ISOLATION AND CHARACTERIZATION OF CERTAIN COMPOUNDS HAVING CYTOTOXIC PROPERTIES FROM *VITEX TRIFOLIA PURPUREA*

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Thesis

Submitted in partial fulfillment of the requirement

for the degree of M. Sc. of Science

(Organic Chemistry)

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2012

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ACKNOWLEDGEMENT

First of all great thanks for ALLAH for giving me the potential and patience to achieve this work.

I would like to express my sincerely grateful and deep appreciation to *Professor Dr. Maher A. Mahmoud,* Professor of Organic Chemistry (***D.Sc***.), Faculty of Science, Ain Shams University, for his kindly supervision, continuous advice and his encouragement during this work.

I would like to express my deepest gratitude and hearty appreciation to *Professor Dr. Mortada M. El-Sayed,* Professor of Medicinal Chemistry, Department of Medicinal Chemistry and Biochemistry, Theodor Bilharz Research Institute, for suggesting this work, his kindly supervision, cooperation, positive support and great help for introduction and discussion of this work.

I would like to express my hearty appreciation and sincere gratitude to *Dr. Mona A. Mohamed* Assistant professor of Medicinal Chemistry, Department of Medicinal Chemistry and Biochemistry, Theodor Bilharz Research Institute, for her kindly supervision, great efforts and advice throughout this thesis.

My deep thanks and gratitude to medicinal chemistry department stuff members and friends for help and supporting me during this work.

Finally, this dissertation is dedicated to my lovely parents, brother Ahmed, sisters Wafaa and Heba for their love, inspiration, encouragement, and always supporting me. Words can not adequately express my gratitude towards my beautiful wife Jasmin, for her unlimited support and continuously encouragement to accomplish this work. I am grateful to my sweet heart son Eiad, for filling my life with joy and motivation.

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SUMMARY

-The present study aimed to discovery of cytotoxic compounds from natural sources. Therefore, the methanolic (85%) extract of *Vitex trifolia purpurea* was prepared and successively fractionated with petroleum ether, chloroform and ethyl acetate.

- The total phenolic contents as well as antioxidant activity of the methanolic (85%) extract, chloroform, ethyl acetate and the remaining residue derived from the methanolic extract after fractionation process were evaluated. The results showed that these extracts have antioxidant activity which was correlated with their phenolic contents.

- The methanolic (85%) extract, chloroform fraction, ethyl acetate fraction and the remaining residue derived from the methanolic extract after fractionation process were evaluated as cytotoxic agents especially against human liver cell lines (Hep-G2). The results indicated that all the tested extracts have high cytotoxic activity (IC_{50} = 10.7, 20.8, 65.8 and 6 µg/ml, respectively).

- Each of the chloroform fraction and the remaining residue was submitted to chromatographic separation using paper chromatography, thin layer chromatography and column

chromatography. Also, the structures of the isolated compounds were elucidated using certain spectroscopic techniques such as: UV, IR and NMR spectra.

- From the chloroform fraction, three compounds (1 – 3) were isolated and their structures were elucidated as:

P-hydroxybenzoic acid (1); β-sitosterol-3-*O*-*β*-D-glucopyranoside (2) and 3-*O*-*p*-hydroxycinnamoyl-23-hydroxyursolic acid (3).

- From the remaining residue, three compounds (4 – 6) were isolated and their structures were elucidated as: **Quercetin-3-*O*-*β*-D-glucopyranoside (4); Luteolin-7-*O*-glucoside (5) and Rutin (6).**

- Evaluation of the isolated compounds as cytotoxic agents against Hep-G2 cell lines revealed that p-hydroxybenzoic acid (1), 3-*O*-*p*-hydroxycinnamoyl-23-hydroxyursolic acid (3), quercetin-3-*O*-*β*-D-glucopyranoside (4) and luteolin-7-*O*-glucoside (5) have high cytotoxic activity (IC_{50} =1.24, 1.75, 1.3 and 1.68 µg/ml, respectively). Therefore, this plant *Vitex trifolia purpurea* could be recommended for further studies in order to discover new anticancer drugs from natural sources.

INTRODUCTION

Cancer, medically called a malignant neoplasm, is a term for a large group of different diseases. In cancer, cells divide and grow uncontrollably forming malignant tumors and invade nearby parts of the body **[Mathess *et al*., 2001; Jamal *et al*., 2011]**. The cancer may also spread to more distant parts of the body through the lymphatic system or blood stream. While cancer can affect people of all ages, the risk of developing cancer generally with age. Rates are rising as more people live to an old age and as mass lifestyle changes occur in the developing world **[Mathess *et al*., 2001; Jamal *et al*., 2011; Kathiresan**, ***et al*., 2006]**. Many things are known to increase the risk of cancer including tobacco use, infection, radiation, lack of physical activity, poor diet, obesity and environmental pollutions. Cancer is usually treated with chemotherapy, radiotherapy and surgery **[Madhuri and Pandey, 2009; Anand and Kunnumakkara, 2008]**.

Liver cancer (Hepatocellular Carcinoma, HCC), is a cancer arising from the liver. It is also known as primary liver cancer or hepatoma. It is the commonest primary malignant cancer of the liver in the world. This type is usually combined to the liver, although occasionally it spreads to other organs. It is more common in men and

occurs mostly in people with liver cirrhosis [**Ramaa *et al*., 2010; El-Zayendi *et al*., 2005]**. The risk factors and the pathogenesis of hepatocellular carcinoma (HCC) include the role of viral hepatitis, toxins such as alcohol and aflatoxins and insulin resistance. The epidemiology of HCC is characterized by marked demographic and geographic variations [**El-Zayendi *et al*., 2005; Gomaa *et al*., 2008]**.

Cytotoxic drugs, sometimes known as antineoplastic, anticancer or cancer chemotherapy drugs include a wide range of chemical compounds. Because of their ability to kill tumor cells by interfering with cell division, they are extensively used to treat cancer and some have other medical applications [**Halta, and Ueoka, 2005; Chabner *et al*., 2005]**. Several cytotoxic compounds are available and from experience it is known which drugs are most suitable for a specific cancer type. However, most anticancer drugs lack tumor specificity and cause damage to normal tissues, leading to side effects. Chemotherapeutic drugs are usually used in combination to give a more effective result [**Chabner *et al*., 2005]**. The classical anticancer agents are the alkylating agents (including the platinum compounds), antimetabolites, topoisomerase inhibitors and tubulin- acting agents [**Chabner *et al*., 2005; Musa *et al*., 2011].**The classic chemotherapeutic agents are

effective in many cases, but in view of the fact that many tumors develop resistance to the drugs, there is a need for novel, effective anticancer drugs with new mechanisms of actions. More targeted treatment options are also highly warranted to enable specific eradication of tumor without effecting normal tissues, thus reducing the side effects.

Plants have a great potential for producing new drugs for human benefit. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and even infectious diseases. The increased interest in plant derived drugs is mainly because of the wide spread belief that it is (herbal medicine) safer than costly synthetic drugs which possess side effects. Hence, there is need to screen medicinal plants for promising biological activity. Further, there is a continuous development of resistant strains which pose the need for search and development of new drugs to cure diseases **[Newman and Cragg, 2007; Cragg *et al*., 2009]**. Plants have a long history of use in the treatment of cancer, Hartwell, in his review of plants used against cancer, lists more than 3000 plant species that have been used in the treatment of cancer. The search for anticancer agents from plant sources started with the discovery and development of the vinca alkaloids and isolation of cytotoxic podophyllotoxins. This led to the

discovery of numerous novel chemotherapies showing a range of cytotoxic activities [**Kamr *et al*., 2011].** It is well known that natural products have played an important role in the discovery of useful antitumor agents. Especially clinically relevant anticancer drugs; such as taxol, Camptothecin, vinblastine and vincristine which were discovered from higher plants. Nonetheless, as exemplified by the frequent morbidity and mortality associated with metastatic conditions, there is still clearly a need for the discovery of new agents with higher efficacy **(Mohamed *et al*., 2011)**.

The genus *Vitex* (Family; Verbenaceae) approximately includes 270 known species of trees and shrubs within tropical and sub-tropical regions. It is an interesting source of potential bioactive molecules, as iridoids compounds, flavonoids, diterpenoids derivatives, phytosteroids **[Meena *et al*., 2010]**. *Vitex* species have been reported to be used in traditional medicine to treat a wide range of ailments, such as depression, venereal diseases, malaria, asthma, allergy, wounds, skin diseases, snake bite and body pains **[Nyiligira *et al*., 2007]**. Also, many plants of the genus *Vitex* showed antioxidant, anti-inflammatory, antimicrobial and hepatoprotective activities **[Meena *et al*., 2010]**, as well as cytotoxic activities against different cancer cell lines