Research Article

Edible Bird's Nest Potently Inhibits SARS-CoV-2 Infection: Evidence from an *In Vitro* Study

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Abstract | To date, no effective drug has been approved by regulatory authorities is established for SARS-CoV-2. Consequently, the exploration of natural products and phytopharmaceuticals with potential antiviral properties has become inevitable. This preliminary study investigates the bioactive compounds of acid extract from edible bird's nest (EBN) and their antiviral activity against SARS-CoV-2. EBN samples obtained from East Kalimantan were extracted using an acid extraction method and subsequently lyophilized. Metabolomic profiling of the acid extract EBN was conducted using LC-HRMS. The in vitro cytotoxicity concentration (CC_{50}) was determined using Vero-E6 cells. The antiviral activity of EBN was assessed using three treatment approaches: pre-treatment, co-treatment, and post-treatment, to determine the inhibitory concentration (IC_{50}). The selectivity index (SI) was calculated. Bioactive compounds such as N-acetylneuraminic acid, N-acetyl-D-glucosamine, ketodeoxynonulosonic acid, and N-acetyl-9-O-acetylneuraminic acid were presumed to possess antiviral activity. The in vitro results showed that the CC_{50} value of EBN on Vero-E6 cells was 10.28 mg/mL, while the IC_{50} values were 0.021 ± 0.02 mg/mL (pre-treatment), 0.021 ± 0.01 mg/mL (co-treatment), and 0.505 ± 0.06 mg/mL (post-treatment). The corresponding selectivity index (SI) values were 496.70 ± 39.18, 489.07 ± 21.71, and 20.34 ± 0.26, respectively. In conclusion, the bioactive compounds possess potent antiviral activity against SARS-CoV-2. The interactions between EBN bioactive compounds and key viral proteins of SARS-CoV-2 may involve in the potential mechanism, thereby inhibiting the early stages of the viral replication cycle.

Keywords | Antivirus, Bioactive compound, Swiftlet nest, Metabolomic profiling, COVID-19, SARS-CoV-2

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INTRODUCTION

The global COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, has had a profound impact on public health since its emergence in 2019 (Liyanage *et al*., 2022; Wirawan *et al*., 2021). In 2019. Globally, more than 763 million infections have been recorded, with over 6.9 million deaths, an increase was reported in confirmed cases by 11% across 73 countries primarily from the Eastern Mediterranean, Southeast Asia, and Western Pacific regions as of May 28, 2025 (Murad *et al*., 2025). Several antiviral agents, including favipiravir (FPV), remdesivir (RDV), and the combination of lopinavir and ritonavir (LPV/r), have been tested for their efficacy against SARS-CoV-2 infection (Purwati *et al*., 2021a, b). However, the inconsistent results have been found which ranging from unsatisfactory therapeutic outcomes to unfavorable side effects (Chua *et al*., 2021).

Edible bird's nest (EBN) is a traditional Chinese medicine known for its numerous health benefits, including its antiviral properties (Chua *et al*., 2021). Studies on the potential use of EBN as an antiviral agent have shown its ability to inhibit hemagglutination activity in influenza viruses, suggesting potential efficacy in reducing respiratory viral infections (Chua *et al*., 2021; Dai *et al*., 2021; Sriwilaijaroen *et al*., 2024). Additionally, EBN is also known as a valuable nutritional source with significant bioactive potential, especially in revealing antiviral and anti-inflammatory effects. Haghani and his colleagues reported comparative antiviral efficacy between EBN and commonly used antiviral drugs, such as amantadine and, in the management of influenza cases. EBN was shown to decrease proinflammatory cytokines and chemokines, including TNF-α, CCL-2, NF-κB, NO, and IL-6, while enhancing IFN-γ expression, an important factor in mitigating cytokine storm phenomena often associated with severe viral infections (Haghani *et al*., 2017). Another study was conducted by Chua and his colleagues to analyze the EBN antiviral effects against the influenza virus infections both *in vitro* and *in vivo*. The significance of sialic acid and its derivatives was highlighted in the study, which are key constituents of EBN, as active antiviral components. The findings explained that EBN can hinder viral replication and liberated from host cells, subdue viral proliferation, and decrease the secretion of proinflammatory cytokines (Chua *et al*., 2021).

Although several *in silico* predictive analyses have suggested a role for EBN as an antiviral agent against SARS-CoV-2 infection, these investigations have been limited to computational models (Chua *et al*., 2021; Ningrum *et al*., 2023). The present study is the first to evaluate the antiviral potential of EBN against SARS-CoV-2 infection through in vitro experimentation. It is expected that the findings could be served as a foundation for creating a healthy food from the development of EBN with antiviral activity or as a groundwork for the discovery of novel natural product-based therapeutic agents.

MATERIALS AND METHODS

Study period and location

This research was conducted during April to September 2025 and took place at the Research Center for Vaccine Technology and Development Institute of Tropical Disease, Universitas Airlangga (RCVTD–ITD Unair), Surabaya.

Materials

Vero-E6 cells (ATCC® CRL-1586™) were used in this study. They were cultured in a minimum essential medium (MEM) (Gibco, New York, USA) supplemented with 10% fetal bovine serum (Gibco, New York, USA), 1% penicillin-streptomycin, and 1% amphotericin (Gibco, New York, USA). The virus used was the SARS-CoV-2 strain RCVTD#35 BP38 (Rantam *et al*., 2021), which has been well-defined, and taken from the isolate bank at the Research Center for Vaccine Technology and Development, Institute of Tropical Disease (RCVTD-ITD), Universitas Airlangga. The swiftlet nests functioning as the EBN source in this study were collected from East Kalimantan, Indonesia. The following materials were used for EBN extraction and metabolite profiling, distilled water, 0.4 M H_2SO_4, 1 M NaOH, an analytical column (Thermo Scientific™ Accucore™ Phenyl Hexyl, 100 mm length × 2.1 mm ID × 2.6 µm particle size, Lithuania), MS-grade water, formic acid, and acetonitrile. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT reagent) and DMSO were employed for the colorimetric assay used to determine the antiviral activity of EBN.

Edible bird's nest extraction

An acid extraction method is used for the extraction of the EBN samples according to Tong *et al*. (2020). To reduce moisture content, the bird nests were ground into powder and dried in an oven at 50–55 °C overnight. Distilled water at a concentration of 0.2% (w/v) was used for the EBN powder dissolution and was left to stand for 24 hours. Subsequently, 2% (v/v) of 0.4 M H_2SO_4 was added to the solution, which was then heated at 80 °C for four hours. The acid extract EBN was allowed to cool, and 1 M NaOH was added to neutralize the pH to 7.0–7.2. The extract was centrifuged at 2500 ×g at 4 °C. The supernatant was separated and stored at 4 °C. The resulting extract was then lyophilized using a Lyovapor L-200 Freezer Dryer (Buchi Labortechnik AG, Switzerland). The lyophilized extract was kept at 4 °C until further study.

Untargeted metabolomic profiling using LC-HRMS

The qualitative analysis of the acid extract EBN was performed using Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS). The sample was prepared by adding HPLC-grade methanol to 100 mg of the acid extract EBN, followed by homogenization for two minutes using a vortex mixer. The sample was then filtered through a 0.20 µm nylon filter and injected into a liquid chromatography system (Thermo Scientific™ Vanquish™ Horizon UHPLC with Binary Pump, Germering, Germany) equipped with an analytical column (Thermo Scientific™ Accucore™ Phenyl Hexyl, 100 mm length × 2.1 mm ID × 2.6 µm particle size, Lithuania). Two mobile phases which were solvent A (MS-grade water with 0.1% formic acid) and solvent B (MS-grade acetonitrile with 0.1% formic acid) used here. The gradient elution started with 5% solvent B, progressively increasing to 90% over 16 minutes, sustained at 90% for 4 minutes, and then returned to 5% solvent B by 25 minutes. The separation parameters were as follows: flow rate of 0.3 mL/min, column temperature at 40 °C, and injection volume of 5 µL. The separated components were subsequently ionized and analyzed using a high-resolution mass spectrometer (Thermo Scientific™ Orbitrap™ Exploris 240 HRMS, Bremen, Germany) at a scanning resolution of 60,000 m/z for both positive and negative ions under full MS/dd-MS^2 mode (scan range: 70–800 m/z; maximum injection time: 100 ms; intensity threshold: 5000; charge state: 1; mass tolerance: 5 ppm; dd-MS^2 resolution: 30,000 FWHM; normalized collision energy: 30, 50, 70; collision gas: nitrogen). Ionization was carried out using an OptaMax™ NG Heated Electrospray Ionization (H-ESI) source with the following parameters: spray voltage positive mode (3500 V), negative mode (2500 V); sheath gas (35 AU); auxiliary gas (7 AU); sweep gas (1 AU); ion transfer tube temperature (300 °C); and vaporizer temperature (320 °C). The resulting data were analyzed using Thermo Scientific Compound Discoverer 3.3 software (San Jose, USA), mzCloud Library, ChemSpider Database, and Mass List Search.

Cytotoxicity assay

Vero-E6 cells at passage 43 were cultured in 96-well plates with the density of 7.5×10^3 cells per well using complete growth medium. Two-fold serial dilution ranging from 100 mg/mL to 0.10 mg/mL was prepared for the test solution consisting of the extract of EBN. 100 µL of each test solution was added to the corresponding wells (n = 4 as a technical replication) after 24 hours of incubation. The treated Vero-E6 cells were then incubated for 24 hours, followed by a colorimetric MTT assay as performed in previous studies (Delaiah *et al*., 2024; Kuncorojakti *et al*., 2024). Shortly, after incubation, the medium and test solutions were removed and washed with PBS. Then, 100 µL of MTT reagent (0.5 mg/mL) was added to each well and incubated for 30 minutes at 37 °C with 5% CO_2. The MTT reagent was discarded after incubation, and PBS rewashed the cells. 100 µL of DMSO was added to each well to dissolve the formazan crystals. The plate was placed on an orbital shaker at 150 rpm for five minutes. Colorimetric quantification was used by measuring the optical density (OD) using a microplate reader at a wavelength of 570 nm. In the end, a percent inhibition curve was used to determine the cytotoxic concentration (CC_{50}) of the acidic EBN extract.

Antiviral assay

Previously established method was performed to analyze the antiviral activity assay of the EBN extract. The EBN test solution was prepared through a two-fold serial dilution ranging from 12.5 to 0.006 mg/mL. Vero-E6 cells is used to evaluate the antiviral activity (the range of doses were based on cytotoxicity of EBN). The cells were cultured in 96-well plates at a density of 1×10^4 cells per well and incubated for 24 hours. On the following day, the cells were subjected to three different treatments. The pre-treatment group, Vero-E6 cells were exposed to 100 µL of the EBN test solution and incubated for an hour. After incubation, 100 µL of SARS-CoV-2 viral suspension with a multiplicity of infection (MOI) of 0.01 (0.1 mL from 1 x 10^3 PFU/mL virus stock) was added to each well. The cells were then incubated for four days until cytopathic effects (CPE) were observed (Kuncorojakti *et al*., 2024). Cell viability was assessed using the colorimetric MTT assay, following previously described procedures and normal cells group (non-infection and un-treated) were used to calculated the percent of viable cell. The co-treatment group, 100 µL of the EBN test solution was mixed with 100 µL of viral suspension with MOI 0.01 in an Eppendorf tube and incubated for an hour before being added to the wells. After four days of incubation, the cell viability was evaluated. In the post-treatment group, Vero E6 cells were first incubated with the viral suspension for an hour, after which the EBN extract test solution was added to each well. It took other four-days to incubated the cells. All experiments related to the antiviral activity assay of EBN were performed under Biosafety Level 3 (BSL-3) laboratory conditions. The inhibitory concentration (IC_{50}) of the EBN extract was calculated in five replicates (n = 5).

Selectivity index determination

The selectivity index (SI) was determined as the ratio of the cytotoxic concentration (CC_{50}) to the inhibitory concentration (IC_{50}).

Data analysis

The data in this study are presented descriptively in of tables

and figures. The CC_{50} and IC_{50} values were determined through nonlinear regression analysis. Differences among the three treatment groups in the antiviral activity assay of EBN were analyzed using a one-way ANOVA, followed by Duncan's multiple range test. The level of statistical significance was set at $p < 0.05$. All statistical analyses were performed using GraphPad Prism Ver. 9.5.1. (GraphPad Software, San Diego, California, USA).

RESULTS AND DISCUSSION

Metabolomic profiling of acidic edible bird's nest extract

In the initial phase of this study, the metabolite profile of the acidic EBN extract was mapped using LC-HRMS. The total chromatogram of the acid extract EBN is presented in Figure 1.

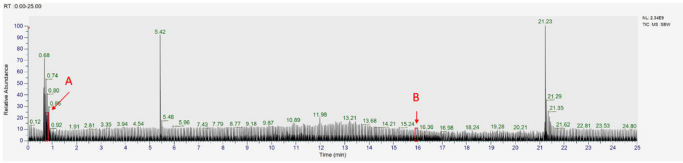

Figure 1: Chromatogram profile of acid extract EBN. Sialic acid derivatives were identified at RT 0.745–0.773 (A) and RT 15.909 (B).

As shown in Figure 1, the LC-HRMS chromatogram of the acid extract EBN revealed the presence of sialic acid derivatives, which were identified at retention times of 0.745–0.773 and 15.909 minutes. Thermo Scientific Compound Discoverer 3.3 software was used to do the further analysis of the chromatographic data (San Jose, USA), integrated with the mzCloud Library, ChemSpider Database, and Mass List Search. Further analysis based on the aforementioned retention times identified four sialic acid derivatives that potentially present antiviral activity against SARS-CoV-2, as shown in Figure 2.

The untargeted metabolomic profiling analysis of the acid extract EBN revealed a total of 68 metabolites. Among these, five metabolites were identified as having potential antiviral activity against SARS-CoV-2. These metabolites included N-acetylneuraminic acid (RT= 0.759), N-acetylneuraminic acid (RT= 0.745), ketodeoxynonulosonic acid (RT= 0.773), N-acetyl-D-glucosamine (RT= 0.767), and N-acetyl-9-O-acetylneuraminic acid (RT= 15.909). The chromatographic profiles of these five metabolites are presented in Figure 2. The results of the sialic acid derivates metabolomic profiling of the acid extract EBN are summarized in Table 1. (The complete metabolomic profiling was present in Supplementary Table 1).

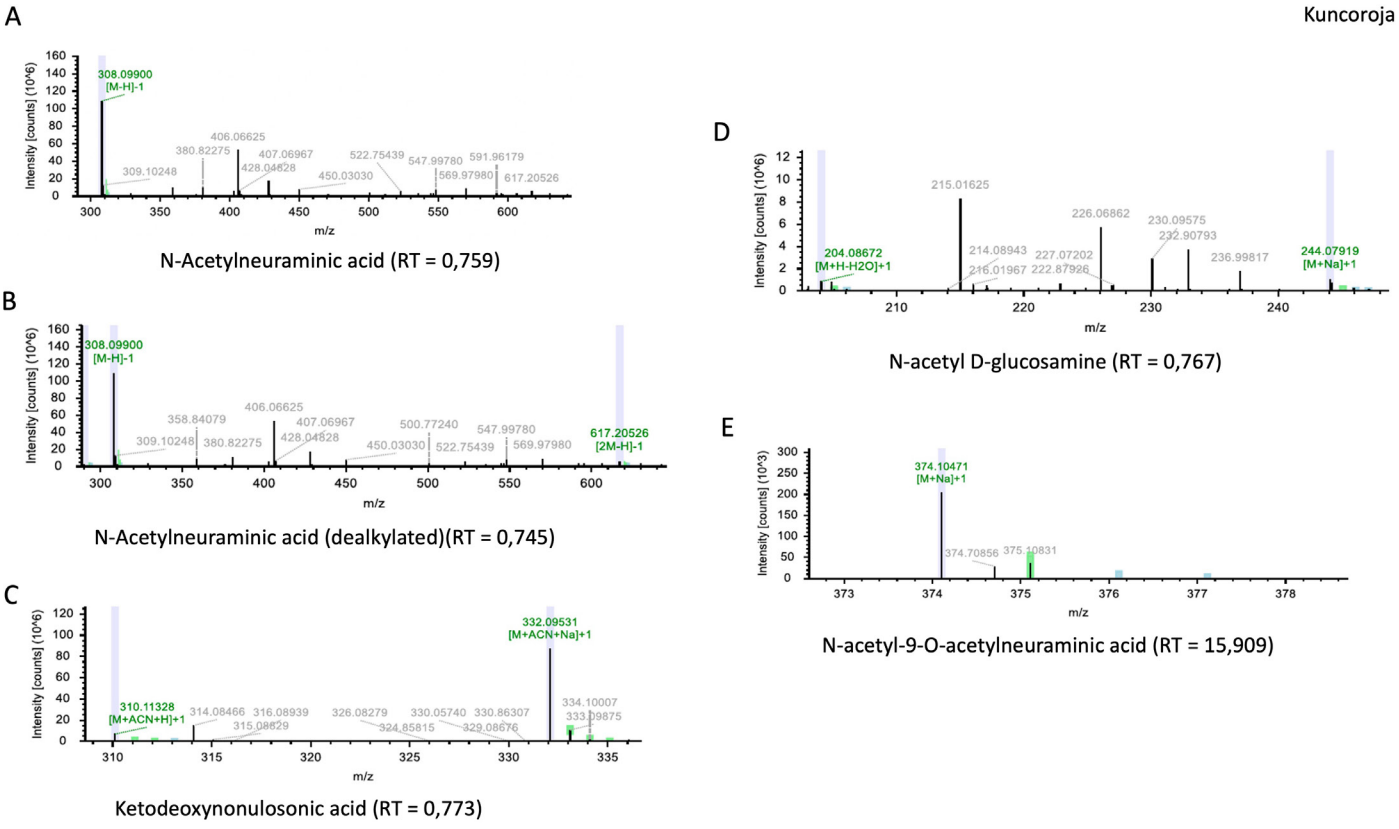

Figure 2: Chromatogram analysis of acid EBN extract for the identification of sialic acid and its derivatives. Based on metabolomic profiling using LC-HRMS, five sialic acid derivatives were identified from the acid extract of EBN: N-acetylneuraminic acid (A and B), ketodexynonulosonic acid (C), N-acetyl-D-glucosamine (D), and N-acetyl-9-O-acetylneuraminic acid (E).

Table 1: Metabolite profiling of sialic acid derivates in EBN extract using LC-HRMS.

No.	Metabolite name	Formula	m/z	RT (Min)
1	N-Acetylneuraminic acid	C11 H19 N O9	308.099	0.759
2	N-Acetylneuraminic acid	C11 H19 N O9	308.099	0.745
3	Ketodeoxynonulosonic acid	C9 H16 O9	332.095	0.773
4	N-Acetyl D-glucosamine	C8 H15 N O6	204.087	0.767
5	N-acetyl-9-O-acetylneuraminic acid	C13 H21 N O10	374.105	15.909

Edible bird's nest (EBN) is a well-known biological product derived from the saliva of swiftlets belonging to two primary genera, Aerodramus and Collocalia, which are predominantly found in Southeast Asia (Tong *et al*., 2020). EBN is also recognized as a traditional Chinese medicine with various health benefits (Ningrum, 2023), including its antiviral properties (Chua *et al*., 2021). To confirm the presence of sialic acid and its derivatives in EBN, the extract was analyzed using metabolomic profiling. One of the "omics" approaches, Metabolomics, presents as a rapid tool for characterizing metabolites within biological systems and is applied to investigate the metabolome, or low-molecular-weight compounds (less than 1500 Daltons) found in tissues, cells, and biological fluids (Chen *et al*., 2022). In the present study, a total of 68 metabolites were identified from metabolomic profiling of the acid extract EBN. Similar research has shown that the extraction method greatly influences both the number of metabolites and the therapeutic potential of EBN. For example, enzymatic extraction methods have been reported to relent between 69 and 775 detectable metabolites (Tong *et al*., 2020). Different extraction methods have been demonstrated in several studies that affect the therapeutic potential of EBN. Water extraction, for example, has been reported with increased wound healing through enhanced corneal keratocyte proliferation (Zainal *et al*., 2011), while enzymatic extraction using pancreatic enzymes produces the extracts of EBN with strong antiviral activity against the influenza virus (Guo *et al*., 2006). Conversely, acid extraction methods produce EBN extracts with notable anti-inflammatory properties (Aswir and Wan Nazaimoon, 2011). Although these extraction methods differ in their therapeutic potential, all have been shown to successfully extract sialic acid and its derivatives. Similarly, in the present study, five sialic acid derivatives were successfully identified in the acidic EBN extract using LC-HRMS analysis.

Determination of the cytotoxic concentration (CC_{50}) of edible bird's nest

The cytotoxic concentration (CC_{50}) of the acid extract edible bird's nest (EBN) was morphologically evaluated. After 24 hours of incubation, cell growth and morphology were assessed descriptively under an inverted microscope. Microscopic observations revealed that 24 hours after treatment, Vero E6 cells exhibited morphological changes beginning at an EBN extract dilution of 6.25 mg/mL. At this concentration, cell density started to decrease, and the cells appeared smaller in size. The pattern of morphological alteration was progressively more apparent with increasing extract concentrations, indicating a dose-dependent effect. At 12.5 mg/mL, morphological changes became more pronounced, with Vero E6 cells appearing rounded and markedly reduced in density. In the highest concentration group (100 mg/mL), morphological alteration was clearly evident; the cells could no longer maintain their normal structure, and the overall cell density was significantly lower compared to untreated control cells. Based on these microscopic observations, the cytotoxicity of the acid extract EBN increased proportionally with extract concentration. The complete pattern of morphological changes in Vero E6 cells is presented in Figure 3.

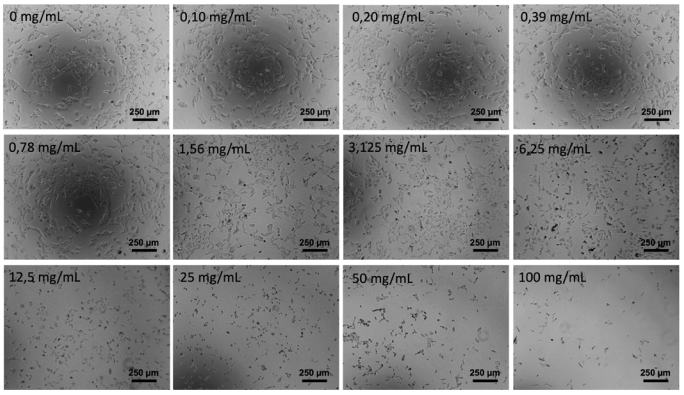

Figure 3: Photomicrograph of cytotoxicity assay of acid EBN extract using Vero E6 cells.

Further, the quantitative assessment of the cytotoxicity assay for the acid extract EBN to determine the CC_{50} value was conducted using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The MTT assay measures cell viability based on mitochondrial activity (Hibono, 2023; Plumeriastuti *et al*., 2022; Roeslan and Tasha, 2021). The formation of purple formazan crystals results from the reduction of tetrazolium salts by the nicotinamide adenine dinucleotide phosphate (NADPH) enzyme. The intensity of the purple color, which corresponds to the amount of formazan produced, was quantified as optical density (OD) using a plate reader or spectrophotometer at a wavelength of 570 nm. Figure 4

presents the non-linear regression curve used to determine the CC_{50} value. The non-linear regression analysis revealed that the cytotoxic concentration (CC_{50}) of the acidic EBN extract was 10.28 mg/mL.

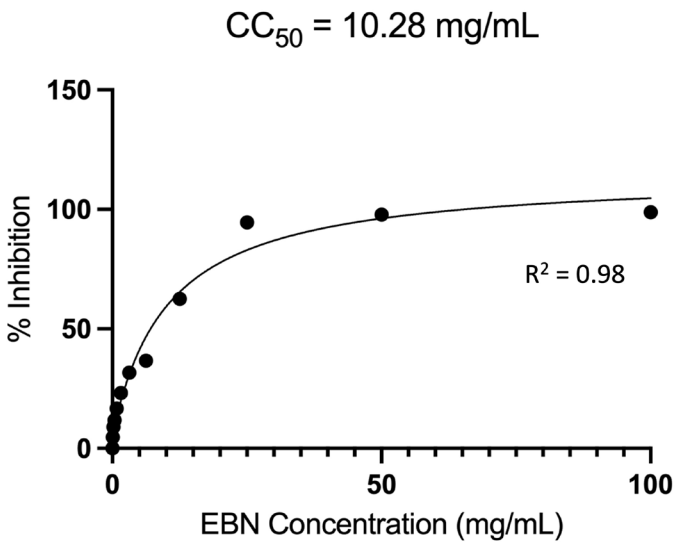

Figure 4: The curve of EBN extract with Cytotoxicity concentration (CC_{50}).

The cytotoxic effects of EBN have been widely investigated by numerous researchers. In vitro assays have been predominantly employed due to their advantages, including cost and time efficiency as well as the ease of optimization (Rashed *et al*., 2021). The MTT assay is a colorimetric method used to evaluate cellular mitochondrial activity. This assay is considered a safe and accurate approach for assessing the cytotoxic effects of substances in vitro (Ghassem *et al*., 2017; Rashed *et al*., 2021; Sul'ain *et al*., 2025). In general, EBN is categorized as a non-toxic substance in healthy cells. Several studies have reported no significant toxic effects even at high concentrations in healthy cell lines. In contrast, EBN demonstrates selective, apoptosis-induced toxicity in certain cancer cells (Li *et al*., 2022). In the present study, the acid extract EBN exhibited low cytotoxicity toward Vero E6 cells. Similar findings have been reported in studies employing water extraction methods, which revealed no significant cell death even at high concentrations in healthy cell lines such as SH-SY5Y (Yew *et al*., 2014) and HepG2 (Yida *et al*., 2014). Other studies have also demonstrated the protective effects of EBN against oxidative stress-induced damage in normal cells. EBN has been reported to mitigate oxidative stress, thereby reducing cell death caused by reactive oxygen species (ROS) (Lee *et al*., 2021; Loh *et al*., 2022). The antioxidant properties of EBN are attributed to 13 identified peptides with molecular weights ranging from 514.29 to 954.52 Daltons (Ghassem *et al*., 2017). Additionally, the sialic acid content in EBN has been shown to attenuate mitochondrial dysfunction in SH-SY5Y cell lines (Rashed *et al*., 2021). Another proposed mechanism suggests that EBN promotes proliferation in human adipose-derived mesenchymal stem cells (hADMSC) through increased expression of IL-6 and VEGF genes, mediated by the activation of transcription factors NF-κB and AP-1 via the p44/42 MAPK and p38 MAPK signaling pathways (Roh *et al*., 2012). A study on selective cytotoxicity has also demonstrated that EBN induces cell death and apoptosis in various cancer cell lines, including human hepatocellular carcinoma (Hep2B) and human breast cancer (MCF-7) cells (Roh *et al*., 2012). According to the National Cancer Institute, a substance is considered to have potential anticancer activity if its CC_{50} value from a crude extract is less than 20 µg/mL (Nguyen *et al*., 2020).

Antiviral activity of edible bird's nest (EBN) against SARS-CoV-2

The antiviral activity of edible bird's nest (EBN) was evaluated based on the inhibitory concentration (IC_{50}) value, determined following the assessment of the cytotoxic concentration (CC_{50}) of the acidic EBN extract. Vero-E6 cells were subsequently treated with SARS-CoV-2 under three different exposure conditions: pre-treatment, co-treatment,

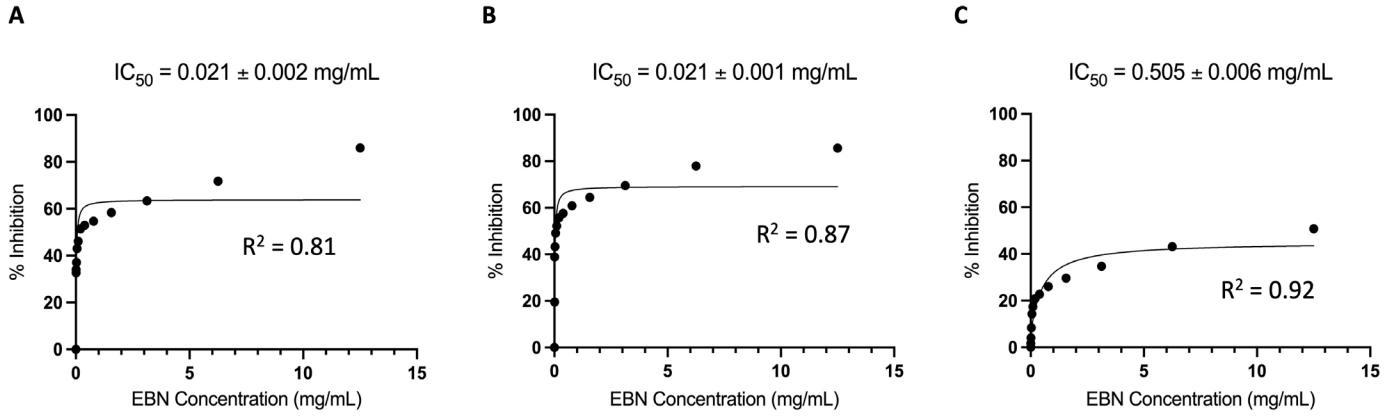

Figure 5: Inhibitory concentration–50 (IC_{50}) curve of acid EBN extract against SARS-CoV-2. IC_{50} evaluation of EBN was conducted based on three viral exposure models: pre-treatment (A), co-treatment (B), and post-treatment (C) against SARS-CoV-2.

and post-treatment. Microscopic evaluations were performed daily for four consecutive days post-treatment to observe the development of cytopathic effects (CPE). Once CPE was identified on the fourth day, cell viability was determined using the MTT assay to quantify the percentage of living cells. The IC_{50} values were calculated through nonlinear regression analysis, as presented in Figure 5. The IC_{50} values were 0.021 ± 0.02 mg/mL (pre-treatment), 0.021 ± 0.01 mg/mL (co-treatment), and 0.505 ± 0.06 mg/mL (post-treatment). The corresponding selectivity index (SI) values were 496.70 ± 39.18, 489.07 ± 21.71, and 20.34 ± 0.26, respectively. The selectivity index (SI), defined as the ratio of CC_{50} to IC_{50}, demonstrated varying results among the different viral exposure models. The values for the pre-treatment and co-treatment groups showed no significant difference, measured at 496.70 39.18 and 489.07 ± 21.71, respectively. In contrast, viral exposure prior to EBN treatment (post-treatment) resulted in a significantly lower value of 20.34 ± 0.26 compared to the pre- and co-treatment models (Figure 6).

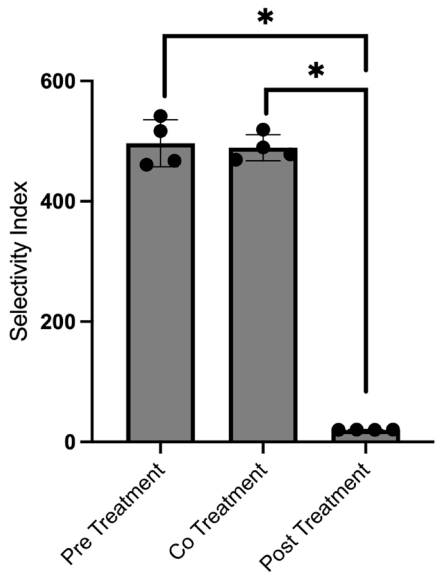

Figure 6: Selectivity index of antiviral activity of acid extract EBN. (Asterisks indicate a significant difference ($p < 0.05$) compared to the pre- and co-treatment groups).

To date, no drugs have been reported to be both effective and officially approved by regulatory authorities for combating SARS-CoV-2 infection. Due to this limitation, the exploration of natural products and phytochemicals with potential antiviral properties has become imperative. Currently, no studies have been reported regarding the use of EBN against SARS-CoV-2. Most existing research on the antiviral activity of EBN has focused on enveloped viruses, such as the avian influenza virus (Sriwilaijaroen *et al.*, 2024). Previous in silico studies have revealed that bioactive compounds in EBN, particularly N-acetylneuraminic acid, exhibit strong negative binding affinities toward several key SARS-CoV-2 viral proteins, including MPro, Spike, and Helicase with binding energies of −6.1, −5.3, and −7.1 kcal/mol, respectively (Ningrum *et al.*, 2023). These findings show that one of the possible antiviral mechanisms of EBN may involve the direct binding of its bioactive compounds to viral structural or functional proteins. The findings from current in vitro study using Vero-E6 cells pre-treated with EBN presented notable antiviral potential. This indicates that EBN treatment could possibly have an interference with the early stages of the viral replication cycle, such as viral attachment, internalization, fusion, or uncoating (Xian *et al.*, 2020). This effect is further supported by the results from the co-treatment group, which showed similar antiviral activity. The results suggested that EBN bioactive compounds may interact directly with viral proteins that contribute to virulence, thereby inhibiting viral internalization into host cells. On the other hand, no significant antiviral activity was observed in the post-treatment group from the effect of EBN against SARS-CoV-2. Similar findings have been reported for other natural compounds, such as curcumin, which demonstrated antiviral activity primarily in pre- and co-treatment conditions against SARS-CoV-2 (Bormann *et al.*, 2021; Marín-Palma *et al.*, 2021; Nicoliche *et al.*, 2024). The high SI observed in this study further supports the potential of EBN as an antiviral agent against SARS-CoV-2. An SI value greater than 3 indicates that the bioactive compound exhibits high selectivity, making it a promising candidate for further in vivo evaluation using animal models (Mahmood *et al.*, 2023).

CONCLUSION

Bioactive compounds such as N-acetylneuraminic acid, ketodeoxynonulosonic acid, N-Acetyl-D-glucosamine, and N-acetyl-9-O-acetylneuraminic acid contained in the acid extract EBN exhibit potent antiviral potential against SARS-CoV-2. The potential mechanism may involve direct interactions between EBN bioactive compounds and key viral proteins of SARS-CoV-2, thereby inhibiting the early stages of the viral replication cycle. This study serves as a preliminary investigation into the antiviral potential of acid extract EBN. Therefore, further mechanistic studies, including investigations into the inhibition of viral replication processes and the immunomodulatory effects of EBN, are needed to be conducted.

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NOVELTY STATEMENT

This study is the first study reported the potency of acid extract EBN against SARS-CoV-2 in vitro. The bioactive compound of EBN such as N-acetylneuraminic acid possess antiviral potential through direct binding between EBN bioactive compound and major protein of SARS-CoV-2.

AUTHOR'S CONTRIBUTION

SK: Designed, conceptualized, supervised, analyzed data and wrote the manuscript. AK, DD and HS: Performed the experiment, analyzed data and wrote the manuscript. YP, EPH, LRY, HMR and WR: Analyzed the data, wrote manuscript. All authors have read and approve the final manuscript.

Ethical approval

Human and animal subjects or animal tissue were not involved, and the *in vitro* was completely conducted in this study.

Generative AI and AI-assisted technology statement

Generative AI was used only for minor editorial purposes, including grammar refinement and language clarity. No AI tools were used for data analysis, interpretation, or generation of scientific content. The authors are fully responsible for the accuracy and integrity of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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Supplementary Table 1: Metabolite profiling of EBN extract using LC-HRMS.

No.	Metabolite name	Formula	m/z	RT (Min)
1	(2R)-2-Hydroxy-3-(phosphonooxy)-propanal	C3 H7 O6 P	171.006	21.239
2	(2S,3S)-N,N′-Dihexadecyl-2,3-dimethoxy-N,N,N′,N′-tetramethyl-1,4-butanediaminium	C42 H90 N2 O2	653.694	0.682
3	(2′E)-2,2′-(4-ethyl-6-methylcyclohex-1-yl-2-ylidene)dihydrazinecarboxamide	C11 H22 N6 O2	271.188	7.745
4	(E)-4-phenyl-3-(pyridine-2-yl)but-2-en-1-ol	C15 H15 N O	226.123	8.888
5	(Nitroimino)dimethanol	C2 H6 N2 O4	123.040	0.669
6	1-Boc-3-(aminomethyl)piperidine	C11 H22 N2 O2	215.175	4.482
7	1-O-(2R-hydroxy-hexadecyl)-sn-glycerol	C19 H40 O4	355.282	1.681
8	2,2,6,6-Tetramethyl-1-piperidinol (TEMPO)	C9 H19 N O	158.154	7.436
9	2-Furoic acid	C5 H4 O3	111.009	1.036
10	27-Norcholestanehexol	C26 H46 O6	472.363	13.271
11	3,6,9,12,15-Pentaoxahexacosan-1-ol	C21 H44 O6	415.303	11.296
12	3-(2-{4-[(4-tetradecylpiperazin-1-yl)carbonyl]phenyl}ethyl)-1,2,4-oxadiazol-5(4H)-one	C29 H46 N4 O3	516.389	13.152
13	4-(Trifluoromethyl)-3,4,5,6-tetrahydro-2-pyridinamine	C6 H9 F3 N2	167.079	0.769
14	4a-Carbinolaminetetrahydrobiopterin	C9 H13 N5 O3	240.108	12.623
15	5,8-tetradecadienal	C14 H24 O	209.190	9.277
16	5-Acetylamino-6-formylamino-3-methyluracil	C8 H10 N4 O4	225.062	4.271
17	6-Gingerol	C17 H26 O4	293.176	9.628
18	[(4E)-2-(Hydroxymethyl)-4-(3-isobutyl-5-methylhexylidene)-5-oxotetrahydro-2-furanyl]methyl octanoate	C25 H44 O5	447.308	14.038
19	[6]-Gingerol	C17 H26 O4	293.176	8.963
20	Acetyl tyrosylarginine cetyl ester	C33 H57 N5 O5	604.442	12.969
21	Amines, C12-14-tert-alkyl, ethoxylated propoxylated	C14 H31 N O	230.248	8.999
22	Azelaic acid	C9 H16 O4	187.098	4.993
23	Betaine	C5 H11 N O2	118.086	0.750
24	C12E5	C22 H46 O6	429.319	11.908
25	C12E7	C26 H54 O8	512.416	11.844
26	Campechic acid A	C40 H70 O8	340.260	5.429
27	CerPE(d14:2(4E,6E)/20:1(11Z)(2OH))	C36 H69 N2 O7 P	690.515	14.380
28	Certonardosterol M	C28 H50 O6	500.395	14.434
29	Citric acid	C6 H8 O7	191.020	1.036
30	Citric acid, tri(2-ethylhexyl)ester, acetate	C32 H58 O8	588.447	14.092
31	Decanophenone	C16 H24 O	233.190	12.622
32	Di(2-ethylhexyl)4,5-epoxycyclohexane-1,2-dicarboxylate	C24 H42 O5	428.337	13.431
33	Dibenzylamine	C14 H15 N	198.128	4.362
34	ELK	C17 H32 N4 O6	389.239	2.389
35	GibberellinA19	C20 H26 O6	361.167	10.472
36	Haplofungin C	C34 H62 O9	632.473	13.989
37	Hexamethylenetetramine	C6 H12 N4	141.113	21.234
38	Isoleucine	C6 H13 N O2	132.102	1.071
39	JBIR-81	C24 H34 N4 O3	425.258	11.273
40	Malevamide D	C40 H68 N4 O8	750.536	13.207

Table continues on next page.....

No.	Metabolite name	Formula	m/z	RT (Min)
41	MGDG(18:3(9Z,12Z,15Z)/14:0)	C41 H72 O10	723.503	5.448
42	N-[(9E)-1-(β-D-Glucopyranosyloxy)-3,4-dihydroxy-9-tetradecen-2-yl]tridecanamide	C33 H63 N O9	618.458	13.487
43	N-acetyl-L-leucyl-N-{(2R)-6-[(tert-butoxycarbonyl)amino]-1-oxohexan-2-yl}-D-leucinamide	C25 H46 N4 O6	497.335	4.573
44	N-Acetylneuraminic acid	C11 H19 N O9	308.099	0.759
45	N-benzyl-1-(5-methyl-2-furyl)but-3-en-1-amine	C16 H19 N O	242.154	4.483
46	N-Boc-L-cyclohexylglycinol	C13 H25 N O3	242.176	7.229
47	N-Methyl-N-nitroso-1-tetradecanamine	C15 H32 N2 O	257.259	6.846
48	N-Nonanoylmorpholine	C13 H25 N O2	228.196	8.066
49	N-{7-[4-(Aminomethyl)phenyl]heptanoyl}-L-seryl-N-(2-cyclohexylethyl)-L-lysinamide	C31 H53 N5 O4	560.416	13.076
50	Noroxycodone-d3	C17 H16 [2]H3 N O4	305.157	2.670
51	O-(Aminomethyl)-L-homoseryl-L-leucyl-L-leucylglycine	C19 H37 N5 O6	432.280	3.647
52	Octaethylene glycol	C16 H34 O9	388.254	3.349
53	Palmitamidopropyl dimethylamine	C21 H44 N2 O	341.353	10.508
54	PEG n5	C10 H22 O6	261.131	2.267
55	Pendecamaine	C23 H46 N2 O3	399.358	10.793
56	Persin	C23 H40 O4	403.282	14.210
57	Pestalpolyol E	C22 H38 O4	389.266	13.565
58	Phallusialide A	C18 H27 Cl N2 O6	401.149	9.261
59	Rhabdopeptide 1	C32 H55 N5 O4	574.431	13.618
60	Rhabdopeptide K	C30 H51 N5 O4	546.400	12.505
61	Scymnol	C27 H48 O6	486.379	13.862
62	Solvent Red 26	C25 H22 N4 O	393.172	10.651
63	Trimetaphosphate	H3 O9 P3	238.892	0.729
64	β-Fluoroaspartic acid	C4 H6 F N O4	152.036	21.240
65	N-Acetylneuraminic acid	C11 H19 N O9	308.099	0.745
66	Ketodeoxynonulosonic acid	C9 H16 O9	332.095	0.773
67	N-acetyl D-glucosamine	C8 H15 N O6	204.087	0.767
68	N-acetyl-9-O-acetylneuraminic acid	C13 H21 N O10	374.105	15.909