Phytochemical and Biological Studies on *Albizia anthelmintica* Family Fabaceae

A Thesis Submitted
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I dedicate this work to the soul of

My mother Aziza

Who taught me the meaning of hard work, how to be

Self-dependent and patient, without her prayers and love

I wouldn't have achieved anything in my life

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Dina Mohammed Bahagt Seif

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List of Abbreviations

ALP	Alkaline Phosphatase
ALT	Alkaline Transaminase
APT	Attached Proton Test
Ara	Arabinose
AST	Aspartate Transaminase
A2780	Human Ovarian Carcinoma Cell Line
A549	Human Lung Epithelial Carcinoma Cell Line
brs	Broad singlet
BEL 7402	Hepatocellular Carcinoma Cell Line
BGC 823	Human Gastric Cancer Cell Line
CC	Column Chromatography
^{13}C-NMR	Carbon-13 Nuclear Magnetic Resonance
CD_3OD	Deutrated methanol
conc.	Concentrated
COSY	Correlation Spectroscopy
d	Doublet
dd	Doublet of doublet
dil.	Diluted
DMSO-d_6	Deutrated Dimethyl Sulfoxide
ELISA	Enzyme-Linked Immuno-Sorbent Assay
EDTA	Ethylene Diamine Tetra Acetic acid
FBS	Fetal Bovine Serum
Fig.	Figure
GGT	Gamma Glutamyl Transferase
Glc	Glucose
^{1}H-NMR	Proton Nuclear Magnetic Resonance

HCT-8, HCT-116	Human Colon Carcinoma Cell Line
HT-29	Human Colorectal Adenocarcinoma Cell Line
HepG-2	Human Hepatocellular Carcinoma Cell Line
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High Performance Liquid Chromatography
HRESIMS	High Resolution ElectroSpray Ionization Mass Spectrometry
HSQC	Heteronuclear Single Quantum Correlation
Hz	Hertz
IC_{50}	Inhibitory Concentration showing 50 % inhibition
***J* value**	Coupling Constant
KB	Oral Carcinoma Cell Line
LC_{50}	Lethal Concentration to 50%
LC_{90}	Lethal Concentration to 90%
LD_{50}	Lethal Dose showing 50% inhibition
LED	Light Emitting Diode
m	multiplet
MCF 7	Human Breast Adenocarcinoma Cell Line
mp	Melting point
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MS	Mass Spectrometry
m/z	Mass to charge ratio
OA	Oleanolic Acid
OD	Optical Density
ppm	Part per million
RPMI	Roswell Park Memorial Institute medium
R_f	Retardation factor
Rha	Rhamnose
s	Singlet

SRB	Sulphorhodamine B
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
Xyl	Xylose
µg/mL	Microgram per milliliter
µM	Micromole
UV	Ultraviolet
δ	Chemical shift by delta value
λ	Wave length

Introduction

Medicinal plants are considered a treasure house of potential drugs and recently there has been an increasing awareness about their importance. This interest in drugs of natural origin is due to several reasons, including the frequent inefficiency of conventional medicine, possible development of side effects of synthetic drugs and also large percentage of the world's poor population doesn't have access to these synthetic drugs. Furthermore, the long history of use of folk medicine suggests that natural products have fewer side effects compared to synthetic drugs. It has been estimated that in developed countries such as the United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as China and India, the contribution is as much as 80% [Kokila *et al*., 2013]. The plant kingdom offers a unique and renewable resource for the discovery of potential new drugs and important lead compounds against various pharmacological targets including pain, cancer, HIV, Alzheimer's disease and malaria [Farag *et al.,* 2013].

In Egypt, many plants have been used in folk medicine. The ancient Egyptians were fa?miliar with many medicinal herbs and aware of their usefulness in the treatment of various diseases. They used the plant organs such as the roots, rhizomes, flowers, leaves, fruits and seeds. They applied their medicaments in the form of powders, pills, suppositories, creams, pastes and ointments. However, scientific evidence for the medicinal properties of such plants was not always demonstrated [Mohamed *et al*., 2013].

Family Fabaceae (legume or bean family) is a large and economically important family of flowering plants. It includes trees, shrubs, and perennial or annual herbaceous plants, which are easily recognized by their fruit (legume) and their compound stipulated leaves. The family is widely distributed and is the third-largest land plant family in terms of number of species [Stevens, 2006]. Legumes are economically and culturally important plants due to their extraordinary diversity and abundance, they comprise a wide variety of edible vegetables that contain oil and fats with different uses. Their medicinal value is due to their effectiveness in the treatment of a wide variety of human diseases. The diversity of chemically active constituents, such as tannins, flavonoids, alkaloids and terpenes found in the plants of this family are known to have a potent biological activity [Molares and Ladio, 2011]

Albizia is a large genus belonging to the family Fabaceae, which comprises about 150 species that are widely distributed in the tropics, with the great diversity in Africa and Central South America. They are commonly called silk plants or silk trees. The generic name honors the Italian nobleman Filippo degli Albizzi, who introduced *Albizia julibrissin* to Europe in the mid-18th century. Albizias are important forage, timber, medicinal plants and many are cultivated as ornamentals for their attractive flowers. *Albizia* species have been reported to be rich in phenolic compounds, steroidal and triterpenoidal saponins [Joycharat *et al*., 2013]. *Albizia*, Tree of Happiness is used in traditional Chinese medicine for relieving depression, insomnia, irritability and stress. [Chen and Chen, 2001]. It is also used by native tribes in tropical and subtropical regions of Asia and Africa for the treatment of skin problems, malaria, cough, dysentery, ameobiasis and as an anthelmintic [Kokila *et al*., 2013].

Albizia anthelmintica Brong. or worm cure *Albizia* is a bush that is common in western part of Sudan and also cultivated in Egypt. It is an attractive tree in bloom, with fluffy-cream coloured, scented flowers and is suitable for ornamental purposes. Its wood is used for manufacturing furniture. The stem bark has been used in Sudanese folk medicine for the treatment of tape worm infection, stomach troubles, amoebic dysentery and less frequently for malaria [Broun and Massey, 1929]. The plant is widely used in East Africa by small holder farmers to treat their livestock against internal parasites [Anon, 1996].

The main objective of this study was to investigate the cytotoxic bioactivity of *A. anthelmintica* leaves and to determine its phytochemical composition, in order to identify the compounds responsible for this bioactivity. The selected plant leaves was chosen based on preliminary phytochemical screening which revealed the presence of saponins and phenolic compounds. Though many phytochemical constituents and promising pharmacological activities were reported from different plants belonging to this genus, yet nothing could be traced concerning the saponin content of *A. anthelmintica*.

In addition, few studies were traced in the literature on the pharmacological activity of *A. anthelmintica,* mainly about the anthelmintic activity of the bark [Koko *et al*., 2000; Gradé *et al*., 2008], and the antioxidant activity of the leaves [Mohamed *et al*., 2013]. Therefore, it was found interesting to carry out another perspective of biological and phytochemical investigation on *A. anthelmintica* leaves.

The steps of the protocol were done as follows:

1. Collection, identification and drying of plant material.

2. Phytochemical screening of the plant.

3. Preparation of the plant extract.

4. Fractionation of the plant extract using various chromatographic techniques.

5. Determination of the biological activity of the extract, fractions and isolated compounds.

6. A phytochemical study of the most biologically active fraction using chromatographic techniques to isolate the compounds responsible for the bioactivity.

7. Structural identification of the isolated compounds using spectroscopic and spectrometric techniques.