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Research Article

A current insight and future perspective of edible bird nest as caviar of the east

Muhammad Andika Haraharap[1], Osfar Sjofjan[1], Lilik Eka Radiati[1], Muhammad Halim Natsir[1], Rony Abdi Syahputra[2], Fahrul Nurkolis[3]

1 *Faculty of Animal Sciences, Universitas Brawijaya, Malang, Indonesia*
2 *Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia*
3 *Department of Biological Sciences, Faculty of Sciences and Technology, State Islamic University of Sunan Kalijaga (UIN Sunan Kalijaga), Yogyakarta, Indonesia*

Corresponding author: Osfar Sjofjan (osfar@ub.ac.id)

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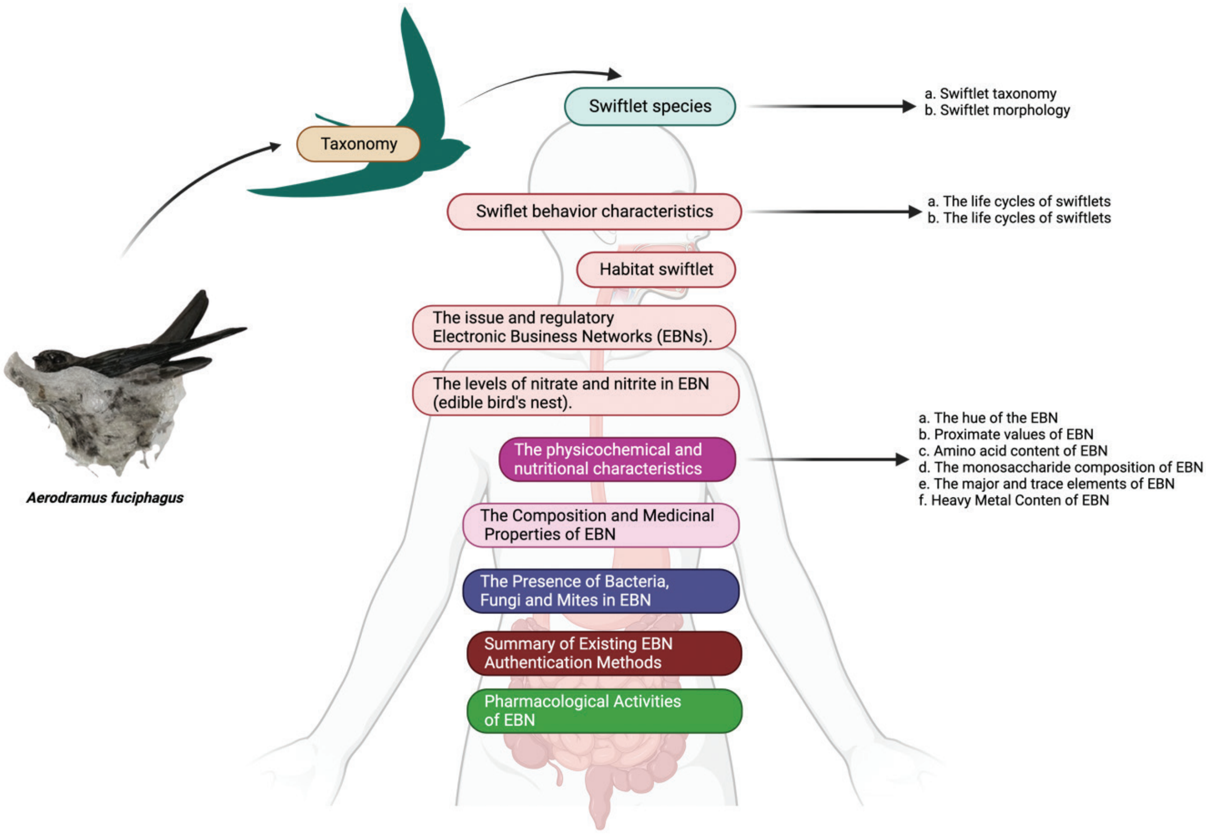
Abstract

Edible bird's nest (EBN) is a highly valuable food product obtained from swiftlet nests, primarily those of the *Aerodramus* genus. Due to its purported health benefits and exceptional taste, EBN is often referred to as the "caviar of the East." This abstract presents a comprehensive review of the current state of EBN research, focusing on its chemical composition, nutritional value, pharmacological effects, and safety considerations. The chemical composition of EBN is intricate and influenced by various factors, including bird species, geographic origin, nest collection time, and processing methods. It is primarily composed of proteins, polysaccharides, minerals, lipids, and a wide range of bioactive compounds such as sialic acid, amino acids, and antioxidants. Additionally, EBN has demonstrated antioxidant, anti-inflammatory, anti-tumor, and anti-aging properties attributed to these bioactive constituents. While EBN is generally considered safe for human consumption, it is essential to address concerns related to potential contaminants like heavy metals, microbial pathogens, and allergens. This review offers a comprehensive overview of previous research conducted on residual impurities that may be present in edible bird's nests (EBNs). The review encompasses various aspects, including, the regulatory framework and associated concerns regarding EBNs, the levels of nitrite and nitrate detected in EBNs, the presence of bacteria, fungi, and mites in EBNs, the identification of allergenic substances in EBNs, and the presence of heavy metals and excessive mineral content at different stages of EBN processing, including raw uncleaned (RUC) EBNs, raw cleaned (RC) EBNs, and EBNs after undergoing treatment.

Graphical abstract:



Keywords

Edible bird's nest (EBN), swiftlet nests, chemical composition, nutritional value, pharmacological effects, safety considerations

Introduction

Edible bird's nest (EBN) is a remarkable and highly prized food product derived from the nests of swiftlets, a small bird species belonging to the *Aerodramus* genus (Gausset 2004). These swiftlets construct their nests using solidified saliva, creating a gel-like structure. EBN has a rich history of consumption and cultural significance, particularly in countries like China, Malaysia, Indonesia, and Thailand (Tan et al. 2014). EBN primarily comes from two main swiftlet species: *Aerodramus fuciphagus* and *Aerodramus maximus*. These birds are known for their exceptional nest-building skills, creating nests in dark caves, crevices, or specially designed structures (Hamzah et al. 2013). Harvesting occurs during specific periods when swiftlets have finished breeding but before their eggs hatch, ensuring the nests are of the highest quality. The chemical composition of EBN is intricate and influenced by factors like bird species, geographic origin, harvest time, and processing methods (Quek et al. 2015). EBN is primarily composed of proteins, polysaccharides, minerals, lipids, and various bioactive compounds. Proteins are a significant component, contributing to its unique texture and nutritional value. Polysaccharides, including glycoproteins and glycosaminoglycans, give EBN its gel-like properties (Ramlan et al. 2018). EBN is rich in essential and non-essential amino acids, with notable levels of cysteine and arginine. Cysteine, in particular, is abundant and contributes to the distinctive aroma and flavor of EBN. Moreover, EBN contains minerals like calcium, potassium, and iron, enhancing its nutritional profile. Various bioactive compounds, such as sialic acid, amino acids, and antioxidants, have been identified in EBN (Vercruysse et al. 2005). Sialic acid, a unique sugar found in EBN, is of particular interest due to its potential health benefits, including its role in brain development, cognition, and immune function. Amino acids like glycine, proline, and lysine contribute to both the nutritional quality and potential therapeutic effects of EBN (Srivastava et al. 2012). EBN has been the subject of numerous scientific studies exploring its potential pharmacological activities. It exhibits antioxidant properties, thanks to the presence of bioactive compounds such as amino acids and antioxidants (Kim et al. 2012). These properties underlie its potential anti-aging, anti-inflammatory, and immune-modulating effects. Furthermore, EBN has shown promise in traditional and modern medicinal applications, including the treatment of respiratory disorders, gastrointestinal conditions, and skin ailments. While EBN is generally considered safe for consumption, it is vital to address safety concerns. Potential contaminants, including heavy metals, microbial pathogens, and allergens, have been identified as areas of concern. Implementing quality control measures, such as adhering to good manufacturing practices during harvesting and processing, is essential to ensure the safety and purity of EBN products (Chen et al. 2014). In conclusion, this review of edible bird's nest (EBN) highlights its unique properties, nutritional composition, and potential health benefits. Further research is needed to understand the mechanisms underlying its therapeutic effects and to establish comprehensive guidelines for safe consumption. Reviewing the current state of EBN research is crucial for advancing our knowledge of this unique food product and its potential health advantages. The following points underscore the significance of conducting a comprehensive review, uncovering Chemical Composition: EBN is a complex natural product with a diverse chemical makeup. Reviewing existing research helps elucidate its precise components, including proteins, polysaccharides, minerals, lipids, and bioactive compounds (Kim et al. 2012). Understanding the chemical composition provides a deeper insight into EBN's nutritional profile and potential therapeutic effects. Evaluating Nutritional Value: Assessing the nutritional value of EBN is essential for determining its dietary importance and potential health benefits. A review of studies allows researchers to identify essential amino acids, vitamins, and minerals present in EBN, contributing to a comprehensive understanding of its nutritional content (Dai et al. 2021). Exploring Pharmacological Activities: EBN has long been associated with various pharmacological activities, including antioxidant, anti-inflammatory, anti-tumor, and immune-modulating effects (Hou et al. 2021). Conducting a review of existing research enables the evaluation of scientific evidence supporting these activities. It also helps identify potential mechanisms of action, paving the way for future studies to explore the therapeutic potential of EBN in various health conditions. Identifying Bioactive Compounds: EBN contains several bioactive compounds, such as sialic acid, amino acids, and antioxidants, contributing to its potential health benefits (Rohaizan 2017). Reviewing the literature aids in identifying and understanding the roles of these bioactive compounds in EBN. This knowledge can facilitate targeted research investigations and potential applications in nutrition and health. The objective of this review is to comprehensively analyze the current state of research on edible bird's nest (EBN), focusing on its chemical composition, nutritional value, pharmacological activities, and safety considerations. The review aims to synthesize existing knowledge, identify knowledge gaps, and evaluate methodologies, ultimately providing a deeper understanding of EBN and guiding future research in this field. While the nutritional value of edible bird's nest (EBN) has been discussed earlier, it is equally important to consider the safety aspects associated with its consumption. This review offers a comprehensive overview of previous research conducted on residual impurities that may be present in edible bird's nests (EBNs). The review encompasses various aspects, including, the regulatory framework and associated concerns regarding EBNs, the levels of nitrite and nitrate detected in EBNs, the presence of bacteria, fungi, and mites in EBNs, the identification of allergenic substances in EBNs, and the presence of heavy metals and excessive mineral content at different stages of EBN processing, including raw uncleaned (RUC) EBNs, raw cleaned (RC) EBNs, and EBNs after undergoing treatment.

Taxonomy

Swiftlet species

Swiftlet taxonomy

The swiftlet, a small avian species belonging to the Collocalini tribe within the swift family (Apodidae), presents challenges in bird taxonomy due to limited observable variations in physical characteristics (Chantler and Driessens 2000). Initially, Gray (1840) suggested grouping all swiftlet species under one genus, *Collocalia*, a classification that persisted for over a century. The discovery of echolocation in specific swiftlet species by Medway (1957) prompted Brooke (1970, 1972) to propose a taxonomic revision of the genus *Collocalia*. Brooke's work resulted in the division of this genus into three distinct genera: *Collocalia* for non-echolocating swiftlets, *Hydrochous* for non-echolocating gigantic swiftlets, and Aerodramus for echolocating swiftlets. Subsequent studies have raised alternative taxonomic proposals. Some researchers, including Chantler and Driessens (2000) and Salomonsen (1983), have suggested merging these three genera back into a single genus, *Collocalia*. Others have proposed further subdivisions and the inclusion of new sister groups like Chaeturini and Apodini based on different morphological and behavioral characteristics (Sibley and Monroe 1990). To address the challenges of morphological identification, Lee et al. (1996) conducted a molecular sequencing analysis of cytochrome-b mitochondrial DNA in swiftlets to reorganize their taxonomy with molecular data. However, this study focused on a limited segment (406 bp) of the cytochrome-b DNA, leaving several questions unanswered. Thomassen et al. (2003) conducted a more comprehensive sequencing of the entire cytochrome-b gene, providing evidence in favor of the monophyly (shared ancestry) of swiftlets. However, they encountered variability in the cytochrome-b gene among different *Hydrochous* species, making it difficult to determine *Hydrochous*' position in the swiftlet evolutionary tree. In a subsequent study, Jordan Price et al. (2004) expanded the inclusion of swift and swiftlet species and introduced the use of an additional genetic marker, the NADH dehydrogenase subunit-2 gene (ND2), to improve taxonomic arrangement and analysis. This study confirmed swiftlet monophyly and classified swiftlets into two distinct tribes: *Aerodramus* and *Collocalia*. Notably, it challenged the previous notion that these tribes were incapable of echolocation. Jordan Price et al. (2004) proposed that the capacity for echolocation should not be limited to a single genus. They found that the pygmy swiftlet (*Collocalia troglodytes*), previously considered a non-echolocating species within the *Collocalia* genus, also possessed echolocation abilities, suggesting that echolocation is not a reliable criterion for swiftlet taxonomy. In a separate investigation, Thomassen et al. (2005) expanded the cytochrome-b sequence dataset by including two additional sequences, 2S rRNA (12S) and nuclear non-coding b-fibrinogen intron 7 (Fib7), to explore the phylogenetic relationships between *Hydrochous gigas* and other swiftlet species. Their findings supported Gray's (1840) proposal to classify swiftlets into a unified genus, *Collocalia*. The taxonomy and nomenclature of swiftlet species remain uncertain, and further research is needed to determine the monophyletic grouping of these avian species.

Swiftlet morphology

The Collocaliini Tribe comprises 36 distinct swiftlet species, categorized into four genera: *Aerodramus*, *Hydrochous*, *Schoutedenapus*, and *Collocalia*. Among these, seven species, specifically *Collocalia* esculenta and *Collocalia linchi* from the *Collocalia* genus, as well as *Aerodramus fuciphagus*, *Aerodramus germani*, *Aerodramus maximus*, *Aerodramus unicolor*, and *Aerodramus francicus* from the *Aerodramus* genus, are known to produce edible bird's nests (EBN) (Zuki et al. 2012). Swiftlet species found in Malaysia pose challenges for morphological identification due to significant similarities. A study in Malaysia identified seven distinct swiftlet species: *Aerodramus francica*, *Aerodramus vestita*, *Aerodrames brevirustis*, *Aerodramus fuciphaga*, *Aerodramus maximus*, *Collocalia esculenta*, and *Hydrochous gigas*. However, reliable identification is often limited to *C. esculenta*, distinguished by its unique color pattern, and *Hydrochous gigas*, recognized for its larger body size. The remaining five species exhibit superficial similarities and are occasionally found in similar habitats. White-nest swiftlets (*Aerodramus fuciphagus*) and black-nest swiftlets (*Aerodramus maximus*) in Malaysia construct nests primarily from saliva, which are collected for commercial use. Several other swiftlet species build nests using materials such as vegetation, grass, feathers, and mud, but these nests lack significant economic value. *Aerodramus fuciphagus* and *Aerodramus maximus* share nearly identical physical characteristics, differing mainly in the glossiness of their feathers and the presence of tarsal feathering, which requires careful observation. Swiftlets possess large, darkly pigmented eyes and relatively short beaks, with uniform black coloration in their beaks, legs, and feet. Some swiftlet species have lost their perching abilities, relying on hanging from their nests or standing on their hock joints, which do not require the use of metatarsal structures. The white-nest swiftlet, *Aerodramus fuciphagus*, is smaller in size with a blackish-brown upper body and variable rump coloration ranging from whitish to brownish. It has shorter wings, a deeper tail-notch, and darker underparts compared to the black-nest swiftlet. *Aerodramus fuciphagus* and *Aerodramus maximus* exhibit specific measurements in various body parameters, including body length, tail length, wing cord length, tarsus length, and expanded wing length (Looi et al. 2015).

Swiflet behavior characteristics

The life cycles of swiftlets

The life cycles and behaviors of swiftlets in various habitats have been meticulously observed and extensively studied over the past century. As noted by Viruhpintu et al. (2002), swiftlets are known for their monogamous behavior and exhibit a strong preference for nesting sites, showing high levels of loyalty to their chosen locations. According to Quang et al. (2002), swiftlets typically begin their breeding activities when they reach one year of age. However, there is considerable variation in breeding seasons and the timing of breeding activities, including nest-building, egg laying, egg incubation, and young rearing, among different species and geographical locations. These observed variations could potentially be influenced by climatic factors such as precipitation levels, atmospheric humidity, and the availability of food resources (Langham 1980). Table 1 presents the life cycles of the swiftlet species *Aerodramus fuciphagus* and *Aerodramus maximus*.

Table 1. Swiftlet life cycles.

Parameters	Species		References
	White-nest Swiftlet (*Aerodramus fuciphagus*)	Black-nest Swiftlet (*Aerodramus maximus*)	
Reproductive cycle of a swiftlet species: egg	roughly 92 to 120 days, a solitary egg clutch, with an estimated egg size ranging from 16 to 25 mm	roughly 92 to 120 days, two eggs per clutch, with an approximate egg size of 10 to 15 mm	(Langham 1980; Lim 2002; Viruhpintu et al. 2002).
The incubation and fledging durations	23±3 days	43±6 days	(Medway 1957; Langham 1980; Lim 2002)
Swiftlets engage in year-round breeding activities	between the months of October and February		(Langham 1980)
Construction of a single nest by swiftlets	typically requires around 30–45 days during the breeding season, but in the non-breeding season, this process takes approximately 60–80 days		(Aowphol et al. 2008)
Construction of nests	mostly undertaken by male swiftlets throughout a span of around 35 days. both male and female swiftlets engage in the process of nest building		(Lim 2002; Marcone 2005)

Growth of swiftlets

The research conducted by Reichel et al. in 2007 focused on Mariana swiftlets in the Saipan region, revealing specific developmental milestones in nestlings. Newborn nestlings were observed to have pink skin and lacked any natal down. Tiny pin feathers were detected under the skin on their dorsum and wings around days 4–6. By the ninth day, pin feathers were visible in all tracts, and by the thirteenth day, they began emerging through the skin. Feather emergence occurred between days 17 and 19. Nestlings started opening their eyes around the twentieth day, and their flight feathers reached about 50% growth by the thirty-seventh day. Around days 45–47, nestlings had a full set of feathers and could engage in short-distance flights. Typically, swiftlet species have a fledging period ranging from approximately day 39.8 to 53.3, although this duration may vary among species and regions. Initially, a newly hatched chick had a wing length of about 6 mm, which gradually increased until primary pin feathers emerged around day 12–13. Wing length exhibited continuous linear growth from day 13 to day 45. Similar to wing development, tail length displayed linear growth between day 15 and day 45. Nestling weight on the first day was recorded at 1.11±0.06 grams, with gradual growth leading to an approximate weight of 8.21 grams by day 29. It's important to note that incubation periods, fledging age, clutch size, and growth rates may vary among different swiftlet species and regions (Table 2). The swiftlet reproduction process involves mating, egg production, nest construction using saliva, incubation, hatching, and parental care, including feeding the offspring until they are ready for flight (Nugroho and Budiman 2013) (Fig. 1).

Scholarly sources indicate that the growth and reproductive processes of swiftlets are influenced by specific environmental parameters. These conditions include a relative humidity of approximately 90%, temperatures ranging from 28 to 30 degrees Celsius, and the availability of an adequate food supply (Dai et al. 2021). Consequently, swiftlets are typically found in favorable habitats, such as Indonesia, Malaysia, and similar regions. Table 3 provides a comprehensive list of the primary countries and locations that are prominent in EBN production. Taxonomists strive to classify birds in the Apodidae family by examining their physical characteristics, behavior, genetic traits, and additional empirical data. However, there is still a lack of consistent findings. Swiftlets are identified by their small size, low weight, elongated wingtips, and relatively short legs. When their wings are folded, the wingtips extend beyond the tail ends. As mentioned in the cited source, swiftlets have small, curved appendages and relatively weak musculature, making them unable to support their own body weight (Zuki et al. 2012). These birds cannot assume an upright position, which aligns with the etymology of their family name, Apodidae, derived from the Greek term meaning "devoid of feet." Swiftlets are known for their remarkable speed and agility, primarily engaging in extended periods of aerial activity. These avian species have short beaks characterized by broad gaps, which are essential for effectively capturing airborne arthropods. Additionally, they rely on echolocation as a vital sensory mechanism in their pursuit of prey.

Table 2. Growth rate of various swiftlet species.

Reproductive parameters of different swiftlet species					
Species	**Incubation period (days)**	**Age at fledging (days)**	**Clutch size**	**Source and location**	**Reference**
Mariana Swiftlet (*Aerodramus bartschi*)	22.95	47	1	Saipan	(Reichel et al. 2007)
White-nest Swiftlet (*Aerodramus fuciphagus*)	25.1 ± 0.3	39.8 ± 2.6	2	Singapore	(Lee and Kang 1994)
White-nest Swiftlet (*Aerodramus fuciphagus*)	23.0 ± 3.0	43.0 ± 6.0	2	Malaysia	(Langham 1980)
Black-nest Swiftlet (*Aerodramus maximus*)	25.5 ± 2.2	45.9 ± 2.6	1	Singapore	(Lee and Kang 1994)
Black-nest Swiftlet (*Aerodramus maximus*)	28.0	58.5	1	Sarawak	(Medway 1957)
Mossy-nest Swiftlet (*Aerodramus vanikorensis*)	23.0	48.5	1–2	Sarawak	(Medway 1957)
White-rumped Swiftlet (*Aerodramus spodiopygius*)	23.0	46.0	2	Fiji	Turburton (1986)
Mountain Swiftlet (*Aerodramus hirundinaceus*)	NA	53.3 ± 1.2	1	New Guinea	Turburton (2003)
Clossy Swiftlet (*Collocalia esculenta*)	21.5	42	2	Sarawak	(Medway 1957)

Table 3. The man producing countries regions of EBN.

Country	Main region	Mainly Types of EBN
Indonesia	Kalimantan, Sumatra, Java, Sulawesi	Cave EBN, House EBN
Malaysia	Sarawak and Sabah, East and west coasts of Malay Peninsula, (e.g. Kuala Lumpur)	Cave EBN, House EBN
Thailand	South, Cent Apart	Cave EBN, House EBN
Vietnam	Quy Nhon	Cave EBN
The Philippines	Palawan island	Grass EBN
Burma	Tanintharyi	House EBN
Combodia	Koh Kong	House EBN
China	Guangdong Province, Yunnan Province, Dazhou Island	House EBN

Figure 1. Illustrates the sequential stages involved in the process of swiftlet nest production and breeding, leading up to the point where the nests are considered suitable for harvesting: **A.** The process of collecting swiftlet nests through gentle tapping. **B.** Eggs laid by swiftlets. **C.** Recently hatched swiftlet offspring. **D.** Swiftlet chicks at the age of 10 days. **E.** Swiftlet chicks at the age of 17 days. **F.** Swiftlet chicks aged between 21 and 30 days. **G, H.** The swiftlet offspring have reached the stage of development when they are capable of flight. Additionally, the swiftlet nests have reached a state of readiness where they may be collected for harvesting.

Habitat swiftlet

Swiftlets exhibit natural breeding behavior within limestone caves, where they adhere to the walls and ceilings (Langham 1980; Baker and Genty 1998; Lim 2002). Numerous scholars have conducted investigations on the impact of different characteristics of nest sites and the correlations between nesting success and environmental conditions (Phach and Voisin 1998; Sankaran 2001; Viruhpintu et al. 2002; Jehle et al. 2004). For example, a study conducted by Jehle et al. (2004) examined nesting success by making daily observations starting from the day of egg laying in various environmental conditions. In Viruhpintu et al.'s study (2002), it was observed that both *Aerodramus fuciphagus* and *Aerodramus maximus* exhibited distinct nest construction behaviors within the cave wall to minimize competition for nestling space between the two species. Furthermore, the selection of nestling places by both species was found to be based on specific traits rather than random placement. *Aerodramus fuciphagus* is typically found in natural caves at low altitudes, ranging up to 1280 meters, as well as in buildings. On the other hand, *Aerodramus maximus* tends to roost at elevations ranging from sea level up to 1830 meters (Lim 2002). The ability of *A. maximus* to fly and inhabit greater altitudes has been attributed to its larger body size and larger wingspan. Several factors related to the nest site can impact breeding success. These factors include the texture of the rock surface at the nest site (rough, slightly rough, or smooth), the presence or absence of nest support, the inclination of the cave wall (flat, inwardly inclined, or outwardly inclined), and micrometeorological conditions like temperature and relative humidity. Typically, avian species prefer nesting at elevated locations characterized by an inward-inclining, smooth, and concave surface, as opposed to lower areas with rugged cave walls (Sankaran 2001). A smooth and concave surface provides better support for nest building and increases the chances of successful nestling rearing. Additionally, locations that are taller and inward-inclining serve as a deterrent to predators, protecting both eggs and nestlings (Viruhpintu et al. 2002). mThe temperature range conducive to successful nestling development falls between 26 °C and 35 °C, with elevated temperatures potentially harming eggs and reduced temperatures affecting

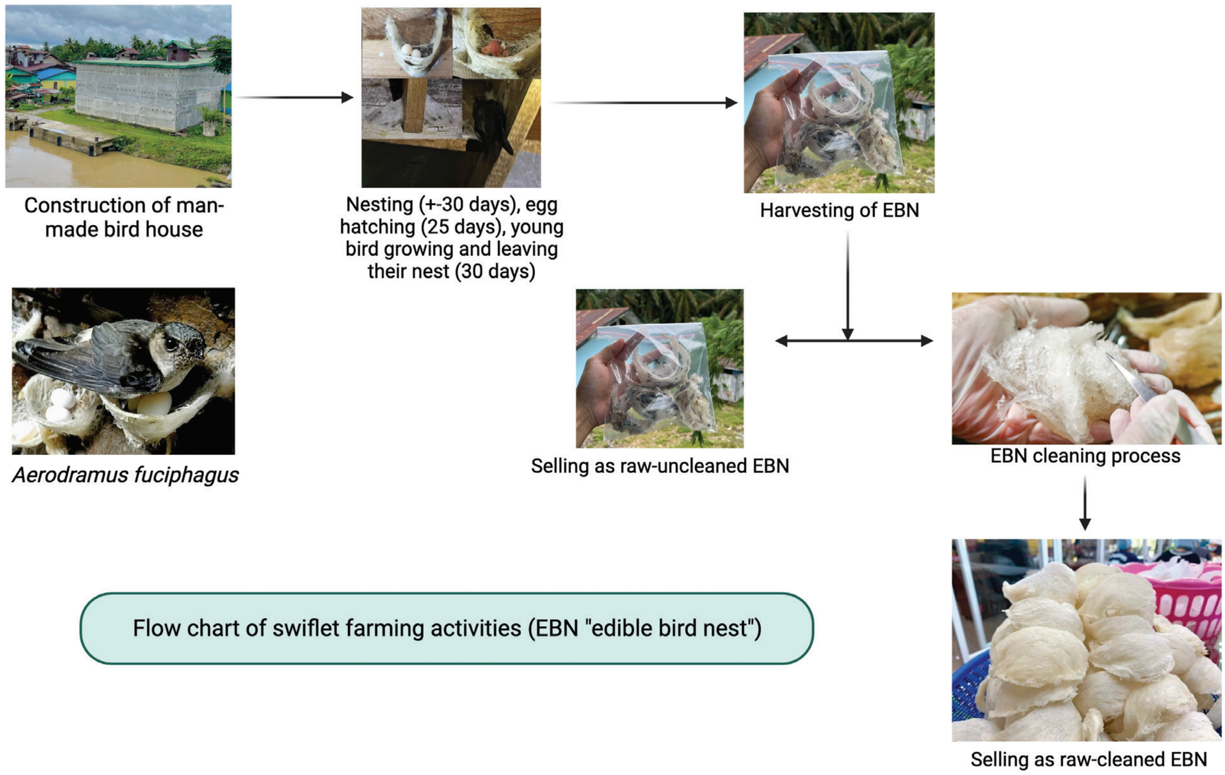


Figure 2. Flow chart of swiftlet farming activities EBN.

featherless juvenile swiftlets (Quang et al. 2002). An optimal humidity range for nesting is typically between 80% and 90%, as excessive humidity can lead to fungus growth, while insufficient humidity can compromise nest adhesion to the cave wall (Medway 1962; Jehle et al. 2004) (Fig. 2).

The physicochemical and nutritional characteristics

The hue of the EBN

The nests of swiftlets come in three distinct colors: white, yellow (gold), or red (Saengkrajang et al. 2013; Chua and Zukefli 2016). The coloration of edible bird's nests (EBN) varies significantly depending on where they are harvested, either in caves or buildings. In cave environments, the two protrusions that serve as attachment points for the nest cup to the cave wall typically exhibit a fresh EBN stain resembling the color of blood. However, over time, these protrusions darken and turn brown following the harvesting process (Sims 1961). There were different beliefs regarding the cause of the reddish coloration of the Edible-Nest Swiftlet (EBN). One perspective suggested that the saliva of the swiftlets, which they used to construct their nests, contained traces of blood, resulting in the red hue. Conversely, another viewpoint proposed that the reddish tint was a result of the swiftlets consuming lotus seeds, seaweeds, or mollusks, with the pigments from these sources mixing with the bird's saliva. Research investigations have shed light on the red coloration of edible bird's nests (EBN), revealing that it can be attributed to the process of nitrate oxidation occurring within the droppings of swiftlets (But et al. 2013). The coloration of EBN is significantly influenced by the presence of nitrate and nitrite. Cave EBN, in particular, exhibits elevated levels of these minerals, resulting in a slightly yellowish to reddish or deeper hue compared to house EBN. The presence of sodium nitrite in EBN can lead to the generation of aryl-C-N and NO_2 side groups in the aromatic amino acids in white EBN, causing a change in color from white to red (Quek et al. 2015). House EBN typically appears pale or whitish in color, which can be attributed to its relatively lower levels of nitrate and nitrite compounds (Paydar et al. 2013).

Proximate values of EBN

The proximate values of edible bird's nest (EBN) compounds obtained from swiftlet nests in various countries were examined, as presented in Table 4. The protein composition of EBN sourced from Malaysia, Indonesia, Thailand, and the Philippines falls within the range of 59.8% to 66.9%, while the carbohydrate content ranges from 25.6% to 31.4%. There is no substantial variation in the moisture and fat content of EBN across different locations, with fat consistently exhibiting the lowest value among the proximate content of EBN. A significant protein content serves as an indicator that the swiftlets are situated in a favorable feeding environment at the given site (Hamzah et al. 2013; Saengkrajang et al. 2013; Hun et al. 2015). The water activity values of EBN exhibit a low range of 0.68 to 0.80, which explains the absence of mold growth in the nest despite prolonged exposure to a humid environment. Furthermore, the levels of protein, carbohydrates, moisture, and

Table 4. This research examines the proximate values of edible bird's nest (EBN) collected from birds habitats in different countries.

No.	Proximate (%)	Malaysia (Marcone 2005)	Thailand (Saengkrajang et al. 2013)	Philippine (Hamzah et al. 2013)	Indonesia (Hamzah et al. 2013)
1	Protein	62	62.58	–	65.8
2	Carbohydrate	27.26	29.66	16	10
3	Moisture	7.5	19.82	5.58	10.87
4	Ash	2.1	6.72	1.5	1.5
5	Fat	0.14	0.96	0.05	0.04

Table 5. Summary of EBN compotition.

Component	Content	References
***Proximate analysis* (%)**		Yu-Qin et al. 2000; Marcone 2005
Moisture	7.5–12.9	
Ash	2.1–7.3	
Fat	0.14–1.28	
Protein	42–63	
Carbohydrate	10.63–27.26	
Total nitrogen	25.62–27.26	
Protein	60.93	(Hui Yan et al. 2022)
Moisture	16.04	
Fat	0.09	
Ash	1.96	
Carbohydrate	20.98	

fat exhibit similarities between the cave and house EBN samples. EBN demonstrates commendable stability in its proximate values, especially in conditions characterized by reduced visibility (Tan et al. 2014).

Edible bird's nest (EBN) is indeed renowned for its high protein content and its rich composition of essential amino acids and monosaccharides, making it a unique and valuable food item. The typical composition of EBN from the genus Aerodramus includes lipid (0.14–1.28%), ash (2.1%), carbohydrate (25.62–27.26%), and protein (62.0–63.0%) (Table 5). Among the amino acids present in EBN, serine, threonine, aspartic acid, glutamic acid, proline, and valine are the most prevalent (Kathan and Weeks 1969). Additionally, EBN contains essential elements such as calcium (1298 ppm), sodium (650 ppm), magnesium (330 ppm), potassium (110 ppm), phosphorous (40 ppm), zinc, and iron (30 ppm) (Marcone 2005). One of the remarkable components of EBN is sialic acid (N-acetylneuraminic acid), which constitutes a significant proportion of essential sugars in EBN, making up approximately 9% of the total essential sugars. Sialic acid is known for its potential neurological and cognitive benefits, especially in infant brain development, as it is a functional constituent of brain gangliosides (Chau et al. 2003; Colombo et al. 2003; Wang et al. 2019). Sialic acid also plays a role in modulating the immune system by affecting the viscosity of mucus and promoting cell detachment from microbes and parasites (Lehmann et al. 2006). Table 7 provides a summary of the proximate nutritional composition of raw EBN based on various studies. It consistently shows that protein is the major nutrient in EBN, accounting for around 60–63% of its composition, followed by carbohydrates at approximately 20–28%. The glycoprotein in EBN is the primary component responsible for these high protein and carbohydrate levels, making EBN a unique and valuable dietary resource (Marcone 2005; Babji et al. 2015). Determining the composition of protein, carbohydrate, moisture, fat, and ash in raw EBN is essential for various purposes, including the hydrolysis process for the extraction of its valuable components.

According to (Table 6), the composition of Edible Bird's Nest (EBN) can indeed vary, particularly in terms of protein content, depending on factors such as the geographic location where it is harvested and the species of swiftlet that produces it. Table 8 shows that the protein content in the EBN samples studied ranged from 53% to 56%, which is slightly lower than the protein content reported in EBN from Thailand and a local study, where protein levels ranged from 60.9% to 66.9% and 56% to 61.5%, respectively. On the other hand, the protein content in your study exceeded that of samples from Penang and Indonesia, which ranged from 24% to 49%, as well as those from Batu Pahat, Johor, which measured 35.8%. It's worth noting that variations in EBN composition can be influenced by factors such as the swiftlet species, the specific location where the nests are harvested, and potentially the presence of adulterants. Adulteration can lead to a decrease in the overall crude protein content, as indicated in a recent study by Marcone (2005), which found reductions ranging from 1.1% to 6.2% in protein content due to adulterants. The consistency in protein content across different regions in Malaysia, as observed in your study, could be attributed to the fact that the saliva used to construct the nests is produced by individuals of the same swiftlet species, despite differences in environmental habitats. This suggests that the swiftlet species themselves play a significant role in determining the nutritional composition of EBN.

The differences in the nutritional composition between a half cup of Edible Bird's Nest (EBN) and stripe-shaped EBN can be attributed to the unique structure and cleaning process of each type of EBN.

A half cup of EBN is primarily composed of a mucin-rich glycoprotein that solidifies upon exposure to air, resulting in the formation of a cup-shaped nest with minimal contaminants. This composition contributes to the higher protein and carbohydrate content observed in a half cup of EBN. The mucin glycoprotein in EBN possesses characteristics of both proteins and carbohydrates, making it a unique and nutrient-rich substance. On the other hand, stripe-shaped EBN refers to fragments obtained from the periphery of the EBN nest. These fragments attach to the surface of the bird's home and have a hardened texture, which tends to accumulate a higher amount of contaminants. The cleaning process for stripe-

Table 6. This study examines the proximate composition, safety profile, and microorganism profile of various samples of edible bird's nest (EBN) collected from different regions in Malaysia. The samples were obtained from the following locations: A – Alor Setar, Kedah; B – Sibu, Sarawak; C – Rompin, Pahang; D – Kuala Selangor; E – Johor Bahru; F – Jerantut, Pahang; and G – Port Klang, Selangor.

Parameters	Regions							Tolerance level
	A	B	C	D	E	F	G	
Proximate analysis (%) (Tan et al. 2020)								
Protein	54.3	53.9	53.0	53.7	54.4	55.5	56.4	NA
Carbohydrate	29.7	30.5	28.0	31.7	28.6	28.6	28.8	NA
Moisture	10.8	12.4	12.3	13.1	14.0	12.1	12.1	<15%
Ash	2.8	2.7	3.4	2.7	2.9	2.8	2.2	NA
Crude fat	0.1	0.1	0.1	0.1	0.1	0.1	0.1	N

Table 7. The proximate composition and total dietary fiber content of the half cup and stripe-shaped edible bird's nest (EBN) were analyzed.

Nutritional composition (%)	Present study		(Tan et al. 2020)	(Ma and Liu 2012b)
	EBN half cup	EBN stripe-shaped	House EBN	House EBN
Crude Protein	56.96	54.70	42.00–63.00	53.00–56.40
Carbohydrate	23.96	22.12	10.63–27.26	28.00–31.70
Moisture	15.92	19.51	7.50–12.90	10.80–14.00
Ash	3.16	3.67	2.10–7.30	2.20–3.40
Fiber	3.89	19.96	NA	NA
Crude Fat	ND	ND	0.14–1.28	0.1
Caloric value	331.00	349.50	NA	NA

Not detected (lower than Limit of Detection, LOD), NA: Not available.

Table 8. The amino acids present in edible bird's nest (EBN) sourced from Indonesia, Malaysia, Thailand, and china are of interest for academic investigation.

Type of Amino Acid (mg/g)		Content			
		Malaysia(Marcone 2005)	Thailand(Saengkrajang et al. 2013)	Indonesia(Elfita 2014)	China(Hui Yan et al. 2022)
Essential Amino Acid (EAA)					
Histidine	his	3.3	0.08	2.3	2.02
Threonine	thr	4.4	1.24	3,8	3.8
Tyrosine	tyr	10.1	0.53	3.9	3.62
Valine	val	10.7	1.08	3.9	4.16
Methionin	met	0.8	8.35	0.5	0.47
Lysine	lys	3.5	0.82	2.3	2.09
Isoleucine	ile	10.1	1.1	3.8	1.83
Leucine	leu	3	–	3.8	3.83
Phenylalanine	phe	6.8	0.53	4.5	3.54
Tryptophan	trp	–	–	–	0.70
Non Essential Amino Acid (N-EAA)					
Hydroxyproline	hyp	–	–	3.6	0.92
Aspartate	asp	9.5	4.06	4.5	4.71
Serine	ser	15.4	1.88	4.5	5.05
Glutamate	glu	7	14.56	3.6	3.58
Glycine	gly	5.9	1.52	1.8	2.05
Arginine	arg	5.4	–	3.9	3.19
Alanine	ala	4	0.92	1.3	1.43
Proline	pro	–	0.98	3–6	4.08
Cysteic Acid	cya	–	8.35	–	1.71
Total EAA		48.4	12.49	20.3	26.4

shaped EBN can be laborious and may result in a significant loss of nutrients during the procedure. The increased ash content in stripe-shaped EBN may be attributed to the inclusion of feathers and other extraneous substances embedded within the solidified structure of the nest, as well as the removal of water-soluble minerals during the cleaning procedure. Stripe-shaped EBN may also contain fibrous proteins, such as keratin, collagen, or plant-based material derived from the swiftlet's diet in its specific habitat. The caloric value of EBN is influenced by its protein, carbohydrate, fat, and fiber composition. The higher caloric content observed in stripe-shaped EBN compared to half cup EBN can be attributed to the elevated fiber content present in the stripe-shaped EBN. Dietary fiber contributes to the caloric value of a food item, and the differences in fiber content between the two types of EBN account for the variations in caloric content (Table 7).

Amino acid content of EBN

The amino acid composition of Edible Bird's Nest (EBN) can indeed vary depending on factors such as the region of harvest, geographical location, and even the country of origin. The data presented in Table 8 indicates differences in the amino acid profiles of EBN from Thailand and Malaysia. In the analysis of EBN from Thailand, it was found that arginine, leucine, and tryptophan were absent or present in very low concentrations. Conversely, EBN from Malaysia exhibited the absence of proline, tryptophan, cysteine, and cystine. There were also variations in amino acid content within different regions of Malaysia, with some regions displaying deficiencies in proline and cystine compared to others. It's important to note that the levels of essential amino acids in EBN, regardless of the source, are generally lower than those of non-essential amino acids. Essential amino acids are amino acids that the human body cannot synthesize on its own and must obtain from dietary sources, making them crucial for nutrition. The data also indicates that Malaysia had the highest concentration of total essential amino acids among the countries mentioned, followed by China, Indonesia, and Thailand. This suggests that the amino acid composition of EBN can indeed vary significantly based on its source, which may be influenced by factors such as the swiftlet species, diet, and environmental conditions in each region.

The monosaccharide composition of edible bird's nest (EBN) is of interest

The monosaccharide composition of Edible Bird's Nest (EBN) is characterized by a notable presence of galactose and N-acetylhexosamine, as indicated in Table 9. An examination of EBN from various regions, including Vietnam, Indonesia, and Thailand, revealed significant levels of N-acetylglucosamine (ranging from 4.76% to 5.64%), N-acetylgalactosamine (ranging from 3.79% to 4.54%), and galactose (ranging from 4.58% to 5.43%) (Tung et al. 2008). These findings are consistent with previous research. In addition, the analysis detected the presence of rhamnose at a concentration of 0.02% and xylose at a concentration of 0.21%. These variations in monosaccharide composition within EBN may be attributed to the removal of proteoglycans during the cleaning process. The diverse range of monosaccharides found in EBN can potentially offer health benefits to consumers. The significant concentration of sialic acid is particularly important as it is a key component of cerebral gangliosides, which are associated with neurological development and brain maturation in infants (Chau et al. 2003; Colombo et al. 2003; Wang and Brand-Miller 2003). Sialic acid also plays a role in mucus by preventing the colonization of harmful microorganisms, potentially contributing to the protection of the respiratory system (Lehmann et al. 2006).

The study focuses on major and trace elements in EBN

Table 10 provides an analysis of major and trace components in both half cup and stripe-shaped edible bird's nest (EBN) and compares these findings with data from other cited studies (Ma and Liu 2012; Mohamad Ibrahim et al. 2021). In both forms of EBN, there are notable concentrations of macronutrients, including calcium, sodium, sulfur, magnesium, and potassium, as well as various other macro and trace elements. Specifically, calcium, magnesium, and sodium exhibited statistically significant differences in content between the half cup and stripe-shaped EBN samples. Additionally, the trace elements iron and zinc also displayed significant differences in content between these two sample types. These variations in the content of macro and trace elements may have implications for the nutritional value and potential health benefits associated with different forms of EBN. Further research is warranted to explore the significance of these differences and their potential impact on human health.

Heavy metal content of EBN

Table 11 presents the concentrations of heavy metals in both half cup and stripe-shaped edible bird's nest (EBN) samples, comparing them to the maximum allowable levels of heavy metals in specified food (EBN) as established by the Food Act 1983 and the provisional tolerable weekly intake (PTWI) determined by the Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA). The findings indicate that there was no statistically significant difference in lead levels between the half cup and stripe-shaped EBN samples. Furthermore, the lead levels in both types of EBN were below the permissible threshold of 300.00 ppb. In contrast, the concentration of arsenic showed a statistically significant difference between the two shapes, with higher levels observed in the stripe-shaped EBN. However, it's important to note that both concentrations remained below the permissible threshold for EBN, which is 150.00 ppb. A minimal concentration of cadmium was observed exclusively in the half cup EBN sample, while no traces of cadmium were detected in the stripe-shaped EBN sample. Notably, no traces of additional heavy metals, such as mercury and nickel, were found in any of the EBN samples. These findings suggest that the heavy metal concentrations in the tested EBN samples are within permissible limits and do not pose significant health risks based on established standards and guidelines.

The presence of heavy metal contamination in edible bird's nest (EBN) can have various sources, including both the swiftlet house environment and the processing/manufacturing procedures of EBN. These potential sources of contamination include, construction Materials: Heavy metals like lead can leach into the swiftlet house environment from materials such as rusty iron bars, pressure-treated wood, and lead-based paints used during construction, Processing and Manufacturing: The procedures involved in cleaning and processing EBN may involve the use of chemicals and materials that can intro-

Table 9. The monosaccharides present in edible bird's nest (EBN) derived from various geographical locations.

Monosaccharide	Monosaccharide content (%)		
	(Kathan and Weeks 1969)	(Tung et al. 2008)	(Chua et al. 2015)
Galactose	16.9	4.58–5.43	11.19
Mannose	–	0.48–0.99	1.05
Fucose	0.7	0.36–0.78	1.14
N-acetylglucosamine	5.3	4.76–5.64	–
N-acetylgalactosamine	7.2	3.79–4.54	–
Sialic acid	8.6	–	–
Rhamnose	–	0.13–0.33	0.02
Xylose	–	–	0.21
Total monosaccharide	38.7	14.10–17.71	13.61
Galactose	16.9	4.58–5.43	11.19
Mannose	–	0.48–0.99	1.05

Table 10. The present study investigates the major and trace element composition of two distinct forms of edible bird's nest (EBN), namely the half cup and stripe-shaped EBN.

Element Content (mg/100 g)		Present Study (Ma and Liu 2012b; Mohamad Ibrahim et al. 2021)			RNI- Intake Level (mg/day)(Noor et al. 2005)
		Half Cup EBN	Stripe-Shaped EBN	House EBN	
Major element	Boron	0.03	0.04	NA	Not Set
	Calcium	735.45	652.95	123.10–859.80	2500.00
	Magnesium	105.97	129.57	88.30–152.80	350.00
	Potassium	16.72	22.00	3.64–35.20	Not Set
	Phosphorus	1.95	4.10	0.03–6.79	4000.00
	Sodium	504.90	682.14	263.80–670.80	2300.00
	Sulfur	240.16	211.32	624.40–884.00	Not Set
Trace element	Chromium	0.03	0.04	0.01–0.06	Not Set
	Cobalt	0.001	0.001	0.00–0.06	0.04
	Copper	0.88	2.34	0.47–11.06	10.00
	Iron	0.68	1.13	0.16–1.94	45.00
	Molybdenum	0.002	0.002	0.00–0.09	2.00
	Selenium	0.01	0.01	0.01–0.04	0.40
	Zinc	0.44	0.76	0.05–2.26	45.00
	Manganese	0.14	0.14	0.02–0.59	9.00

NA: Not available.

Table 11. The present study examines the concentration of heavy metals in both the half cup and stripe-shaped edible bird's nest (EBN) samples.

Heavy metal content (pbb)	Present study (Chen et al. 2014)			Maximum regulatory
	Half Cup EBN	Limit	House EBN	
Arsenic	8.76	23.81	0.06–34.35	150.00 *
Cadmium	3.15	ND	0.06–1.87	1000.00 **
Lead	116.61	115.21	2.24–592.84	300.00 *
Mercury	ND	ND	0.06–70.18	70.00 *
Nickel	ND	ND	56.14–400.00	500.00 ***

* Values are permissible levels set (Act 1983)** Values are permissible levels set (Noordin et al.)*** Values obtained (Chen et al. 2014).

duce heavy metals into the final product, Swiftlet Feathers: Swiftlets can be exposed to heavy metals in the external environment, which can accumulate in their feathers. This contamination can potentially transfer to the nest material, Nest Contaminants: Even after the cleaning process, contaminants may persist in the nest material, including traces of heavy metals, The study's findings indicate that the concentrations of metals tested in both half cup and stripe-shaped EBN samples are below the permissible limits, suggesting that EBN is safe for human consumption in terms of heavy metal contamination. However, it's essential for continued monitoring and quality control to ensure that EBN products maintain these safety standards.

The composition and medicinal properties of Edible Bird's Nest (EBN)

Research on the medicinal benefits of Edible Bird's Nest (EBN) has involved extensive analysis of its composition over an extended period. The primary aim of this research is to identify the bioactive compounds present in EBN and understand their potential mechanisms of action. Here is a summary of the key components found in EBN: Protein Content: EBN is rich in protein, with a protein content ranging from 62.0% to 63.0% (Table 12). Amino Acids: The most prevalent amino acids in EBN include serine, threonine, aspartic acid, glutamic acid, proline, and valine. Lipid Content: EBN typi-

Table 12. Summary of EBN compotition as medicinal properties of Edible Bird's Nest (EBN).

Component	Content	References
***Amino acid* (*molar percent basis*)**		(Yu-Qin et al. 2000; Marcone 2005)
Aspartic + asparagines	2.8–10.0	
Threonine	2.7–5.3	
Serine	2.8–15.9	
Glutamic + glutamine	2.9–7.0	
Glycine	1.2–5.9	
Alanine	0.6–4.7	
Valine	1.9–11.1	
Methionine	0–0.8	
Isoleucine	1.2–10.1	
Leucine	2.6–3.8	
Tyrosine	2.0–10.1	
Phenylalanine	1.8–6.8	
Lysine	1.4–3.5	
Histidine	1.0–3.3	
Arginine	1.4–6.1	
Tryptophan	0.02–0.08	
Cysteine	2.44	
Proline	2.0–3.5	
***Fatty acid analysis* (%)**		(Marcone 2005)
(P) Palmitric C16:0	23–26	
(O) Steric C18:0	26–29	
(L) Linoleic C18:1	22	
(Ln) Linolenic C18:2	26	
***Triacylglycerol* (%)**		(Marcone 2005)
PPO	14–16	
OOL	13–15	
PLnLn	18–19	
Monoglycerides	27–31	
Diglycerides	21–26	
Vitamin		(Yu-Qin et al. 2000)
Vitamin A (IU/mg)	2.57–30.40	
Vitamin D (IU/mg)	60.00–1280.00	
Vitamin C (mg/100g)	0.12–29.30	
***Elemental analysis* (*ppm*)**		(Yu-Qin et al. 2000; Marcone 2005)
Sodium (Na)	330–20554	
Potassium (K)	110–2645	
Calcium (Ca)	798–14850	
Magnesium (Mg)	330–2980	
Phosphorus (P)	40–1080	
Iron (Fe)	30–1860	
Sulfur (S)	6244–8840	
Barium (Ba)	4.79–41.09	
Strontium (Sr)	4.25–21.90	
Silicon (Si)	8.34–62.02	
Aluminium (Al)	15–2368	
Manganese (Mn)	3.58–12210	
Zinc (zn)	19.95–72.40	
Copper (Cu)	4.68–110.65	
Molybdenum (Mo)	0–0.94	
Cobalt (Co)	0–0.63	
Germanium (Ge)	0.05–0.97	
Selenium (Se)	0.12–0.77	
Nickel (Ni)	0–0.47	
Vanadium (V)	0.03–2.84	
Chromium (Cr)	0–7.45	
Lead (Pb)	0.50–4.08	
Cadmium (Cd)	0–0.83	
Mercury (Hg)	0.001–0.160	
Hormone determination		(Ma and Liu 2012a)
Testostrone (T) (ng/g)	4.293–12.148	
Estradiol (E2) (pg/g)	802.333–906.086	
Progesterone (P) (ng/g)	24.966–37.724	
Luteinizing hormone (LH) (mIU/g)	1.420–11.167	
Follicle-stimulating hormone (FSH) (mIU/g)	0–0.149	
Prolactin (PRL) (ng/g)	0–0.392	

cally contains low lipid levels, ranging from 0.14% to 1.28%. Ash Content: The ash content in EBN is approximately 2.1%.

Carbohydrate Content: Carbohydrates make up about 25.62% to 27.26% of EBN's composition. Essential Elements: EBN contains essential elements such as calcium (1298 ppm), sodium (650 ppm), magnesium (330 ppm), potassium (110 ppm), phosphorus (40 ppm), zinc, and iron (30 ppm). Sialic Acid: EBN is notably rich in sialic acid (N-acetylneuraminic acid), contributing to around 9% of its total essential sugars. Sialic acid plays a significant role in brain development and neurological enhancement. Oligosaccharides: EBN contains oligosaccharide sequences, including sialic acid, which have the ability to affect cell detachment from microbes and parasites. Sialic acid is also associated with immune system modulation and mucus viscosity. Overall, the composition of EBN is characterized by its high protein content, essential amino acids, essential elements, and bioactive compounds like sialic acid and oligosaccharides, which may contribute to its potential health benefits. Researchers have been exploring these components to better understand EBN's medicinal properties.

Sialic acid, a prominent component of Edible Bird's Nest (EBN), offers several health benefits, including, LDL Cholesterol Reduction: Sialic acid has been shown to reduce low-density lipoprotein (LDL) levels, contributing to improved cardiovascular health, Enhancement of Fertility: Sialic acid may play a role in fertility enhancement, Blood Coagulation Regulation: Sialic acid has properties that can regulate blood coagulation. In addition to sialic acid, EBN contains other glyconutrients, including:, N-Acetylgalactosamine (GalNAc): GalNAc is involved in synaptic functions between nerve cells and may impact memory. N-Acetylglucosamine (GlcNAc): GlcNAc serves as a precursor for glycosaminoglycans, which are essential for joint cartilage health. Glucosamine deficiency has been linked to conditions like arthritis and cartilage deterioration, Galactose: Galactose is another monosaccharide found in EBN, Fucose: Fucose is present in smaller amounts. The glycoproteins and proteoglycans found in EBN are known to contribute to various health benefits, including, Enhanced Bone Strength: EBN extract, when administered orally, has been associated with increased bone strength and higher calcium concentrations. Cartilage Flexibility: Proteoglycans in EBN contribute to cartilage flexibility and joint health. Epidermal Growth Factor (EGF) Activity: EBN extracts have demonstrated the ability to enhance the activity of epidermal growth factor (EGF), which plays a role in cellular processes and cell growth. These bioactive compounds and glyconutrients found in EBN contribute to its potential health-promoting properties and have been the subject of scientific research to better understand their effects on human health.

The presence of bacteria, fungi and mites in EBN

The consumption of edible bird's nests (EBNs) carries potential health risks due to the presence of various microorganisms (Table 13). These microorganisms can lead to foodborne illnesses, posing a significant concern. Among the microorganisms identified in EBNs, several deserve particular attention. Coliform bacteria are used as key indicators in food microbiology. They include various Gram-negative, rod-shaped bacteria such as *Citrobacter*, *Enterobacter*, *Escherichia*, and *Klebsiella*. Their presence in EBNs suggests issues with hygiene and sanitation during the harvesting and processing stages. Certain strains of *E. coli* are known foodborne pathogens, causing gastrointestinal illnesses. Their presence in EBNs raises concerns about contamination and food safety. *Salmonella* is a widely recognized foodborne pathogen responsible for causing serious illnesses, including salmonellosis. The presence of *Salmonella* in EBNs is a significant food safety risk. *Staphylococcus aureus* is capable of producing toxins that can lead to food poisoning. Its presence in EBNs can result in gastrointestinal symptoms and foodborne illnesses. Yeast and mold are common contaminants in various foods, including EBNs. While not always harmful, some molds produce mycotoxins that can be detrimental to health. In addition to these bacteria and fungi, other potential contaminants of EBNs include mites, which are known allergens and may trigger respiratory allergies and anaphylactic reactions in sensitized individuals, whether the EBNs are consumed heated or unheated. To ensure the safety of EBNs for consumption, rigorous hygiene practices must be

Table 13. Bacteria, fungi and mites associated with EBNs.

No	References	Type of samples	Microbes	Microbes after treatment
1	(Wong et al. 2018)	Raw uncleaned (house nest)	Bacteria (isolates) *Acinetobacter* sp., *Brevibacterium* sp., *Bacillus subtilis*, *Bacillus shackletonii*, *Bacillus* sp., *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus flexus*, *Bacillus circulans*, *Bacillus cereus*, *Bacillus aryabhattai*, *Deinococcus* sp., *Enterococcus faecalis*, *Enterococcus* sp., *Listeria fleischmannii*, *Microbacterium* sp., *Paenibacillus* sp., *Paenibacillus* sp. 23-13, *Paenibacillus agglomerans*, *Paenibacillus alvei*, *Staphylococcus nepalensis*, *Staphylococcus Kloosi*, *Staphylococcus* sp., *Staphylococcus sciuri*, *Staphylococcus* sp. Y3 *Virgibacillus halophilus*	Double boiling *Bacillus subtilis*, *Bacillus* sp.
		Raw cleaned (commercial EBNs)	Bacteria (isolates) *Acinetobacter* sp., *Acinetobacter radioresistens*, *Acinetobacter calcoaceticus*, *Brevibacillus* sp., *Brevibacterium* sp., *Bacillus* sp., *Bacillus badius*, *Bacillus cereus*, *Bacillus flexus*, *Bacillus lichniformis*, *Caryphanon* sp., *Deinococcus* sp., *Enterobacter cloacae*, *Enterobacter hormaechei Exiguobacterium* sp., *Solibacillus silvestris*, *Staphylococcus* sp., *Staphylococcus pasteuri*, *Staphyloccus saprophyticus*, *Staphylococcus sciuri*, *Sporosarcina saromensis*	Double boiling *Brevibacillus* sp., *Brevibacillus agri*, *Bacillus* sp.
2	(Kew et al. 2015)	Raw and commercial nests	Mites (Isolates) *Eustathia cultrifer*, *Pteroherpus garrulacis*, *Pterodectes amaurochalinus*, *Laminalloptes* sp., *Berlesella alata*, *Neochauliacia* sp., *Suidasia* sp., *Austroglycyphagus* sp., *Aleuroglyphus ovatus*, *Dermanyssus* sp., *Cheyletus* sp., *Tarsonemid*, *cunaxid mites*, *Collocalidectes* sp., *Streetacarus* sp., *Hemisarcoptes* sp. *and unidentified oribatid mites*	N/A
3	(Sien et al. 2013)	Swiftlet feces in swiftlet farm houses	Bacteria (Isolates) *Bacillus* sp., *Dermacoccus* sp. 103, *Enterococcus harae strain ss33b*, *Escherichia coli*, *Leucobacter iarius strain* 40, *Lysinibacillys* sp. B4, *Paenibacillus* sp. Gh-134, *Proteus* sp., *Pseudomonas aeruginosa strain* 123, *Sporasarcina* sp., *Staphylococcus* sp.	N/A
4	(Sani et al. 2015)	Raw cleaned EBN	Mold (Isolates) Aspergillus spp. and Penicillium spp.	
5	(Chen et al. 2015)	Raw uncleaned (house nest)	Fungi (Isolates) Soil Fungi: *Blastobotrys* sp., *Lichtheimia* sp., *Nigrospora* sp., *Paecilomyces* sp., *Perenniporia* sp., *Phialosimplex* sp., *Syncephalatrum* sp., *Sagenomella* sp., *Stephanoascus* sp. *Talaromyces* sp, *Plant Fungi*: *Coprinellus* sp., *Fomitopsis* sp., *Lasiodiplodia* sp., *Lenzites* sp., *Letendraea* sp., *Polyporales* sp., *Rigidoporus* sp. *Environmental Fungi*: *Aspergillus* sp., *Candida* sp., *Cladosporium* sp., *Neurospora* sp., *Penicillum* sp., *Eurotium* sp.	Double boiling Soil Fungi: *Phialosimplex* sp. Plant Fungi: – Environmental Fungi: *Aspergillus* sp., *Candida* sp., *Cladosporium* sp., *Neurospora* sp., *Penicillum* sp., *Eurotium* sp.
		Raw cleaned (commercial EBNs)	Fungi (Isolates) Soil Fungi: *Chrysosporium* sp., *Nigrospora* sp., *Sagenomella* sp., *Sebanicales* sp. *P*, Plant Fungi: – Environmental Fungi: *Aspergillus* sp., *Candida* sp., *Cladosporium* sp., *Neurospora* sp., *Penicillum* sp.	

followed throughout the entire production and processing chain. This includes thorough cleaning and sanitization of equipment and facilities, as well as proper drying and storage conditions to prevent microbial growth. Routine testing and monitoring for contaminants, such as bacteria and molds, are essential to identify and mitigate potential risks. Consumers should also exercise caution by sourcing EBNs from reputable suppliers and ensuring that they have undergone appropriate processing and quality control measures to minimize health risks associated with microbial contamination.

Table 13 and Table 14 offer insights into the identification and concentrations of mites, fungi, and bacteria in edible bird's nests (EBNs). Kew et al. (2014) employed scanning electron microscopy to explore the structure of EBNs, revealing the presence of mites, fungi, bacteria, and even feathers on both raw uncleaned (RUC) and cleaned (RC) EBNs. RUC EBNs contained mite egg-shells, fecal particles, remnants of arthropods, and various bacterial and fungal structures. Similar contaminants, including mites, bacteria, and fungi, were found on RC EBNs. In another study by Sien et al. (2013), 500 bacterial isolates were successfully obtained from swiftlet feces in Sarawak, Malaysia. These isolates fell into 11 categories, with 96% being Gram-positive bacteria and the remaining 4% categorized as Gram-negative. Staphylococcus sp. was the most prevalent bacterium in the fecal samples. Analyzing Tables 13, 14, it's evident that double boiling significantly reduces bacterial types and quantities in EBNs. However, this method may not be effective against thermophilic bacteria like Bacillus sp. and Brevibacillus sp. Surprisingly, repeated boiling did not significantly reduce fungal diversity and abundance, suggesting fungi's resistance to heat. Notably, gamma radiation effectively reduces yeast and mold populations. Microwave sterilization has shown promise in decreasing Salmonella and E. coli in EBN drinks. Heat sterilization successfully eliminates various contaminants, including yeast, mold, coliform, E. coli, Salmonella, and Staphylococcus aureus in EBN beverages, with no detectable growth. Zhang et al. (2020) demonstrated that low-energy X-ray irradiation at a dosage of 350–400 Gy reduced E. coli O157:H7 and S. Typhimurium in dry EBNs, eliminating these pathogens to undetectable levels.

Summary of existing EBN authentication methods

Irresponsible makers frequently include adulterants, such as tremella fungus (*Tremella fuciformisis*), karaya gum (*Sterculia urens*), red seaweed, pig skin, egg white, and vermicelli rice, into edible bird's nest (EBN) in order to augment its net weight and dimensions, hence enhancing its commercial value. The utilization of these chemicals as adulterants is attributed to their resemblance in terms of color, taste, and texture to the authentic bird's nest salivary cement. This natural cement is known for its challenging detectability by unaided human vision, as noted by Marcone (2005). Consequently, a diverse range of instruments and analysis techniques have been developed for the authentication of EBN, utilizing empirical measures, composition analysis, microscopic examination, and molecular biology-based technology (Table 15). The utilization of distinct methodologies serves specific purposes and goals in the authentication process of swiftlet nests. These methods are based on comprehensive reviews of prior research, enabling the quantitative and qualitative verification of nutritional content, secondary metabolites, heavy metal contamination, nitrate and nitrite contamination, as well as microbiological content within swiftlet nests.

Pharmacological activities of Edible Bird's Nest

The effects of EBN extract are presented in a comprehensive manner, providing particular details, as outlined in Table 16, presents a comprehensive overview of the preclinical investigations carried out to examine the therapeutic properties of EBN extract.

Recommendations and conclusion

This study has highlighted the substantial differences in nutritional content between half-cup and stripe-shaped edible bird's nests (EBNs), emphasizing the importance of considering these distinctions when evaluating EBN quality. It's crucial to recognize that various forms of EBN can have distinct nutritional compositions due to factors like shape (half-cup or stripe-shaped), texture (soft or firm), and the presence of impurities. Importantly, both forms of EBN appear safe for consumption, as the levels of heavy metals detected in this study remained within permissible limits. To address the inherent subjectivity in human judgment, incorporating nutritional content as a grading criterion for Evidence-Based Nutrition (EBN) is advisable. This critical review has identified several knowledge gaps concerning potential residual pollutants in EBNs. These gaps encompass the need for further investigation into nitrite and nitrate levels, the presence of bacteria, fungi, mites, heavy metals, and other contaminants, as well as their impact on EBN color changes and allergenicity. Specific areas that warrant exploration include. Comparative studies of potential residual contaminants in different types of RC EBN products, such as cup-shaped EBN and instant cook EBN. An examination of various processing methods, including cleaning, drying, and sterilization, to assess their effectiveness in removing and mitigating contaminants during the transition from RUC EBN to RC EBN. Research on the effects of bleaching during the cleaning process. Investigations into the factors influencing color changes during the drying process. Collaboration between researchers and industry stakeholders in the EBN sector is essential to address these knowledge gaps effectively. Furthermore, policymakers can play a pivotal role in developing evidence-based regulations that align with industry realities. It is recommended to include heat-resistant bacteria, fungi, and allergens in the formulation of RC EBN standards. In summary, there is a compelling need for additional research to gain a deeper understanding of potential lingering contamination in EBNs.

Table 14. The composition of edible bird's nests (EBNs) includes bacteria, fungi, and mites.

No	References	Type of samples			Enumeration method	Source of samples
		Raw uncleaned EBN	Raw cleaned EBN	After treatment/ Others		
1	(Tan et al. 2014)		ND E. coli, S. Aureus and Salmonella		Australian Standard- Escherichia coli, Samonella spp., Coliform,	RC: Seven RUC house nest samples from
			Total Plate Count: 2.3*105–25*105 cfu/g, Coliform ND- 43 cfu/g		and total plate count. Official AOAC method- Staphylococcus aureus	different regions in Malaysia then cleaned in lab. Seven regions include:
			Mould <10–140 cfu/g		Bacteriological Analytical Manual	Alor Setar, Kedah; Sibu,
			Yeast <10–10 cfu/g		of Food and Drug Authority- mould and yeast	Sarawak; Rompin, Pahang; Kuala Selangor; Johor Bahru; Jerantut, Pahang; and Port Klang, Selangor
2	(Wong et al. 2018)	6.0*102–1.02*105 CFU/0g		Double boiling 0–2.4*102 CFU/g	Total Plate Count	RUC: Five Malaysia house nest from Kuala Sanglang, Pantai Remis, Kluang, Kajang and Kota Bharu
			4.0*101–1.5*105 CFU/g	Double boiling		RC: Six commercial
				0–2.4*102 CFU/g		sample purchase from five different Chinese traditional medicine shops from Malaysia and one from Medan, Indonesia
3	(Sien et al. 2013)			Swiftlet feces 6.03–9.22 log10 CFU/g	Total Plate Count	Swiftlet feces from swiftlet houses located in ten places, including Kota Samarahan, Saratok, Semarang, Betong, Sarikei, Sibu, Sepinang, Maludam, Kuching and Miri in Sarawak
4	(Sani et al. 2015)		7.64–7.66 log CFU/g	Gamma Irradiation 20 kGy <2 log CFU/g	Total Plate Count	RC: Raw cleaned samples from Pahang and Terengganu
			5.61–5.95 log CFU/g	Gamma irradiation 5 kGy <2–4.64 log CFU/g	Plate count -agar Brilliance Coliform-Coliforms	
			2.47–2.67 log CFU/g	Gamma irradiation 1 kGy <2 log CFU/g	Plate count agar Brilliance E. coli- E. coli	
			4.55–4.66 log CFU/g	Gamma irradiation 5 kGy <2 log CFU/g	Plate count – agar Rabbit Plasma; Fibrinogen – Staphylococcus aureus	
			4.8–5.10 log CFU/g	Gamma irradiation 5 kGy <3 log CFU/g	Plate count-agar Dichloran rose; Bengal chloramphenicol- Yeast and molds	
			Not detected	N/A	Plate count- agar xylose lysine deoxycholate agar and brilliant green agar- Salmonella spp.	
5	(Chen et al. 2015)	40–18,080 CFU/g	40–2,640 CFU/g	N/A	Plate count- Sabouraud Detrose Agar- Fungi	RUC and RC: Same sample batch with (Wong et al. 2018) exclude sample from Medan
6	(Kew et al. 2015)	Live 0–66.4 mites/g. Dead 15.9–2,613 mites/g. Total 18–2,613 mites/g.	Live 0 mites/g. Dead 0–88 mites/g. Total 0–88 mites/g.	N/A	Stereomicroscope- Mite	RUC and RC: Same sample batch with (Wong et al. 2018) exclude sample from Medan.

Table 15. Authentic EBN detection methods.

Method	Content	Problem of detection methods	References
Empirical measures	Visual examination, burning tests, colouring checks based on observation and experience	Subjective and non-measureable	(Liu and Zhang 1995; Leh 2000; Chen et al. 2014)
Composition analysis	Composition of lipids, proteins and hormones based on the range of	These constitutes are commonly found in most mammalian cells. The composition can be adulterated by materials that contain the same chemical compound, making this method nonspecific.	(Marcone 2005; Ma and Liu 2012b)
Optical microscopy	Characteristics of down feathers, nest powder, and nest texture from observation	Relies on operator experience and require specific operation technique	(Sam et al. 1991; Liu and Zhang 1995; Marcone 2005; Chan et al. 2018)
Scanning electron microscopy	Fibre array of micro-structure from high-resolution image		(Sam et al. 1991; Marcone 2005)
Fluorescence method	EBN give out blue-green luorescence at ultraviolet light at 365 nm	Although there was a significant difference of chemical fingerprint determined between the EBN and other materials, there is still very limited information on the EBN collected from different geographical areas which makes these methods operate under a small dynamic range.	(Deng et al. 2006)
Modified xanthoproteic nitric acid test	Proteins with amino acids carrying aromatic groups, especially in the presence of tyrosine and tryptophan, can be measured using a reflectance colour-meter which could detect adulterant at the concentration down to 1% in EBN		(Marcone 2005)
Gas chromatography (GC)	Composition of amino acids and oligosaccharide chain of five monoses such as D-mannitose, D-galactose, D-mannose, N-acetyl-D- galactosamine, N-acetyl-D-glucosamine, and N-acetylneuraminate		(Yu-Qin et al. 2000; Marcone 2005)
Capillary Gas chromatography (GC)	Amino acids composition		(Wang et al. 2019)
Infrared spectrometry (IR) and Fourier transform infrared spectroscopy (FTIR)	Characteristics of amino acids, proteins, and carbohydrates based on their functional groups	Carbohydrates and amino acids exist in most adulterants and are not specific characteristic ingredients in EBN, making these methods prone to inaccurate results.	(Sun et al. 2001; Deng et al. 2006)
High performance thin layer chromatography (HPTLC)	Amino acids composition		(Teo et al. 2013)
SDS-PAGE	Number and characteristics of protein bands	Adulterants in EBN samples cannot be determined unless the protein band or point is specific and identifiable.	(Wu et al. 2007)
Atomic absorption	Analysis of minerals	The minerals of EBN may be varied due to the geological factors which cause complication in developing the detection standard.	Marcone (2005)
Molecular biological technology	Detection of fibrinogen gene and cytochrome b gene	The detection of specific genes may be an efficient method to identify far homolog adulterant. However, it is not able to detect close homolog adulterant due to gene conservation. Generally, molecular detection method such as real- time PCR may require specific operation skill.	(Lin et al. 2009; Wu et al. 2010)
A modified xanthoproteic nitric acid test	Measure crude protein content (62%–63%) using a reflectance colorimeter which could detect adulterant at the concentration down to 1% in EBN		(Marcone 2005)
Genetic identification	An independent molecular phylogeny using cytochrome b mitochondrial DNA sequences		(Act 1983; Thomassen et al. 2003; Lin et al. 2009; Wu et al. 2010)
Hyphenated PCR and gel electrophoresis	The combination of DNA based PCR and protein based two-dimensional gel electrophoresis methods (down to 0.5% EBN in a mixture)		(Wu et al. 2010)

Tabel 16. Summary the effects that have been investigated through the utilization of EBN extract.

Pharmacological activities	Sample preparation	Model	Control group	Results	Proposes mechanism and suggested acting compound	References
Antiviral effects	Water extract (Enzyme extraction)	Madin-darby canine kidney cells (MDCK)	Non-hydrolyzed EBN and untreated cells/ mice	EBN, after being hydrolyzed with pancreatin F, exhibited potent antiviral properties in MDCK cells and inhibited the binding of the virus' hemagglutinin surface protein to erythrocytes.	By inhibiting the viral genes (NA and NS1), the bioactive compounds (sialic acid or thymol derivatives) in EBN demonstrated the potential for antiviral activity. Suggestion for an agent: Sialic acid or derivatives of thymol	(Haghani et al. 2016)
Eye care effects	Water extract	Rabbit corneal keratocytes cell	Untreared cells	Low concentrations of EBN stimulated cell proliferation synergistically, particularly in serum-containing media. With the addition of EBN, corneal keratocytes retained their phenotypes, as demonstrated by both phase contrast micrographs and gene expression analysis.	BN induces corneal cell proliferation and is able to preserve their phenotypes and functionality by synthesizing stromal constituents. This was demonstrated by an increase in the functional gene expression of corneal keratocyte proliferation factors collagen type 1, ALDH, and lumican. The active substance neither proposed nor evaluated	(Yew et al. 2018)
Antioxidant effects	Water extract	Human neuroblastoma cell (SH-SY5Y)	Untreated cells	On SH- SY5Y cells, EBN demonstrated protective properties against hydrogen peroxide-induced toxicity and oxidative stress. Lactoferrin and ovotransferrin have antioxidant properties in SH-SY5Y cells as well.	EBN and its constituents reduced cytotoxicity induced by hydrogen peroxide and decreased reactive oxygen species (ROS) through increased scavenging activity. Lactoferrin and ovotransferrin within EBN may contribute to the overall functional properties of EBN Suggestion for an agent: Lactoferrin and ovotransferrin are components of egg whites.	(Tsai et al. 2018)
Neuroprotective effects	Water extraction	Human neuroblastoma cell (SH-SY5Y)	Untreated cells	Observations of morphological and nuclear staining indicate that EBN treatment decreases the level of 6-hydroxydopamine-induced apoptotic alterations in SH-SY5Y cells.	EBN extract is more efficacious in enhancing reactive oxygen species (ROS) accumulation, early apoptotic membrane phosphatidylserine externalization, and caspase-3 cleavage inhibition. This study obviously demonstrated that EBN extracts inhibiting apoptosis may induce neuroprotective effects against 6-hydroxydopamine-induced degeneration of dopaminergic neurons.	(Careena et al. 2018)
The augmentation of skeletal integrity	Enzyme extraction	Female sprague-dawley rats	Fed an AIN93G-based normal diet	The femur of rats who had ovariectomy and were administered with EBN extract exhibited an increase in calcium levels and enhanced bone strength. The administration of EBN extract resulted in an increase in dermal thickness, whereas there was no significant effect on serum estradiol content.	The synthesis of estrogen leads to accelerated bone loss during the initial ten years following menopause. There has been a suggestion that the use of EBN extract may have a positive impact on enhancing bone strength, while also potentially regulating the levels of serum estrogen.	(Matsukawa et al. 2011; Chua et al. 2013; Hou et al. 2021)

Pharmacological activities	Sample preparation	Model	Control group	Results	Proposes mechanism and suggested acting compound	References
	Hot-water extraction	Human articular chondroytes cell (HACs)	Untreated cells	The application of EBN extract resulted in an augmentation of HACs proliferation, a decrease in the expression of catabolic genes, and a reduction in the formation of prostaglandin E2 (PGE2). The present study observed an elevation in the gene expression of type II collagen, aggrecan, and SOX-9, as well as an increase in the creation of total sulfated glycosaminoglycan during the investigation of anabolic activity.	The EBN extract has the ability to decrease the expression of matrix metalloproteinases (MMP), cytokines, and other catabolic mediators. This reduction in expression can effectively mitigate the degradation of cartilage and slow down the degenerative process of osteoarthritic cartilage.	
Erectile dysfunction	Water extract (enzyme extraction)	Castrated male wistar rats	Un-castrated rats	The castrated rat that received a dosage of 9 mg/kg/day of EBN extract demonstrated a notable increase in testosterone and luteinizing hormone levels. Additionally, a considerable elevation in the penis index was noted.	The authors engaged in speculation regarding the potential impact of an elevated dosage of EBN extract (9 mg/kg/day) on sexual functions. The recommended active ingredient is testosterone; however, there is no available data to support the presence of EBN extract in it.	(Ma and Liu 2012b)

Conflict of interest

The authors declare there is no conflict interest.

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