Biochemical Studies on Some Natural Products Isolated from Orange Peels

A THESIS
SUBMITTED BY

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بسم الله الرحمن الرحيم

قال تعالى :" و ما أوتيتم من العلم إلا قليلا "

صدق الله العظيم

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رســـالة دكتـوراه الفلسفه فى العلوم

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Abstract

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Biochemical Studies on Some Natural Products Isolated from Orange Peels.

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Four higher plants were chosen from ten examined plants and investigated for their constituents and antimicrobial activities. The most antimicrobial bioactive powder of the examined plants was orange peels. Egyptian baladi orange peels powder (*Citrus sinensis*) exhibited higher significant antimicrobial activity on bacteria, yeast and moderate activity on fungi. It contains 64.4 mg/100g dry matter soluble sugars using glucose as a standard and 792.4 mg/100g total phenol using rutin as a standard. The average content of pectin was 157 mg / g dry weight. Total fiber content of orange peels powder was 55 %. Extracts of cold and hot water, ethanol (95%) and ethanol (50%), chloroform and ether were obtained from the dried orange peels. Cold water which was considered as the most active fraction and was further purified by column chromatography on silica gel and Sephadex LH-20 columns, yielded fractions with considerable antimicrobial activities. IE and IIE fractions had the lowest MIC values against tested bacteria and fungi ranged from 13 –275 μg / ml (*Candida albicans* is the most sensitive strain with MIC equal to 130 μg/ml). Total flavonoid compounds were identified and quantified by HPLC. It has been also demonstrated that cold water extract and its fractions exerted high antioxidant capability, insecticidal activity and were non toxic. All extracts were found to contain mainly high amounts of soluble phenolic compounds. The most active fractions were further purified by polyamide S column, TLC and Sephadex LH-20 column. The molecular weight of each compound of IE and IIE fractions was determined by using Sephadex G-25 column equilibrated by 50 % aqueous acetone. Further confirmation was done by studying m.p., $^{1}H_{1}$ NMR and IR. Didymin, naringin, hesperidin, naringenin, tetra-O-methylscutellarein, sinensetin, nobiletin, and tangeretin were the main compounds isolated and purified from fractions IE and IIE.

Keywords: Baladi orange peels, Cold water extract, Antimicrobial activity, Silica gel column, Sephadex LH-20 column, Flavonoids, Insecticidal activity, Antioxidant activity.

Aim of the work

After screening of different plants for their contents and studying their antimicrobial properties, the present study was designed to fulfill following goals:-

►Preparation of successive extracts by different solvents of orange peels powder for selection the extract which has the most potent antimicrobial activity towards bacteria, yeast and fungi strains.

►Purification of the selected extract of orange peels powder by column chromatography using suitable methods.

►Studying the antimicrobial activity of the isolated and purified fractions besides their toxicity, insecticidal activity and their antioxidant effect.

►Identification of the compounds isolated from the purified fractions by the spectral analysis i.e. $^{1}H_{1}$-NMR, IR, UV spectrum and determination of the melting point.

Introduction

Throughout the ages, plants have been used by humans as a source of food, cosmetics, medicine, clothing and even shelter. Plants have served as the basis of sophisticated traditional medicine systems for thousands of years in countries such as China and India. These plant-based systems continue to play an essential role in health care. It has been estimated by the WHO that about 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care. Plant products also play an important role in the health care systems of the remaining 20 % of the population who mainly reside in developed countries, **Kumarasamy *et al*., 2002.**

Fruits and vegetables have had conferred to be capable of delivering health benefits besides fulfilling physiological needs, **Kaur and Kapoor, 2001** and **Esra *et al*., 2006.**

The world production of citrus fruits in 1990 was 16% higher than in 1980. This fact has promoted a rapid growth of the citrus fruit industry, as well as an increased development of the technology for by-products utilization. Production of oranges is the highest among citrus fruits, contributing 71% to the total amount, followed by mandarins (13%), lemons and limes (10%) and grapefruit (6%), **Kale and Adsule, 1995.** According to the **FAO, 1994** the world production of orange fruit is 58.7 million tons per year, where the Egyptian production is 1.3 million tons per year.

Citrus peel has been reported to be a good source of pectin and dietary fiber in general, with an equilibrated proportion of soluble and insoluble fractions, **Baker *et al*., 1995 and Larrauri *et al*., 1994**. Approaches to the development of products with increased dietary benefits from citrus peel have placed emphasis not only on the recovery of carbohydrates and pectin, **Baker, 1994** but also on the production of potentially important secondary metabolites, such as polyphenols, **Manthey and Grohmann, 1996**.