Effect of Propolis and Bee Venom against Some Pathogenic Bacteria

Thesis

Submitted for the degree of Master of Science as a Partial fulfillment for requirements of the Master of Science

By

Basma Ibrahim Mohamed Hussein

B.Sc. Entomology/Microbiology
Faculty of Science, Ain Shams University

Supervised by

Prof. Amany Zaky Mohamed Kamal Salem

Prof. of Entomology Department
Faculty of Science – Ain Shams University

Prof. Elsayed Ibrahim Haggag Badair

Prof. of Beekeeping, Agriculture Research Center, Giza

Prof. Wael Refat Abd Elhameed Hablas

Prof. of Clinical Pathology
Faculty of Medicine –Al-Azhar University

Faculty of Science

Ain Shams University

2015

وَلَوْلَا فَضْلُ اللَّهِ عَلَيْكَ وَرَحْمَتُهُ لَهَمَّتْ
طَّآئِفَةٌ مِّنْهُمْ
أَن يُضِلُّوكَ وَمَا يُضِلُّونَ إِلَّا أَنفُسَهُمْ
ۖ
وَمَا يَضُرُّونَكَ مِن شَيْءٍ ۚ وَأَنزَلَ اللَّهُ
عَلَيْكَ الْكِتَابَ وَالْحِكْمَةَ وَعَلَّمَكَ مَا
لَمْ تَكُن تَعْلَمُ ۚ وَكَانَ فَضْلُ اللَّهِ عَلَ
يْكَ عَظِيمًا ﴿١١٣﴾

Acknowledgments

First I feel so thankful to Allah *for showing me the right ftath and helping me to complete this work.*

My appreciation to Prof. Amany Zaky Mohamed Kamal Salem, *ftrof. of Entomology Department, Faculty of Science, Ain Shams University for supporting me with the facilitations and the help she offered to me.*

My sincere gratitude to my advisor Prof. Elsayed Ibrahim Haggag Bedair, *ftrof. of Beekeeping, Agriculture Research Center, Giza, for the continuous support, his welcome to me at any time I need him. Also my sincere thanks to* Prof. Wael Refat Abd Elhameed Hablas *ftrof. and Head of clinical pathology Department, Faculty of Medicine Al-Azhar University for his cooperation, support and supervision.*

Special thanks to Dr. Abir Elfiky, *Director of ANDI Center in Vacsera, for her helpful manner And advices, also many thank to* Prof. Dr Ahmed Abdel-Hady *plant protection Institute for encouraging me to continue my study in the master degree.*

Last but not least, I can't forget to thank all members of my Family, especially my Parents, and my Husband for pushing me forward in every step in the journey of my life.

Candidate

Basma

List of Contents

Subject	*Page No.*
List of Abbreviations	**i**
List of Tables	**ii**
List of Figures	**iii**
Abstract	**iv**
Introduction	**1**
Literature Review	**6**
Materials and Methods	**30**
Results	**43**
Discussion	**65**
Conclusion	**81**
Summary	**82**
References	**84**
Arabic Summary	-
Arabic Abstract	-

List of Abbreviations

B.subtilis	*Bacillus subtilis*
BV	Bee venom
BVA	Bee venom acupuncture
C.albicans	*Candida albicans*
Conc.	Concentration
E.coli	*Escherichia coli*
EEHP	Ethanol Extracted Haramaya Propolis
EEP	Ethanol extract of Propolis
F.gigantica	*Fasciola gigantic*
GC/ MS	Gas chromatography / Mass spectrometry
HPLC	High performance liquid chromatography
Hr	Hour
Labs	Laboratories
MIC	Minimum inhibitory concentrations
MRSA	methicillin- resistant *Staphylococcus aureus*
R.Sphaeroides	*Rhodobacter sphaeroides*
TEM	transmission electron microscope
µg	Microgram

List of Tables

Tab. No.	Title	Page
1	The identified compounds of Propolis sample by GC/MS	46
2	Effect of Propolis of different concentrations	48
3	Effect of Propolis (300µl/well) on the selected pathogens	51
4	HPLC analysis of Bee venom sample test	58
5	Bee venom potency in mice	60

List of Figures

Fig. No.	Title	Page
1	Chromatogram for Propolis sample (EEP) analyzed by GC-MS	45
2	Inhibitory zones of clinical isolates of different concentrations of propolis	48
3	The effect of 2% conc. of propolis on *Salmonella sp*	49
4	The effect of 2% conc. of propolis on MRSA	50
5	The effect of 2% conc. of propolis on *E.coli*	50
6	The effect of 2% conc. of propolis on *Proteus*	51
7	Inhibitory zones of the bacterial strains of 1% conc. (300 µl /well) of propolis	52
8	Effect of propolis on MRSA of 1% conc. (300 µl /well) of propolis	53
9	Inhibition zones exhibited by propolis 1%conc	54
10	TEM results control images	56
11	TEM results from the treated MRSA	57
12	HPLC chromatogram of Bee venom	59
13	Inhibitory zones of clinical isolates at different bee venom conc.	61

14	The inhibition zones of the 4 bacterial strains (5% bee venom conc.)	**63**
15	The inhibition zones of the 4 bacterial strains (2.5% bee venom conc.)	**64**
16	The inhibition zones of the 4 bacterial strains (1.25% bee venom conc.)	**65**

Abstract

This thesis aimed to evaluate the antibacterial effect of Propolis and bee venom of *Apis mellifera carnica* on selected human pathogenic bacteria (methicillin resistant *staphylococcus aureus* (MRSA) represented Gram positive bacteria, while *Proteus sp., E.coli, and Salmonella sp.* represented Gram negative bacteria. Chemical analyses of Propolis & bee venom were performed using GC/MS and HPLC, respectively.

For Propolis analysis, the major component was Hexamethyl- cyclotrisiloxane which was found to be recent to the Egyptian Propolis constitution; also esters of fatty acids were dominant in the sample.

Qualitative HPLC analysis of honey bee venom cleared that the sample contained the major components which were melittin (52.1%), phospholipase A2 (15.91%), apamin (2.3%).

Agar well diffusion method was used to evaluate the antibacterial activity of both Propolis and bee venom. The results showed that Propolis and bee venom had significant effect on (MRSA) in low conc. and had a weak activity against Gram-negative bacteria on (*Proteus sp., E.coli* ,and *Salmonella sp*.) in low conc.

The mode of action of Propolis on MRSA was found out by transmission electron microscope, the images showed clear inhibition of cell line division causing non-separation of daughter cells.

Introduction

Introduction

Apitherapy is the use of honey bee products for medical purposes, this include bee venom, true honey, royal jelly, pollen, Propolis, and beeswax. Their use in human treatment traced back thousands of years and healing properties are included in many religious texts including the Veda, Bible and Quran **(Ali, 2012).**

The most attracting bee products are Propolis and Bee venom for their biological studies and antimicrobial activities, both contain pharmacologically important constituents **(Bankova, 2005, Prakash & Bhargava, 2014).**

Propolis is a resinous substance with a complex chemical composition that is collected from various species of plants by *Apis mellifera* bees, which results in a material of different colors and consistencies **(Alencar *et al.*, 2007).** It has been long used in folk medicine of different nations as early in Egypt as 3000B.c **(Haile & Dekebo, 2013).**

It is mainly composed of resin (50%),wax(30%),essential oils (10%),pollen(5%) and other organic compounds (5%)(**Gomez-caravaca etal.,2006).**

Bees use it in the construction and protective layer as bacteria, viruses, and fungi.Bee venom is synthesized in the venom glands of worker bees and virgin queen and is stored in the venom sac and injected through the sting apparatus during the stinging process (**Schmidt and Buchman, 1999)**.The main components of Bee venom are enzymes: phospholipase A2, hyaluronidase, peptides: melittin (approx. 50%), mast cell degranulating (MCD) peptide, apamin, and promelittin **(Haghi *et al.*, 2012).**

The widespread use of antibiotics and chemicals against harmful microorganisms has increased and lead to the microbial resistance for many of them **(Davies, 2010).**

The emergence and rapid spread of Multidrug Resistance bacterial infections, especially methicillin-resistant *Staphylococcus aureus* (MRSA), has increased the rate of patient mortality and morbidity and become

A major challenge in clinical treatment of patients with conventional antibiotics **(Phougat *et al*., 2014).**

The aim of this thesis is to investigate the antibacterial effects of Propolis and bee venom on some selected human pathogenic bacteria specially the most common human pathogenic bacteria found in hospitals causing serious diseases to man; which are methicillin- resistant *Staphylococcus aureus* (MRSA),. *Escherichia coli, Salmonella sp. and Proteus sp*.

Selection of the bacterial strains of this study depended on its impact in the microbiological labs and the Egyptian hospitals as they are considered to be most common human pathogenic bacteria.

Staphylococcus aureus (Gram positive) is a significant human pathogen causing healthcare-associated and community acquired infections **(Rasigade and Vandenesch, 2014)**.Antibiotics effectively treat these infections, however, the emergence of methicillin-resistant *S. aureus* (MRSA) currently presents a challenge to healthcare systems worldwide **(Taylor,2013)** also it is difficult to treat using available antibiotic agents **(Choi *et al*., 2015)** and can cause a variety of serious infections that are often difficult to control, It is responsible for many skin and soft tissue

infections as well as a serious form of pneumonia. abscesses, endocarditis and infections of surgical sites **(Fierobe et *al*., 1999, Rubio *et al*., 1999, Taylor, 2013).**MRSA has become the leading cause of invasive illness, resulting in a high rate of mortality worldwide **(Goldrick, 2004, Todd 2006, Hebert & Weber, 2011).**

Proteus species is a Gram-negative bacteria belonging to the Enterobacteriaceae family. It is an opportunistic pathogen capable of causing urinary tract infections and best known for its pattern of swarming differentiation on agar plates, as well as for its association with the development of renal stones in patients with urinary tract infection (**Mobley & Belas. 1995, Coker *et al*., 2000).**

Salmonellae are gram-negative bacteria that cause gastroenteritis and enteric fever **(Ohl & Miller, 2001).**Enteric fever is a prolonged systemic illness that results from infection with the exclusively human pathogens, *S. typhi* and *S. paratyphi.*Clinical manifestations include fever, abdominal pain, transient diarrhea or constipation, and occasionally a maculopapular rash.

Escherichia coli are normal inhabitants of the human gastrointestinal tract and are among the bacterial species most frequently isolated from stool cultures. When *E. coli* strains acquire certain genetic material, they can become pathogenic. *E.*

coli are among the most frequent bacterial causes of diarrhea and are classified by clinical syndrome they produce **(Nataro & Kaper, 1998)**.For these aspects the present study was carried out in order to assess the effect of ethanolic extract of propolis and honeybee venom on these selected human pathogenic bacteria.

Plan of work

1- Extraction of ethanolic extract of Propolis of *Apis mellifera carnica* samples.

2- Chemical analysis of the ethanolic extract of Propolis samples by GC/MS.

3- Qualitative analysis of HPLC of the honey bee venom sample.

4- Making different concentrations for both honey bee products.

5- Collection of determined clinical isolates from Al-Azhar hospital.

6- Agar well diffusion method was used to evaluate the antibacterial activities of both Propolis and Bee venom.

7- Investigation the mode of action of methicillin-resistant *Staphylococcus aureus* treated with Propolis by Transmission electron microscope.