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Edible Bird's Nest Extract: A Review of its Composition and Biomedical Impact on Skin

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Abstract

Edible bird's nest (EBN) is an ingredient that has been consumed for a long time in Asia as a soup. Not only for soup but also used as a cosmetic. Edible bird's nest has an international Nomenclature Cosmetic Ingredient (INCI) name of Swiftlet nest extract with listed function as skin conditioning. We summarized and analyzed EBN and its biomedical impact on the skin in recent 5 years to serve as a foundation for future research on the effects of EBN. The EBN contains protein, sialic acid, and Epidermal Growth Factor Hormone (EGF) as important compositions. Not only contain some beneficial compounds, but EBN also contains nitrite and nitrate that consider ingredients that could produce harmful substances. EBN has long been famous for its pharmacological effect on the skin whether as enhancing skin complexion, anti-aging or whitening skin. This article reviews the composition and effect on the skin of EBN. Finally, this article proposed research directions to serve as a foundation for more research on EBN.

Keywords:edible bird's nest; composition; skin

1. Introduction

Edible Bird's Nest (EBN) known as cubilose is dried saliva from swiftlet, like Aerodramus maximus and Aerodramus fuciphagus (genus Aerodramus). It can be also produced by swiftlet from the genus Collocalia [1]. The swiftlet makes its nest from its saliva to protect the eggs and nestling [2]. Freshly made, When exposed to air, the nested cement (glutinous secretion/mucin glycoprotein) eventually dries and hardens from its soft, viscous state[3]. Global market demand for EBN is still increasing due to its medicinal and economic value, its nutrition, and also it has long quality guarantee period [2]. EBN has been used in traditional Chinese medicines and cuisine. It is a good source of carbohydrates and protein but it also contains some trace elements, nitrate, and nitrite contents. EBN also contains EGF and it was discovered for the first time in 1987. EGF-like peptide promotes cell proliferation both having the potential to improve monthly irregularities and benefit women's health. It is well known that human EGF can promote cell division and development. It has a mitogenic effect and stimulates the growth of certain tissues like epidermal and epithelial. EBN is therefore thought to have regenerative and antiaging qualities [4]. Besides EGF, EBN contains sialic acid that has a pharmacological function. The EBN industry In China has rapid growth due to the properties on therapeutic, nutritional, and cosmetic [2].

The skin functions as the body's main defense against damage from the outside, but extrinsic factors like UV rays, wounds, or inflammatory triggers can harm the skin, causing dryness, pigmentation, epidermal thickness, and wrinkles with a delayed return to skin health. UV is the main cause of skin aging, and ultraviolet B (UVB) can seriously damage cells by causing cellular oxidative stress when it reaches the dermis layer of skin, where fibroblasts are found. Skin inflammation and photoaging are brought on by this loss of oxygen balance. It has been hypothesized that excessive expression of matrix metalloproteinases (MMPs), particularly MMP-1, causes collagen, a major component of the extracellular matrix, to break down, causing skin photoaging [5].

Modern science and technology have also demonstrated the nutritional benefits and therapeutic

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qualities of EBN, including anti-aging benefits by reducing UVB-induced skin photoaging, anti-inflammatory benefits by reducing TNF/IFN-stimulated inflammation, and wound healing benefits [5]. EBN's moisturizing, whitening, and anti-oxidant properties are used externally as skincare items to improve skin [6]. According to reports, EBN increases antioxidant capacity and has an anti-oxidant action against oxidative stress. The public has generally recognized EBN's skincare properties. The high protein content and discovery of EGF-like activity were previously used as the key justifications for EBN's unproven skincare benefits. Studies today suggest that EBN may have a whitening effect on the skin. The amount of free NANA was inversely proportional to the whitening effect of EBN [7]. Thus, this review provided information related to EBN and its effect on skin.

2. Classification of EBN

EBN can be classified based on some criteria, such as physical, geographic, and quality criteria. EBN physics can be obtained from its variety of colors and shapes. Its color can be white, golden, or red. EBN Shape can be a cup, triangle, strip, or cake. EBN can be collected from the cave, where the swiftlet natural nesting activities, or even in a house, where a nest is constructed in the man-made swiftlet house, with geographic are mostly in South Asia (Indonesia, Malaysia, Thailand, Vietnam, Cambodia, Brunei, Singapore, and Philippines) [2], [3]. Cave EBN has more valuable than house EBN, even in reality cave EBN contains more feathers and foreign materials than house EBN [8]. EBN quality criteria are seen from grade (A to C) and its purity (premium, feather, grass, raw) [2]. For cost-saving purposes, the EBN crumble should be taken into account while manufacturing EBN goods in bulk, because study shows that they have a comparable level of NANA release [9].

3. Proximate Analysis of EBN

The physicochemical characteristics of EBN have been linked to its therapeutic and dietary advantages [10]. The composition of EBN is protein, carbohydrate, ash, and lipid. EBN has nutrient content that is affected by geo-origins variation [11]. The variation in the nutritional composition of EBN is affected by different locations such as different environments (like origin (man-made house or caverns) and place (Malaysia region or adjacent countries)), climates, microorganisms (e.g., fungi, bacteria, protozoa), insect populations, quality (clean process, and contaminants) and color (white, yellow, or red), have been considered in a few studies that have reported on the nutritional composition of EBN [2], [10], as table 1.

The shape of EBN also gives contribute to its composition. The full structure of the nest, which was nearly entirely made of pure mucin-rich glycoprotein that hardens in contact with air, making a cup-shaped nest with little contaminants, can possibly be explained by the greater protein and carbohydrate content in half-cup EBN. Stripe-shaped EBN, on the other hand, is made up of fragments that were taken from the edge of the nest and stick to the surface of the birdhouse, trapping more contaminants in its dense texture. Stripe-shaped EBN required a laborious cleaning procedure that resulted in some nutritional loss [10].

4. Nitrate and nitrite content in EBN

Nitrite (NO2) and nitrate (NO3) exist naturally in our water and food. Nitrate is unlikely to cause harm since it is an insert and stable. On the other hand, nitrite can react and become nitrosamine, a harmful substance. Nitrite content found in bird nests is directly related to soil contamination from the swiftlet's nesting site's mountain cave or harvest environment. Soil bacteria can produce nitrite and nitrate sources from ammonia through anaerobic fermentation. Under the presence of the nitrate reductase enzyme, microbial action quickly converts nitrate ion (NO3-) to nitrite ion (NO2) [2], [14].

The color of EBN can be changed, from white to yellow, red, or brown. These color changes are found due to the content of the nitrite and nitrate as well as the ferric ion and drying process that involved high temperature. The cleaning process that conducts properly can reduce the concentration of nitrite and nitrate in EBN. One of the steps to lighten the EBN color is by using bleach-like hypochlorite, but no study shows the effect of this EBN bleaching process on humans [2].

The uncontrolled environment or conditions (temperature, RH, and sanitation) in stone caves contribute to the elevation of nitrite and nitrate levels in EBN. Possibility through anaerobic bacteria fermentation or nitrification. Nitrite and nitrate contents in cave EBN seem higher than the house EBN [8].

When compared to EBN hydrolysates, the raw EBN had significantly lower nitrate and nitrate concentrations. The elevated nitrite content of EBN hydrolysates may result from bactericidal nitrite reductase's enzymatic conversion of nitrate in a natural environment. Higher concentrations in the EBN hydrolysates result from the trapped nitrate and nitrite being released during the enzymatic hydrolysis process that breaks down the EBN glycoproteins [14].

Table 1. Proximate analysis from different classification and sources

Classification of EBN	Source of EBN	Proximate analysis	Reference
Half-cup and stripe-shaped EBN	Malaysia	- The protein content of the half-cup EBN (56.96 ± 0.09%) and stripe-shaped EBN (54.70 ± 0.16%), - The carbohydrate content of the half-cup EBN (23.96 ± 0.13%) and stripe-shaped EBN (22.12 ± 0.18%).	[10]
	Malaysia	- The protein content of the half-cup EBN (56,96 ± 0.09%) and stripe-shaped EBN (54.70 ± 0.16%)	[12]
House and cave EBN	Southern Thailand	- The protein content of the house EBN (52.68–54.73%) and the cave EBN (52.65–55.25%) - The carbohydrate content of the house EBN (20.27–21.54%) and the cave EBN (20.05–23.16%) - Ash content of the house EBN (6.88–7.92%) and the cave EBN (6.07–9.67%) - The fat content of the house EBN (0.19–0.57%) and the cave EBN (0.15–0.33%)	[8]
	Malaysia	- The protein content of the house EBN (66.97 ± 2.80%) and the cave EBN (68.01 ± 4.64%) - The carbohydrate content of the house EBN (26.23 ± 2.76%) and the cave EBN (22.52 ± 3.61%) - Ash content of the house EBN (6.64 ± 0.14%) and the cave EBN (9.42 ± 2.16%) - The fat content of the house EBN (0.16 ± 0.19%) and the cave EBN (0.05 ± 0.07%)	[11]
The house EBN	Malaysia, Thailand, Philippine, Indonesia	- The protein content of EBN from Malaysia (62%), Thailand (62.58%), Philippine (-), and Indonesia (65.8%) - The carbohydrate content of EBN from Malaysia (27.26%), Thailand (29.66%), Philippine (16%), and Indonesia (10%) - Ash content of EBN from Malaysia (2.1%), Thailand (6.72%), The philippine (1.5%), and Indonesia (1.5%) - The fat content of EBN from Malaysia (0.14%), Thailand (0.96%), Philippine (0.05%), and Indonesia (0.04%)	[12]
	Indonesia (5 areas)	- The protein content is 53.09 – 56.25% - The carbohydrate content is 19.84 - 23.04 % - The ash content is 5.44 – 6.25 % - The fat content is 0.07 – 0.57 %	[13]
Cleaned EBN and its alcalase hydrolysis product	Malaysia	- The peptide contents of the cleaned EBN (1.77 $\pm$ 0.05 %) and the hydrolyzed EBN (12.10 $\pm$ 0.34 %) - The total polysaccharide contents of the cleaned EBN (1.52 $\pm$ 0.37 %) and the hydrolyzed EBN (13.84 $\pm$ 0.16 %)	[14]

Table 2 summarized the nitrate and nitrite content of EBN from different sources. Different bird farm environments, including humidity, pH, and climate, as well as harvesting contamination or the cleaning of the collected EBN, are likely to be responsible for the variations of nitrite and nitrate content [4].

5. Sialic acid is one of the important components of EBN

Sialic acid is a family with more than 20 compounds derived from neuraminic acid, where the predominant form is N-acetylneuraminic acid (NANA). Due to the highly charged property, NANA is involved in retaining water on the cell surface and enhances cellular fluid uptakes. NANA could be a good quality control marker for EBN. The majority of NANA exists in conjugated forms as oligosaccharides and glycoproteins. After the actions of neuraminidase or acid hydrolysis, a free form of NANA will be released [6]. Table 3 summarized several research methods used to measure total NANA and free NANA. To analyze the content of NANA in EBN, spectrophotometry, HPLC, or LC-MS/MS are used. The difference in measuring total NANA or free NANA is that there is prior hydrolysis treatment using acid in the analysis method for total NANA content.

One of the compounds in EBN is acetamide, a straightforward amide with the chemical formula C_2H_5NO. It can be produced artificially or naturally as a byproduct of other processes. The abundance of N-acetylated moieties on sugar, amino acids, and proteins, which is NANA, may be the cause of the presence of acetamide in the EBN samples. A low molecular weight organic acid called ethyl 2-hydroxy-propanoate is naturally present in a wide range of foods and has properties that include skin hydration, skin renewal, skin whitening, pH regulators, and anti-acne activity. This may likely explain how EBN, together with other bioactive

components in the extracts, has the ability to brighten skin and have an anti-aging impact [10].

A study to evaluate the NANA content on different shapes of EBN, mainly cups and strip has been carried out. White EBN showed no significant changes in the amount of free NANA between various shapes. Cup EBN has the greatest free NANA content of all the shape EBN, which is consistent with the conventional classification. Only red EBN could show this distinction; white EBN, according to their shape, was insignificant. The removal of feathers and other foreign objects, air drying, and baking are all steps in the processing of EBN, which may be one of the factors reducing the amount of free NANA. It takes a lot of time to mold and immerse the strip and cake of EBN in water during these manufacturing phases. Since free NANA is extremely water soluble and breaks down quickly in an acidic environment [7].

6. Epidermal growth factor in EBN

EBN is highly identified EGF as its compound. During photoaging, EGF can cause pro-proliferative and anti-inflammatory effects. EGF can also protect the skin from inflammation caused by Staphylococcus aureus, TNF/IFN, and atopic dermatitis. Hence, skin disease treatments like wound healing that lead to skin regeneration may use EBN extracts that are sialic acid and EGF enriched. Also, the present study demonstrated that EBN-B with increased sialic acid and EGF concentration exerted stronger biological effects, such as anti-inflammatory and wound-healing activities [5].

7. EBN extraction method

It has been noted that distinct EBN extracts prepared using various extraction techniques displayed various therapeutic benefits. Because the extraction of an active component depends greatly on the extraction technique used, this could be one explanation [15]. EBN extraction is commonly used in water because it contains a large number of water-soluble compounds [10]. In classic natural therapies, aqueous extracts made from various plant parts are frequently employed [16] . Procedure for extracting EBN using water started by soaking it in water for over a night. This procedure is meant to soften and loosen the protein strands [17].

Different extraction methods are used like stew, full stew, sonification, and hot water. It is normal practice to wash and dry EBNs before manufacturing and packaging in order to extend their shelf life, reduce their water activity, and make storage easier. As a result, no foreign or undesired elements, such as mites, fungus, or feather strands, were found [12]. Stewing by the double boiling process has a big contribution to the distribution of SA in EBN. Increasing the stewing temperature, prolonging the stewing time, and increasing the liquid-material ratio can increase the content of free SA in cooking EBN. Recommendation conditions for stewing EBN are in temperature below 100^0C with time of about more than 30 min and some studies show overcooking time for over 1 hour [18], [19]. Consistent with the results of other studies, The quality of the extracted phytochemical compounds was enhanced by raising the extracting temperature [16].

A study on evaluate the effect of four extraction methods, the boiled water extraction (eHMG), the spray-dried water extraction method (pHMG), acid extraction (using 0,4 M sulfuric acid), and pancreatin extraction (using 0.5 mg/ml pancreatin) shows that due to the variances in the metabolites natural physicochemical qualities, the identities of the extracted metabolites are distinct among the four different extracts, indicating that there isn't a single extraction technique that could extract all sorts of metabolites. Not only eHMG and pHMG show the highest total number of metabolites among others, but also the eHMG extraction method found have a greater number of metabolites and about 57.14% of the metabolites from the pHMG extraction method were identical to the eHMG metabolites. As sialic acid is a key component in EBN, eHMG extraction method yielded sialic acid [15], [20]. More polar solvents are superior to less polar solvents for extracting both organic and inorganic substances [16].

7.1. EBN Extraction using acid and enzymatic hydrolysis

Not only heat extraction method for EBN, but also hydrolysis by enzyme and acid. Protein and NANA are major compounds in EBN, where these proteins have large molecular sizes (>100 kDa) resulting in not being easily absorbed. Meanwhile, the majority of NANA is in conjugated form and it needs to be released as free form since it shows better absorption and function [9]. Some studies show that EBN extracted using acid or enzymatic hydrolysis has better health benefits than EBN itself. Proteins are hydrolyzed to create peptides that are less secondary structural and have lower molecular sizes than the original proteins [21].

The study that investigates the chemical properties of EBN with different pretreatment and enzymes shows that the efficiency of the hydrolysis process of EBN is determined by a combination of pretreatment and enzyme. This study showed that heat pretreatment like boiling, boiling ultrasound, and autoclave was crucial to unfold the glycoprotein, on enzymatic hydrolysis can increase the degree of hydrolysis (DH) of EBN [22]. Using proteases, the glycoproteins can be hydrolyzed into glycopeptides [14].

Pancreatin, compared to other proteases such as papain, pepsin, and trypsin, displays multiple

hydrolytic actions and it is more effective at simultaneously cleaving the peptide and glycosidic bonds of EBN glycoprotein to produce a hydrolysate that contains significant amounts of small molecule glycopeptides and, possibly, sialic acid. In EBN, sialoglycoprotein is the primary macronutrient. Two noteworthy nutrients in EBN that considerably increase bioactivity are peptide and sialic acid [22]. In order to create EBN hydrolysate with the maximum DH, a combination of autoclave pretreatment at 120 °C and enzymatic hydrolysis by pancreatin was considered to be the optimal method [22].

Another study comparing the hydrolysis effect of EBN on different protease shows that alkaline protease has the highest free and oligosaccharide-bound SA content (about 7%). Optimization has been carried out and the optimal parameters are enzymatic hydrolysis time of 2 h and temperature of 600C, alkaline protease dosage of 12.000 U/g, pH 11, and liquid to the material ratio of 60:1 [18].

The pharmacological effect of EBN is affected by the way of extraction. A study on hydrolyzing EBN using alcalase, papain, and papaya juice at 0,5 to 3 hours shows that the rate of DH and soluble protein digestion rises with an increase in hydrolysis time. Hydrolyzing using alcalase and papain at 3 hours shows the highest solubility of the protein, meanwhile papaya juice at 2 hours [23]. The reaction of an enzyme that hydrolyzed EBN glycoproteins with complex structure (i.e., tertiary structure) into EBN glycopeptides with a simpler structure and more readily soluble in water may account for the increased solubility of the EBN hydrolysates compared to the raw EBNs [14]. The antioxidant activity also shows highest on papain in 1 to 2 hour hydrolysis time. EBN's functional qualities had been enhanced by enzymatic hydrolysis, and the results suggested that EBN may be used to create natural antioxidants [23]. The hydrolysis conjugates and antioxidant effect show significantly correlated [24].

Another study that using alcalase and papain for hydrolysis shows an elevation in DPPH radical scavenging activity and ABTS Radical Scavenging Activity compare to unhydrolyzed EBN [21]. Other studies using simulated gastric fluid (SGF), pepsin, papain, and flavourenzym as enzymes for EBN hydrolysis show that papain has the highest digestion rate after 8 hours and pepsin can release the conjugated NANA after 48 hours. In term of its activity, EBN digest show 100-fold better inhibition activity on tyrosinase than EBN extract [9].

The terminals of the glycoproteins and the reduced peptide content of the raw EBNs suggest that the EBN glycoproteins are larger in molecular size. However, because enzymes, like protease, convert the EBN glycoprotein into glycopeptides during enzymatic hydrolysis, the primary amine groups—which take the form of lower molecular size glycopeptides—are widely distributed in the EBN hydrolysates. This demonstrates the efficacy of the enzyme that hydrolyzes the glycoprotein to release the bioactive peptides capable of physiological action. Bioactive peptides are short-chain protein fragments that are inactive but can be made active using a variety of techniques, including enzyme hydrolysis [14]. Some parameters significantly affect the efficiency of enzymatic hydrolysis, such as hydrolysis time, substrate concentration, enzyme concentration, type of enzyme, etc [22].

8. Pharmacological effects on skin

8.1. Antioxidant activity

In the human body, biological and chemical systems are attacked by reactive oxygen species (ROS). Antioxidants have been employed to neutralize free radicals and stop the oxidation of biomolecules brought on by excessive ROS generation [23]. EBN's functional properties, including anti-aging and skin renewal, are strongly correlated with its potent antioxidant actions [11].

Antioxidant activity is currently measured using spectrophotometric methods based on single electron transfer (SET) processes and hydrogen atom transfer (HAT). These assays included the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay, the 2,2-diphenyl-1,(2,4,6-trinitrophenyl)hydrazyl (DPPH) radical scavenging activity assay, the oxygen radical absorbance capacity test, the ferric reducing antioxidant potential test (FRAP), the cupric reducing antioxidant capacity test, and the oxygen radical absorbance capacity test (CUPRAC) assay [25].

A study that evaluates the antioxidant activity of EBN has been carried out by observing the DPPH and ABTS radicals scavenging activity between hydrolyzed EBN and unhydrolyzed EBN. DPPH and ABTS define as IC50 value, the antioxidant's concentration for 50% scavenging of DPPH/ABTS radicals in the specific time period. The lower IC50 value shows higher antioxidant activity. IC50 of DPPH and ABTS radicals scavenging activity of papain-treated EBN (2.9073 ± 0.20 mg/g and 1.3972 ± 0.10 mg/g) and alcalase-treated EBN (1.6185 ± 0.06 mg/g and 0.2643 ± 0.05 mg/g) higher than unhydrolyzed ones (4.0240 ± 0.20 mg/g and 4.4273 ± 0.11 mg/g) [21].

Other studies show that DPPH inhibitory activity of EBN extract is not detected, but by hydrolyzing EBN using alcalase (13.7 + 2.3 %), papain (29.9 + 4.9 %) and papaya juice (17.2 + 1.0 %) in 2 hours show an increase of inhibition activity and comparable to ascorbic acid as standard [23].

Another study also shows that the DPPH scavenging activity of EBN was 1.57–3.76 mg AAE/g. The sulfhydryl group in amino acids and the phenolic hydroxyl group in total phenolic content are likely connected with and contribute to the strong free radical scavenging activity in EBN. The FRAP value ranged between 1.10 to 2.51 mg AAE/g. The

house EBN shows a stronger antioxidant effect than the cave [14].

8.2. Tyrosinase inhibition activity

EBN has long been famous for its ability to whiten skin [9]. The inhibitory effect of EBN on tyrosinase could serve as an indicator of skin whitening function [26].

EBN protein hydrolysates could be used to make antioxidant peptides. Compared to undigested EBN, digested EBN showed a stronger inhibition of tyrosinase activity and melanogenesis in cultivated B16 cells. Melanin production is associated with the skin's ability to whiten. Melanin overproduction can result in skin conditions such as freckles, skin discoloration, and chloasma. Under the catalysis of tyrosinase, melanin is produced through a sequence of processes including hydroxylation, oxidation, and intramolecular cyclization. Melanin uses L-tyrosine as a substrate. Hence, antioxidant activity and tyrosinase inhibitory activity can be used to assess the whitening action of the samples [27], [28].

Study shows that rate of tyrosinase inhibitory activity is affected by free sialic acid (63,43%) rather than glycan sialic acid [27]. Table 4 summarizes studies on tyrosinase inhibitor activity assay of EBN.

8.3. Moisturizing effect

EBN has the potential to become a moisturizing ingredient for use in cosmetics. Natural moisturizing factors (NMFs) are present in corneocytes and neatly structured intercellular lipids operate as a barrier against trans-epidermal water loss (TEWL) and maintain the skin's water balance by retaining moisture in the epidermal layer stratum corneum. Fillagrin prevented TEWL by binding to keratin fibers in epithelial cells. In addition to filaggrin, filaggrin-2, a member of the filaggrin family also contributes to the development of NMFs. This study also identifies that filaggrin and filaggrin-2 expression is regulated by EBN extract and EBN digests through p38-MAPK signaling. According to a study using mouse skin *ex vivo*, the epidermal level of skin after treatment of EBN extract and digest is increased but not the thickness. This study concludes that EBN digest expresses more moisturizing effect than EBN extract [19].

Besides filaggrin, EBN causes targeted genes relating to skin-moisturizing mRNA expressions. It has effects on the GATA3, PPARα, PPARβ, PPARγ, and CASP14 in keratinocytes after 24-h treatments of EBN extract in doses of 1, 10, and 100 ug/ml. Positive controls are used IL4 (GATA3), linoleic acid (PPARα), linoleic acid (PPARβ), linoleic acid (PPARγ), and vitamin D3 (CASP14). The transcriptional factor GATA3 is essential for controlling the production of filaggrin and filaggrin-2. The mRNA levels of PPARα, PPARβ, and PPARγ are determined to evaluate the participation of PPARs as a target in the EBN-mediated regulation of filaggrin and filaggrin-2. The Ca2+-dependent cysteine protease caspase 14 (CASP14) is implicated in the senescence of epidermal keratinocytes. In order to promote keratinocyte differentiation and the production of the cornified membrane, CASP14 functions in the proteolysis and deamination of the proteins profilaggrin and filaggrin-2. The effectiveness of EBN to increase CASP14 expression was evaluated using an RT-PCR test and shows the CASP14 expression levels elevated in a dose-dependent manner [12].

8.4. Photoaging activity

Photoaging is skin aging caused by UV irradiation. Deep wrinkles, pigmentation, dry, rough skin, and a loss of elasticity are signs of aging skin [29]. UVB radiation accelerates MMP production, which breaks down collagen and other ECM proteins during the photoaging process. UVB-induced MMP-1 overexpression is a key factor in the physiological mechanisms underlying skin photoaging by triggering the breakdown of type I and type III collagen [5].

Study shows that EBN reduced MMP 1 and promoted collagen expression [5]. Another study that used EBN extract treated with protease from Bacillus amyloliquefaciens shows activity in preventing ultraviolet B irradiation-mediated oxidative stress and photoaging that was conducted using *in vitro* and *in vivo* models. This EBN extract activates the synthesis of hyaluronic acid and ceramide in UVB-Irradiated hairless mice and HaCaT Cells. In UVB-irradiated hairless mice, EBN extract treated with protease prevented wrinkle development, uneven skin tone, moisture loss, and lowered antioxidant activities, demonstrating that it can protect skin from UVB irradiation by exerting an antioxidant effect. Hyaluronic acid is involved in physiological water balance regulation. By up-regulating hyaluronic acid, it can keep the skin moisturized [29]. Meanwhile, ceramide can increase anti-mitotic and pro-cell death characteristics in keratinocytes [30].

8.5. Miscellaneous studies

A study evaluating the sun protective factor (SPF) of EBN extracts using *in vitro* method shows that in range concentration of 2000 to 7000 ppm SFP values were 7.8 to 22.24. EBN contains peptides that consist of amino acids that acted as an antioxidant due to its phenol group. Phenol has chromophore groups that could absorb UV light and reduce its intensity on the skin [31].

Another *in vitro* study evaluated the formulation of EBN in the preparation of o/w and w/o cream as lightning cream using 40% steamed EBN. The results of product testing on the lightening effect and moisturizing test using a mouse show that there is a

potential for lightening and moisturizing compared to control but there is no difference between w/o and o/w cream [32].

A clinical trial study on EBN extract consumption has been carried out. This study evaluated the functional improvement in skin health, including wrinkles, elasticity, hydration, and whitening, as well as safety after women consumed a commercial product containing 20% hydrolyzed swiftlet nest extract for 12 weeks. It shows a significant decrease in the skin wrinkle value compared to the placebo group. On the other hand, elasticity and skin moisture before and after intake show decreased in both groups but no significant difference [33].

9. Safety

Studies using culture B16 cells show that there was no cytotoxicity or proliferation-inducing effect in any of the samples from 10 to 1000 μg mL−1 of white, yellow, and red EBN [9].

According to clinical trial studies, consumption of products containing 20% hydrolyzed swiftlet nest extract for 12 weeks shows no adverse reaction [33].

Table 2. the nitrate and nitrite content of EBN

Classification of EBN	Source of EBN	Nitrite and nitrate content	Reference
House and cave EBN	Southern Thailand	- The nitrite contents of the house EBN (28.69–47.83 mg/kg) and The cave EBN (120.85–218.18 mg/kg) - The nitrate contents of the house EBN (225.58–1944.25 mg/kg) and the cave EBN (8982.44–21769.4 mg/kg)	[8]
Cleaned EBN and its alcalase hydrolysis product	Malaysia	- The nitrite contents of the cleaned EBN (0.09 ± 0.04 mg/kg) and the hydrolyzed EBN (0.77 ± 0.03 mg/kg) - The nitrate contents of the cleaned EBN (1.10 ± 0.06 mg/kg) and the hydrolyzed EBN (10.48 ± 0.14 mg/kg)	[14]
House EBN	Indonesia	- The nitrite content was 3.11 – 18.28 % - The nitrate content was 650.11 – 1,051.06 %	[13]

Table 3. Methods for analyzing sialic acid (NANA)

	Method	References
Total sialic acid		
Spectrophotometry	Sialic acid quantitation kit (sigma)	[7]
	Resorcinol method	[34]
	Ninhydrin method	[35]
HPLC	- Detector: Fluorescent detector - Column: RP Agilent HC-C18 Column (4.5 x 250 mm, 5 μm). - Mobile phases: Methanol: acetonitrile: water solution (7:8:85) - Wavelength: excitation 373 nm and emission 448 nm. - Hydrolysis using sodium bisulfate and derivatization using O-Phenylenediamine HCl	[12]
	- Detector : UV detector - Column : 300SCX Cation exchange chromatographic column (4.6 mm x 250 mm, 5 μm) or equivalent - Mobile phases: Acetonitrile – 0.1% phosphoric acid aqueous solution (90:10 v/v) - Wavelength: excitation 205 nm. - Hydrolysis using acetic acid	[36]
	- Detector : UV detector - Column: NA - Mobile phases: Methanol (8% v/v), acetonitrile (9% v/v) in water - Wavelength: excitation 230 nm. - Preparation procedures are not clear	[37]
LC-MS/MS	- Detector: UPLC QQQ - Column: The Imtakt Unison UK-Amino C18 column (Supleco, 100 mm × 2 mm, 3 μm) - Mobile phase: 10 mM ammonium formate: Acetonitrile	[5]
Free sialic acid		
HPLC	- Detector: fluorescence detector Column: X-Bridge C18 (4.6 mm X 250 mm, 5 μm) - Mobile phase : 1,0% (v/v) tetrahydrofuran (containing 0,5% (v/v) phosphoric acid and 0,15% (v/v) N-butylamine) : acetonitrile (95:5,v/v) - Wavelength: excitation 230 nm and emission 425 nm. - Derivatization using O-Phenylenediamine HCl	[27]
	- Detector: fluorescence detector. - Column: reversed phase Agilent HC-C18 column (4.5 × 250 mm, 5 μm)	[11]

	- Mobile phases: methanol, acetonitrile, and water solution (7: 8: 85) - Wavelength: excitation 373 nm and emission 448 nm. - Hydrolysis using trifluoroacetic acid and derivatization using DMB (4,5-dimethyl-1,2-phenylenediamine)	
	- Detector: diode array Coloumn: SCX Cation exchange (4.6 mm X 250 mm, 5 µm) - Mobile phase: acetonitrile: 0,1% phosphoric acid (78:22) - Wavelength: 205 nm	[18]
LC-MS/MS QQQ	- Detector: LC-MS/MS QQQ with negative electron spray ionization mode. The capillary voltage is 3.5 kV and the cone voltage is 10 V with source temperature is 100 °C, and the desolvation temperature is 325 °C. - Column: Eclipse XDB - C18 (2.1 X 100 mm, 3.5 µm) - Mobile phase: solvent A (contain water and 0.1% formic acid) and solvent B (contain acetonitrile and 0.1% formic acid) - Wavelength: 205 nm	[9]
Glycan-form sialic acid		
HPLC	- Detector: fluorescence detector Column: X-Bridge C18 (4.6 mm X 250 mm, 5 µm) - Mobile phase : 1,0% (v/v) tetrahydrofuran (containing 0,5% (v/v) phosphoric acid and 0,15% (v/v) N-butylamine) : acetonitrile (95:5,v/v) - Wavelength: excitation 230 nm and emission 425 nm. - Mix using sevage reagent and derivatization using O-Phenylenediamine HCl	[27]
	- Detector: diode array Coloumn: SCX Cation exchange (4.6 mm X 250 mm, 5 µm) - Mobile phase: acetonitrile: 0,1% phosphoric acid (78:22) - Wavelength: 205 nm	[18]
	- Detector: fluorescence detector. - Column: reversed phase Agilent HC - C18 column (4.5 × 250 mm, 5 µm) - Mobile phases: methanol: acetonitrile: water (7: 8: 85) - Wavelength: excitation 373 nm and emission 448 nm. - Hydrolysis using trifluoroacetic acid and derivatization using DMB (4,5-dimethyl-1,2-phenylenediamine)	[11]
Protein-form sialic acid		
HPLC	- Detector: fluorescence detector Column: X-Bridge C18 (4.6 mm X 250 mm, 5 µm) - Mobile phase : 1,0% (v/v) tetrahydrofuran (containing 0,5% (v/v) phosphoric acid and 0,15% (v/v) N-butylamine) : acetonitrile (95:5,v/v) - Wavelength: excitation 230 nm and emission 425 nm. - Hydrolyzed in boiling water and derivatization using O-Phenylenediamine HCl	[27]
	- Detector: diode array Coloumn: SCX Cation exchange (4.6 mm X 250 mm, 5 µm) - Mobile phase: acetonitrile: 0,1% phosphoric acid (78:22) - Wavelength: 205 nm	[18]
	- Detector: fluorescence detector. - Column: reversed phase Agilent HC-C18 column (4.5 × 250 mm, 5 µm) - Mobile phases: methanol: acetonitrile: water (7: 8: 85) - Wavelength: excitation 373 nm and emission 448 nm. - Hydrolysis using sulpho acid and derivatization using DMB (4,5-dimethyl-1,2-phenylenediamine)	[11]

Table 4. Tyrosinase inhibition activity assay of EBN

Standard	Result	Source of EBN	Method	Reference
Vitamin C	10 mg/ml water extract of EBN inhibited the formation of dopachrome by at least 50%	Not determined	Mushroom tyrosinase activity assay	[7]
	2.0 mg/ml white EBN extract and 2.0 mg/ml yellow EBN extract, each, inhibited 50% activity of tyrosinase, while red EBN extract inhibited nearly 40%. After digested using simulated gastric fluid (SGF) for 48 hours, all EBN extracts show stronger inhibition than non-digested EBN	EBN purchased from the Hong Kong market with the country of origin from Indonesia, Vietnam, Thailand, and Malaysia	Mushroom tyrosinase activity assay	[9]

	1000 ug.mL^{-1} of EBN extract show inhibition of melanin formation to the control, while EBN digest from 1-10 ug.mL^{-1}		B16 melanogenesis assay	
Not determined	The EC_{50} value of digested EBN was 7.22 mg/mL and for the tyrosinase activity of stewed EBN, its EC_{50} value was 18.74 mg/mL	EBN From Indonesia (undigested and digested)	Intracellular tyrosinase activity assay using B16 cell	[27]

Table 5. The summary of EBN's composition and its impact on the skin

Composition	Activity	Possibly mechanism
Lipid	Moisturizing effect	Lipid deposits between corneocytes thus gives softness and smoothness
Protein	Antioxidant	Scavenging free radical
	Moisturizing effect	Increasing fillaggrin and fillagrin-2 expressions at mRNA and protein levels
	Sun protective factor	Chromophore groups at phenol content could absorb UV light and reduce its intensity on the skin
Sialic acid	Tyrosinase inhibitor	Inhibits tyrosinase by non-competitive inhibition.
	Antiaging	Through antioxidant mechanism as a hydroxyl radical scavenger
Ethyl 2-hydroxy-propanoate	Skin hydration, skin renewal and skin whitening	Penetrate to the sebaceous follicle ducts and hydrolysed into ethanol and lactic acid.
Epidermal growth factor	Antiaging	Protect the skin from inflammation caused by Staphylococcus aureus, TNF/IFN, and atopic dermatitis

10. Discussion

EBN is a famous nutritional ingredient in China. It has several active ingredients such as protein, sialic acid and EGF.

Protein, where EBN is a rich source of it, has contribute to the free radical scavenging activity. This activity due to the sulfhydryl group in amino acids and the phenolic hydroxyl group in total phenolic content [14]. Protein in EBN has moisturizing effect through p38-MAPK signaling by increasing fillagrin and fillagrin-2 expression, where it can prevent TEWL [19]. EBN also causes targeted genes relating to skin-moisturizing mRNA expression [12]. Photoaging occurs because UVB radiation could accelerate the breakdown of collagen and other ECM proteins. UVB-induced MMP-1 overexpression is a key player in the physiological mechanisms underlying skin photoaging as it initiates collagen breakdown by cleaving type I and type III collagen [5]. EBN extract has chromophore groups on its phenol group of amino acids. It could absorb ultraviolet [31].

Sialic acid as one of the important glycoprotein, is consider as ingredient that contribute to tyrosinase inhibitor through non-competitive inhibition [6]. Non-competitive inhibitor are able to bind to both free enzymes and enzyme-substrate complexes on the same equilibrium constant [26].

Ethyl 2-hydroxy-propanoate also known as ethyl lactate, is one of the composition of protein in EBN. It penetrate to the sebaceous follicle ducts and hydrolysed into ethanol and lactic acid. Naturally, lactic acid presents in the skin, as part of Natural Moisturizing Factor (NMF) and thus has hydrating effect to skin. Lactic acid also a tyrosinase inhibitor [38], [39].

Lipids content in EBN act as emollient and softening substances that give skin its smooth, supple texture. The ability of oily substances to deposit between corneocytes, smoothing out their edges, is what gives skin its softness and smoothness. Since they depend on how much light is reflected by the skin surface, skin radiance and luminosity [40].

EGF is a mitogenic polypeptide that is in charge of protecting and maintaining the epithelia [41]. On the oxidative events in skin aging and delayed wound healing, the growth factor is slow down, including EGF production. EBN contains EGF that can protect the skin from inflammation caused by Staphylococcus aureus, TNF/IFN, and atopic dermatitis [5].

Table 5 contains the summary of EBN's composition and its impact on the skin.

Apart from containing beneficial ingredients, there are compounds that need attention in EBN, namely nitrites. It can react and become nitrosamine, a harmful substance [42]. The cleaning of the collected EBN, is one of the way to be responsible for the variations of nitrite and nitrate content [4].

11. Conclusion

EBN composition especially protein, sialic acid, and EGF act as active ingredients. There have been many studies on EBN extract that have shown effects on the skin due to its composition. Not only on its advantage effects that has been carried out, but also its safety.

Studies on a clinical trial of EBN extract when used via topical need to be carried out to understand the direct effect on human skin.

12. Conflicts of interest

There are no conflicts to declare.

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