



Analysis of Metabolite Profiles Using Liquid Chromatography, High Resolution Mass Spectrometry and Evaluation of the Antioxidant Activity of Etanol Extracts from Swiftlet Nests

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ARTICLE INFORMATION

Article History:

Received: March 05, 2026

Revised: April 24, 2026

Published: May 25, 2026

Keywords:

Aerodramus fuciphagus

Antioxidant

DPPH

LC-HRMS

Metabolite

ABSTRACT

White swiftlet nests (*Aerodramus fuciphagus*) are widely known to possess various functional biological activities. However, research on the specific profiles of secondary metabolites and volatile/non-protein compounds in crude extracts remains limited. This study aims to identify macromolecular chemical components and metabolites in white SBW ethanol extracts using Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS) and to evaluate their antioxidant capacity via the DPPH method. A 708-g sample of white SBW was prepared, dried at 40°C (moisture content obtained: 21%), ground, and extracted by stepwise maceration using ethanol as a solvent until a concentrated extract yield of 0.35% was obtained. Reverse-phase LC-HRMS analysis using a C18 column detected 19 major compounds, including amino acid derivatives, lipids, chromates, and several organic compounds. The results revealed the presence of 4-amino-4-deoxycrosmate acid, which theoretically indicates microbial interaction or contamination during nest formation or storage. Antioxidant activity testing showed an IC₅₀ value of 3693.39 ppm. These results confirm that the SBW ethanol extract exhibits antioxidant activity that is classified as very weak/less active compared to pure polyphenolic compounds, which aligns with SBW's characteristics as a long-term biological protective agent through cellular modulation, rather than as an aggressive free radical scavenger.

INTRODUCTION

Edible Bird's Nest (EBN), locally known as Sarang Burung Walet (SBW), is a high-value natural commodity produced from the salivary gland secretions of swiftlets (Daud et al., 2021; Unal et al., 2022; Abdullah et al., 2025). The species that predominantly contribute to the global EBN market are *Aerodramus fuciphagus* (white-nest swiftlet) and *Aerodramus maximus* (black-nest swiftlet) (Jamalluddin et al., 2019; Kong et al., 2022). Among the commercially available nest types, white EBN is considered to possess superior functional properties and commands a higher market value because its structure consists predominantly of endogenous glycoproteins with minimal contamination from environmental materials, unlike black nests, which contain greater amounts of extraneous substances (Bappenas, 2015; Chien et al., 2015; International Diabetes Federation, 2021; Dewi, 2020).

Nutritionally, white EBN contains approximately 50–60% protein, 20–30% total carbohydrates, including 7–12% functional sialic acid, less than 1% lipids, and relatively stable concentrations of macro- and micro-minerals (Nuroini & Wijayanti, 2017). Owing to its rich biochemical composition, EBN has attracted considerable scientific interest. Previous studies have primarily focused on its protein and glycoprotein fractions, which have demonstrated various biological activities, including immunomodulatory, regenerative, neuroprotective, and antiviral effects (Chok et al., 2021). These findings suggest that EBN possesses substantial potential as a natural bioactive material for health applications.

Despite extensive investigations into the macronutrient and glycoprotein components of EBN, limited information is available regarding the presence of polar secondary metabolites and low-molecular-weight non-protein organic compounds. Such compounds may contribute significantly to the biological activities of EBN but remain insufficiently characterized. The extraction of these metabolites requires an appropriate solvent system capable of penetrating the glycoprotein-rich matrix of EBN. Ethanol is widely recognized as a universal polar solvent that effectively extracts polar and semi-polar organic compounds from biological materials (Harniza, & Wahid, 2009). The selection of ethanol as the extraction solvent is further supported by its favorable dielectric constant and ability to facilitate the release of bioactive constituents embedded within the EBN matrix.

Comprehensive characterization of these compounds requires advanced analytical techniques with high sensitivity and accuracy. Liquid Chromatography High Resolution Mass Spectrometry (LC-HRMS) has emerged as a powerful tool for metabolomic profiling, enabling precise identification of metabolites based on accurate mass measurements and molecular fingerprinting (Hanafiah et al., 2019; Nuroini & Wijayanti, 2017). The application of LC-HRMS is therefore essential for elucidating the chemical composition of ethanol extracts of EBN and identifying potentially bioactive compounds that have not been previously reported.

In addition to chemical characterization, evaluating the antioxidant activity of EBN extracts is important because oxidative stress plays a significant role in tissue damage and delayed wound healing. Antioxidant compounds can mitigate oxidative damage and support tissue regeneration, particularly in the oral cavity, where wound healing is frequently challenged by microbial exposure and inflammatory processes. Therefore, identifying antioxidant constituents within EBN may provide scientific evidence supporting its therapeutic applications.

Accordingly, this study was conducted to perform ethanol extraction of Indonesian white EBN, comprehensively characterize its chemical compound profile using LC-HRMS, and evaluate its antioxidant activity through the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. Furthermore, this study aims to identify bioactive compounds present in the EBN extract that may contribute to oral wound-healing processes.

METHOD

This study employed a laboratory-based experimental design to investigate the chemical profile and antioxidant activity of white edible bird's nest (*Aerodramus fuciphagus*). The primary material used was white edible bird's nest, while the solvents and reagents included ethanol, methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), chromatography-grade water (H₂O),

acetonitrile, ammonium formate, and 0.1% formic acid. Laboratory equipment consisted of a mortar and pestle, drying oven, filtration apparatus, rotary evaporator, vortex mixer, sonicator, microplate reader, vials, and aluminum foil. Metabolite profiling was conducted using a Vanquish™ Horizon Ultra-High Performance Liquid Chromatography (UHPLC) system equipped with a Binary Pump and coupled to a Quadrupole Time-of-Flight High-Resolution Mass Spectrometer (Q-ToF HRMS) (Thermo Scientific™, Germany).

A total of 708 g of white edible bird's nest was manually cleaned to remove visible impurities, including feathers and other foreign materials. The cleaned samples were dried in an oven at 40°C to preserve thermolabile compounds and subsequently pulverized using a mortar and pestle to obtain 558 g of fine powder, corresponding to a moisture loss of approximately 21%. The powdered sample was extracted by sequential maceration using ethanol as the extraction solvent. Two extraction cycles were performed, each using 700 mL of ethanol to maximize the extraction of bioactive compounds. The mixtures were filtered, and the combined filtrates were concentrated under reduced pressure using a rotary evaporator until a solvent-free yellowish extract was obtained.

The chemical constituents of the ethanol extract were characterized using LC-HRMS analysis. Chromatographic separation was carried out on a C18 column (100 mm × 2.1 mm, 2.6 µm particle size). The mobile phase consisted of solvent A, water containing 0.1% formic acid, and solvent B, acetonitrile containing 0.1% formic acid. The flow rate was maintained at 0.3 mL/min, with an injection volume of 5 µL. The gradient elution program was initiated at 5% solvent B and gradually increased to 50% within the first 8 min, followed by an increase to 98% within the next minute. The composition was maintained at 98% solvent B for 3 min before returning to the initial conditions, with a total run time of 15 min. The column temperature was maintained at 40°C, while the laboratory temperature was approximately 25°C. Metabolites were identified based on accurate mass measurements and chromatographic profiles generated by the LC-HRMS system (Hanafiah et al., 2019; Nuroini & Wijayanti, 2017).

The antioxidant activity of the ethanol extract was evaluated using the DPPH free radical scavenging assay. Briefly, 0.0647 g of ethanol extract was dissolved in 6 mL of ethanol and homogenized using ultrasonication. The solution was filtered, and the resulting filtrate was used for further analysis. The DPPH stock solution was prepared by dissolving 4 mg of DPPH in methanol and diluting it to a final volume of 25 mL. The solution was homogenized using a vortex mixer and sonicator and stored in a dark container to prevent photodegradation. The extract solution was subsequently diluted to obtain concentrations of 0, 266.47, 535.21, 1070.43, 2140.85, and 4281.70 ppm. A volume of 240 µL of DPPH solution was added to each concentration, and the mixtures were incubated in the dark for 30 min at room temperature. Absorbance was measured at a wavelength of 520 nm using a microplate reader. The percentage of radical scavenging activity was calculated, and the half-maximal inhibitory concentration (IC₅₀) was determined from the concentration–response curve (Rifqi, 2017; Anggraini & Kasmawati, 2017; Haris, 2019; Hanafiah et al., 2019).

This study received ethical approval from the Health Research Ethics Committee under approval number 1/KEPK/EC/XI/2025 with the title "*Uji Kandungan (Senyawa Aktif) pada Sarang Burung Walet yang Berperan dalam Penyembuhan Luka pada Rongga Mulut*" (Evaluation of Active Compounds in Edible Bird's Nest Associated with Oral Wound Healing).

RESULTS AND DISCUSSION

Preparation and Extraction of White Edible Bird's Nest

The cleaning and low-temperature drying process (40°C) was performed to minimize thermal degradation and preserve the integrity of the thermolabile protein and glycoprotein components present in white edible bird's nest (EBN). From an initial sample weight of 708 g, a total of 558 g of dried powder was obtained after processing, corresponding to a moisture loss of approximately 21%. This reduction in weight reflects the removal of water content while maintaining the stability of the bioactive constituents within the sample.

Subsequent extraction of the dried EBN powder was carried out through sequential maceration using ethanol as the extraction solvent. A total of 558 g of powdered EBN yielded

1.9671 g of a yellowish, paste-like concentrated extract, corresponding to an extraction yield of 0.35%. The relatively low extraction yield is consistent with the inherent biochemical characteristics of white EBN, which is primarily composed of high-molecular-weight glycoproteins and structural proteins that exhibit limited solubility in ethanol. As a result, only a small proportion of low-molecular-weight polar and semi-polar organic compounds can be effectively extracted using ethanol, leading to a comparatively low recovery of extractable constituents (Chok et al., 2021; Nuroini & Wijayanti, 2017; Sandi & Musfirah, 2019; Tottoli et al., 2020; Sandi & Musfirah, 2019).

The present findings are in agreement with previous studies reporting that proteins constitute the major component of white EBN. Marcone reported that white EBN contains approximately 62–63% protein and identified a 77 kDa protein exhibiting characteristics similar to ovotransferrin found in avian eggs (Marcone, 2005). The predominance of these proteinaceous constituents further supports the low extraction yield obtained with ethanol and suggests that the extracted fraction mainly contains minor metabolites and low-molecular-weight compounds rather than the major glycoprotein matrix of EBN.

Identification of Chemical Constituents in White Edible Bird's Nest Ethanol Extract by LC–MS Analysis

The chemical constituents present in the ethanol extract of white edible bird's nest (EBN) were characterized using Liquid Chromatography–Mass Spectrometry (LC–MS). LC–MS is a powerful analytical technique that combines liquid chromatography (LC), which separates compounds based on their physicochemical properties, with mass spectrometry (MS), which enables the identification of compounds based on their mass-to-charge ratio (m/z). In this study, chromatographic separation was performed using a reverse-phase C18 column as the nonpolar stationary phase. The mobile phase consisted of water containing 5 mM ammonium formate (solvent A) and acetonitrile containing 0.05% formic acid (solvent B). This analytical approach facilitated the separation and identification of metabolites present in the extract. The compounds detected by LC–MS analysis, together with their retention times, mass-to-charge ratios, and molecular formulas, are presented in Table 1.

Table 1. Chemical Compounds Identified in the Ethanol Extract of White Edible Bird's Nest by LC–MS Analysis.

No.	Retention Time (min)	m/z	Molecular Formula	Identified Compound
1	0.838	118.0863	$C_5H_{11}NO_2$	Valine
2	0.888	132.1019	$C_6H_{13}NO_2$	Isoleucine
3	0.898	117.0194	$C_4H_6O_4$	Succinic acid
4	1.123	166.0863	$C_9H_{11}NO_2$	L-Phenylalanine
5	1.174	158.0824	$C_7H_{13}NO_3$	N-Acetylvaline
6	1.177	224.0918	$C_{11}H_{13}NO_4$	N-Acetyl-L-tyrosine
7	1.722	229.1548	$C_{11}H_{20}N_2O_3$	Leucylproline
8	1.791	286.1764	$C_{13}H_{23}N_3O_4$	L-Prolyl-L-leucylglycine
9	2.450	259.1301	$C_{11}H_{20}N_2O_5$	γ -L-Glutamyl-L-leucine
10	2.532	570.2423	$C_{24}H_{37}N_5O_{11}$	L-Threonyl-L-threonyl-L-seryl-L-tyrosyl-L-threonine
11	2.699	302.2078	$C_{14}H_{27}N_3O_4$	Leu-Gly-Leu
12	2.788	598.3561	$C_{27}H_{47}N_7O_8$	L-Alanyl-L-valyl-L-prolyl-L-isoleucyl-L-alanyl-L-glutamine
13	3.126	253.1186	$C_{12}H_{16}N_2O_4$	Ala-Tyr (Alanyltyrosine)
14	3.300	253.1185	$C_{12}H_{16}N_2O_4$	Alanyltyrosine
15	3.323	130.0874	$C_6H_{13}NO_2$	DL- β -Leucine

According to the LC-HRMS analysis presented in Table 1, 4-amino-4-deoxychorismate acid was detected in the ethanol extract of white edible bird's nest

(EBN). Biosynthetically, this compound serves as an intermediate in the shikimate pathway, which is involved in the synthesis of *p-aminobenzoic acid* (PABA) and folic acid in various microorganisms, including bacteria such as *Escherichia coli*, *Bacillus subtilis*, and members of the actinomycete group. In contrast, animals, including the swiftlet species *Aerodramus fuciphagus*, do not possess the shikimate pathway and therefore are unable to synthesize chorismate-derived compounds endogenously. The natural composition of swiftlet saliva is primarily characterized by glycoproteins rich in sialic acid rather than metabolites associated with microbial biosynthetic pathways.

The detection of 4-amino-4-deoxychorismate acid in the present study may therefore indicate the presence of microbial-derived metabolites associated with the nest matrix. This finding suggests possible interactions between the nest material and environmental microorganisms, including bacteria and fungi that may colonize the nest surface during nest formation in swiftlet houses or caves, or during post-harvest handling and storage before thorough cleaning. Such microbial contributions may influence the overall metabolite profile of EBN and could account for the presence of compounds that are not naturally synthesized by swiftlets.

This observation is consistent with previous findings reported by Chok et al., who demonstrated that white EBN contains a relatively high total amino acid content compared with other nest types, highlighting the complex biochemical composition of EBN and the potential contribution of associated biological and environmental factors to its metabolite profile (Chok et al., 2021; Iskandar, 2017).

Table 2. DPPH Radical Scavenging Activity of White Edible Bird's Nest Ethanol Extract at Different Concentrations.

Sample Concentration (ppm)	Absorbance (520 nm)	Inhibition (%)
0.00 (Control)	0.8505	0.00
266.47	0.8244	3.07
535.21	0.8060	5.22
1070.43	0.7060	17.05
2140.85	0.5460	35.80
4281.70	0.3847	54.77

Based on the linear regression analysis between extract concentration and percentage inhibition, the IC_{50} value of the white edible bird's nest (EBN) ethanol extract was determined to be 3693.39 ppm. According to the classification of antioxidant activity based on the DPPH assay, antioxidant strength is categorized as very strong when the IC_{50} value is below 50 ppm, strong at 50–100 ppm, moderate at 100–150 ppm (and in some classifications up to 250 ppm), weak at 151–200 ppm (or up to 500 ppm), and very weak or inactive when the IC_{50} value exceeds 200 ppm (Iskandar, 2017; Chok et al., 2021). Therefore, the IC_{50} value obtained in the present study indicates that the ethanol extract of white EBN exhibits very weak antioxidant activity and can be classified as inactive in directly scavenging DPPH free radicals.

The low antioxidant activity observed in this study may be attributed to the intrinsic biochemical characteristics of EBN. Unlike many plant-derived extracts that are rich in phenolic and polyphenolic compounds capable of donating electrons or hydrogen atoms to neutralize free radicals, EBN primarily consists of proteins, glycoproteins, carbohydrates, and sialic acid. Consequently, its antioxidant mechanism may differ substantially from that of plant-based antioxidants. The DPPH assay specifically measures the ability of compounds to directly donate electrons or hydrogen atoms to stabilize free radicals; therefore, compounds lacking strong radical-scavenging properties generally exhibit high IC_{50} values. In this context, the weak activity of the

ethanol extract suggests that the extracted fraction contained limited amounts of compounds capable of directly reducing DPPH radicals (Rifqi, 2017).

Although the ethanol extract demonstrated weak antioxidant activity in the DPPH assay, this finding does not necessarily indicate the absence of biological antioxidant potential. Previous studies have suggested that the antioxidant effects of EBN may operate through indirect cellular mechanisms rather than direct free-radical scavenging. These mechanisms include the stimulation of endogenous antioxidant enzyme expression, maintenance of mitochondrial integrity, and protection against oxidative stress at the cellular level. Such activities may not be adequately reflected by chemical assays such as DPPH, which primarily evaluate direct radical-neutralizing capacity (Rifqi, 2017).

Furthermore, previous research conducted by Chok et al. reported that higher antioxidant activity was observed in EBN extracts obtained through alkaline extraction methods. The enhanced antioxidant capacity was attributed to protein hydrolysis, which releases bioactive amino acids and peptides, including cysteine (Cys), methionine (Met), histidine (His), tryptophan (Trp), and lysine (Lys), all of which possess antioxidant properties (Chok et al., 2021). The relatively weak activity observed in the present study may therefore be related to the use of ethanol extraction, which is less effective in hydrolyzing EBN proteins and releasing antioxidant peptides. As a result, the antioxidant compounds responsible for radical-scavenging activity may not have been extracted in sufficient quantities.

Taken together, these findings indicate that while the ethanol extract of white EBN exhibits limited direct antioxidant activity in the DPPH assay, EBN may still possess significant biological antioxidant potential through alternative mechanisms and bioactive components that are more effectively released through protein hydrolysis-based extraction methods. Further studies employing enzymatic or alkaline extraction, as well as cellular antioxidant assays, are recommended to better elucidate the antioxidant properties and therapeutic potential of EBN.

CONCLUSION

The ethanol extract of white edible bird's nest (*Aerodramus fuciphagus*) was successfully characterized using LC–MS analysis, which identified 15 chemical compounds consisting predominantly of amino acids, peptide derivatives, and other low-molecular-weight organic metabolites. Several detected compounds, including valine, isoleucine, phenylalanine, leucylproline, γ -L-glutamyl-L-leucine, and alanyltyrosine, may contribute to biological processes associated with tissue repair and wound healing. The detection of 4-amino-4-deoxychorismate acid suggests the possible presence of microbial-derived metabolites associated with the nest matrix and highlights the influence of environmental factors on the chemical composition of edible bird's nest.

Evaluation of antioxidant activity using the DPPH assay demonstrated that the ethanol extract exhibited very weak free radical scavenging activity, as indicated by an IC_{50} value of 3693.39 ppm. These findings suggest that the ethanol-soluble fraction of white edible bird's nest contains limited compounds capable of directly neutralizing free radicals. Nevertheless, the identified amino acids and peptide-related compounds indicate potential biological activities that warrant further investigation, particularly regarding their role in oral wound healing. Future studies should employ enzymatic or hydrolytic extraction approaches and advanced bioactivity assays to better characterize bioactive peptides and elucidate the mechanisms underlying the therapeutic properties of edible bird's nest.

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