Ain Shams University
Faculty of Science
Botany Department

SCREENING ANTITUMOR ACTIVITIES IN CERTAIN EGYPTIAN PLANTS

Thesis
Submitted for the Partial Fulfillment of the Degree of
Master of Science in Botany

By
Marwa Mohamed Mounir Mohamed Aly Ahmed
B.Sc. in Science (Botany-Chemistry)
(2002)

Ain Shams University
Faculty of Science
Botany Department

2010

Ain Shams University
Faculty of Science
Botany Department

SCREENING ANTITUMOR ACTIVITIES IN CERTAIN EGYPTIAN PLANTS

Thesis
Submitted for the Partial Fulfillment of the Degree of
Master of Science in Botany

By
Marwa Mohamed Mounir Mohamed Aly Ahmed
B.Sc. in Science (Botany-Chemistry)
(2002)

Supervised By

Prof. Dr. Sayed Farag Khalifa
Professor of Plant Taxonomy and Flora, Botany Department,
Faculty of Science, Ain Shams University

Prof. Dr. Seham Mohamed Aly Moustafa
Professor of Plant Physiology, Botany Department,
Faculty of Science, Ain Shams University

Prof. Dr. Bassem Mohamed S. EL-Menshawi
Professor of Pharmacognosy, Pharmacognosy Department,
National Research Center

Ain Shams University
Faculty of Science
Botany Department
2010

Approval Sheet

Title of the Thesis: Screening Antitumor Activities in Certain Egyptian Plants

Degree: Master of Science in Botany

Name: Marwa Mohamed Mounir Mohamed Aly Ahmed

This Thesis for Master of Science Degree has been approved by:

1. ***Prof. Dr. Safya Mohamed Ghazi***
   Professor of Plant Physiology, Botany Department,
   Faculty of Science, Helwan University

2. ***Prof. Dr. Mymona Abd El Aziz Krd***
   Professor of Plant Physiology, Botany Department,
   Faculty of Science, Cairo University

3. ***Prof. Dr. Seham Mohamed Aly Moustafa***
   Professor of Plant Physiology, Botany Department,
   Faculty of Science, Ain Shams University

Date of examination: / / 2010

بسم الله الرحمن الرحيم

الحمد لله الذى هدانا لهذا و ما
كنّا لنهتدى لولا أن هدانا الله

صدق الله العظيم
(سورة الأعراف الآية ٤٣)

ACKNOWLEDGMENT

First of all, all thanks & praise are to *Allah* for giving me prosperity & strength to fulfill this work.

I would like to express my gratitude to Late *Professor Dr. Sayed Farag khalifa* (Botany Department, Faculty of Science, Ain Shams University) for supervision, generous guidance & sincere help throughout this work.

My sincere appreciation is expressed to *Professor Dr. Bassem S.El-Menshawi* (Prof. of Pharmacognosy, National Research Centre) for suggesting the research point, supervising this work, and offering me the opportunity to join his project's team, where this study was conducted under the project "Plant Drug Discovery", funded by the Academy of Scientific Research and Technology.

Thanks are also due to *Prof. Dr. Seham Mohamed Aly Moustafa* for guidance and valuable advices throughout accomplishment of this thesis.

I am also grateful to *Prof. Dr.Amira A.Hassanein***,** Head of Botany Department, Faculty of Science, Ain Shams University, for her support.

I would like to express my sincere gratitude to *Professor Dr. Lotfy Boulos*, Professor of Botany (Visiting Professor, Alexandria University) and *Agronomist Teresa Labib* (Orman Garden) for the identification and authentication of the plant species, and to *Dr. Ashraf Tawfeek Soliman*, Assistant Professor of Ecology, Faculty of Science, Cairo University, for his kind help in the collection of the wild species specimens and the in-field identification.

I would like also to thank *Dr.Khaled Mahmoud Hanafy* and *Dr. Salwa El-Haloty*, Researchers, Pharmacognosy department, NRC, for their help and support.

I would like also to express my personal gratitude and sincere thanks to *Mrs May Aly Hassan El-Manawaty* Ass. Researcher, Pharmacognosy department, NRC, for her sincere guidance; and to *Dr. Dina Nadeem Abdel Shafy*, Researcher, Virology department, NRC, for her help.

A special "Thank you" is due to all my colleagues at the *In Vitro* Bioassay-Cell Culture lab and Pharmacognosy Research laboratory-NRC for their help and sincere support throughout the years.

Many thanks to the staff members & Head of Botany Department, Faculty of Science, Ain Shams University for their help in this work.

ABSTRACT

ABSTRACT

One hundred and twenty Egyptian plants were randomly collected from different localities in Egypt. Botanical voucher specimens for all samples collected were prepared for the confirmation of taxonomical identification. Crude methanolic extracts were prepared for different organs of the plant species under investigation.

Biological screening was carried out to the respective extracts using two *in vitro* bioassays [brine shrimp lethality and hepatocellular human tumor cell line (HepG2) cytotoxicity] with the aim of identifying active antitumor plant extract(s) that can represent a possible candidate drug with cytotoxic/antitumor properties.

The four most active methanolic plant extracts on brine shrimp lethality *in vitro* bioassay were chosen as promising bioactive materials and their LC_{50} and LC_{90} were calculated and afterward subjected to initial fractionation schemes. These plants were *Casimiroa edulis* (branches), *Cedrela odorata* (leaves), *Citrus sinensis* (branches) and *Tabernamontana coronaria* (branches).

The five most active extracts on hepatocellular human tumor cell line (HepG 2) *in vitro* cytotoxicity bioassay were chosen as promising materials and their LC_{50} and LC_{90} were calculated. These extracts were subjected to initial fractionation schemes. These promising extracts were those of *Alkanna orientalis* (herb), *Amberboa lippii* (herb), *Haplophyllum tuberculatum* (herb), *Primula boveana* (herb) and *Verbascum sinaiticum* (herb).

Key words:

Egyptian plants, taxonomical identification, extraction, fractionation, cytotoxicity, antitumor, *in vitro* bioassay, brine shrimp, human tumor cell lines (HepG 2).

CONTENTS

PREFACE

Cancer refers to a group of diseases characterized by abnormal cell proliferation with a tendency to invade adjacent tissues and produce metastases (WHO, 2005). It can seriously threaten human health and it may progress until it causes death (Amara *et al*., 2008 and American Cancer Society, 2002a). Cancer causes about 13% of all deaths worldwide (WHO, 2006). According to the American Cancer Society, 7.6 million people died from cancer in the world during 2007 (American Cancer Society, 2007). The mechanisms of formation and spreading of cancer are still not well understood but both external factors (e.g., tobacco smoking, chemicals, radiation and infections) and internal ones (e.g., inherited metabolism mutations, hormones and immune conditions) are believed to be relevant. These factors may act together or sequentially to initiate and promote carcinogenesis where it may take more than 10 years from the initiation of cell mutation to the formation of detectable cancer. It should be mentioned that there is still a lack of thorough cure for cancers. A worthy goal at the present is to develop treatments that produce remission and/or palliation. A

cancer is said to be in remission when all clinical evidence of cancer has disappeared (Feng and Chien, 2003).

Modern cancer therapies have significantly prolonged the average life of the cancer patient, but still have not succeeded in reducing cancer mortality in certain types of cancer (Amin and Mousa, 2007). Most cancers can be treated and some cured, depending on the specific type, location and stage. Once diagnosed, effective treatments include surgery, radiotherapy, chemotherapy, hormone therapy and immunotherapy. Each of these treatments has advantages and disadvantages, and their combination is usually needed to produce the most effective results (American Cancer Society, 2002b). As research develops, treatments are becoming more specific for the type of cancer pathology.

Throughout the ages, humans have relied on nature for their basic needs for the production of foodstuffs, shelter, clothing, means of transportation, fertilizers, flavors and fragrances, and, not least, medicines, where nature continues to be an abundant source of novel chemotypes and pharmacophores (Cragg and Newman, 2005b).

Natural products have been a source of medicinal agents for thousands of years and an impressive number of

modern drugs have been isolated from natural sources, particularly plants (Cragg and Newman, 2005a). Thus, there is an unquestionable demand for novel cytotoxic agents bringing not only gains in efficacy but decreasing the frequency of adverse effects and offering a better convenience in administration (Ismael *et al*., 2008). Consequently, the need has been expressed for industrial drug production from plants in order to increase the economic and health potentials as well as the social benefits from natural resources (Elujoba *et al*., 2005).

INTRODUCTION

Cancer or neoplasm is a malignant growth, which is characterized by unregulated proliferation of cells in the body. It can arise in any organ of the body such as lung, breast, ovary, intestine, leukocytes, etc. Cancerous cells propagate from a single cell and multiply without control to develop into tumor tissues. These cancerous cells can invade nearby tissues and can spread through the bloodstream and lymphatic system to other parts of the body (metastasis) (Nygren, 2001).

It poses a real global problem, accounting for 12.5% of all death cases worldwide in 2005; by 2020, new cases of cancer are expected to reach about 15 million every year; 70% of which will be in developing countries, including over one million in the African region (WHO, 2005).

MEDICINAL PLANTS AND NATURAL PRODUCTS

Currently there is considerable scientific and commercial interest in the continuing discovery of new anticancer agents from natural product sources (Kinghorn *et al*., 2003). The search for natural products to be used in cancer therapy represents an area of great interest in which the plant kingdom has been the most important source,

providing many anti-tumor agents with novel structures and unique mechanism of action (Chang *et al*., 1999).

Natural Products, especially plants, have been used for the treatment of not only cancer but a variety of diseases for thousands of years. Terrestrial plants have been used as medicines in Egypt, China, India and Greece from ancient times and an impressive number of modern drugs have been developed from them (Shoeb, 2006). Analyses of the new drugs marketed during the period between 1981 and 2002 show that about 50 % owe their origin in one way or another from natural sources and in some disease areas well over 60 % are derived from natural products (79 % of the antibacterial, 62 % of the anticancer and 74 % of the antihypertensive drugs are derived from natural products) (Newman *et al*., 2003).

Drug discovery from medicinal plants has played an important role in the treatment of cancer and, indeed, most new clinical applications of plant natural metabolites and their derivatives over the last half century have been applied towards combating cancer (Newman *et al*., 2000&2003; Butler, 2004). Of all available anticancer drugs between 1940 and 2002, 40% were natural products or natural product-derived (Newman *et al*., 2003).

WHO (2001) defined natural products from medicinal plants as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes, which might be used as active constituents in immediate consumption or as a basis for herbal products.

Africa has a long and comprehensive list of medicinal plants based on local knowledge (Ethnobotanical evidence). Phytochemical screening of these plants revealed the presence of secondary metabolites such as alkaloids, saponins, flavonoids and phenols with biological activity. The plant parts where these secondary metabolites are found include the leaves, flowers, seeds, roots, barks and stems and are used for preparing medicines or "herbal drugs" (Okigbo *et al*., 2009).

Phytochemicals are chemical compounds formed during the plant normal metabolic processes. These chemicals are often referred to as "secondary metabolities" and more recently natural products, of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids (Harborne, 1973; Okwu, 2004).