A Comparative study on the Chemical and Biological Profiling of *Acacia* species growing in Egypt

A thesis submitted by

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Bachelor Degree of Pharmaceutical Sciences and Drug Design
Ain Shams University, 2013

In Partial Fulfillment of the Requirements for the Degree of Masters in Pharmaceutical Sciences (Pharmacognosy)

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2018

Acknowledgments

First of all, I would like to extend due praise and thanks to ALLAH, the source of knowledge and may his peace and blessings be upon all his prophets; for helping and guiding me throughout my life. In my quest to complete this thesis I have been blessed with an abundance of support, endless patience and guidance throughout this gratifying learning experience.

I would like to express my deep sense of gratitude and respectful regards to my thesis supervisor **Prof. Abdel-Nasser Singab**; for his unfailing support and encouragement through the process of researching and writing this thesis. His expertise and useful feedbacks on my work contributed to the final result of this work.

I would like to deeply thank **Prof. Amira Abdel Motaal** for steering me into a path of intellectual growth and encouraging me to always exploit my potential to the maximum. I would sincerely like to thank **Dr. Sherweit El-Ahmady** for her invaluable guidance, patience and insightful comments that added considerably to my experience. Her office was always open for me throughout the journey and her consistent "you are almost done!" pulled me through.

I am highly thankful to **Prof. Rudolf Bauer**, Professor of Pharmacognosy, Institute of Pharmaceutical Sciences, University of Graz for hosting me in his lab for a period of 6 months and providing me with an intellectual encouraging research environment.

I am grateful to **Dr. Rania Hathout** for her valuable and insightful input in this work**. Dr. Mohamed Ashour** and **Dr. Ahmed Elissawy**, thank you for your much appreciated assistance.

I must express my deep and sincere gratitude to my parents for their unparalleled love, providing me with selfless, unfailing support and encouragement throughout the years of study. I am forever indebted to my parents for giving the opportunities and experiences that have made me who I am. I am sincerely thankful to my sister Dina for always being there for me when I needed her and sharing with me this rollercoaster ride. I want to also sincerely thank my friends for providing me with unlimited encouragements.

I would like to dedicate this thesis to my late aunt, Abir, her husband, Dr Tarek and their son Mohamed. May their soul rest in peace.

Abstract

Three Acacia species: *A. farnesiana, A. tortilis and A. longifolia*, were investigated for seasonal variations of the phytochemical constituents and their influence on in-vitro anti-inflammatory activities. Samples of leaves and bark of the three species were collected in two different seasons and extracted using 50% and 70% ethanol. LC-MS analysis revealed the presence of rutin in the leaves of all three species, while catechin was commonly present in the bark. Other major components included vicenin 2, isoquercitrin, kaempferol 3-*O*-rutinoside and isorhamnetin 3-*O*-rutinoside in *A. farensiana* leaves, while *A. longifolia* leaves contained myricetin 3-O-rhamnoside, quercitrin and luteolin. The level of rutin varied according to the flowering period being highest in winter in *A. tortilis* (245.4 mg/mg) and *A. longifolia* (3.0 mg/mg). The content of total phenolics (TPC) showed a marked variation among the three species, which was reflected to some extent in the COX-1 and COX-2 enzymes inhibition. The anti-inflammatory activity of the 50% AF bark (s) extract was assessed at two different doses adopting the carrageenan induced rat hind paw edema method. The maximum inhibition of edema of the bark extract was 34% at 120min and 37.8% at 240min for doses of 100 and 200 mg/kg, respectively. Optimization modeling was performed for better visualization and utilization of the data showing a strong inverse correlation between TPC and the mean inhibition of COX-2 in case of the leaves (r^2 0.783). Finally, cluster analysis of the data obtained from the leaf samples reflected on the taxonomical classification of the species; that of *A. farnesiana* and *A. tortilis* on one hand and that of *A. longifolia* in another class. This is in coherence with the grouping of the former speci in the *Vachellia* taxon while that of *A. longifolia* in the *Acacia* taxon.

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Abbreviation list

AF	*Acacia farensiana*
AFLs	*Acacia farnesiana* leaves collected in spring
AFLw	*Acacia farensiana* leaves collected in winter
AT	*Acacia tortilis*
ATLs	*Acacia tortilis* leaves collected in spring
ATLw	*Acacia tortilis* leaves collected in winter
AL	*Acacia longifolia*
ALLs	*Acacia longifolia* leaves collected in spring
ALLw	*Acacia longifolia* leaves collected in winter
Ara	Arabinose
CMC	Carboxymethylcellulose
COX -1	Cyclooxgenase -1 enzyme
COX-2	Cyclooxygenase-2 enzyme
d	Doublet
dd	Doublet of doublet
ETOH	Ethanol
ESI-MS	Electron Spray Ionization Mass Spectrometer
Fig.	Figure
Glu	Glucose
HM	Herbal medicine

HR	High resolution
HCA	Hierarchal cluster analysis
^{1}H-NMR	Proton Nuclear Magnetic Resonance
HPLC/PDA	High performance liquid chromatography -Photodiode array
HRESIMS	High Resolution Electrospray Ionisation Magnetic Resonance
LC-ESI-MS	Liquid chromatography- electron spray ionization- mass spectrometer
NMR	Nuclear magnetic resonance
MS	Mass spectrometry
m/z	Mass to charge ratio
NS-398	N- [2-(cyclohexyloxy) 4-nitrophenyl] methane sulfonamide
PCA	Principle Component Analysis
Rham	Rhamnose
TPC	Total polyphenolic content
TLC	Thin Layer Chromatography
UV	Ultraviolet
UHPLC	Ultra High Performance Liquid Chromatography
WHO	World Health Organization

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INTRODUCTION

Introduction

Herbal medicines (HM) and their preparations has been developed thousands of years ago to relief symptoms or to treat diseases all over the world. Herbal products are dispensed either as single herbs or as a combination of several herbs in composite formulae. A good record of plants used is available for Traditional Chinese Medicine, Kampo medicine, Ayurvedic medicine, European medicine, and traditional medicines of Africa, Australia and Americas (Wink, 2015). The ancient Egyptians were familiar with many medicinal plants and were aware of their usefulness in treatment of various diseases. They used the plants organs such as roots, rhizomes, flowers, leaves, fruits, seeds and oils where they applied their medicaments in the form of powders, pills, suppositories, creams, pastes and ointments. Scientific evidence for the medicinal properties of such plants have not been all reported; only a few naturally occurring active compounds from plants have been developed by the pharmaceutical industry into products and are presently found in the market (Abdel-Azim et al., 2011).

The past decade has seen an unprecedented growth in the popularity of herbal medicines for preventing and treating diseases (Hennell, 2012). However, as the popularity of complementary medicines continues to grow, serious concerns have been raised about their quality and safety. In 2000 the World Health Organization (WHO) still indicated the lack of adequate or accepted research methodology for evaluating traditional medicines and since then, much effort is put in the quality control and research of herbal products. Quality control of products of natural origin is required because the variability of the active compounds in herbs is large. Depending on the species and on environmental factors, such as the cultivating region, the climate (temperature, humidity, light, wind) and the harvest time, the active compounds and their concentrations can vary widely. Differences are also caused by the way the plants are treated, e.g. the way of drying, washing, crushing and pulverizing, the storage and the conservation (Alaerts et al., 2010). For the above reasons herbal medicine quality control (QC) poses a great challenge, as the large variety of complex chemicals and the compositional variation makes analysis especially difficult. In monographs of the WHO (Guidelines for the Assessment of Herbal Medicine et al.), the European Pharmacopoeia (The European Pharmacopoeia online et al.), the United States Pharmacopeia (The United States Pharmacopeia 28 and The United States Pharmacopeial Convention: Rockville) and the Pharmacopoeia of the People's Republic of

China (hui, 2000) markers are often used for identification and quality control of bulk herbal material besides macroscopic and microscopic identification. These markers are compounds used for quality control, regardless their therapeutic properties. However, given the complexity of herbs concerning their composition, markers are not always sufficient for the identification and quality control of the global herb (Xie et al., 2006). Additionally, studying just a few markers does not reflect the complexity of the biological samples and ignores synergic effects between compounds. The lack of unique chemical compounds (markers) for the identification of certain herbs is also a real problem to qualitatively differentiate them.

To counter the uprising problem of marker identification, it seems necessary to determine a profile of the phytochemical constituents of herbal products in order to ensure the reliability and repeatability of pharmacological and clinical research, to understand their bioactivities, possible side effects and to enhance product quality control (Bauer, 1998). To develop the characteristic herbal profile or 'fingerprint' several analytical techniques including Spectroscopy, High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), Capillary Electrophoresis (CE) and Thin Layer Chromatography (TLC) can be applied. Such techniques are generally powerful and can provide rich information on complex samples and mixtures. Fingerprint approaches rely on the inherent relationships between multiple compounds to display the chemical pattern of herbal sources. Hence, multivariate analysis is used to facilitate practically impossible comparison of complex data. This includes searching similarities, matching, discrimination, classification and finding relationships between data and hence it becomes the preferred method of sample comparison for quality assurance requirements. Multivariate data handling of herbal fingerprints involves pattern recognition and multivariate calibration data analysis where pattern recognition is subdivided into Exploratory Data Analysis, Unsupervised Pattern Recognition and Supervised Pattern Recognition.

Within the Fabaceae family with about 730 genera, the genus *Acacia* is one of the largest generas with regard to the number of species included. More than 1450 species are found distributed in Africa, the Americas, Asia and Australia as well as the Middle East where several members of the genus date from the very early times. The ancient Egyptians had a hieroglyphic symbol for *Acacia*, and the Bible describes the Ark of the Tabernacle as being made of *Acacia* wood (Murphy, 2008). *Acacias* have a variety of secondary compounds, some of which are a

good source of food or food additives, whereas others have potential industrial applications. Secondary metabolites includes amines and alkaloids, cyanogenic glycosides, cyclitols, fatty acids and seed oils, fluoroacetate, gums, non-protein amino acids, terpenes including essential oils, diterpenes, phytosterol and triterpene genins and saponins, hydrolyzable tannins, flavonoids and condensed tannins *(Elansary et al., 2011)*. Various *Acacia* species have been reported to be effective against a variety of diseases including malaria, leprosy and most concerning cancer. The fresh plant parts of different *Acacia* species are considered as astringent, spasmolytic, demulcent, anthelmintic and abortifacient in Indian traditional medicine system (Ahmad et al., 2011).

Although several studies have been published indicating the seasonal variation of secondary metabolites but no compiled information representing the optimum season for the collection of pharmaceutically important constituents from different plant sources is available (Soni et al., 2015). The main objective of this study was to determine whether seasonal variation and the extracting solvent are significant parameters affecting the chemical composition and consequently the anti-inflammatory activity of the three *Acacia* species in question; *Acacia farnesiana, Acacia tortilis* and *Acacia longifolia*. Chemometric approaches were applied on the obtained data for quality authentication of the species using chemometric software, including data presentation in two dimensional models as Principle Component Analysis (PCA) and Hierarchal Cluster Analysis (HCA).

The steps of protocol were done as follows

1. Collection of different organs of the studied species in different seasons.
2. Phytochemical screening of the plant.
3. Preparation of extracts using different solvents of different organs.
4. Profiling of the different extracts using the appropriate analytical method.
5. Determination of the biological activity of the extracts.
6. Optimization modeling to determine the best collection season and extracting solvent.
7. Using multivariate analysis to compare the species and to associate the results of biological activity to the chemical profiling.

Review of literature