Faculty of science
Ain-Shams University

Evaluation of Anticancer and Antioxidant Activities of Propolis, Pollen and Probiotic Bacteria as Functional Food Ingredients

A thesis
Submitted For the Degree of Doctor of Philosophy in Science
(Microbiology)

By

Sara Adel Amer Mahmoud

M.Sc. of Microbiology, Ain Shams Univ., 2012.

Microbiology Department
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Approval Sheet

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كلية العلوم
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تقييم نشاط صمغ النحل و حبوب اللقاح وبكتريا البروبيوتك المثبط للسرطان والمضاد للأكسدة كمكونات أغذية وظيفية

رســـــاله

مقدمة للحصول علي درجة دكتــوراة الفلسـفة في العلوم

في الميكروبيولوجـــي

من

ســـــارة عادل عامر محمود

(ماجيستير العلوم في الميكروبيولوجي-جامعة عين شمس - ٢٠١٢)

قسم الميكروبيولوجي

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ثم

كامل الشكر و التقدير و الإمتنان إلى الأساتذة الذين قاموا بالإشراف على هذا العمل حتى يظهر بصورة لائقة وهم:

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ثم أتقدم بخالص الشكر إلى أعضاء قسم الاغذية الخاصة والتغذية بمعهد بحوث تكنولوجيا الأغذية و كل من ساعدنى بالقول أو بالفعل أثناء قيامى بهذا العمل لإخراجه فى أحسن صورة.

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Dedication

This thesis is dedicated to:

The sake of Allah, my Creator and my Master,

My great teacher and messenger, Mohammed (May Allah bless and grant him), who taught us the purpose of life

My great parents, who never stop giving of themselves in countless ways,

My dearest husband, who leads me through the valley of darkness with light of hope and support,

My beloved sisters

My lovely kids: Larin & Lamar.

To all my family, the symbol of love and giving,

To My friends who encourage and support me,

To all the people in my life who touch my heart, I dedicate this research.

Declaration

I hereby declare that this thesis is my own work and effort and that it has not been submitted anywhere for any award. Where other sources of information have been used, they have been acknowledged

Signature: Sara Adel Amer

Abstract

Apiculture products and probiotic exhibits valuable pharmacological and functional properties. Our aim of this study was combining propolis, pollen and probiotic as a natural, nutritive and functional ingredients in order to capitalize most of their desired health benefits. So the present study was firstly planned to determine the chemical composition of propolis and pollen extracted by different ethanol/water ratio (100% ethanol, 70% ethanol/water and 100% water). The second step was evaluating the pharmaceutical activity of the prepared extracts of propolis and pollen or their mixture (antibacterial, antioxidant and anticancer activity). The third objective was exploring the effect of addition of propolis, pollen extracts or their mixtures on the antibacterial and antioxidant activities of the tested lactic acid bacterial strains. The final objective was selecting the best bee product extracts and lactic acid bacterial strains, in order to be used in manufacturing of stirred biofermented milk fortified with chosen bee product extracts.

Propolis and pollen extracts were obtained by different concentrations of ethanol. 26 phenolic compounds and 10 flavonoids from propolis and pollen extracts were identified. The major phenolic compounds were ethyl vanillin and hisperidin. Moreover, Propolis and pollen have found to contain a variety of vitamins, minerals and sugars.

The obtained results indicated that the tested extracts have antibacterial activities against foodborne pathogenic bacteria.

MICs of extracts for food-borne pathogenic bacteria ranged between 20-2.5 mg/ml. Meanwhile, there was no recorded MIC against LAB strains. Moreover, some LAB strains have been stimulated at lower concentrations of extracts. The antibacterial activity of propolis extracts was higher than pollen extracts. In synergism assay, the antibacterial activity of some tested LAB strains have been remarkably improved by the presence of some extracts of propolis and pollen or their mixtures.

The antioxidant activity of propolis and pollen extracts was comparable. However, there was a significance difference between the IC_{50} of the extracts. 70% ethanolic extracts of propolis (EEP70) and combined mixtures of ethanolic propolis and pollen extracts (EPP70) have found to exhibit high antioxidant activity.

All tested extracts show cytotoxic activity against the two tested cancer cell lines: breast cancer (MCF-7) and liver cancer (Hep-G2) cell lines. Generally, 70% ethanolic extract of propolis (EEP70), water extract of propolis (WEP) and combined mixture of water extracts of propolis and pollen (WPP) show the highest cytotoxic activity.

Lactobacillus acidophilus TISTR 450 was selected for production of stirred acidophilus milk fortified with water extract of propolis (WEP) or with mixture of water extracts of propolis and pollen (WPP). These fermented products were found to contain a verity of vitamins and organic acids. The best organoleptic properties were obtained with WPP acidophilus milk followed by WEP acidophilus milk. In addition, the viable

cell counts of probiotic bacteria in fermented milk product were satisfactory, maintaining above a level of 7 log cfu/ml during storage at 4°C within designated shelf-life. The highest number of probiotic strain was achieved with WPP fermented acidophilus milk. This indicates that potential health benefits could be obtained by regular consumption of acidophilus milk fortified with WPP. This study may be useful in developing functional foods with high dietary antioxidant content or chemopreventive anticancer drugs with a potential to influence tumor cell progression.

Keywords: Propolis, Pollen, Lactic Acid Bacteria, Antioxidant, Antibacterial, anticancer, Polyphenols, MIC, Fermented Milk.

Contents