Borneo Journal of Pharmacy
Vol 8 Issue 1 February 2025
Pages 15 – 24

https://journal.umpr.ac.id/index.php/bjop/article/view/6595
DOI: https://doi.org/10.33084/bjop.v8i1.6595
e-ISSN: 2621-4814

Research Article

Antioxidant Activity of Edible Bird's Nest (*Aerodramus fuciphagus*) from Central Borneo, Indonesia

Dita Ayulia Dwi Sandi [1*]

Eka Fitri Susiani [2]

Satrio Wibowo Rahmatullah [1]

I Ketut Adnyana [3]

Pratiwi Wikaningtyas [3]

[1] Department of Pharmacy, Universitas Lambung Mangkurat, Banjarbaru, South Borneo, Indonesia
[2] Department of Pharmacy, Universitas Borneo Lestari, Banjarbaru, South Borneo, Indonesia
[2] School of Pharmacy, Institut Teknologi Bandung, Bandung, West Java, Indonesia

*email: dita.sandi@ulm.ac.id; phone: +628115031489

Keywords:
CUPRAC
DPPH
FRAP
NO Method

Abstract

Edible bird's nests (EBNs) from *Aerodramus fuciphagus* have been traditionally used in Asia for their various health benefits, including antioxidant properties. Previous studies have reported antioxidant activity in EBN water extracts using methods like ABTS and ORAC. However, the antioxidant activity can vary significantly depending on factors such as the source of EBN, extraction methods, and the specific assay used. This study aimed to comprehensively evaluate the antioxidant potential of EBN water extract using a range of *in vitro* assays. The antioxidant activity was assessed using four different methods: 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, nitric oxide (NO) radical scavenging, ferric reducing antioxidant power (FRAP), and cupric reducing antioxidant capacity (CUPRAC). Results demonstrated that the EBN water extract exhibited moderate NO radical scavenging activity with an IC_{50} value of 116.86 µg/mL. While weak activity was observed in the DPPH assay, the FRAP and CUPRAC assays showed promising antioxidant potential with an ascorbic acid equivalent (AAE) of 4.567±0.30 and 3.487±0.095 µg AAE/mL, respectively. These findings suggest that the antioxidant activity of EBN water extract may be primarily attributed to its protein content. However, further investigations are warranted to elucidate the specific mechanisms of action and isolate the bioactive compounds responsible for the observed antioxidant effects. These findings have implications for the development of EBN-based functional foods, nutraceuticals, and cosmeceuticals with enhanced antioxidant properties.

Received: January 16th, 2024
1st Revised: May 15th, 2024
2nd Revised: September 25th, 2024
Accepted: January 13th, 2025
Published: February 28th, 2025

INTRODUCTION

Edible bird's nest (EBN), a highly valued natural product, is geographically distributed across a broad region encompassing the western Indian Ocean islands, the Philippines, northern Australia, the western and southwestern Pacific, and southern continental Asia[1]. Currently, the primary production of EBN is concentrated in Southeast Asia, with Indonesia, Malaysia, and Thailand collectively accounting for over 95% of the global supply. Notably, Indonesia stands as the world's largest EBN producer, with significant contributions originating from the islands of Kalimantan, Sumatra, Java, and Sulawesi. This concentrated production in specific regions highlights the ecological and economic importance of EBN within Southeast Asia[2].

Previous research has indicated a correlation between swiftlet habitats and edible bird's nest (EBN) production. Yacoob *et al.*[3] demonstrated that swiftlets inhabiting forest areas produce a higher yield of EBN compared to those in residential

How to cite: Sandi DAD, Susiani EF, Rahmatullah SW, Adnyana IK, Wikaningtyas P. Antioxidant Activity of Edible Bird's Nest (Aerodramus fuciphagus) from Central Borneo, Indonesia. Borneo J Pharm. 2025;8(1):15-24. doi:10.33084/bjop.v8i1.6595

settings. This finding, combined with satellite data analysis quantifying forest extent in Central Kalimantan, Indonesia, as conducted by Ito *et al*.[4], suggests a significant contribution of the Kalimantan forest ecosystem to EBN production. Notably, the South Barito Regency, with its capital in Buntok, has been identified by the Central Kalimantan Provincial Government as possessing substantial potential for forest-based EBN cultivation. This potential warrant further investigation to understand the specific ecological factors within this region that influence swiftlet populations and EBN production.

Edible bird's nest has a long-standing history of traditional use in Asia for health maintenance, with documented applications including antioxidant and anti-inflammatory effects, as well as bone strengthening. Recent scientific investigations have further explored the therapeutic potential of EBN, demonstrating its efficacy as a tonic and stimulant. Studies have also reported its skin-related benefits, such as whitening and moisturizing effects, potential ultraviolet protection, and the acceleration of wound healing in diabetic mice[5-8]. These diverse findings suggest that EBN possesses a range of bioactive compounds with significant therapeutic implications, warranting further investigation into its specific mechanisms of action and clinical applications.

Edible bird's nest exhibits regional variations in nutritional profiles, particularly within Indonesia. Edible bird's nest sourced from West and South Sumatra, West Java, Kalimantan, and Sulawesi demonstrates distinct compositions[9]. Notably, protein constitutes the primary component of EBN, typically exceeding 60%. Indonesian EBN, specifically, presents a high protein content, ranging from 53.09% to 56.25%, which is comparable to, and in some cases surpasses, that of Thai EBN (52.65% to 55.65%)[10]. Previous research by Helmi *et al*.[11] further highlighted these regional differences by comparing EBN protein content between Java and Kalimantan. Their findings revealed that Kalimantan EBN (0.221 to 0.634 µg/µL) generally exhibits higher protein concentrations than Java EBN (0.059 to 0.122 µg/µL). Within Kalimantan, EBN from Central Kalimantan (0.634 µg/µL) demonstrated a higher protein content than that from South Kalimantan (0.221 µg/µL), emphasizing the need for detailed regional characterization of EBN's nutritional composition.

Protein plays a crucial role in cellular antioxidant defense by stimulating the synthesis of enzymatic antioxidants and elevating antioxidant concentrations within tissues, thereby mitigating oxidative stress[12]. Furthermore, sialic acid, a component of blood vessel endothelium, exhibits direct antioxidant activity through free radical scavenging. Notably, Indonesian EBN has been reported to contain a higher concentration of N-acetylneuraminic acid, the primary constituent of sialic acid, compared to EBN from Thailand and Vietnam. Specifically, Indonesian EBN has demonstrated an N-acetylneuraminic acid content of up to 12.12%[13]. This unique compositional characteristic suggests a potentially enhanced antioxidant capacity of Indonesian EBN, warranting further investigation into its implications for oxidative stress-related conditions.

Antioxidants function by neutralizing reactive free radicals through electron donation, thereby stabilizing these unstable species and preventing oxidative damage. They achieve this by inhibiting the initiation or propagation of chain oxidation reactions[14]. While previous studies have explored the antioxidant potential of edible bird's nest (EBN) water extracts using ABTS and ORAC assays, reporting relatively low activity (1% at 1000 µg/mL)[15], the observed variations in antioxidant activity can be attributed to differences in antioxidant chemical structures, free radical sources, and the physicochemical properties of sample preparations from diverse geographical origins[16]. Consequently, a targeted activity analysis using methods specific to the sample type is essential. This study aims to comprehensively evaluate and compare the antioxidant activity of EBN water extract from Central Borneo using a suite of assays, including 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO) scavenging, ferric reducing antioxidant power (FRAP), and cupric reducing antioxidant capacity (CUPRAC). These methods, selected for their distinct mechanisms of action, will provide a robust assessment of the antioxidant potential of EBN from this region.

MATERIALS AND METHODS

Materials

This study utilized a range of laboratory equipment and reagents. The tools employed included: Erlenmeyer flasks (Iwaki, Japan), a vortex mixer (DLab, China), a magnetic stirrer (DLab, China), a hot plate (Maspion, Indonesia), micropipettes (DLab, China), a freeze dryer (Eyela, Japan), a UV-Vis spectrophotometer (PG Instruments Limited, Germany), glassware (Pyrex, Germany), and a pH meter (Ionix, Thailand). The primary ingredient, EBN, was sourced from Buntok, Central

Borneo, Indonesia, and identified as *Aerodramus fuchipagus* (Certificate No. 2400/IPH.1.02/KS.02.03/VII/2019, Research Center for Biology, Indonesian Institute of Sciences). Reagents used in the study were: vitamin C (p.a, Merck, Germany), DPPH solution (p.a, Merck, Germany), sodium nitroprusside (p.a, Merck, Germany), 50 mM phosphate-buffered saline (p.a, Brataco, Indonesia), Griess reagent (p.a, Brataco, Indonesia), 0.2 M phosphate buffer (pH 6.6) (p.a, Brataco, Indonesia), 1% potassium ferricyanide [$K_3Fe(CN)_6$] (p.a, Brataco, Indonesia), trichloroacetic acid (TCA) (p.a, Brataco, Indonesia), 0.1% ferric chloride ($FeCl_3$) (p.a, Brataco, Indonesia), 0.01 M copper(II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$) (p.a, Brataco, Indonesia), 0.0075 M neocuproine solution (p.a, Merck, Germany), 1 M ammonium acetate buffer solution (pH 7) (p.a), and aluminum foil (WITA, Indonesia).

Methods

Preparation of EBN water extract

Authentic EBN were meticulously selected and cleaned to remove any extraneous materials. A 1000 g sample of the cleaned EBN was then dissolved in 33.9 L of ultrapure water. The resulting solution was homogenized by continuous stirring for 30 minutes, followed by heating at 45°C for an additional 30 minutes to facilitate extraction. The mixture was subsequently filtered using filter paper to obtain the crude extract. This filtrate was then subjected to lyophilization (freeze-drying) using an Eyela® freeze dryer to yield a dry powder of the EBN water extract. The final yield of the EBN water extract was 43.165 g, representing a 4.32% recovery.

DPPH method

To assess antioxidant activity, a DPPH radical scavenging assay was performed. Water extracts of EBN were prepared at concentrations of 50, 500, 750, 1000, and 1250 µg/mL. Ascorbic acid was used as a positive control, with concentrations ranging from 1 to 5 µg/mL. For each sample, 2 mL of the respective extract or ascorbic acid solution was combined with 2 mL of a DPPH solution in a test tube. The resulting mixture was vortexed and incubated at 37°C in the dark for 30 minutes. Absorbance was then measured at 516 nm using a UV-Vis spectrophotometer.

Nitric oxide method

The nitric oxide scavenging activity of EBN water extract was evaluated using the Griess reagent method. Solutions of EBN water extract, prepared at concentrations of 20, 40, 60, 80, and 100 µg/mL, were tested alongside vitamin C standards at 2, 4, 6, 8, and 10 µg/mL. For each sample, 0.75 mL of the solution was combined with 0.25 mL of 50 mM sodium nitroprusside in phosphate-buffered saline. The resulting mixture was incubated at 16-20°C, protected from direct sunlight, for 15 minutes. Subsequently, 1 mL of the incubated mixture was combined with 1 mL of Griess reagent and incubated for 45 minutes at room temperature (16-20°C), shielded from light. The absorbance of the resulting solution was measured at 525 nm using a UV-Vis spectrophotometer.

FRAP method

The ferric reducing antioxidant power of EBN water extract (1000 µg/mL) and vitamin C standards (5, 10, 15, 20, and 25 µg/mL) was determined using the FRAP assay. Briefly, 1.25 mL of each sample solution was combined with 1.25 mL of 0.2 M phosphate buffer and 1.25 mL of 1% $K_3Fe(CN)_6$. The reaction mixture was incubated at 50°C for 20 minutes. Subsequently, 1.25 mL of 10% TCA was added, and the mixture was vortexed. A 2.5 mL aliquot of the resulting solution was then combined with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. Following a 10-minute incubation period at room temperature in the dark, the absorbance of the solution was measured at 706 nm using a UV-Vis spectrophotometer.

CUPRAC method

The antioxidant capacity of EBN water extracts and vitamin C solutions was determined using the CUPRAC assay. Working solutions of EBN water extracts at concentrations of 750, 1000, 1500, 2000, and 3000 µg/mL, as well as vitamin C solutions at 20, 40, 60, 80, and 100 µg/mL, were prepared. For each sample, 1 mL of the respective solution was combined in a test tube with 1 mL of 0.01 M $CuCl_2 \cdot 2H_2O$, 1 mL of 0.0075 M neocuproine solution, and 1 mL of 1 M ammonium acetate buffer (pH 7). Subsequently, 0.1 mL of distilled water was added to each mixture. The resulting solutions were thoroughly vortexed and incubated at room temperature for 30 minutes. Absorbance was then measured at 450 nm using a UV-Vis spectrophotometer.

Data analysis

Statistical analysis was performed using Microsoft Excel software. All quantitative data are presented as the mean ± SD of triplicate measurements (n=3). The percentage inhibition for both DPPH radical scavenging assay and NO inhibition assay was calculated using the following **Equation 1**. The average %inhibition was used to create a linear regression to obtain **Equation 2**, in which y = %inhibition and x = concentration.

$$\%inhibition = \frac{Absorbance\ of\ Blank - Absorbance\ of\ Sample}{Absorbance\ of\ Blank} \text{x} 100\% \quad [1]$$

$$y = bx + a \quad [2]$$

The IC_{50} values, representing the concentration of the test compound required to inhibit 50% of DPPH radical activity, were calculated using linear regression analysis as defined by **Equation 2**. Specifically, the IC_{50} was determined by setting the 'y' value in the regression equation to 50 and solving for 'x'. Subsequently, the antioxidant activity of each compound was categorized based on its respective IC_{50} value. This classification, outlined in **Table I**, provided a structured framework for comparing the antioxidant potential of the tested compounds.

Table I. Antioxidant ability category[17].

IC_{50} (µg/mL)	Antioxidant Category
<50	Very strong
50-100	Strong
100-150	Moderate
>150	Weak

For the FRAP and CUPRAC assays, standard curves were generated using varying concentrations of vitamin C, with corresponding absorbance values. Linear regression analysis was then performed to derive **Equation 3**, which established the relationship between absorbance and vitamin C concentration in, which y = %inhibition and x = concentration. Subsequently, the absorbance values obtained from the EBN samples (y-value in **Equation 3**) were used to calculate the Ascorbic Acid Equivalent (AAE) using this established linear regression equation.

$$y = bx + a \quad [3]$$

RESULTS AND DISCUSSION

The DPPH assay, a widely utilized method for evaluating antioxidant capacity in plant extracts, was employed in this study. This assay leverages the stable free radical DPPH, simplifying the procedure to a single reagent. However, the thermolabile and photolabile nature of DPPH necessitates careful handling to ensure accurate results. The assay quantifies the scavenging capacity of antioxidants by measuring the reduction of DPPH radicals, which can be directly monitored without additional substrates[18]. In this study, the characteristic dark purple color of the DPPH solution, observed at a wavelength of approximately 516 nm, diminished to pale yellow upon reduction by hydrogen donors from antioxidant compounds. The decrease in absorbance exhibited a linear correlation with increasing extract concentration, as depicted in **Figure 1**. Ascorbic acid, utilized as a positive control, demonstrated potent antioxidant activity with an IC_{50} value of 3.28 µg/mL. In contrast, the ethanolic extract of EBN exhibited significantly weaker antioxidant activity, with an IC_{50} value of 1135.61 µg/mL (**Table II**). These results suggest a notable difference in the antioxidant potential between ascorbic acid and the EBN extract.

The NO radical scavenging assay, based on the inhibition of NO generated from sodium nitroprusside in a buffered saline phosphate solution (pH 7.2), was employed to assess the antioxidant potential of the EBN sample. Sodium nitroprusside, known to decompose in aqueous solutions at physiological pH, releases NO, which subsequently reacts with oxygen under aerobic conditions to form stable nitrates and nitrites. The quantity of these products was determined using the Griess reagent, resulting in a color change from colorless to varying shades of pink and purple, indicative of NO inhibition. While the NO assay is less frequently utilized due to its reagent-intensive nature and prolonged preparation time, it offers the advantage of demonstrating high reactivity with proteins and diverse free radicals[19]. Given that the EBN sample from Central Kalimantan exhibited a quite high, approximately 53.09-56.25% substantial protein content (0.634 µg/µL), exceeding

that of samples from South Kalimantan and Java Island, this method was deemed particularly relevant[11]. As depicted in **Figure 2**, a linear decrease in absorbance was observed with increasing concentrations of both ascorbic acid (control) and the EBN sample, indicating a dose-dependent scavenging effect. The IC_{50} value for ascorbic acid, representing a potent antioxidant, was determined to be 10.94 µg/mL. In contrast, the IC_{50} value for the EBN sample was 116.86 µg/mL, suggesting moderate antioxidant activity (**Table III**). These results highlight the ability of the EBN sample to scavenge NO radicals, potentially attributed to its high protein content and the presence of other bioactive compounds.

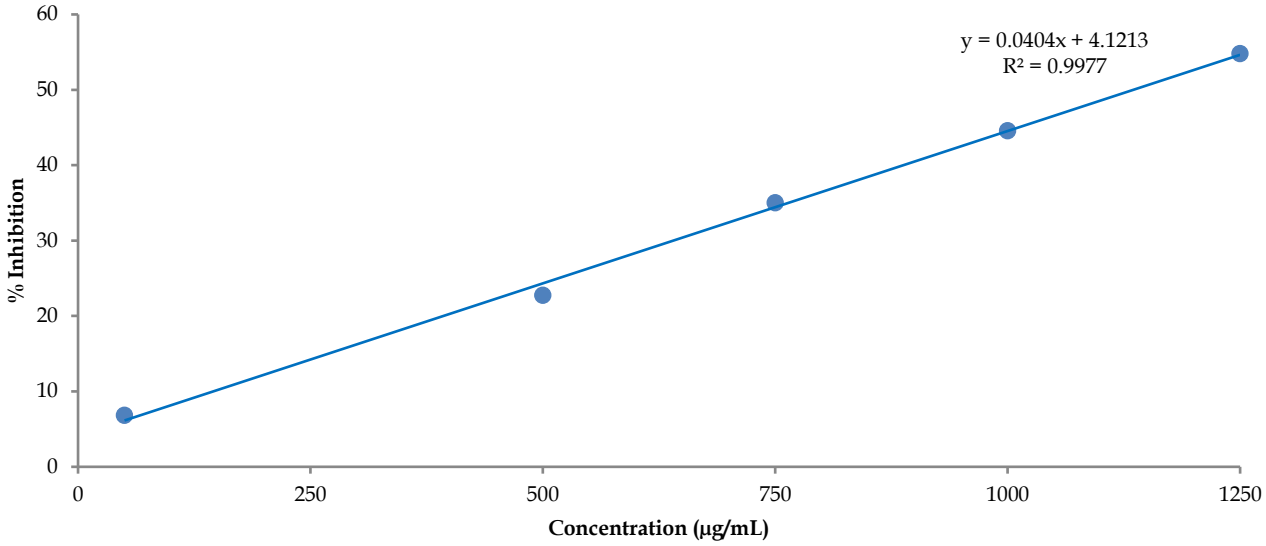

Figure 1. Graph of concentration and %inhibition of EBN samples with DPPH assay.

Table II. The IC_{50} value of ascorbic acid and EBN water extract using DPPH assay.

Samples	IC_{50} (µg/mL)	Category
Ascorbic acid	3.28	Very strong
EBN water extract	1135.61	Weak

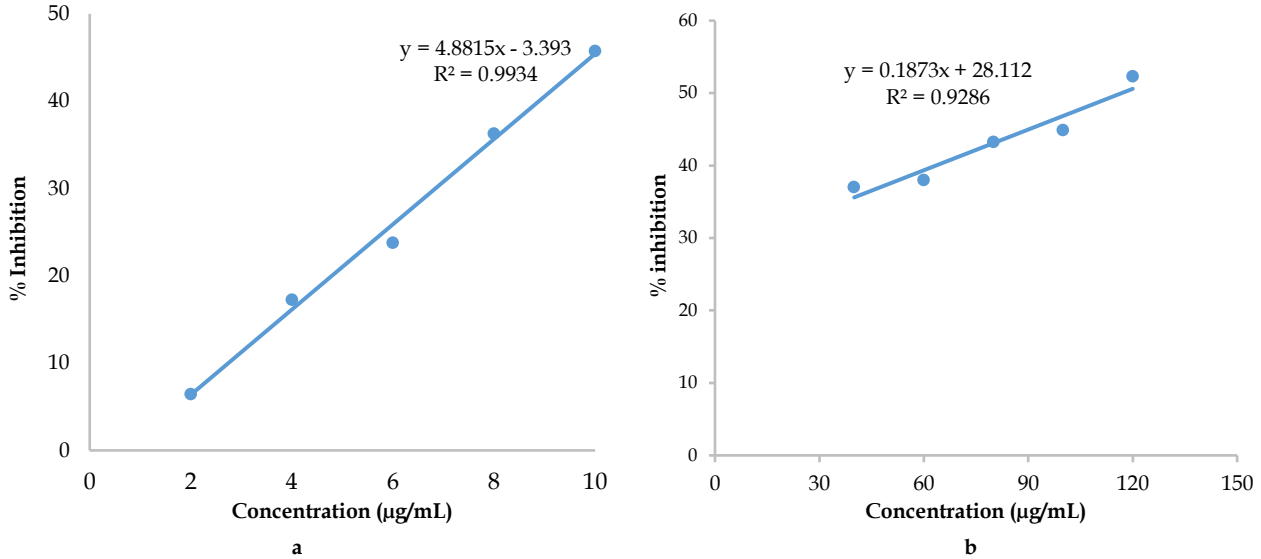

Figure 2. Graph of concentration and %inhibition of (**a**) ascorbic acid and (**b**) EBN samples with NO assay.

Table III. The IC_{50} value of ascorbic acid and EBN water extract using NO assay.

Samples	IC_{50} (µg/mL)	Category
Ascorbic acid	10.94	Very strong
EBN water extract	116.86	Moderate

As presented in **Tables II** and **III**, ascorbic acid as a positive control exhibited potent antioxidant activity in both the DPPH and NO assays, reflecting its established efficacy. In contrast, the water extract of EBN demonstrated a concentration-dependent antioxidant effect. The IC_{50} value of EBN in the DPPH assay was 1135.61 µg/mL, indicating weak antioxidant capacity. However, in the NO assay, EBN showed a significantly lower IC_{50} of 116.86 µg/mL, classifying it as a moderate antioxidant. This discrepancy suggests that the EBN extract may possess a higher affinity for scavenging NO radicals compared to DPPH radicals. This could be attributed to the inherent differences in the reaction mechanisms of the two assays. Specifically, the NO assay involves interactions with various proteins and other free radicals, potentially allowing for a wider range of reactive compounds within the EBN extract to contribute to its antioxidant activity[15].

The FRAP assay was employed to assess the antioxidant capacity of the samples due to its cost-effectiveness, utilizing readily available reagents. This method quantifies the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) via a single electron transfer (ET) reaction. ET-based assays rely on redox reactions, where the change in oxidant state serves as an endpoint indicator. In this study, the formation of Fe^{2+}, indicative of antioxidant activity, occurred through the reduction process at a controlled pH of 6.6, achieved by the addition of phosphate buffer. This specific pH ensures optimal reaction conditions for the reduction of Fe^{3+} by antioxidant compounds, providing a reliable measure of their reducing power[20].

The reducing power of the tested compounds was assessed by their ability to convert Fe^{3+} to Fe^{2+}. This conversion was monitored spectrophotometrically by measuring the formation of a dark green complex resulting from the reaction of Fe^{2+} with $FeCl_3$. The intensity of the dark green color, directly proportional to the amount of Fe^{2+} produced, indicates the reducing capacity of the compounds. Thus, a change in solution color from light green to dark green signifies the compound's ability to reduce Fe^{3+}, reflecting its antioxidant potential. The greater the intensity of the dark green color, the higher the reducing power of the compound, and consequently, the stronger its antioxidant activity[21]. To establish a standard curve for the FRAP assay, ascorbic acid, a known antioxidant, was used as a reference. As depicted in **Figure 3**, a linear relationship was observed between increasing ascorbic acid concentrations and absorbance, demonstrating a reliable dose-response. The antioxidant activity of the experimental sample, a water extract of EBN, was then quantified and expressed as AAE.

The CUPRAC assay quantifies antioxidant activity through the spectrophotometric measurement of the Cu(I)-neocuproine (Nc) chelate formed during the reduction of Cu(II)-Nc by antioxidants. This method leverages the redox reaction between chain-breaking antioxidants and the CUPRAC reagent. The resulting bis(neocuproine)copper(I) chelate, exhibiting maximum absorbance at 450 nm, manifests as a color change from light blue to orange-yellow. Notably, the CUPRAC assay operates at physiological pH (7), facilitated by an ammonium acetate buffer, offering a significant advantage over the acidic pH requirements of the FRAP assay. This physiological pH compatibility enhances the assay's relevance for biological samples. Furthermore, the CUPRAC reaction reaches completion within 30 minutes, and due to the absence of radical reagents, the assay demonstrates robustness against variations in physical parameters such as temperature, sunlight, pH, and humidity. These characteristics render the CUPRAC method a simple, versatile, and reliable tool for evaluating antioxidant capacity[22].

The antioxidant capacity of the EBN water extract, as determined by the CUPRAC assay, exhibited a dose-dependent linear relationship. As illustrated in **Figure 4**, increasing concentrations of the EBN extract resulted in a corresponding linear increase in antioxidant capacity. The effective concentration required to achieve 50% antioxidant capacity (EC_{50}) using the CUPRAC method was calculated to be 1325.58 µg/mL. Notably, this value was statistically comparable to the IC_{50} obtained using the DPPH assay, which was 1135.61 µg/mL. This congruence between the EC_{50} and IC_{50} values across two distinct antioxidant assays reinforces the robust antioxidant potential of the EBN water extract. The consistent results suggest that the EBN extract contains compounds capable of effectively scavenging free radicals and reducing cupric ions, highlighting its potential as a natural antioxidant source.

The antioxidant activity of the EBN water extract was quantified using both the FRAP and CUPRAC assays, yielding slightly varying results. The FRAP assay determined an AAE value of 4.567±0.30 µg AAE/mL extract, while the CUPRAC assay yielded 3.487±0.095 µg AAE/mL extract (**Table IV**). These values indicate that 1 mL of the EBN water extract possesses antioxidant capacity equivalent to 4.567±0.30 µg and 3.487±0.095 µg of ascorbic acid, respectively, depending on the assay employed. The observed difference in AAE values between the two methods may be attributed to variations in the reaction mechanisms and sensitivities of the FRAP and CUPRAC assays towards different antioxidant compounds present in the extract. This highlights the importance of employing multiple assays to comprehensively assess the antioxidant potential of natural extracts[23].

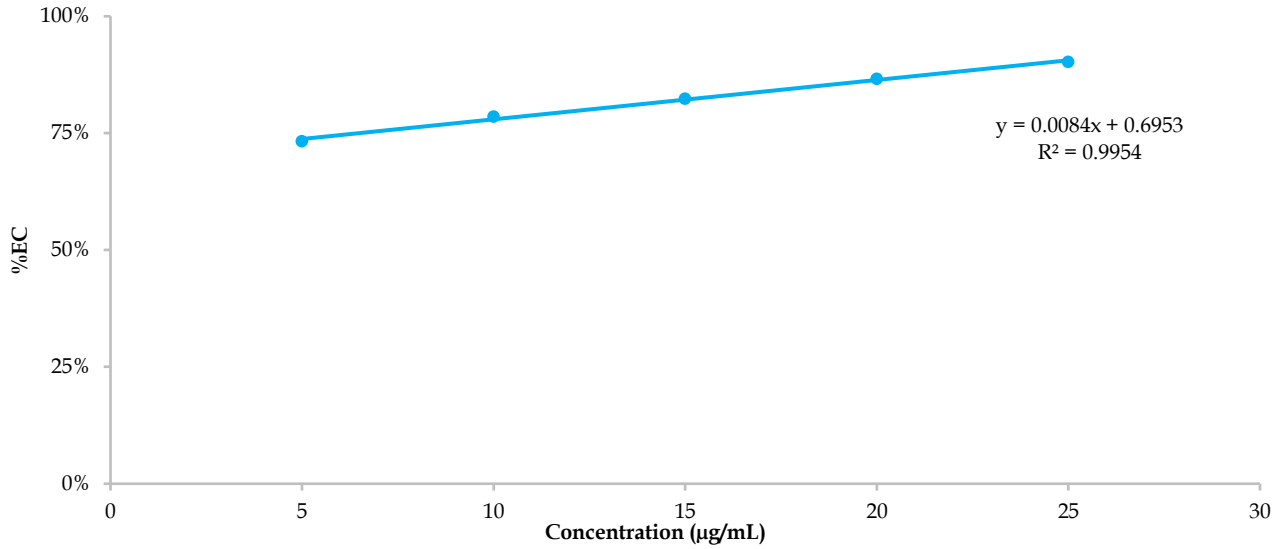

Figure 3. Graph of concentration and %EC of ascorbic acid with FRAP assay.

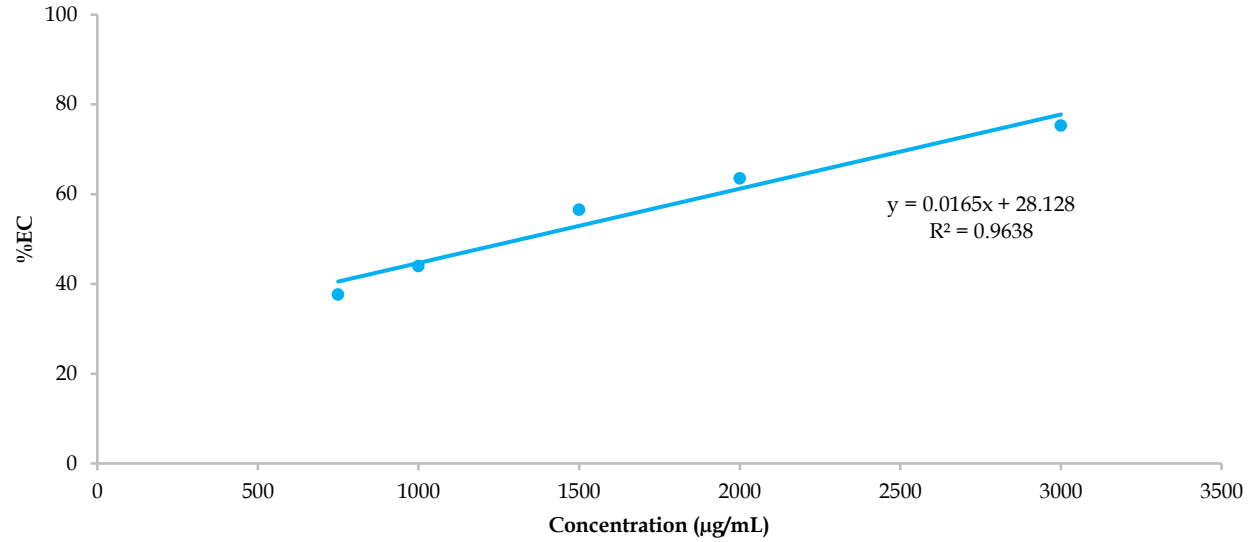

Figure 4. Graph of concentration and %EC of EBN samples with CUPRAC assay.

Table IV. The AAE value of EBN water extract using FRAP and CUPRAC assays.

Samples	AAE±SD (µg/mL)	
	FRAP	CUPRAC
EBN water extract	4.567±0.30	3.487±0.095

Edible bird's nest exhibits notable antioxidant potential, attributed to its rich protein and amino acid composition. Specifically, EBN contains proteins capable of stimulating enzymatic antioxidant synthesis and increasing tissue antioxidant concentrations, thereby mitigating oxidative stress[12]. Furthermore, EBN contains glutathione, a potent antioxidant tripeptide composed of cysteine (0.5%), glutamate (2.72%), and glycine (1.54%). Amino acid profiling revealed serine (3.83%), aspartic acid (3.27%), tyrosine (2.96%), proline (2.94%), and leucine (2.8%) as the most abundant proteins, while methionine (0.10%) was the least prevalent[9]. Cysteine, a key component, forms thiol compounds with sulfhydryl groups, which act as electron donors to neutralize free radicals. This mechanism effectively quenches free radical activity. Additionally, other essential amino acids like phenylalanine and methionine, along with non-essential amino acids such as serine and aspartic acid, contribute to the overall antioxidant capacity of EBN[24]. These findings suggest that the diverse amino acid profile of EBN plays a crucial role in its antioxidant properties, potentially offering therapeutic benefits against oxidative stress-related conditions.

In addition to protein content, sialic acid was identified in EBN from both Kalimantan and Java. Notably, the EBN sample from Central Kalimantan exhibited the highest protein band intensity within the 107-127 kDa range, closely aligning with the known molecular weight of sialic acid-associated proteins (106-128 kDa). This band intensity was significantly greater than that observed in EBN samples from Java or South Kalimantan, as corroborated by previous research[11]. Beyond its structural role in protein, sialic acid has been increasingly recognized for its antioxidant properties, functioning as a free radical scavenger, which is particularly crucial for maintaining endothelial integrity in blood vessels[25]. The higher sialic acid content in Central Kalimantan EBN, as indicated by protein band intensity, may therefore contribute to enhanced antioxidant potential, potentially offering greater health benefits. Further studies are needed to quantify sialic acid levels directly and explore the specific mechanisms underlying its antioxidant effects in EBN.

Limited research has explored the antioxidant potential of Indonesian EBN. In a study by Rifqi[26] using an water extract of EBN from Palu, Central Sulawesi, weak antioxidant activity was observed via the DPPH assay, yielding an antioxidant activity index (AAI) of 0.010-0.013. This finding aligns with the present study, which demonstrated a high IC_{50} value of 1135.61 µg/mL, indicating low radical scavenging capacity. Conversely, Quek *et al.*[27] reported a FRAP value range of 1.10 to 2.51 mg AAE/g for EBN samples from Peninsular Malaysia. In contrast, the current research revealed a significantly higher FRAP value of 4.567 ± 0.30 µg AAE/mL for Indonesian EBN. These discrepancies may be attributed to variations in geographical origin, extraction methods, and analytical techniques. The higher FRAP value observed in this study suggests a potentially greater reducing capacity of Indonesian EBN compared to samples from other regions, warranting further investigation into the specific compounds responsible for this activity.

CONCLUSION

This study demonstrated that the water extract of EBN exhibits significant antioxidant activity, particularly as evidenced by the NO scavenging assay. These results suggest that the antioxidant mechanism of EBN water extract may be attributed, at least in part, to its protein content. Given the established role of antioxidants in mitigating oxidative stress and reducing the risk of chronic diseases, including cancer, EBN water extract holds promise as a potential source of natural antioxidants. Furthermore, its potential application in cosmeceutical formulations, such as sunscreens and anti-aging products, warrants exploration. Natural antioxidants can offer protection against ultraviolet radiation-induced skin damage, including roughness, wrinkles, and carcinogenesis. Future research should focus on elucidating the specific antioxidant mechanisms of EBN fractions and isolating the bioactive compounds responsible for these effects. Investigating other relevant antioxidant pathways will also provide a more comprehensive understanding of EBN's potential therapeutic applications.

ACKNOWLEDGMENT

The authors express their sincere gratitude to the Ministry of Research and Higher Education of Indonesia for their generous financial support, which made this research possible. We also extend our appreciation to the entire research team for their dedicated efforts and valuable contributions throughout this project. This research was conducted in collaboration with the School of Pharmacy, Bandung Institute of Technology, and was funded by the Ministry of Research and Higher Education of Indonesia in the Higher Education Collaborative Research scheme under grant number 661/K11/KM/2019.

AUTHORS' CONTRIBUTION

Conceptualization: Dita Ayulia Dwi Sandi
Data curation: Dita Ayulia Dwi Sandi, Eka Fitri Susiani, Satrio Wibowo Rahmatullah
Formal analysis: Dita Ayulia Dwi Sandi, Eka Fitri Susiani
Funding acquisition: Dita Ayulia Dwi Sandi
Investigation: Dita Ayulia Dwi Sandi, Eka Fitri Susiani, Satrio Wibowo Rahmatullah
Methodology: Eka Fitri Susiani, I Ketut Adnyana, Pratiwi Wikaningtyas
Project administration: Eka Fitri Susiani, Satrio Wibowo Rahmatullah

Resources: -
Software: -
Supervision: I Ketut Adnyana, Pratiwi Wikaningtyas
Validation: I Ketut Adnyana, Pratiwi Wikaningtyas
Visualization: Dita Ayulia Dwi Sandi, Eka Fitri Susiani
Writing - original draft: Dita Ayulia Dwi Sandi, Satrio Wibowo Rahmatullah
Writing - review & editing: I Ketut Adnyana, Pratiwi Wikaningtyas

DATA AVAILABILITY

All data are available on request from the corresponding author.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

REFERENCES

1. Lee TH, Wani WA, Lee CH, Cheng KK, Shreaz S, Wong S, et al. Edible Bird's Nest: The Functional Values of the Prized Animal-Based Bioproduct From Southeast Asia-A Review. Front Pharmacol. 2021;12:626233. DOI: 10.3389/fphar.2021.626233; PMCID: PMC8089372; PMID: 33953670

2. Thorburn C. The Edible Birds' Nest Boom in Indonesia and South-east Asia. Food Cult Soc. 2014;17(4):535-53. DOI: 10.2752/175174414X14006746101439

3. Yacoob MR, Khairy WIW, Munirah AR, Nabilah JNF, Zulhazman H. Suitable habitat and environmental conditions for successful edible bird nest swiftlet houses. AIP Conf Proc. 2021;2347:020197. DOI: 10.1063/5.0054174

4. Ito Y, Matsumoto K, Usup A, Yamamoto Y. A sustainable way of agricultural livelihood: edible bird's nests in Indonesia. Ecosyst Health Sust. 2021;7(1):1960200. DOI: 10.1080/20964129.2021.1960200

5. Sandi DAD, Musfirah Y. Wound Healing Effects of Edible Bird's Nests Oinment (Aerodramus fuciphagus) in Alloxan-Induced Male Rats. Majalah Obat Tradisional. 2019;24(1):33–9. DOI: 10.22146/mot.39072

6. Sandi DAD, Rahmatullah SW. Pengujian Efek Tonikum Sarang Burung Walet Putih (Aerodramus fuchipagus) pada Mencit Putih Jantan dengan Metode Ketahanan Lama Waktu Berenang. J Pharmascience. 2016;3(2):29–35. DOI: 10.20527/jps.v3i2.5735

7. Sandi DAD, Susiani EF. Formulation of edible bird's nest (Aerodramus fuciphagus) from Central Kalimantan as skin whitening and moisturizing cream. J Pharm Bioallied Sci. 2021;13(1):39–45. DOI: 10.4103/jpbs.jpbs_276_19; PMCID: PMC8142912; PMID: 34084047

8. Sandi DAD, Rahmatullah SW. Stimulant Effect of Edible Bird's Nest (Aerodramus fuchipagus) from Borneo on White Mice. Res J Pharm Biol Chem Sci. 2018;9:927–30.

9. Elfita L, Wientarsih I, Sajuthi D, Bachtiar I, Darusman HS. The diversity in nutritional profile of farmed edible bird's nests from several regions in Indonesia. Biodiversitas. 2020;21(6):2362–8. DOI: 10.13057/biodiv/d210604

10. Chantakun K, Kishimura H, Kumagai Y, Benjakul S. Physicochemical properties of house and cave edible bird's nest from Southern Thailand. ScienceAsia. 2022;48(2):136–43. DOI: 10.2306/scienceasia1513-1874.2022.017

11. Helmi, Subekti DT, Mranata B, Sudarnika E, Lukman DW, Wibawan IWT. Protein Profile of Edible Bird's Nest Origin Kalimantan AndJava Islands Indonesia. IOSR J Agric Vet Sci. 2018;11(5):69–73. DOI: 10.9790/2380-1105026973

12. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition. 2002;18(10):872–9. DOI: 10.1016/s0899-9007(02)00916-4; PMID: 12361782

13. Tung CH, Pan JQ, Chang HM, Chou SS. Authentic determination of bird's nests by saccharides profile. J Food Drug Anal. 2008;16(4):86–91. DOI: 10.38212/2224-6614.2339

14. Chaudhary P, Janmeda P, Docea AO, Yeskaliyeva B, Razis AFA, Modu B, et al. Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. Front Chem. 2023;11:1158198. DOI: 10.3389/fchem.2023.1158198; PMCID: PMC10206224; PMID: 37234200

15. Yida Z, Imam MU, Ismail M. In Vitro Bioaccessibility and Antioxidant Properties of Edible Bird's Nest Following Simulated Human Gastro-Intestinal Digestion. BMC Complement Altern Med. 2014;14:468. DOI: 10.1186/1472-6882-14-468; PMCID: PMC4289220; PMID: 25475744

16. Maesaroh K, Kurnia D, Al Anshori J. Perbandingan Metode Uji Aktivitas Antioksidan DPPH, FRAP dan FIC terhadap Asam Askorbat, Asam Galat dan Kuersetin. J Chim Nat Acta. 2018 Aug 26;6(2):93-100. DOI: 10.24198/cna.v6.n2.19049

17. Zamzani I, Triadisti N. Limpasu Pericarpium : an Alternative Source of Antioxidant From Borneo with Sequential Maceration Method. J Profesi Medika J Kedokteran Kesehatan. 2021;15(1):60-8. DOI: 10.33533/jpm.v15i1.2820

18. Gulcin I, Alwasel SH. DPPH Radical Scavenging Assay. Processes. 2023;11(8):2248. DOI: 10.3390/pr11082248

19. Sadeer NB, Montesano D, Albrizio S, Zengin G, Mahomoodally MF. The Versatility of Antioxidant Assays in Food Science and Safety-Chemistry, Applications, Strengths, and Limitations. Antioxidants. 2020;9(8):709. DOI: 10.3390/antiox9080709; PMCID: PMC7464350; PMID: 32764410

20. Nwachukwu ID, Sarteshnizi RA, Udenigwe CC, Aluko RE. A Concise Review of Current In Vitro Chemical and Cell-Based Antioxidant Assay Methods. Molecules. 2021;26(16):4865. DOI: 10.3390/molecules26164865; PMCID: PMC8400447; PMID: 34443459

21. Gülçin I. Fe(3+)-Fe(2+) transformation method: an important antioxidant assay. Methods Mol Biol. 2015;1208:233-46. DOI: 10.1007/978-1-4939-1441-8_17; PMID: 25323511

22. Özyürek M, Güçlü K, Tütem E, Başkan KS, Erçağ E, Çelik SE, et al. A comprehensive review of CUPRAC methodology. Anal Methods. 2011;3(11):2439-53. DOI: 10.1039/C1AY05320E

23. Sukhtam T, Jones A, Soliven A, Dennis GR, Shalliker RA. A comparison of the performance of the cupric reducing antioxidant potential assay and the ferric reducing antioxidant power assay for the analysis of antioxidants using reaction flow chromatography. Microchem J. 2019;149:104046. DOI: 10.1016/j.microc.2019.104046

24. Klompong V, Benjakul S, Yachai M, Visessanguan W, Shahidi F, Hayes KD. Amino acid composition and antioxidative peptides from protein hydrolysates of yellow stripe Trevally (Selaroides leptolepis). J Food Sci. 2009;74(2):C126-33. DOI: 10.1111/j.1750-3841.2009.01047.x; PMID: 19323726

25. Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, et al. Essential of Glycobiology. 2nd edition. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2009. PMID: 20301239; NBKID: NBK1908

26. Rifqi A. Perbandingan metode ekstraksi dan uji aktivitas antioksidan ekstrak sarang burung walet (collocalia i.-uciphaga) dengan metode DPPH (2,2-Difenil-l-l- Pikrihidrazil) [bachelor's thesis]. Jakarta: Universitas Islam Negeri Syarif Hidayatullah; 2017. Available from: http://repository.uinjkt.ac.id/dspace/handle/123456789/37323

27. Quek MC, Chin NL, Yusof YA, Law CL, Tan SW. Characterization of edible bird's nest of different production, species and geographical origins using nutritional composition, physicochemical properties and antioxidant activities. Food Res Int. 2018;109:35–43. DOI: 10.1016/j.foodres.2018.03.078; PMID: 29803459