# Table of content

# List of papers 2017

This thesis is based on the following papers, which are referred to in the text by their Arabic numbered.

| | |
|---|---|
| **1** | El-Seedi, H.R., **Abd El-Wahed, A.,** Khalifa, S.M., Ali, H.B., Kita, M., Kigoshi, H., Alajlani, M., Backlund, A., Göransson, U. and Verpoorte, R. Chemistry, chemical property space, activity and synthesis of macrocyclopeptide alkaloids. ***Journal Natural Products***, 2017. Accepted**. IF= 3.662** |
| **2** | Hesham R. El-Seedi , **Aida A. Abd El-Wahed**, Bassem Y. Sheikh, Shaden A. M. Khalifa, Aamer Saeed, Ufuk Koca-Caliskan, Mohammad F. A-Ajmi, Rob Verpoorte. Bee venom composition; unraveling the biodiversity and the potential biological activities. Chapter for published by Elsevier Science Publishers, The Netherlands (**Accepted**) |

## Conferences and posters

1. Medical Congress 2013 - Pain and Pain from 12 -13 November, 2013, **Stockholm** Waterfront Congress Centre.
2. Norcdic Natural Products conference (NNPC):"Natural Products Research - Past, Present &Future" 15th - 16th June 2015, **Visby**, Sweden with one poster.
3. 2I SCE 2015 in Stockholm 23st Conference for the International Society for Chemical Ecology (ISCE 2015), **Stockholm**, 29th -5th July 2015 with two posters.

## Workshop

- Attended for workshop in **Malaya University (UM)** presented by El-Sevier Publishing Campus in November 25, 2015.

## Author's contribution

Participated in the planning experiments, reviewing the literature, chemical analysis, evaluation, and writing.

# Financial Support


- Thanks and appreciation to Division of Pharmacognosy, Department of Medicinal Chemistry, Faculty of Pharmacy, Uppsala University, Sweden for my invitation to visit the laboratory for doing the experiments and enormous number of HPLC, LC-MS and MS-MS analysis in addition to synthesis of the peptides by solid phase synthesizer (SPPS) of my thesis and within the framework of joint co-operation between both **Prof. Hesham El-Seedi** as Egyptian side and **Prof. Ulf Göransson** as Swedish side in the field of scientific research and based on the confidence of the Swedish Professor in the Egyptian side.
- Thanks and appreciation to the working group in both Sweden and Egypt, to their role in the discussions and scientific guidance, which still for hours throughout the days.
- The financial support for conducting experiments and carrying all technical and biological tests in the lab and other labs.
- Thanks and appreciation also to Prof. Hesham El-Seedi who allocated a monthly budget to cover the costs of solvents, equipment and field trips. This opportunity given to post-graduate students to entirely exclude them and their families from the extra costs related to education and/or research.

# Acknowledgment

First and foremost thanks are to ALLAH, the most beneficent and merciful. By the grace of ALLAH, the present work was realized and ended in this form.

From the guideline" who doesn't thank people groups, he doesn't express gratitude toward Allah". I might want to express my earnest appreciation to everybody who has supported and encouraged me. I might want to thank every one of them, as the accompanying: My exceptional supervisor, **Prof. Hesham Rushdy El-Seedi**, Prof. of Natural Products Chemistry, Faculty of Science, El-Menoufia University whom taught me how to focus in my goals, dealing with good behavior, he allowed me to contact with other culture (via traveling to Sweden and Malaysia). I'm indebted for you for all your help and provide all facilities under my disposal that the work need. I would like to thanks my supervisor, **Prof. Hamdy Taher Abou El-Enain**, Department of Bee Research, Plant Protection Research Institute, Agricultural Research Centre for his kind supervision, and encouragement are also appreciated. I extend my humble work for the spirit of my teacher; I hope to be in balance Good deeds.

I would like gratefully to express my deep thanks to **Prof. Ulf Göransson**, Division of Pharmacognosy, Department of Medicinal Chemistry, Faculty of Pharmacy, Uppsala University, Sweden for his kind acceptance to work at his lab in Sweden, all discussion related to the work. He was very kind as provided all facilities to me in his lab and the other labs. **Dr. Robert Burman**, Division of Pharmacognosy, Department of Medicinal Chemistry, Faculty of Pharmacy, Uppsala, University, Sweden for helping me in analysis, his excellent contribution in testing cytotoxicity of the compounds in professional way. He was very kind and co-operative as well as Adam for his contribute in antimicrobial assay. **Dr. Sunithi Gunasekera**, Division of Pharmacognosy, Department of Medicinal Chemistry, Faculty of Pharmacy, Uppsala, University, Sweden for long discussion about synthesis of peptide. **Erik and Cammilla**

(PhD student) explains many things and asked him many times about different things, explained and gave me a lot of time for discussion.

My colleagues in Natural Products laboratory "**Nermeen, Eman, Rehan, Amal, Zahran, Islam, Ahmed, Adel, Shimaa, Amira, Neseren, Asmaa, Mohamed, and Fawkya**" Thanks to all of you for always being there when you were most needed.

Special thanks to **Dr. Naglaa Al-Ahmady**, Department of Bee Research, Plant Protection Research Institute, Agriculture Research Centre for his great support and encouragement during all research work. Also my friends at the work "**MS. Mona, Abeer, Sobhia, Jaber, Salah, Shaaban, Mhagob, Hayam, Hanan, Fikri, Mohamed and Ekbal**" for their support and co-operation: All of them helped me with all they could do. Thank you very much for all.

Finally, I would like to thank **my family** for their spiritual help and patience for their concern, encouragement and financial support of me during all research work.

Last but not least, to every one who gives me a support or was a reason of help I want to thank him from deep of my heart.

**Aida A. Abd El-Wahed**

# Abbreviations

| | |
|---|---|
| ACN | Acetonitrile |
| ACPs | Anti-cancer Peptides |
| Ala | Alanine |
| AMPs | Anti-microbial Peptides |
| ANN | Artificial Neural Network |
| Api m 2 | Hyaluronidase |
| Api m 3 | Acid Phosphatase |
| Api m 5 | Dipeptidylpeptidase IV |
| Api m 7 | CUB Serine Protease |
| Arg | Arginine |
| Asn | Asparagine |
| Asp | Aspartic Acid |
| BV | Bee Venom |
| BOC | *t*-Butoxycarbonyl |
| FMOC | 9-Fluorenylmethyloxycarbonyl |
| CNS | Central Nervous System |
| Cys | Cysteine |
| Da | Dalton |
| DA | Discriminant Analysis |
| DCM | Dichloromethane |
| DIPEA | Diisopropylethylamine |
| DMF | *N*,*N*-Dimethylformamide |
| DMSO | Dimethyl Sulfoxide |
| E-value | Expect Value |
| ESI-MS | Electrospray Ionization-Mass Spectrometry |
| EtOH | Ethanol |
| FA | Formic Acid |
| FDA | Food and Drug Administration |
| FD | Fluroescein Diacetate |
| FMCA | Fluorometric Microculture Cytotoxicity Assay |
| Gln | Glutamine |
| Glu | Glutamic Acid |
| Gly | Glycine |

| | |
|---|---|
| HBTU | *N*,*N*,*N*′,*N*′-Tetramethyluroniumhexafluorophosphate |
| His | Histidine |
| HIV-1 | Human Immune Deficiency Virus 1 |
| HPLC | High Performance Liquid Chromatography |
| HSV-1 | Herps Simplex Virus 1 |
| IARC | International Agency for Research on Cancer |
| $IC_{50}$ | Inhibitory Concentration 50% |
| Ile | Isoleucine |
| LC-MS | Liquid Chromatography-Mass Spectrometry |
| Leu | Leucine |
| LMW | Low Molecular Weight |
| Lys | Lysine |
| NPs | Natural Product |
| µM | Micromolar |
| *m/z* | Mass-To-Charge Ratio |
| MALDI | Matrix-Assisted Laser Desorption/Ionization |
| MCD | Mast Cell Degranulating |
| Met | Methionine |
| MICs | Minimum Inhibitory Concentrations |
| MRJPS | Major Royal Jelly Proteins |
| MS | Mass Spectrometry |
| MS-MS | Mass Spectrometry-Mass Spectrometry |
| MW | Molecular Weight |
| PBS | Phosphate Buffered Saline |
| Phe | Phenylalanine |
| PLA2 | Phospholipase A2 |
| Pro | Proline |
| QTOF-MS | Quadrupole Time-Of-Flight- Mass Spectrometry |
| RF | Random Forest |
| RJ | Royal Jelly |
| RT | Room Temperature |
| S-S bond | Disulfide Bond |
| Ser | Serine |

| | |
|---|---|
| SI | Survival Index |
| SPPS | Solid Phase Peptide Synthesis |
| SVM | Support Vector Machines |
| TFA | Triflouroacetic Acid |
| Thr | Threonine |
| TIPS | Trimethylsilylisopropane |
| Trp | Tryptophan |
| TSB | Tryptic Soy Broth |
| Tyr | Tyrosine |
| Val | Valine |
| WHO | World Health Organization |

# List of tables

# List of figures

# Summary

The work present in this thesis was carried out mainly at the Division of Pharmacognosy, Department of Medicinal Chemistry, Faculty of Pharmacy, Uppsala University, Sweden, Division of Clinical Pharmacology, Uppsala University Hospital, Sweden, a laboratory of Natural Products Chemistry, Department of Chemistry, Faculty of Science, EL-Menoufia University, Egypt and Department of Bee Research, Plant Protection Research Institute, Agricultural Research Centre, Egypt.

This study focus in isolation, characterization and identification for bioactive peptide (s) from royal jelly (RJ) and bee venom (BV), in addition to synthesis of secapin and royalisin from BV and RJ, respectively.

**Specific objectives of this study were:**

- Biologically evaluate for RJ and BV as antimicrobial and anticancer agents, respectively.
- Isolation and structure elucidation of bioactive peptide(s) from RJ and BV.
- Synthesis of bioactive peptide of secapin and royalisin from BV and RJ, respectively.

The work carried out in this thesis had been summarized in the following chapters:

## Chapter 1:

1. In this part, it is focus on cancer, antimicrobial diseases and role of natural products in cure of these diseases. Additionally, some current drugs from nature sources as anticancer and antimicrobial.

2. Gives overview on bee products, their ingredients which act as anti-microbial and anti-cancer activities, finally we focued on RJ and BVas source for bioactive peptide(s).

## Chapter II: Materials, and methodology

This included the materials and reagents were used in addition to the instruments and databases. The chapter is divided into three parts:

**The 1st part:** Isolation and identification for bioactive peptide from RJ as anti-microbial.

**The 2nd part:** Isolation and determination for bioactive peptide from BV as anti-cancer.

**The 3rd part:** Includes synthesis of secapin and royalisin using solid phase peptide synthesis (SPPS).

## Chapter III: Results and discussion

**Part 1:** Enzymatic hydrolyzate for RJ was performed by trypsin with the high yield 51.6%. Forty three peptides were identified using a combination of mass spectrometry and bioinformatics. Hydrolyzate proteins were fractionation using high pressure liquid chromatography (HPLC) into 14

fractions. Six fractions shown antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans*. Bioassay guided isolation for RJ_F11 leads to isolation and structure elucidation of three peptides ((RJHP_1, 2, 3) using RP-HPLC. The peptides were eluted at $R_t$ 32, 36 and 31.5 min, respectively. The amino acid sequence were HPARFENFDDVNFR, VFHPLLKHHQVMNCDK and LLTFDLTTSKLLK, respectively. The molecular weight for isolated peptides were 1762.85, 1945.78 and 1491.31 Da, respectively. The most active peptide was RJHP_3 ($MIC_{100}$ was 216, 108, and 216 µg/ml), respectively compared with melittin ($MIC_{100}$: 5.17, 7.12, 1.78 and 2.2 µg/ml) towards *E. coli, P. aeruginosa* and *S. aureus*, respectively.

**Part 2:** Isolation for four peptide from BV including two new peptides, in addition to melittin, and Mast Cell Degranulating (MCD). The cytotoxic activity of the four peptides were evaluated by FMCA assay, with five different cancer cell lines. Melittin and HF6 peptides were exert promise results as cytotoxic against cancer cell lines ($IC_{50}$ for U-937 GTB, RPMI 8226/s, PRMI 8226/Dox40, CCRF CEM, CEM/ VM-1 at 1.3, 1.1, 1.4, 1.7 and 2.0 µM), respectively, and for melittin while HF6 has $IC_{50}$ at 2.5, 1.8, 2.1, 1.9 and 2.1 µM, respectively.

**Part 3:** Include the synthesis of two peptides; secapin and royalisin from BV and RJ, respectively by SPPS.