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IDENTIFICATION AND CHARACTERIZATION OF ANGIOTENSIN-CONVERTING ENZYME (ACE) INHIBITORY PEPTIDE FROM EDIBLE BIRD'S NEST USING *IN SILICO* AND *IN VITRO* APPROACHES

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In this study, *in vitro* and *in silico* approaches were employed to identify potential bioactive peptides in edible bird's nest (EBN). Proteomic profiling of soluble EBN proteins was performed using liquid chromatography tandem mass spectrometry (LC-MS/MS). Five proteins were selected as potential precursors for bioactive peptides which were: deleted in malignant brain tumours 1, lysyl oxidase 3, acidic mammalian chitinase, NK-lysin and mucin-5AC. Analyses on these proteins using BIOPEP-UWM databases gave six dominant bioactivities which were: angiotensin-converting enzyme (ACE) inhibitor, dipeptidyl peptidase-IV (DPP IV) inhibitor, dipeptidyl peptidase-III (DPP III) inhibitor, antioxidative, stimulating and renin inhibitor. The bioactive peptides with the most potential from EBN proteins were the ACE and DPP IV inhibitors. Meanwhile, for the *in silico* proteolysis of EBN proteins using BIOPEP-UWM, 33 types of enzymes were employed which stem bromelain and pepsin gave the highest degree of hydrolysis and produced the highest number of bioactive peptides. Five tripeptides were generated from these EBN proteins after the gastrointestinal digestion simulation, which were IRA, YPG, MKY, IVR and AVL.

However, none of these tripeptides were novel. Whereas, in the *in vitro* approach, EBN hydrolysis using trypsin was used to produce the ACE inhibitory peptide. The EBN hydrolysate was purified using ultrafiltration membrane, fast protein liquid chromatography (FPLC) and reversed phase-high performance liquid chromatography (RP-HPLC). The fraction with a molecular weight of <3 kDa gave the lowest IC_{50} value and was purified using FPLC. The F2 fraction from FPLC giving the lowest IC_{50} value was further purified using RP-HPC, which gave a PP3 peptide with the lowest IC_{50} value. After purity confirmation with analytical HPLC, the PP3 peptide was identified as VLAMQQMDAR using LC-MS/MS, which is a novel peptide. The PP3 peptide gave an IC_{50} value of 186.83 µM, was stable from pH 6 to 8 and temperature temperature until 90^{o}C as well as stable against gastrointestinal digestion. The PP3 peptide exhibited a mixed mode inhibition, i.e., it gave competitive and non-competitive modes. In conclusion, using *in silico* and *in vitro* approaches, this study showed that EBN is a promising source of bioactive peptides, especially the ACE inhibitory peptide.

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PENGENALPASTIAN DAN PENCIRIAN PEPTIDA PERENCAT *ANGIOTENSIN CONVERTING ENZYME* (ACE) DARIPADA SARANG BURUNG WALET MENGGUNAKAN PENDEKATAN *IN SILICO* DAN *IN VITRO*

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Dalam kajian ini, pendekatan *in silico* dan *in vitro* digunakan untuk mengenalpasti sumber peptida bioaktif yang berpotensi daripada Sarang burung walet (SBW) adalah. Pemprofilan proteomik protein SBW larut telah dijalankan menggunakan kromatografi cecair spektrometri jisim tandem (LC-MS/MS). Lima protein telah dipilih sebagai pelopor berpotensi untuk peptida bioaktif iaitu *deleted in malignant brain tumors 1, lysyl oxidase 3, acidic mammalian chitinase, NK-lysin* dan *mucin-5AC*. Analisis protein tersebut menggunakan pangkalan data BIOPEP-UWM mendapati bahawa protein tersebut menghasilkan enam bioaktiviti iaitu perencat ACE, perencat dipeptidyl peptidase-IV (DPP IV), perencat dipeptidyl peptidase-III (DPP III), antioxidatif, peptida perangsang dan perencat renin. Didapati peptida bioaktif yang paling berpotensi daripada protein SBW adalah perencat ACE dan perencat DPP IV. Sementara itu, proteolisis protein SBW secara *in silico*

menggunakan BIOPEP-UWM telah dijalankan menggunakan 33 jenis enzim. Didapati *stem bromelain* dan pepsin memberikan darjah hidrolisis tertinggi dan menghasilkan bilangan peptida bioaktif tertinggi. Lima tripeptida perencat ACE telah dihasilkan daripada protein SBW selepas menjalani simulasi pencernaan gastrointestin iaitu IRA, YPG, MKY, IVR dan AVL. Walau bagaimanapun, peptida yang dihasilkan daripada pendekatan *in silico* telah dilaporkan dalam kajian lepas dan bukan peptida baharu. Manakala, melalui pendekatan *in vitro*, hidrolisis SBW menggunakan tripsin dijalankan untuk menghasilkan peptida perencat ACE. Hidrolisat SBW yang terhasil ditulenkan menggunakan membran pengultra-turasan, kromarografi cecair protein cepat (FPLC) dan kromatografi cecair berprestasi tinggi – fasa terbalik (RP-HPLC). Fraksi dengan berat molekul <3 kDa memberikan nilai IC_{50} terendah dan ditulenkan selanjutnya menggunakan FPLC. Fraksi F2 daripada FPLC menunjukkan nilai IC_{50} terendah dan ditulenkan seterusnya menggunakan RP-HPLC, yang menghasilkan peptida PP3 dengan nilai IC_{50} terendah. Selepas pengesahan ketulenan menggunakan HPLC analitikal, peptida PP3 dikenal pasti sebagai VLAMQQMDAR dengan menggunakan LC-MS/MS. Nilai IC_{50} PP3 adalah 186.83 µM. Peptida PP3 didapati stabil terhadap pH antara pH 6 hingga pH 8 dan suhu sehingga mencecah 90^{o}C. Peptida PP3 juga stabil terhadap pencernaan gastrointestin. Mod perencatan bagi PP3 adalah mod campuran iaitu bersaing dan tak bersaing. Peptida PP3 yang terhasil daripada pendekatan *in vitro* adalah baharu. Kajian ini menunjukkan SBW merupakan sumber yang baik untuk menghasilkan peptida bioaktif terutamanya peptida perencat ACE.