in fenugreek	17
Table 6: Stilbenes reported in fenugreek	17
Table 7: Miscellaneous Phenolic compounds in fenugreek.....	17
Table 8: Reported volatile compounds in fenugreek.	19
Table 9: Fatty acids reported in fenugreek seeds.....	20
Table 10: Reported <i>in vitro</i> antimicrobial activity of fenugreek	22
Table 11: Reported <i>in vitro</i> anticancer activity of fenugreek	27
Table 12: Application of different chromatographic methods for the quality control of fenugreek.	38
Table 13: Application of different spectroscopic methods for the quality control of fenugreek /trigonelline containing plants.....	44
Table 14: Application of DNA-based techniques for the quality control of fenugreek.....	47
Table 15: Application of chemometrics in quality control of fenugreek.....	51
Table 16: Fenugreek samples with their codes, market sources, and country of origins.	56
Table 17: Extraction yield % (Average of 4 replicates) of fenugreek seeds collected from different geographical origins..	68
Table 18: Extraction yield % (Average of 4 replicates) for cultivated fenugreek seeds.	71
Table 19: Absorbance of different concentrations of gallic acid standard at 765 nm.	80
Table 20: Absorbance of different concentrations of quercetin standard at 415 nm.	82
Table 21: Total polyphenol and total flavonoid content calculated in ($\mu\text{g}/\text{mg}$) dry residue in different fenugreek samples	84
Table 22: Different concentrations of trigonelline standard and their measured peak areas at 265 nm	87
Table 23: Trigonelline and 4-hydroxyisoleucine contents in (mg/g) dry residue from HPTLC analysis of fenugreek samples	96

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
IC₅₀	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