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Proceeding Paper

Characterization of Functional Proteins from Edible Bird's Nest (EBN) Using Proteomic Techniques in Combination with Bioinformatics Analyses †

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Abstract: Edible Bird's Nest (EBN) is a valuable and nutritious food tonic made from the saliva of Swiftlet birds. EBN has been consumed for centuries due to its high nutritional value. Proteins are the main nutrient in EBN. However, studies investigating the functional proteins in EBN are still limited. Therefore, the present study aims to characterize the functional proteins from EBN using proteomic-based techniques. In addition, the molecular characteristics of the functional proteins were analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The results showed that 0.951 ± 0.03 mg/mL of functional proteins were successfully extracted from the EBN. Several distinct protein bands in the range of 35–135 kDa were profiled from the EBN. Using ultrahigh performance liquid chromatography coupled with quadrupole time-of-flight tandem mass spectrometry (UHPLC-Q-TOF-MS-MS), a total of 51 proteins were identified from the EBN. The protein sequences were processed in the BIOPEP database to predict the potential biological activity of EBN proteins. The prediction results suggest that the EBN proteins potentially possess antioxidant, anti-inflammatory, immunostimulatory, antitamnestic, antihypertensive (angiotensin converting enzyme (ACE) inhibitors), antidiabetic (alpha-glucosidase inhibitors, alpha-amylase inhibitors, dipeptidyl peptidase IV inhibitors) and antithrombotic properties. The antioxidant properties [DPPH scavenging activity (20.84% ± 3.43) and ABTS scavenging activity (31.49% ± 1.66)] and anti-inflammatory properties [inhibition of nitric oxide (NO) production (21.30% ± 4.41) and inhibition of albumin denaturation (25.30% ± 0.32)] were experimentally validated. In conclusion, the results suggest that functional proteins from EBN are potentially functional ingredients in the nutraceutical and food industries.

Keywords: Edible Bird's Nest (EBN); Functional Proteins; Proteomic; Bioinformatics

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1. Introduction

EBN is a highly prized delicacy that is made from the solidified saliva of swiftlets. It is known for its unique texture and nutritional value [1]. This delicacy has been consumed for centuries and is often used in traditional Chinese cuisine for its health benefits. Lee et al. [2] reported that the EBN industry is part of Malaysia's National Key Economic Areas (NKEA). As part of this initiative, various efforts have been made to promote EBN production and ensure its sustainability. For instance, studies have been conducted to explore better agronomic practices for EBN production [3]. Additionally, the beneficial properties of EBN have been confirmed by modern science and technology, revealing its nutritional and pharmacological advantages, including antioxidant [4], anti-inflammatory [5], and immune-modulating effects [6]. EBN has been reported that to be rich in proteins (over 50%), with a total amino acid content of 40% [7]. However, relatively little work has been done to characterize functional proteins in EBN using proteomic-based techniques. The present study aims to explore the potential functional properties of EBN proteins and their potential benefits for human health.

2. Materials and Methods

2.1. Chemicals and Materials

The dried EBN used in the study was obtained from a licensed swiftlet facility in Johor, Malaysia. The EBN was ground to powder and stored in an airtight container until further analysis. All chemicals and reagents used in the study were of analytical grade.

2.2. Protein Extraction from EBN

Protein extraction from EBN was performed according to a previously published method by Lee et al. [8]. The protein content of the supernatant was quantified using the Bradford assay [9].

2.3. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The protein sample (0.3 mg/mL) was run on 12% resolving gel and 4% stacking gel using the SDS-PAGE system [8].

2.4. Characterization of Functional Proteins from EBN Using Liquid Chromatography-Mass Spectrometry

The UHPLC-QTOF-MS/MS analytical system (1290 Infinity Series, Agilent Technologies, USA) was used to study the proteins in the EBN. PEAKS Studio X+ (Bioinformatics Solutions Inc., Waterloo, ON, Canada) was used to search and identify protein sequences in the databases (UniProt and Swiss-Prot). The NCBI database was used to identify the unidentified protein sequence. The BIOPEP database (https://biochemia.uwm.edu.pl/en/biopep-uwm-2/, accessed on Day Month Year) was used to process the identified protein sequences for the prediction of the biological activity of EBN.

2.5. Antioxidant and Anti-Inflammatory Activity

The antioxidant (scavenging activities of the ABTS free radical and DPPH free radical) and anti-inflammatory assays (inhibition of nitric oxide (NO) production and inhibition of albumin denaturation) were performed according to the methods reported by Ding et al. [10] and Hu et al. [11], respectively.

2.6. Statistical Analysis

The experiments were performed in triplicate, and the resulting data were analyzed using one-way ANOVA with SPSS (SPSS Inc., Chicago, IL, USA). Statistical significance was considered at a p-value of less than 0.05. The means and standard deviations (SD) were calculated and presented in the results.

3. Results and Discussion

3.1. Identification of EBN Proteins Using SDS-PAGE

After water extraction of protein from EBN, the protein content was 0.951 ± 0.03 mg/mL. The SDS-PAGE technique was used to profile the EBN proteins for quality determination and the electropherogram profile of the EBN protein is depicted in Figure 1. As a result, the protein band occurs between 75–180 kDa (Top), 35–63 kDa (Middle) and below 25 kDa (Bottom). Previous studies have identified several protein bands in EBN samples, with molecular weights ranging from 15 to 150 kDa [12–14]. This wide range of molecular weights indicates the presence of various proteins with different sizes and structures in the EBN. Overall, the different protein bands likely represent different proteins or protein subunits within the EBN sample. To further understand the EBN proteins, mass spectrometry was performed to provide a more complete picture of the EBN proteome.

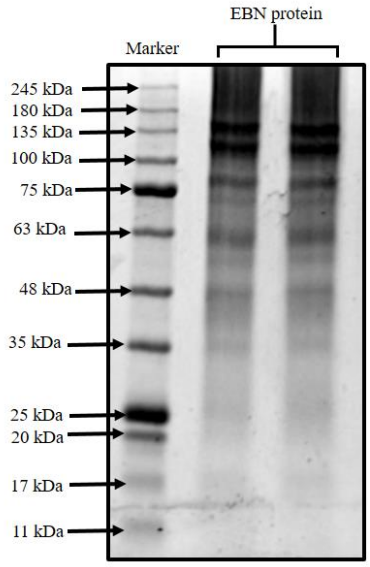

Figure 1. Separation of EBN proteins using Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE).

3.2. Characterization of EBN Proteins and Functional Proteins Prediction

EBN protein was analyzed using LC-MS/MS, generating a total of 51 distinct accumulated proteins (listed in Supplementary File S1). The LC-MS/MS analysis revealed the presence of various proteins associated with EBN. These proteins play crucial roles in various biological processes and could provide valuable insights into the composition and functionality of EBN. Several identified proteins from the LC-MS/MS analysis of EBN are consistent with other studies investigating the protein composition of EBN. For example, three proteins including deleted in malignant brain tumors 1 protein (DMBT1), lysyl oxidase homolog 3 (LOXL3), and acidic mammalian chitinase (AMCase) were found in the EBN crude extract [15,16]. In which, DMBT1 and AMCase were found in EBN to be important for immunomodulation. DMBT1 is a scavenger receptor cysteine-rich protein with functions in innate immunity [17]. Because of the scavenging capacity of DMBT1, it takes part in the immune response by attaching to microbes when triggered by a local inflammatory response [18]. In addition, there have been other studies investigating the role of AMCase which has been found to have immunomodulatory effects by promoting the activation of immune cells and regulating cytokine production [19]. LOXL3 is an enzyme involved in cross-linking collagen and elastin fibers that are important components of the extracellular matrix. Its presence in EBN crude extract suggests that it may play a role in maintaining and repairing tissues, such as stimulating skin rejuvenation and aiding in anti-aging [20]. In order to predict their potential biological activity, the 51 identified

proteins were further processed in the BIOPEP database. The results demonstrated that EBN proteins belonging to 9 functional groups were annotated (Figure 2), including dipeptidyl peptidase (DPP) IV inhibitor (30.36%), ACE inhibitor (29.76%), antioxidative (14.88%), alpha-glucosidase inhibitor (7.74%), antithrombotic (7.14%), antiamnesic (6.55%), anti-inflammatory (1.79%), immunostimulating (1.19%), and alpha-amylase inhibitor (0.60%). These results therefore highlight the multiple dimensions of EBN proteins and their potential role in promoting general health and well-being.

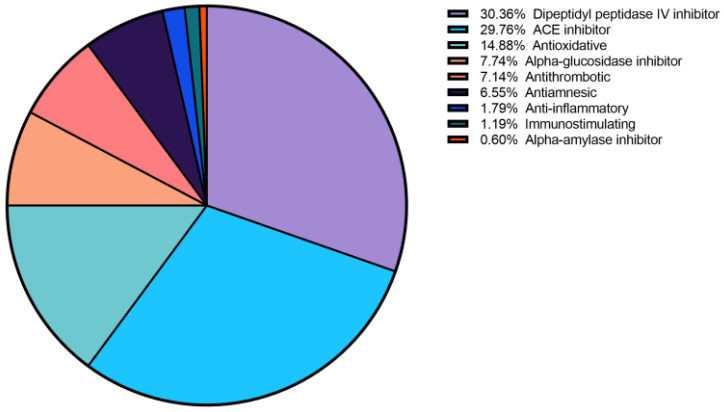

Figure 2. Functional proteins predicted from EBN using BIOPEP database.

3.3. Evaluation of Antioxidant and Anti-Inflammatory Activity of EBN Proteins

Inflammation and oxidative stress are closely related and have been linked to the pathogenesis of many chronic diseases [21]. Therefore, the antioxidant and anti-inflammatory properties of EBN proteins were investigated and the results are shown in Figure 3. As the protein concentration increased, the EBN proteins became more effective in scavenging DPPH and ABTS radicals. At a protein concentration of 100 μg/mL, the DPPH radical scavenging ability and the ABTS radical scavenging ability reached their highest values of 20.84 ± 3.43% (Figure 3A) and 31.49 ± 1.66% (Figure 3B), respectively. For anti-inflammatory effects, the results also showed a similar trend to the antioxidant assays as shown in Figures 3C and 3D, the inhibition increased with increasing protein concentration. A protein concentration of 100 μg/mL showed a high inhibition of albumin denaturation (25.30 ± 0.32%) and inhibition of NO production (21.30 ± 3.43%). This result provides a fundamental understanding of the potential therapeutic use of EBN proteins in scavenging free radicals and suppressing inflammatory diseases.

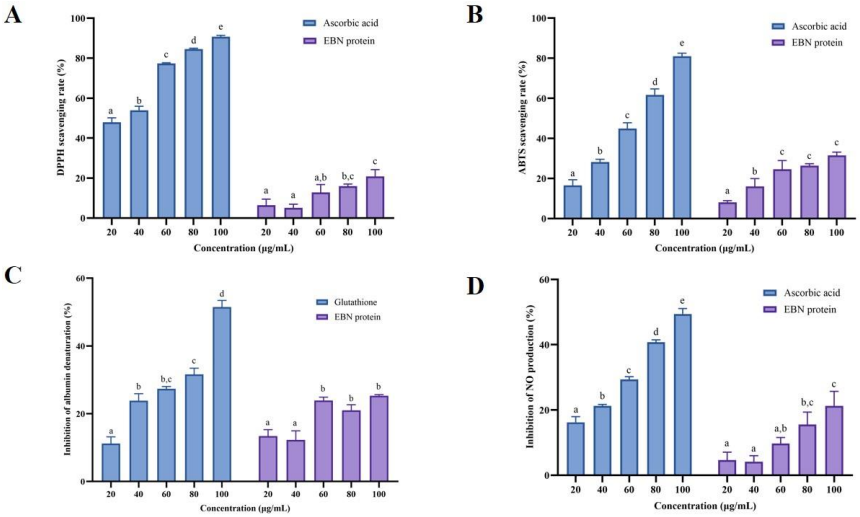

Figure 3. Antioxidant activity [(**A**) DPPH scavenging activity, (**B**) ABTS radical scavenging activity] and anti-inflammatory activity [(**C**) Inhibition of albumin denaturation, (**D**) Inhibition of NO production] of EBN at different protein concentrations. Statistical significance was shown with different letters on the same bars when $p < 0.05$.

4. Conclusions

In conclusion, this research effectively identified and characterized 51 functional proteins found in EBN using proteomic-based methods. Based on the BIOPEP database, the identified proteins were predicted to have potential biological activities such as antioxidant, anti-inflammatory, immunostimulating, antiamnesic, antihypertensive, antidiabetic, and antithrombotic properties. The antioxidant and anti-inflammatory properties of EBN proteins have also been experimentally validated. These findings suggest that the presence of a diverse range of proteins in EBN suggests potential nutritional and functional benefits. Further research is required to isolate and purify functional EBN proteins. In addition, in vitro and in vivo studies should be conducted to explore the potential use of EBN proteins as nutritional supplements in food and traditional medicine. These efforts can further improve our understanding of the functional properties of EBN and broaden its potential applications in various industries.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Supplementary File S1: The list of identified proteins in EBN.

Author Contributions: Conceptualization, C.H.L. and N.H.; methodology, N.H.; software, C.H.L.; formal analysis, C.H.L. and N.H.; investigation, C.H.L.; writing—original draft preparation, C.H.L.; writing—review and editing, B.B.N., S.L.W. and K.Y.W.; supervision, T.H.L. and H.J. All authors have read and agreed to the published version of the manuscript.

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