

Abstract of thesis presented to the Senate of Universiti Malaysia Terengganu in fulfilment of the requirements for the degree of Master of Science

**IDENTIFICATION AND CHARACTERIZATION OF RED SEAWEED
(*PYROPIA VIETNAMENSIS*) PROTEINS AS POTENTIAL PRECURSOR OF
DIPEPTIDYL PEPTIDASE-IV (DPP-IV) INHIBITORY PEPTIDE USING *IN
SILICO* APPROACH**

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Pyropia vietnamensis, a red seaweed from the Bangiaceae family, has a high protein content. Therefore, it has high potential as a substrate for generating bioactive peptides. This study was conducted to determine the proteomic profiles of *P. vietnamensis* proteins through liquid chromatography-mass spectrometry (LC-MS/MS) analysis to assess its potential as the precursor of dipeptidyl peptidase-IV (DPP-IV) inhibitory peptide using *in silico* approach and to characterize the novel synthetic DPP-IV inhibitory peptide predicted from *in silico* approach. This study extracted proteins from *P. vietnamensis* using a phenol-based extraction method followed by LC-MS/MS analysis for proteomic profiling. Based on the identified proteins, five were selected and analyzed using the BIOPEP-UWMTM database. *In silico* proteolysis was conducted using seven enzymes contained in the enzyme action tool of the database. Furthermore, the novel peptides were screened and characterized *in silico* using various bioinformatics tools, including PeptideRanker, PepCalc, PeptideCutter, ToxinPred, and AllerTop. A novel and potential DPP-IV inhibitory peptide was synthesized and further characterized *in vitro* in terms of its inhibitory activity, mode of inhibition, and stability against pH, temperature, and Simulated Gastrointestinal Digestion (SGID). *In silico* analysis revealed potential bioactive

peptides from *P. vietnamensis*, including DPP-IV inhibitory peptide, Angiotensin-Converting Enzyme (ACE) inhibitory peptide, dipeptidyl peptidase-III (DPP-III) inhibitory peptide, antioxidative, and alpha-glucosidase inhibitor, with DPP-IV inhibitory peptide was predicted as the most effective bioactive peptides. Meanwhile, Calpain 2 was predicted to be the most potential enzyme for releasing DPP-IV inhibitory peptides based on the highest A_E value (0.596). Furthermore, the potential novel peptides include CFA (Cysteine-Phenylalanine-Alanine), ACF (Alanine-Cysteine-Phenylalanine), RFPS (Arginine-Phenylalanine-Proline-Serine), DEWG (Aspartic acid-Glutamine-Tryptophan-Glycine), NYCL (Asparagine-Tyrosine-Cysteine-Leucine), CVPR (Cysteine-Valine-Proline-Arginine), and DACF (Aspartic acid-Alanine-Cysteine-Phenylalanine). It was highlighted that the synthesized CVPR offers an IC_{50} of 0.66 mg/ml and exhibited an uncompetitive mode of action towards DPP-IV inhibitory activity. In addition, the synthesized CVPR was reported to be stable in acidic conditions (pH 3-5), heat stable up to 90°C, and survived SGID. Thus, this study suggests that *P. vietnamensis* proteins could serve as potential precursors for DPP-IV inhibitory peptides and provide insight into the antidiabetic properties of the red seaweed through an *in silico* approach.

Abstrak tesis yang dikemukakan kepada Senat Universiti Malaysia Terengganu sebagai memenuhi keperluan untuk Ijazah Sarjana

PENGENALPASTIAN DAN PENCIRIAN PROTEIN (*PYROPIA VIETNAMENSIS*) RUMPAI LAUT MERAH SEBAGAI PELOPOR PEPTIDA PERENCATAN DIPEPTIDYL PEPTIDASE-IV (DPP-IV) MENGGUNAKAN PENDEKATAN *IN SILIKO*

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Pyropia vietnamensis, rumpai laut merah daripada keluarga Bangiaceae, mempunyai kandungan protein yang tinggi. Oleh itu, ia sangat berpotensi sebagai substrat untuk menjana peptida bioaktif. Kajian ini dijalankan untuk menentukan profil proteomik protein *P. vietnamensis* melalui analisis LC-MS/MS, untuk menilai potensinya sebagai pelopor peptida perencatan dipeptidyl peptidase-IV (DPP-IV) menggunakan pendekatan *in siliko*, dan untuk mencirikan peptida perencatan DPP-IV novel sintetik yang diramalkan daripada pendekatan *in siliko*. Kajian ini telah mengekstrak protein daripada *P. vietnamensis* menggunakan kaedah pengekstrakan berasaskan fenol diikuti dengan analisis LC-MS/MS untuk pemprofilan proteomik. Berdasarkan protein yang dikenal pasti, lima protein telah dipilih dan dianalisis menggunakan pangkalan data BIOPEP-UWMTM. Proteolisis *in siliko* telah dijalankan menggunakan tujuh enzim yang terkandung pada *enzyme action tool* dalam pangkalan data tersebut. Tambahan pula, peptida baru telah disaring dan dicirikan secara *in siliko* menggunakan pelbagai alat bioinformatik termasuk PeptideRanker, PepCalc, PeptideCutter, ToxinPred, dan AllerTop. Peptida perencatan DPP-IV yang baru dan berpotensi telah disintesis dan dicirikan selanjutnya secara *in vitro* dari segi aktiviti perencatannya, mod perencatan, dan kestabilan terhadap pH, suhu, dan Simulasi

Pencernaan Gastrosus (SGID). Analisis *in siliko* mendedahkan peptide bioaktif yang berpotensi daripada *P. vietnamensis* termasuk peptida perencatan DPP-IV, peptida perencatan enzim penukar angiotensin (ACE), peptida perencatan dipeptidyl peptidase-III (DPP-III), antioksidatif, dan perencat alpha glukosidase, dengan peptida perencatan DPP-IV diramalkan sebagai peptida bioaktif yang paling berpotensi. Calpain 2 diramalkan sebagai enzim yang paling berkesan untuk membebaskan peptida perencatan DPP-IV berdasarkan nilai A_E tertinggi (0.596). Tambahan pula, peptida novel yang berpotensi ditemui ialah CFA (Sisteina-Fenilalanina-Alanina), ACF (Alanina-Sisteina-Fenilalanina), RFPS (Arginina-Fenilalanina-Prolina-Serina), DEWG (Asid Aspartik-Glutamina-Triptofan-Glisina), NYCL (Asparagina-Tirosina-Sisteina-Leusina), CVPR (Sisteina-Valina-Prolina-Arginina), dan DACF (Asid Aspartik-Alanina-Sisteina-Fenilalanina). Didapati CVPR yang disintesis memberikan nilai IC_{50} 0.66 mg/ml dan mempamerkan cara tindakan yang tidak kompetitif terhadap aktiviti perencatan DPP-IV. Sebagai tambahan, CVPR yang disintesis didapati stabil dalam keadaan berasid (pH 3-5), stabil terhadap haba sehingga 90°C, dan bertahan terhadap SGID. Dengan demikian, kajian ini mencadangkan protein *P. vietnamensis* sebagai pelopor peptida perencatan DPP-IV yang berpotensi dan memberikan gambaran tentang sifat antidiabetes rumpai laut merah melalui pendekatan *in siliko*.