بسم الله الرحمن الرحيم

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تم رفع هذه الرسالة بواسطة / مني مغربي أحمد

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

ملاحظات: لا يوجد

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Investigation of the phytochemical constituents and pharmacological effects of *Alhagi maurorum*, family Fabaceae

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2022

Acknowledgment

First of all, ultimate thanks and praises are to ALLAH. Without your power and mercy, one could do nothing.

I dedicate this work to the soul of my dad. I will always be a proud daughter. Thank you for paving the way and for being an inspirational role model throughout my whole life.

Words will never express my deepest gratitude to all those who helped me during preparation of this study.

Assoc. Prof. Dr. Nada Mostafa, Associate Professor of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, for her kind supervision, efforts, scientific and academic guidance throughout the whole work. It is a great honour to work under her supervision.

Prof. Dr. Mohamed El-Shazly, Professor of Pharmacognosy, Faculty of Pharmacy and Biotechnology, German University in Cairo, for offering the plant to be studied, his constructive guidance and thorough revision of this work till it reached this form. I shall always appreciate his help.

Assoc. Prof. Dr. Noha Swilam, Associate Professor of Pharmacognosy, The British University in Egypt, for always being an older sister and a generous supervisor in designing this study as well as guiding me through all the study till it reached its final form. I greatly appreciate her efforts motivating me all the way through.

And I would like to thank **Assoc. Prof. Dr. Heba Handoussa**, Associate Professor of Pharmacognosy, Faculty of Pharmacy and Biotechnology, German University in Cairo for her valuable help and support in performing the UPLC-PDA-ESI/MS/MS part in this study. **Assoc. Prof. Dr. Esther Menze**, Associate Professor of Pharmacology and Toxicology, Faculty of Pharmacy, Ain Shams University for her great support and guidance throughout the pharmacological antiulcer study, data analysis and results interpretation. **Prof. Dr. Reham Amin,** Professor and head of department of Oral Biology, Faculty of Dentistry, The British University in Egypt for suggesting the immunohistochemical marker to be measured as well as guiding me in the work related to staining and morphometric analysis. **Dr. Nouran Fahmy,** Lecturer of

Pharmacognosy, Faculty of Pharmacy, Ain Shams University. My dear friend and section-mate thanks for always being there for me since day one in college.

I am thankful to the British University in Egypt for providing the laboratory facilities.

A special thank you to my **mom**. I will never be able to repay your endless love, support and sacrifices. Without your support and enthusiasm this thesis would not have been possible. My deep love and appreciation to my life partner the most supportive husband **Mohamed**, thank you for making my life easier. I would like to thank my great brother **Sherif** for his support and for always offering me many opportunities in work and life. I am proud to be your little sister. My soulmate sister **Mariam**, thank you for having you by my side throughout the whole journey. My lovely children **Ali** and **Lana** I promise to spend more time with you. My friend **Dina Moustafa** thank you for all your care and support. Thanks to all my BUE friends and colleagues for their continuous encouragement.

Hala El-Zahar

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List of abbreviations

ALP	Alkaline phosphatase
ALT	Alanine transaminase
ANOVA	Analysis of variance
AST	Aspartate transaminase
CEAM	Crude extract of *Alhagi maurorum*
COX	Cyclooxygenase
DPPH	2,2-Diphenyl-1-picrylhydrazyl
EFAM	Ethyl acetate fraction of *Alhagi maurorum*
ELISA	Enzyme-linked immunosorbent assay
ESI	Electrospray ionization
RF	Radio frequency
FRAP	Ferric reducing antioxidant power
GAE	Gallic acid equivalent
GPx	Glutathione peroxidase
GSH	Reduced Glutathione
HEPES	4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid
H & E	Hematoxylin and eosin stain
HPLC	High performance liquid chromatography
HRP	Horseradish Peroxidase
IL	Interleukin
MCP-1	Monocyte chemoattractant protein-1
MDA	Malondialdehyde
MMP-9	Metalloproteinase-9
MTT	3-(4,5-dimethylthiazol-2-yl_-2,5-diphenyl-2H-tetrazolium bromide
OEC	Oral epithelial cells
PCNA	Proliferating cell nuclear antigen
PLGA	Poly (lactic-co-glycolic acid)
RE	Rutin equivalent
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TMB	Tetra-methyl-benzidine
TNF-α	Tumor necrosis factor-alpha
TPTZ	2,4,6-Tripyridyl-s-triazine)–ferric complex
UPLC	Ultra-performance liquid chromatography

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Introduction

Medicinal plants have been always an inspiring source for the discovery and synthesis of new drugs. Ancient people used to treat different diseases by plants from surrounding nature driven by their intuition and former trials. Thus, folk medicine has a long history as old as the history of humanity (Singh, 2018). In modern medicine, natural products have proven to be one of the valuable sources for developing new lead compounds and scaffolds which help in the discovery of drugs (Yuan *et al.*, 2016).

Family Fabaceae (Leguminoseae) is the third largest family of flowering plants behind Orchidaceae and Asteraceae. It is one of the most diverse plant families in the world with around 20,000 species and 700 genera providing many edible and raw plant materials which are used in drug industry (Tavassoli *et al*., 2020).

Alhagi maurorum, belongs to the genus *Alhagi*, family Fabaceae, is a widely distributed folk medicinal plant. Traditionally, the plant roots, aerial parts, and manna are used to cure different diseases in different areas especially in the Middle East/North Africa (MENA) and Central Asian countries (Ahmad *et al.*, 2015a).

In Egypt, *A. maurorum* was used to treat gastrointestinal, liver, and urinary tract disorders (Awaad *et al.*, 2008). It was traditionally recommended for treatment of the progressive ulcers, haemorrhoids, cough, rheumatic pain, angina, eczema, and gastritis in Pakistan, Uzbekistan, and Saudi Arabia (Tavassoli *et al*., 2020). The manna of *Alhagi* was reported in the Islamic traditional medicine (ITM) books for its laxative, detergent, and antipyretic properties, and as a cure for cough and liver bile diseases (Tavassoli *et al*., 2020).

Aim of the work:

The aim of the work presented in this thesis is:

1. Collection, extraction and fractionation of *Alhagi maurorum* aerial parts using different solvents.
2. Determination of total phenolic and flavonoid content of the crude extract and different fractions.
3. LC-MS/MS analysis and standardization of the polyphenol-rich fraction
4. Isolation of the major compounds from the polyphenol fraction.
5. Evaluation of the *in vitro* antioxidant and cytotoxic activities of the crude extract and its fractions.
6. Assessment of the *in vivo* anti-inflammatory and antiulcerogenic activity of the crude extract and fractions.

Review of literature

Genus *Alhagi* is well known for its rich phytochemical profile with different reported phytochemical constituents as well as numerous biological activities. This encouraged us to perform a comprehensive literature survey of the different *Alhagi* species to study the previously reported data regarding the phytochemistry and biological activities of this genus and to perform more research on *Alhagi* different species.

The review is divided into four main sections:

Section I: Phytochemical review of different phytochemical constituents and their distribution within *Alhagi* species

Section II: Biological review of genus *Alhagi*

Section III: Folk medicine and traditional uses of genus *Alhagi*

Section IV: Oral ulcers.

I. Phytochemical review of genus *Alhagi*

Phytochemical studies performed on the different *Alhagi* species have showed the occurrence of different classes of active constituents, such as polyphenols, alkaloids, polysaccharides, proteins, and lipids. The main phytochemicals formerly identified from different *Alhagi* species were demonstrated in tables (1) as follows:

1. Polyphenols

Polyphenols class was found to be the major phytochemical class in genus *Alhagi* including flavonoids, organic acids, proanthocyanidins and lignans as shown in table (1).

Flavonoids including quercetin (1) was isolated from the aerial parts of *A. maurorum* (Abdel-Mageed *et al.*, 2019), isorhamnetin (4) was isolated from the aerial parts of different *Alhagi* species such as *A. pseudalhagi* (Guijie *et al.*, 2010), and *A. persarum* (Eskalieva and Burasheva, 2002). (Singh *et al.*, 1999) isolated two flavonone glycosides *Alhagi*din (10) and *Alhagi*tin (11) from the whole plant of *A. pseudalhagi* and their structures were chemically and spectroscopically established as hespiritin 7-galactosyl (1→2) [rhamnosyl(1→6)] glucoside and naringenin 5-methylether 4'-glucoside respectively.

(Zhou *et al.*, 2017) isolated for the first time from the ethanol extract of aerial parts of *A. sparsifolia*; formonoetin (23), 3', 4', 7-trihydroxylisoflavone (24) and butin (25). A urease inhibitory octamethoxy-flavanenol (28) was isolated from the ethyl acetate fraction of *A. maurorum* roots (Al-Snafi, 2015).

(Alimova *et al.*, 2010) identified two oligomeric proanthocyanidin glucosides; alhacin (45) and alhacidin (46) from the aerial and underground parts of *A. pseudalhagi.* Their structures and relative configurations were elucidated as 7-O-β-D-Glcp→6 galloyl-(+) catechin-(4α-8) -(-)-