Chinese Herbal Medicines 15 (2023) 383–390

Contents lists available at ScienceDirect

Chinese Herbal Medicines

journal homepage: www.elsevier.com/locate/chmed

Review

Proteins: Neglected active ingredients in edible bird's nest

Man Yuan [a], Xiaoxian Lin [a], Dongliang Wang [a,b,*], Jianye Dai [a,b,*]

[a] Hebei Edible Bird's Nest Fresh Stew Technology Innovation Center, Langfang 065700, China
[b] School of Pharmacy, Lanzhou University, Lanzhou 730000, China

ARTICLE INFO

Article history:
Received 8 November 2022
Revised 15 January 2023
Accepted 21 February 2023
Available online 16 May 2023

Keywords:
acidic mammalian chitinase
bioactivity
edible bird's nest
lysyl oxidase homolog 3
mucin-5AC
proteins

ABSTRACT

Edible bird's nest (EBN) is a kind of natural invigorant with a long history of consumption in Asia, especially in China. EBN is formed by mixing the saliva of swiftlets (*Aerodramus*) with feathers and other components during the breeding season. Proteins are the most important nutrient in EBN. By studying proteins in EBN, we can not only elucidate their components at the molecular level, but also study their bioactivities. Therefore, it is of great significance to study the proteins in EBN. Previous research on the proteins in EBN was preliminary and cursory, and no one has summarized and analyzed the proteins in EBN and correlated the bioactivities of these proteins with the biological functions of EBN. This article focused on the proteins in EBN, listed the proteins identified in different proteomic studies, and introduced the sources, structures and bioactivities of the most frequently identified proteins, including acidic mammalian chitinase, lysyl oxidase homolog 3, mucin-5AC, ovoinhibitor, nucleobindin-2, calcium-binding protein (MW: 4.5×10^4) and glucose-regulated protein (MW: 7.8×10^4). The properties of these proteins are closely related to the bioactivities of EBN. Therefore, this article can provide inspiration for further research on the efficacy of EBN.

Contents

1. Introduction

Edible bird's nest (EBN) is a kind of natural invigorant formed by the saliva secreted by several species of swiftlets (*Aerodramus*) with their feathers and other substances for laying eggs and incubation during the breeding season (Shao et al., 2018, Fig. 1A). When the baby swiftlets fly away as an adult, the nest will be abandoned, then it will be manually collected and processed into EBN. In China, EBN has always been regarded as premium food and invigorant, and it was known as the "Caviar of the East" (Marcone, 2005; Jian et al., 2016a). In the past, EBN has always been the favorite food of the royal family and nobles. Nowadays, EBN is also

* Corresponding authors.
E-mail addresses: dongliang.wang@xxdun.com (D. Wang), daijy@lzu.edu.cn (J. Dai).

https://doi.org/10.1016/j.chmed.2023.02.004
1674-6384/

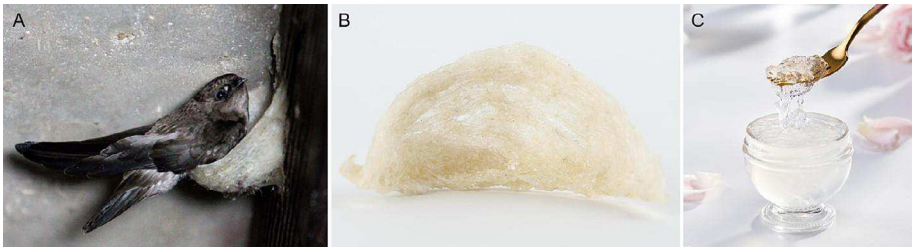

Fig. 1. Swiftlet and EBN (Original photo by Yingzhi Fu). A, swiftlet and nest; B, dried EBN (raw material); C, fresh stewed EBN.

regarded as premium food for important occasions such as state banquets and diplomacy. Besides, the efficacy and medical value of EBN were also recorded in more than 20 classics of Chinese Medicine. According to the *Essential of Materia Medica* ("Ben Cao Bei Yao", in Chinese), EBN can greatly nourish the lung *yin*, relieve phlegm and cough, gently nourish the body, and is very effective medicine to regulate fatigue. Modern researches have also shown that EBN has the functions of immune regulation (Zhao et al., 2016), anti-aging (Yew, Koh, Chye, Othman, & Ng, 2014), and regulation of intestinal flora (Bai, Liu, Zhang, Fan, & Xu, 2020). Therefore, EBN has an important position in premium food and invigorant in China. The market size of EBN in China is about 50–60 billion yuan per year, and the common product types are dried EBN (Fig. 1B) and instant EBN represented by fresh stewed EBN (Fig. 1C).

Different sources, types, picking seasons and other factors of EBN may lead to differences in the chemical composition. Modern research shows that, nutrients of EBN mainly include proteins, sialic acid, amino acids, vitamins, inorganic elements, etc. Among them, sialic acid has been considered as the main nutrient in EBN, but its content only accounts for about 10% of the total mass (Jian et al., 2016b). However, the protein which accounts for 50%–67% (Bai, Liu, Zhang, Fan, & Xu, 2020) of the total mass of EBN has always been neglected. But in fact, proteins are the main nutrient in many animal-derived foods, such as EBN and *Colla CoriiAsini*. The authenticity can be identified based on the protein composition, while the bioactivities of these food can also be studied according to these proteins. Therefore, it is of great significance to identify and study the proteins contained in EBN.

In recent years, some studies have preliminarily identified the proteins in EBN. Through the study of these proteins, it is found that many of them (such as acidic mammalian chitinase) play important biological activities in human body, such as immune regulation. However, no one has systematically summarized these proteins and their biological activities. Therefore, this paper lists the EBN proteins that have been identified so far, and summarizes the sources, structures and biological activities of the most commonly identified proteins in order to provide new ideas for the biological activity research of EBN.

2. Proteins identified in EBN

Previous studies of the proteins in EBN were preliminary and cursory. Few people have analyzed and identified exactly which proteins are contained in EBN. In recent years, as a powerful tool to identify proteins, proteomics played an important role in the study of EBN proteins. Several studies have attempted to correlate EBN properties or biological functions with its protein composition through proteomic methods.

Liu et al. (2012) first used MALDI-TOF-MS in 2012 to analyzed the protein composition in 15 EBN samples. Since then, more and more researchers had carried out the analysis and identification of EBN proteins by different methods such as GPC, RP-HPLC, etc. Statistical analysis showed that a total of 58 proteins have been identified in previous studies (Table 1).

3. Main proteins of EBN and their bioactivities

There were seven kinds of proteins that have been identified three times or more in different EBN protein analysis and identification, including acidic mammalian chitinase, lysyl oxidase homolog 3, mucin-5AC, ovoinhibitor, nucleobindin-2, calcium-binding protein (MW: 4.5×10^4) and glucose-regulated protein (MW: 7.8×10^4). The times of identification and biological activities of these seven proteins have been summarized (Table 2).

3.1. Acidic mammalian chitinase

Acidic mammalian chitinase (AMCase, Fig. 2A) is a glycosidase that belongs to the glycosyl hydrolase family 18, the chitinase class II subfamily. It is expressed in the cytoplasm of most tissues, highly expressed in the stomach, and also detected in the lung. A protein that was highly abundant in all 15 EBN samples had been discovered through MALDI-TOF-TOF/MS (Liu et al., 2012), and it was identified as an AMCase-like protein fragment derived from the purslane. Chitin is an important component in structures such as fungal cell walls and insect exoskeletons. Chitinase can degrade chitin components, and AMCase-like protein is highly expressed in the salivary gland of the swiftlets to secrete saliva for insect digestion (Yew et al., 2019).

AMCase plays an important role in antiviral, anti-inflammatory and cell protection from apoptosis. The study found that AMCase identified in EBN was involved in resisting the invasion of nematodes, fungi and other chitin containing pathogens (Zukefli, Chua, & Rahmat, 2017). It can control the inflammatory response through IL-13, the main regulator of th2 immune response (Hartl et al., 2008), which can be used to explain the anti-inflammatory properties and the inhibition of influenza virus activity of EBN (Mohamad, Mohamad, Abu, Mahmud, & Razak, 2021).

In addition, chitin can promote the development of lung diseases. It has been reported that the chitin polymers in the airways of mice lacking AMCase accumulated continuously, stimulated the expression of fibrogenic cytokines, and developed spontaneous pulmonary fibrosis (Van et al., 2017). More importantly, these symptoms can be improved by restoring AMCase activity in the lungs of these mice. It can be seen that AMCase can prevent or alleviate pulmonary diseases such as pulmonary fibrosis by decomposing chitin.

In addition to the above functions, AMCase can also stimulate pulmonary epithelial cells to produce chemokines and protect them from apoptosis (Hartl et al, 2008; Hartl et al, 2009), which explained the epidermal growth factor (EGF) like effects found in early studies of EBN. It is worth noting that the anti-

Table 1
EBN proteins that have been identified.

Proteins	References
Acidic mammalian chitinase	Kong, Wong, & Lo, 2016; Liu et al., 2012; Ma et al., 2019; Mohamad, Mohamad, Abu, Mahmud, & Razak, 2021; Wong et al., 2017; Wong et al., 2018; Yew et al., 2019; You et al., 2015; Zukefli, Chua, & Rahmat, 2017
Actin, cytoplasmic type 5	Ma et al., 2019
Actin, gamma-enteric smooth muscle	Ma et al., 2019
BEN (bursal epithelium and neurons) glycoprotein precursor	Zukefli, Chua, & Rahmat, 2017
Collagen alpha-2(I) chain	Yew et al., 2019; Zukefli, Chua, & Rahmat, 2017
Collagen alpha-1(VII) chain-like	Zukefli, Chua, & Rahmat, 2017
Carbonic anhydrase 9	Kong, Wong, & Lo, 2016
Coenzyme Q-binding protein COQ10 homolog A, mitochondrial, partial	Zukefli, Chua, & Rahmat, 2017
Carboxypeptidase	Zukefli, Chua, & Rahmat, 2017
Calcineurin-like phosphoesterase domain containing protein 1	Yew et al., 2019
Cytochrome b	Ghassem, Arihara, Mohammadi, Sani, & Babji, 2017
Deleted in malignant brain tumors 1 protein	Ma et al., 2019; Yew et al., 2019
Fibronectin type III domain-containing protein 3B	Yew et al., 2019
Glycosyltransferases	Ghassem, Arihara, Mohammadi, Sani, & Babji, 2017
Hyaluronan mediated motility receptor	Yew et al., 2019
Hemojuvelin	Ma et al., 2019
Integrator complex subunit 1	Yew et al., 2019
Immunoglobulin superfamily member 10-like, partial	Kong, Wong, & Lo, 2016
Ig heavy chain V-III region VH26, partial	Kong, Wong, & Lo, 2016
IgL, partial	Kong, Wong, & Lo, 2016
Keratin, type II cytoskeletal 75	Yew et al., 2019
Lysyl oxidase homolog 3	Ma et al., 2019; Mohamad, Mohamad, Abu, Mahmud, & Razak, 2021; Wong et al., 2017; Wong et al., 2018; Yew et al., 2019; Zukefli, Chua, & Rahmat, 2017
Lysosomal-trafficking regulator	Yew et al., 2019
Leucine-rich repeat-containing protein 9	Zukefli, Chua, & Rahmat, 2017
Mucin-5AC	Kong, Wong, & Lo, 2016; Mohamad, Mohamad, Abu, Mahmud, & Razak, 2021; Wong et al., 2017; Wong et al., 2018; Yew et al., 2019
Mucin-1	Kong, Wong, & Lo, 2016
Mucin-2	Kong, Wong, & Lo, 2016
Mucin-19-like	Kong, Wong, & Lo, 2016
Mucin-22-like	Kong, Wong, & Lo, 2016
Major facilitator superfamily domain containing 11 isoform X4	Zukefli, Chua, & Rahmat, 2017
Nucleobindin-2	Mohamad, Mohamad, Abu, Mahmud, & Razak, 2021; Wong et al., 2018; Yew et al., 2019
Nucleosome assembly protein 1-like 4	Zukefli, Chua, & Rahmat, 2017
NADH dehydrogenase subunit 2	Kong, Wong, & Lo, 2016
Neuropilin and tolloid-like 1	Yew et al., 2019
Non-specific lipid-transfer protein isoform X5	Zukefli, Chua, & Rahmat, 2017
Ovoinhibitor	Mohamad, Mohamad, Abu, Mahmud, & Razak, 2021; Wong et al., 2018; Yew et al., 2019
Ovotransferrin precursor	Ghassem, Arihara, Mohammadi, Sani, & Babji, 2017
Protein arginine N-methyltransferase7	Zukefli, Chua, & Rahmat, 2017
Putative threonine–tRNA ligase 2, cytoplasmic	Yew et al., 2019
Protein lin-9 homolog	Yew et al., 2019
Protein bassoon	Yew et al., 2019
PDZ domain-containing protein GIPC3 isoform X3	Zukefli, Chua, & Rahmat, 2017
Pre-rRNA-processing protein TSR1 homolog isoform X3	Zukefli, Chua, & Rahmat, 2017
Peroxisomal membrane protein PEX16	Zukefli, Chua, & Rahmat, 2017
RGM domain family member B	Yew et al., 2019; Zukefli, Chua, & Rahmat, 2017
Receptor-type tyrosine-protein phosphatase gamma	Zukefli, Chua, & Rahmat, 2017
SANT domain DNA-binding protein	Zukefli, Chua, & Rahmat, 2017
Selenocysteine-specific elongation factor	Yew et al., 2019
Signal transducer and activator of transcription 6-like isoform X5	Zukefli, Chua, & Rahmat, 2017
Small proline-rich protein 3, partial	Kong, Wong, & Lo, 2016
Sushi, von Willebrand factor type A, EGF and pentraxin domain-containing protein 1, partial	Kong, Wong, & Lo, 2016
Transmembrane protein 186	Zukefli, Chua, & Rahmat, 2017
Von Willebrand factor D and EGF domain-containing protein	Kong, Wong, & Lo, 2016
WD repeat-containing protein 72 isoform X5	Zukefli, Chua, & Rahmat, 2017
Zona pellucida sperm-binding protein 2	Yew et al., 2019
ZZ-type zinc finger-containing protein 3 isoform X5	Zukefli, Chua, & Rahmat, 2017
Calcium-binding protein (MW: 4.5×10^4)	Mohamad, Mohamad, Abu, Mahmud, & Razak, 2021; Wong et al., 2018; Yew et al., 2019
Glucose-regulated protein (MW: 7.8×10^4)	Mohamad, Mohamad, Abu, Mahmud, & Razak, 2021; Yew et al., 2019; Zukefli, Chua, & Rahmat, 2017

inflammatory and cytoprotective functions of AMCase are inhibited by allosamidin, but this inhibition will not affect the chitinolytic activity of AMCase, which means that these functions of AMCase may not be related to its chitinase activity.

3.2. Lysyl oxidase homolog 3

Lysyl oxidase (LOX) is a copper-dependent amine oxidase responsible for the formation of lysine-derived crosslinks in extra-

Table 2
Main proteins of EBN and their bioactivities.

Proteins	Bioactivities	Number of times identified
Acidic mammalian chitinase	Degrade chitin. Play a role in antiviral, anti-inflammatory and cell protection from apoptosis. Control the Th2 inflammatory response through IL-13. Prevent or alleviate pulmonary diseases. Stimulate pulmonary epithelial cells to produce chemokines and protect them from apoptosis.	9
Lysyl oxidase homolog 3	Indispensable in normal development. Essential for stabilizing fibril and elastin elasticity. Play a role in the integrity of the blood vessel walls. Enhance integrin signaling in muscle fibers and its adhesion to tendon junctions by catalyzing the oxidation of fibronectin during development. Remove the acetyl and acetamido groups of STAT3 which is helpful to control the inflammatory reaction and immune regulation.	6
Mucin-5AC	Create a tight gel that acts as a protector. Mediate the formation of a bicarbonate gradient and can form a bilayer mucus barrier together with MUC6 that protect epithelial cells from the deleterious effects of luminal acids. Soluble in tear fluid for the capture and removal of foreign debris. Prevent virus and parasites. Defense against some intestinal parasites that invade the lungs.	5
Ovoinhibitor	Involve in antibacterial defense by inhibiting the microbial proteases that constitute the major components. Prevent the shedding of rotavirus antigens and delay the development of rotavirus gastroenteritis. May be the main cause of IgE allergic reactions.	3
Nucleobindin-2	Play a role in preventing insulin resistance, maintaining calcium homeostasis. Promot cell proliferation and inhibit adipocyte differentiation.	3
Calcium-binding protein (MW: 4.5×10^4)	Alleviate endoplasmic reticulum stress. Involve in regulating calcium-dependent activities in the lumen or posterior compartment of the endoplasmic reticulum, which may have an essential function in cellular calcium homeostasis.	3
Glucose-regulated protein (MW: 7.8×10^4)	Alleviate endoplasmic reticulum stress. Anti-apoptosis. May contribute to neuroprotective effect. Prevent the transport of misfolded proteins or protein subunits.	3

cellular matrix proteins such as collagen and elastin. Lysyl oxidase homolog 3 (LOXL3, Fig. 2B), a member of the lysyl oxidase family, is highly expressed in the heart, spleen, uterus, lung, aorta, and coronary arteries, and also expressed in the corneal layer, trabecular meshwork, limbus, and conjunctiva (Battle, Brown, Engelhardt, & Montgomery, 2017; Collaborators, 2013; Dudakova, Sasaki, Liskova, Palos, & Jirsova, 2016; Melé et al., 2015). It has been found that LOXL3-deficient mice exhibited reduced thoracic cavity and pulmonary hypoplasia, indicated that LOXL3 is indispensable in normal development (Zhang et al., 2016). Recent studies of LOXL3 have revealed the role beyond its canonical function as an amino oxidase.

LOXL3 is essential for stabilizing fibril and elastin elasticity. The skin of ovariectomized rats supplemented with EBN was significantly improved compared with other groups (Matsukawa et al., 2011). Besides, the use of LOX family inhibitors β-APN led to an increase in arterioscleroserosis lesions, suggesting that LOXL3 plays an important role in the integrity of the blood vessel walls (Remus et al., 2012). In addition, research has found that LOXL3 enhanced integrin signaling in muscle fibers and its adhesion to tendon junctions by catalyzing the oxidation of fibronectin during development {Kraft-Sheleg, 2016 #37}(Kraft et al., 2016). Moreover, a novel enzymatic activity of LOXL3 in removing the acetyl and acetamido groups of STAT3 has been reported, which is helpful to control the inflammatory reaction and proved the physiological role of LOXL3 in immune regulation (Ma et al., 2017). The above studies indicated that LOXL3 played an important role in maintaining the health of skin, cardiovascular, tendon and immune system.

In addition to the above functions, LOXL3 also plays a role in the pathogenesis of diseases such as Stickler syndrome, Keratoconus, and glaucoma (Sethi, Mao, Wordinger, & Clark, 2011). Up-regulated expression of LOXL3 was detected in the ciliated bronchial epithelium of lung fibrotic tissue compared to control lung tissue (Jones et al., 2018; Aumiller et al., 2017; Chen, Li, & Li, 2019). In addition, all LOX expression levels were elevated in the lungs of patients with idiopathic pulmonary hypertension (Nave et al., 2014). It has been reported that in systemic hypertension, elevated expression of LOXL3 was observed in cardiac fibroblasts and cardiomyocytes (Yu, Horak, & Larson, 2006). *LOXL3* is also one of the genes up-regulated in microarray studies of affected cartilage in osteoarthritis (Yu, Horak, & Larson, 2006). Huang et al. (2016) also confirmed that LOXL3 is involved in osteoarthritis. In addition, LOXL3 is also closely related to a variety of tumors and cancers, and its expression was found in myeloproliferative tumors (Tadmor et al., 2013), breast cancer, ovarian cancer (Sebban, Davidson, & Reich, 2009), and colorectal cancer (Barbazán et al., 2014; Insua et al., 2017), etc. Research has shown that LOXL3 is involved in protein interactions in tumor progression and metastasis as well as genome integrity (Peinado et al., 2005). The above findings suggested that LOXL3 may be a potential therapeutic target for a variety of diseases, but its role in many pathological mechanisms remains to be further studied and verified.

3.3. Mucin-5AC

Mucin is a family of high molecular weight, heavily glycosylated large glycoproteins that are commonly found in saliva and breast milk. Mucin-5AC (MUC5AC, Fig. 2C) is a secreted, gel-forming mucin, mainly expressed in the stomach, eyes, and airways. The highly glycosylated MUC5AC, by virtue of its cysteine-rich repeat sequence, can form intermolecular and intramolecular disulfide bonds, resulting in complex polymers that form a framework of polymerized mucus gels on the surface of epithelial cells. Gel-forming mucins are important components of the mucus layer and function as an epithelial cell barrier that coats and protects the epithelial surface from environmental aggression and prevents the invasion of pathogens (Krishn, Ganguly, Kaur, & Batra, 2018; Okuda et al., 2019). Gastrointestinal digestion of EBN was simulated *in vitro* and the protein (MW: 5.0×10^4) in EBN was sequenced using in-gel digestion combined with MALDI-TOF-TOF/MS, which was found to match multiple mucin sequences from different birds. And the presence of MUC5AC in EBN was further confirmed by Western blot experiments using mucin antibody (Zukefli, Chua, & Rahmat, 2017).

MUC5AC plays a very important role in the health of the stomach and eyes, and its unique characteristics allow it to create a tight gel that acts as a protector. In the stomach, MUC5AC mediates the formation of a bicarbonate gradient and can form a bilayer mucus barrier together with mucin-6 (MUC6) that protects epithe-

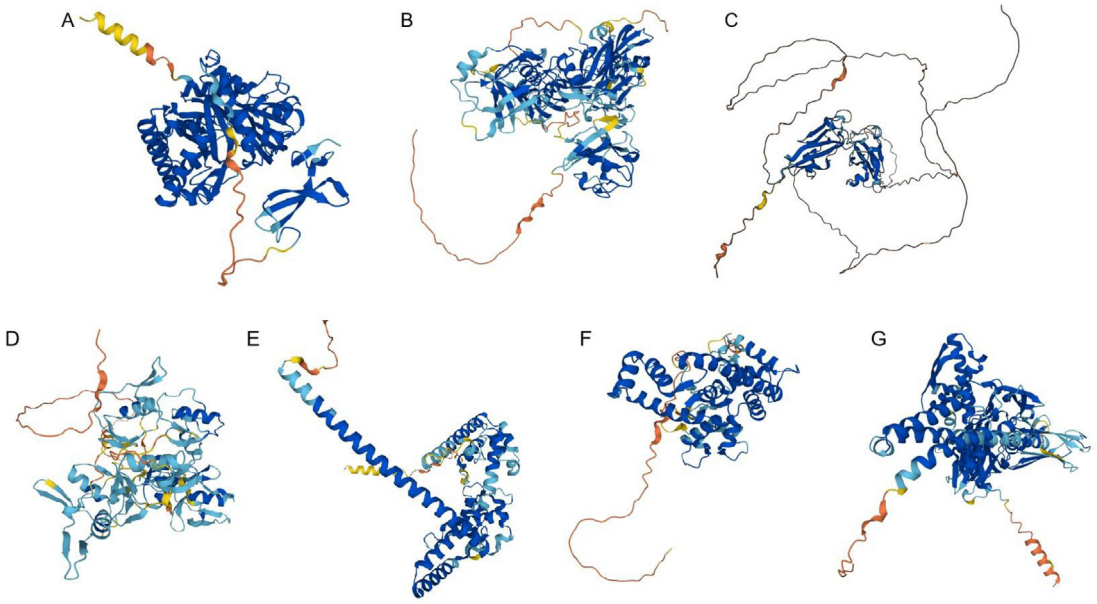

Fig. 2. 3D structures of seven main EBN proteins (Jumper et al., 2021). A: 3D structure of acidic mammalian chitinase (AF-Q9BZP6-F1); B: 3D structure of lysyl oxidase homolog 3 (AF-P58215-F1); C: 3D structure of mucin-5AC (AF-Q14887-F1); D: 3D structure of ovoinhibitor (AF-P10184-F1); E: 3D structure of nucleobindin-2 (AF-P80303-F1); F: 3D structure of 4.5×10^4 calcium-binding protein (AF-Q9BRK5-F1); G: 3D structure of 7.8×10^4 glucose-regulated protein (AF-A0A7P0TAI0-F1).

lial cells from the deleterious effects of luminal acids (Ermund, Schütte, Johansson, Gustafsson, & Hansson, 2013). These findings suggested that the role of EBN in the treatment and prevention of gastric diseases may be derived from MUC5AC.

Mucin loss of the tear film and ocular glycocalyx is thought to be the core pathogenesis of dry eye, and the key point of this mechanism is hyperosmolarity of tear fluid caused by high evaporation or low tear flow. Hypertonic tear fluid can lead to tear film damage, inflammatory response, and loss of surface mucin, which in turn can lead to tear film instability. Mucin is an important component of the tear film and the apical surface of the cornea, and the main mucin expressed by the cup cells of the conjunctiva is MUC5AC. In addition, MUC5AC was shown to be soluble in tear fluid, which in turn moved the soluble mucin to the surface of the eye for the capture and removal of foreign debris (Argüeso, Spurr, Russo, Tisdale, & Gipson, 2003; Gipson, Hori, & Argüeso, 2004).

Some previous studies have suggested that mucus formed by MUC5AC may adversely affect asthma and chronic muco-obstructive pulmonary diseases, and proposed to treat these diseases by inhibiting MUC5AC. But this opinion may be one-sided. Mucins are part of the lung's innate defense system. In the respiratory system, MUC5AC is mainly secreted by surface cells of the larger airways. However, the production of MUC5AC is regulated by the type 1 (viral infections and cigarette smoke) and type 2 (allergens) immune responses, which makes the small airways also begin to secrete MUC5AC. And this is why it is closely related to asthma and chronic muco-obstructive pulmonary diseases (Kesimer et al., 2017; Radicioni et al., 2021). It is speculated that MUC5AC may plays a similar protective role in the lungs as in the stomach. The tight gel formed by MUC5AC facilitates isolation of acute lesions and prevents the migration of invasive pathogens, including viruses, through the lung to other sites. MUC5AC is known to prevent virus that can induce type I inflammation (Hewson et al., 2010) and parasites that can induce type II inflammation (Hasnain et al., 2011), it also critical for defense against some intestinal parasites that invade the lungs (Hasnain et al., 2011). Therefore, it is unwise to blindly treat asthma and chronic muco-obstructive pulmonary diseases by inhibiting MUC5AC. The role of MUC5AC in these pathological mechanisms remains to be further studied and verified.

3.4. Ovoinhibitor

Ovoinhibitor (Fig. 2D) is a serine protease inhibitor belonging to the Kazal family that specifically inhibits serine proteases such as trypsin, chymotrypsin, subtilisin, and porcine elastase (Shechter, Burnstein, & Gertler, 1977; Matsuda, Watanabe, & Nakamura, 1983). Ovoinhibitor was originally purified from egg white (Lineweaver & Murray, 1947) and is highly expressed in the large intestine, liver and uterus of birds, responsible for the secretion of egg white, yolk and eggshell precursors, respectively. Since the EBN is the swiftlet's nest for laying eggs, the ovoinhibitor in EBN may come from the swiftlet's eggs (Wong et al., 2018).

Ovoinhibitor may be involved in antibacterial defense by inhibiting the microbial proteases that constitute the major components. Studies have shown that, purified ovoinhibitor was shown to inhibit trypsin, *Bacillus subtilis*, *Bacillus thuringiensis*, and *Staphylococcus aureus* (Bourin et al., 2011). The above results indicated that ovoinhibitor played an important role in preventing contamination of unfertilized eggs and protecting fertilized chick embryos. Research has found that ovoinhibitor could prevent the shedding of rotavirus antigens and delay the development of rotavirus gastroenteritis in mice model of rotavirus infection (Yolken, Willoughby, Wee, Miskuff, & Vonderfecht, 1987). In addition, it is worth noting that, ovoinhibitor in EBN may be the main cause of IgE allergic reactions, especially in children (Goh, Chew, Chua, Chay, & Lee, 2000).

Serine protease inhibitors in humans are highly expressed in the thymus and stratum corneum, and are also found in the oral mucosa, parathyroid, bartholin, tonsil, and vaginal epithelium, with very low levels detected in the lung, kidney, and prostate (Mägert et al., 1999). Serine protease inhibitors in humans contribute to skin integrity and protective barrier function by modulating defense activation and the activity of desquamation-related protease, which are important for anti-inflammatory and

antimicrobial protection of the mucous epithelium (Deraison et al., 2007).

3.5. Nucleobindin-2

Nucleobindin-2 (NUCB2, Fig. 2E) is a calcium-binding protein that was originally identified in the acute lymphoblastic leukemia cell line KM3 (Barnikol et al., 1994). Nucleonectin-2 is expressed in the cytoplasm of most tissues including immune cells, mainly in the spleen, testis and stomach. It response to endoplasmic reticulum (ER) stress, while activating transcription factor 6 (ATF6) is transported to the Golgi and cleaved by site 1 protease (S1P) (Tsukumo et al., 2007). Nucleonectin-2 plays a role in preventing insulin resistance, maintaining calcium homeostasis, promoting cell proliferation and inhibiting adipocyte differentiation.

Nucleonectin-2 binds to adrenaline to maintain calcium homeostasis. It is post-translationally processed into the biologically active nesfatin-1 peptide, which has been reported to induce satiety, thereby increasing insulin sensitivity. Studies have shown that, loss of nucleonectin-2 exacerbates metabolic inflammation in adipose tissue macrophages in an NF-κB-dependent manner when fed a high-fat diet (Ravussin et al., 2018). The results showed that deletion of nucleonectin-2 did not affect food intake or cause weight gain, but instead caused metabolic inflammation and insulin resistance in mice fed a high-fat diet. It can be seen that nucleonectin-2 plays a regulatory role in insulin secretion and can be a potential contributor to the study of diabetes pathology.

Furthermore, nucleonectin-2 is a positive regulator of EGF-dependent signaling, and it can enhance cell growth and inhibit adipocyte differentiation. It has been reported that endogenous nucleonectin-2 inhibited PP2A activity by controlling intracellular free calcium concentration, thereby enhancing EGF activation of MEK/Erk modules and promoting cell proliferation (Yoshikawa et al., 2000). Therefore, extracellular signal regulated kinases (ERK) activity most likely explains the ability of nucleonectin-2 to enhance cell growth (Ramos, 2008). In addition, the study also found that nucleonectin-2 had the function of adipose suppressor, and its high expression can inhibit the differentiation of 3T3-L1 adipocytes. In summary, it is suggested that the EGF effect and potential weight loss function of EBN may be closely related to nucleonectin-2.

3.6. Calcium-binding protein (MW: 4.5×10^4) and glucose-regulated protein (MW: 7.8×10^4)

Mohamad et al. {Mohamad Nasir, 2021 #24}(2021) have found 4.5×10^4 calcium-binding protein (SDF4, Fig. 2F) and 7.8×10^4 glucose-regulated protein (GRP78, Fig. 2G) in raw EBN and its aqueous extracts. Both proteins can alleviate ER stress, which is closely related to the pathological mechanism of immune-inflammatory diseases. Therefore, these two proteins may have anti-inflammatory effect.

SDF4 is composed of 361 amino acids and is present in brain regions in astrocytes, neurons, oligodendrocyte progenitors, myelinated oligodendrocytes, microglia, macrophages and highly expressed in endothelial cells. Studies have shown that the expression of SDF4 can attenuate ER stress. And it is involved in regulating calcium-dependent activities in the lumen or posterior compartment of the ER, which may have an essential function in cellular calcium homeostasis (Zhu et al., 2021).

GRP78 is a member of the heat shock protein 70 family, exists on the ER membrane and is the main binding protein for Ca^{2+}. GRP78 has anti-apoptotic effects (Ibrahim, Abdelmalek, & Elfiky, 2019; Pfaffenbach & Lee, 2011) and may contribute to neuroprotection in the 6-OHDA-treated neuroblastoma cell model SH-SY5Y (Yew, Koh, Chye, Othman, & Ng, 2014). In normal cells, GRP78 prevents the transport of misfolded proteins or protein subunits. Under ER stress conditions, when unfolded proteins accumulate in the ER lumen, GRP78 activates the UPR and alleviates ER stress by reducing protein translation and increasing ER folding capacity (Aridon et al., 2011; Leak, 2014).

4. Discussion and prospective

Numerous studies have demonstrated the health benefits of EBN, many of which coincide with the bioactivities of proteins identified in EBN, further evidencing that some of the bioactivities of EBN are likely derived from these proteins.

Immune regulation is one of the most studied bioactivities of EBN. The human immune system consists of three lines of defense, and various proteins in EBN participate in the immunity through different mechanisms. The first line of immune defense consists of the skin, mucous membranes and their secretions. The skin covers the surface of the body extensively, keeping pathogens such as bacteria and viruses out of the body. While the mucous membrane mainly covers the oral and nasal cavity, digestive tract, alveoli and other parts, and the mucus and bactericidal substances secreted by it can block, capture and remove foreign substances. AMCase and ovoinhibitors in EBN help maintain skin integrity, while MUC5AC and ovoinhibitors are involved in the antimicrobial protection of mucous epithelium. The second line of immune defense is humoral immunity, including bactericidal substances and phagocytes in body fluids. The AMCase in EBN plays a role in humoral immunity by activating Th2 cells to produce IL-13 that can control the anti-inflammatory response. The first two lines of defense are non-specific immunity, which is not directed against a specific pathogen but has a defensive effect against a variety of pathogens. The third line of defense is specific immunity, which only acts against a specific pathogen, and is mainly composed of immune organs and immune cells with the help of blood circulation and lymphatic circulation. LOXL3 in EBN helps control inflammatory responses by removing the acetyl and acetamido groups of STAT3, while both SDF4 and GRP78 can exert anti-inflammatory effects by alleviating ER stress.

In addition to the above-mentioned immune regulation effects, other bioactivities of EBN are also closely related to the proteins in it. For example, in addition to the sialic acid and EGF, the effect of EBN in maintaining skin elasticity and anti-aging may also benefit from the stabilizing effect of LOXL3 on fibrils and elastin in the skin, and the role of nucleobindin-2 in promoting cell proliferation. While the protective effect of EBN on the stomach and lungs may be derived from the protection of AMCase and MUC5AC on lung epithelium and gastric mucosa. In addition, the other functions of EBN including protective effects on nervous system, cardiovascular, tendon, bone and joint, eyes, and prevention of diabetes and obesity, maintenance of cellular calcium homeostasis, etc. are closely related to the bioactivities of MUC5AC, ncleobindin-2, SDF4, GRP78, etc.

It should be noted that, although the proteins mentioned in this article were identified in EBN, it does not mean that they still have their original bioactivities after being digested and absorbed by the human body. Whether the peptides obtained after the digestion of EBN also have similar bioactivities still needs to be studied through more pharmacological experiments. In addition, the protein analysis of EBN is affected by different extraction methods, and factors such as the source, type, and picking season of EBN may also lead to differences in its chemical composition, including the identified protein components. And there may still be many proteins in EBN that have not been identified by analysis. Therefore, further exploration of the known bioactivities of EBN and the association of proteins, as well as the unknown potential bioactivities, requires more in-depth and detailed research.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work is financially supported by the Fundamental Research Funds for the Central Universities (lzujbky-2022-ct03).

The authors are thankful to Yingzhi Fu from Beijing Xiaoxiandun Biotechnology Co., Ltd. for providing the EBN photos.

References

Argüeso, P., Spurr, M. S., Russo, C. L., Tisdale, A., & Gipson, I. K. (2003). MUC16 mucin is expressed by the human ocular surface epithelia and carries the H185 carbohydrate epitope. *Investigative Ophthalmology & Visual Science, 44*(6), 2487–2495.

Aridon, P., Geraci, F., Turturici, G., D'Amelio, M., Savettieri, G., & Sconzo, G. (2011). Protective role of heat shock proteins in Parkinson's disease. *Neurodegenerative Diseases, 8*(4), 155–168.

Aumiller, V., Strobel, B., Romeike, M., Schuler, M., Stierstorfer, B. E., & Kreuz, S. (2017). Comparative analysis of lysyl oxidase (like) family members in pulmonary fibrosis. *Scientific Reports, 7*, 149.

Bai, W. J., Liu, X. C., Zhang, X. T., Fan, Q. Y., & Xu, D. M. (2020). Research progress on ingredients and efficacy of bird's nest. *Food Science and Technology, 45*(5), 96–100.

Barbazán, J., Muinelo, R. L., Vieito, M., Candamio, S., Díaz, L. A., Cano, A., ... López, R. (2014). A multimarker panel for circulating tumor cells detection predicts patient outcome and therapy response in metastatic colorectal cancer. *International Journal of Cancer, 135*(11), 2633–2643.

Barnikol, W. S., Gross, N. A., Götz, H., Henkel, T., Karabinos, A., Kratzin, H., ... Hilschmann, N. (1994). Human protein NEFA, a novel DNA binding/EF-hand/leucine zipper protein. Molecular cloning and sequence analysis of the cDNA, isolation and characterization of the protein. *Biological Chemistry Hoppe-Seyler, 375*(8), 497–512.

Battle, A., Brown, C. D., Engelhardt, B. E., & Montgomery, S. B. (2017). Genetic effects on gene expression across human tissues. *Nature, 550*(7675), 204–213.

Bourin, M., Gautron, J., Berges, M., Attucci, S., Le, B. G., Labas, V., ... Réhault, G. S. (2011). Antimicrobial potential of egg yolk ovoinhibitor, a multidomain kazal-like inhibitor of chicken egg. *Journal of Agricultural and Food Chemistry, 59*, 12368–12374.

Chen, L., Li, S., & Li, W. (2019). LOX/LOXL in pulmonary fibrosis: Potential therapeutic targets. *Journal of Drug Targeting, 27*(7), 790–796.

Collaborators (2013). The genotype-tissue expression (GTEx) project. *Nature Genetics, 45*(6), 580–585.

Deraison, C., Bonnart, C., Lopez, F., Besson, C., Robinson, R., Jayakumar, A., ... Hovnanian, A. (2007). LEKTI fragments specifically inhibit KLK5, KLK7, and KLK14 and control desquamation through a pH-dependent interaction. *Molecular Biology of the Cell, 18*(9), 3607–3619.

Dudakova, L., Sasaki, T., Liskova, P., Palos, M., & Jirsova, K. (2016). The presence of lysyl oxidase-like enzymes in human control and keratoconic corneas. *Histology and Histopathology, 31*(1), 63–71.

Ermund, A., Schütte, A., Johansson, M. E., Gustafsson, J. K., & Hansson, G. C. (2013). Studies of mucus in mouse stomach, small intestine, and colon. I. Gastrointestinal mucus layers have different properties depending on location as well as over the Peyer's patches. *American Journal of Physiology – Gastrointestinal and Liver Physiology, 305*(5), G341–G347.

Ghassem, M., Arihara, K., Mohammadi, S., Sani, N. A., & Babji, A. S. (2017). Identification of two novel antioxidant peptides from edible bird's nest (*Aerodramus fuciphagus*) protein hydrolysates. *Food & Function, 8*(5), 2046–2052.

Hartl, D., He, C. H., Koller, B., Da Silva, C. A., Kobayashi, Y., Lee, C. G., ... Elias, J. A. (2009). Acidic mammalian chitinase regulates epithelial cell apoptosis via a chitinolytic-independent mechanism. *The Journal of Immunology, 182*(8), 5098–5106.

Gipson, I. K., Hori, Y., & Argüeso, P. (2004). Character of ocular surface mucins and their alteration in dry eye disease. *Ocular Surface, 2*(2), 131–148.

Goh, D. L. M., Chew, F. T., Chua, K. Y., Chay, O. M., & Lee, B. W. (2000). Edible "bird's nest"–induced anaphylaxis: An under-recognized entity? *The Journal of Pediatrics, 137*(2), 277–279.

Hartl, D., He, C. H., Koller, B., Da, S. C. A., Homer, R., Lee, C. G., & Elias, J. A. (2008). Acidic mammalian chitinase is secreted via an ADAM17/epidermal growth factor receptor-dependent pathway and stimulates chemokine production by pulmonary epithelial cells. *Journal of Biological Chemistry, 283*(48), 33472–33482.

Hasnain, S. Z., Evans, C. M., Roy, M., Gallagher, A. L., Kindrachuk, K. N., Barron, L., ... Thornton, D. J. (2011). Muc5ac: A critical component mediating the rejection of enteric nematodes. *Journal of Experimental Medicine, 208*(5), 893–900.

Hewson, C. A., Haas, J. J., Bartlett, N. W., Message, S. D., Laza, S. V., Kebadze, T., ... Johnston, S. L. (2010). Rhinovirus induces MUC5AC in a human infection model and *in vitro* via NF-κB and EGFR pathways. *European Respiratory Journal, 36*(6), 1425–1435.

Huang, Z. M., Du, S. H., Huang, L. G., Li, J. H., Xiao, L., & Tong, P. (2016). Leptin promotes apoptosis and inhibits autophagy of chondrocytes through upregulating lysyl oxidase-like 3 during osteoarthritis pathogenesis. *Osteoarthritis and Cartilage, 24*(7), 1246–1253.

Ibrahim, I. M., Abdelmalek, D. H., & Elfiky, A. A. (2019). GRP78: A cell's response to stress. *Life Sciences, 226*, 156–163.

Insua, Y. V., Cámara, J., Vázquez, E. B., Fernández, A., Rivera, F. V., Silva, M., ... Alonso, A. L. (2017). Predicting outcome and therapy response in mCRC patients using an indirect method for CTCs detection by a multigene expression panel: A multicentric prospective validation study. *International Journal of Molecular Sciences, 18*(6), 1265.

Jian, Y. Y., Li, Q. W., Huang, C. C., Huang, Z. J., Xie, Y., Zeng, B. D., & Zang, Y. (2016). Analysis and comparison of nutrient composition of dried bird's nest and stewed bird's nest. *The Light & Textile Industries of Fujian, 3*, 32–38.

Jian, Y. Y., Li, Q. W., Huang, Z. J., Huang, C. C., Zheng, L. J., Fu, W. Q., ... Zang, Y. (2016). Research progress on nutritional efficacy and authenticity of bird's nest. *Subtropical Agriculture Research, 12*(2), 136–144.

Jones, M. G., Andriotis, O. G., Roberts, J. J., Lunn, K., Tear, V. J., Cao, L., ... Davies, D. E. (2018). Nanoscale dysregulation of collagen structure-function disrupts mechano-homeostasis and mediates pulmonary fibrosis. *Elife, 7*, e36354.

Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., ... Hassabis, D. (2021). Highly accurate protein structure prediction with Alpha Fold. *Nature, 596*(7873), 583–589.

Kesimer, M., Ford, A. A., Ceppe, A., Radicioni, G., Cao, R., Davis, C. W., ... Boucher, R. C. (2017). Airway mucin concentration as a marker of chronic bronchitis. *New England Journal of Medicine, 377*(10), 911–922.

Kong, H. K., Wong, K. H., & Lo, S. C. (2016). Identification of peptides released from hot water insoluble fraction of edible bird's nest under simulated gastro-intestinal conditions. *Food Research International, 85*, 19–25.

Kraft, S. O., Zaffryar, E. S., Genin, O., Yaseen, W., Soueid, B. S., Kessler, O., ... Hasson, P. (2016). Localized LoxL3-dependent fibronectin oxidation regulates myofiber stretch and integrin-mediated adhesion. *Developmental Cell, 36*(5), 550–561.

Krishn, S. R., Ganguly, K., Kaur, S., & Batra, S. K. (2018). Ramifications of secreted mucin MUC5AC in malignant journey: A holistic view. *Carcinogenesis, 39*(5), 633–651.

Leak, R. K. (2014). Heat shock proteins in neurodegenerative disorders and aging. *Journal of Cell Communication and Signaling, 8*(4), 293–310.

Lineweaver, H., & Murray, C. W. (1947). Identification of the trypsin inhibitor of egg white with ovomucoid. *Journal of Biological Chemistry, 171*(2), 565–581.

Liu, X., Lai, X., Zhang, S., Huang, X., Lan, Q., Li, Y., ... Yang, G. (2012). Proteomic profile of edible bird's nest proteins. *Journal of Agricultural and Food Chemistry, 60*(51), 12477–12481.

Ma, L., Huang, C., Wang, X. J., Xin, D. E., Wang, L. S., Zou, Q. C., ... Chin, Y. E. (2017). Lysyl oxidase 3 is a dual-specificity enzyme involved in STAT3 deacetylation and deacetylimination modulation. *Molecular Cell, 65*(2), 296–309.

Ma, X., Zhang, J., Liang, J., Ma, X., Xing, R., Han, J., ... Chen, Y. (2019). Authentication of edible bird's nest (EBN) and its adulterants by integration of shotgun proteomics and scheduled multiple reaction monitoring (MRM) based on tandem mass spectrometry. *Food Research International, 125*, 108639.

Mägert, H. J., Ständker, L., Kreutzmann, P., Zucht, H. D., Reinecke, M., Sommerhoff, C. P., ... Forssmann, W. G. (1999). LEKTI, a novel 15-domain type of human serine proteinase inhibitor. *Journal of Biological Chemistry, 274*(31), 21499–21502.

Marcone, M. F. (2005). Characterization of the edible bird's nest the "Caviar of the East". *Food Research International, 38*(10), 1125–1134.

Matsuda, T., Watanabe, K., & Nakamura, R. (1983). Ovomucoid and ovoinhibitor isolated from chicken egg white are immunologically cross-reactive. *Biochemical and Biophysical Research Communications, 110*(1), 75–81.

Matsukawa, N., Matsumoto, M., Bukawa, W., Chiji, H., Nakayama, K., Hara, H., & Tsukahara, T. (2011). Improvement of bone strength and dermal thickness due to dietary edible bird's nest extract in ovariectomized rats. *Bioscience, Biotechnology and Biochemistry, 75*(3), 590–592.

Melé, M., Ferreira, P. G., Reverter, F., DeLuca, D. S., Monlong, J., Sammeth, M., ... Guigó, R. (2015). Human genomics. The human transcriptome across tissues and individuals. *Science, 348*(6235), 660–665.

Mohamad, N. N., Mohamad, I. R., Abu, B. M. Z., Mahmud, R., & Razak, N. A. (2021). Characterization and extraction influence protein profiling of edible bird's nest. *Foods, 10*(10), 2248.

Nave, A. H., Mižíková, I., Niess, G., Steenbock, H., Reichenberger, F., Talavera, M. L., ... Morty, R. E. (2014). Lysyl oxidases play a causal role in vascular remodeling in clinical and experimental pulmonary arterial hypertension. *Arteriosclerosis, Thrombosis, and Vascular Biology, 34*(7), 1446–1458.

Okuda, K., Chen, G., Subramani, D. B., Wolf, M., Gilmore, R. C., Kato, T., ... Boucher, R. C. (2019). Localization of secretory mucins MUC5AC and MUC5B in normal/healthy human airways. *American Journal of Respiratory and Critical Care Medicine, 199*(6), 715–727.

Peinado, H., Del, C., Iglesias, M., Csiszar, K., Fong, K. S., Vega, S., ... Portillo, F. (2005). A molecular role for lysyl oxidase-like 2 enzyme in snail regulation and tumor progression. *EMBO Journal, 24*(19), 3446–3458.

Pfaffenbach, K. T., & Lee, A. S. (2011). The critical role of GRP78 in physiologic and pathologic stress. *Current Opinion in Cell Biology, 23*(2), 150–156.

Radicioni, G., Ceppe, A., Ford, A. A., Alexis, N. E., Barr, R. G., Bleecker, E. R., ... Kesimer, M. (2021). Airway mucin MUC5AC and MUC5B concentrations and the

initiation and progression of chronic obstructive pulmonary disease: An analysis of the SPIROMICS cohort. *The Lancet Respiratory Medicine, 9*(11), 1241–1254.

Ramos, J. W. (2008). The regulation of extracellular signal-regulated kinase (ERK) in mammalian cells. *International Journal of Biochemistry and Cell Biology, 40*(12), 2707–2719.

Ravussin, A., Youm, Y. H., Sander, J., Ryu, S., Nguyen, K., Varela, L., ... Dixit, V. D. (2018). Loss of nucleobindin-2 causes insulin resistance in obesity without impacting satiety or adiposity. *Cell Reports, 24*(5), 1085–1092.

Remus, E. W., O'Donnell, R. E., Rafferty, K., Weiss, D., Joseph, G., Csiszar, K., ... Taylor, W. R. (2012). The role of lysyl oxidase family members in the stabilization of abdominal aortic aneurysms. *American Journal of Physiology Heart and Circulatory Physiology, 303*(8), H1067–H1075.

Sebban, S., Davidson, B., & Reich, R. (2009). Lysyl oxidase-like 4 is alternatively spliced in an anatomic site-specific manner in tumors involving the serosal cavities. *Virchows Archiv, 454*(1), 71–79.

Sethi, A., Mao, W., Wordinger, R. J., & Clark, A. F. (2011). Transforming growth factor-beta induces extracellular matrix protein cross-linking lysyl oxidase (LOX) genes in human trabecular meshwork cells. *Investigative Ophthalmology & Visual Science, 52*(8), 5240–5250.

Shao, J. H., Ding, Q., Wang, S., Zhao, F. Z., Luo, B. Z., & Liao, X. Y. (2018). Research status of edible bird's nest in southeast Asia. *Journal of Food Safety and Quality Inspection, 9*(5), 958–973.

Shechter, Y., Burnstein, Y., & Gertler, A. (1977). Effect of oxidation of methionine residues in chicken ovoinhibitor on its inhibitory activities against trypsin, chymotrypsin, and elastase. *Biochemistry, 16*(5), 992–997.

Tadmor, T., Bejar, J., Attias, D., Mischenko, E., Sabo, E., Neufeld, G., & Vadasz, Z. (2013). The expression of lysyl-oxidase gene family members in myeloproliferative neoplasms. *American Journal of Hematology, 88*(5), 355–358.

Tsukumo, Y., Tomida, A., Kitahara, O., Nakamura, Y., Asada, S., Mori, K., & Tsuruo, T. (2007). Nucleobindin 1 controls the unfolded protein response by inhibiting ATF6 activation. *Journal of Biological Chemistry, 282*(40), 29264–29272.

Van, D. S. J., Liang, H. E., Naikawadi, R. P., Woodruff, P. G., Wolters, P. J., Erle, D. J., & Locksley, R. M. (2017). Spontaneous chitin accumulation in airways and age-related fibrotic lung disease. *Cell, 169*(3), 497–509.

Wong, C. F., Chan, G. K. L., Zhang, M. L., Yao, P., Lin, H. Q., Dong, T. T. X., ... Tsim, K. W. K. (2017). Characterization of edible bird's nest by peptide fingerprinting with principal component analysis. *Food Quality and Safety, 1*(1), 83–92.

Wong, Z. C. F., Chan, G. K. L., Wu, L., Lam, H. H. N., Yao, P., Dong, T. T. X., & Tsim, K. W. K. (2018). A comprehensive proteomics study on edible bird's nest using new monoclonal antibody approach and application in quality control. *Journal of Food Composition and Analysis, 66*, 145–151.

Yew, M. Y., Koh, R. Y., Chye, S. M., Othman, I., & Ng, K. Y. (2014). Edible bird's nest ameliorates oxidative stress-induced apoptosis in SH-SY5Y human neuroblastoma cells. *BMC Complementary and Alternative Medicine, 14*, 391.

Yew, M. Y., Koh, R. Y., Chye, S. M., Zainal, A. S. A., Othman, I., & Ng, K. Y. (2019). Neurotrophic properties and the *de novo* peptide sequencing of edible bird's nest extracts. *Food Bioscience, 32*, 100466.

Yolken, R. H., Willoughby, R., Wee, S. B., Miskuff, R., & Vonderfecht, S. (1987). Sialic acid glycoproteins inhibit *in vitro* and *in vivo* replication of rotaviruses. *Journal of Clinical Investigation, 79*(1), 148–154.

You, Y., Cao, Y., Guo, S., Xu, J., Li, Z., Wang, J., & Xue, C. (2015). Purification and identification of α 2–3 linked sialoglycoprotein and α 2–6 linked sialoglycoprotein in edible bird's nest. *European Food Research and Technology, 240*(2), 389–397.

Yu, Q., Horak, K., & Larson, D. F. (2006). Role of T lymphocytes in hypertension-induced cardiac extracellular matrix remodeling. *Hypertension, 48*(1), 98–104.

Zhang, J., Liu, Z., Zhang, T., Lin, Z., Li, Z., Zhang, A., ... Gao, J. (2016). Loss of lysyl oxidase-like 3 attenuates embryonic lung development in mice. *Scientific Reports, 6*, 33856.

Zhao, R., Li, G., Kong, X. J., Huang, X. Y., Li, W., Zeng, Y. Y., & Lai, X. P. (2016). The improvement effects of edible bird's nest on proliferation and activation of B lymphocyte and its antagonistic effects on immunosuppression induced by cyclophosphamide. *Drug Design, Development and Therapy, 10*, 371–381.

Zhu, T., Su, Q., Wang, C., Shen, L., Chen, H., Feng, S., ... Chen, J. (2021). SDF4 is a prognostic factor for 28-days mortality in patients with sepsis via negatively regulating ER stress. *Frontiers in Immunology, 12*, 659193.

Zukefli, S. N., Chua, L. S., & Rahmat, Z. (2017). Protein extraction and identification by gel electrophoresis and mass spectrometry from edible bird's nest samples. *Food Analytical Methods, 10*(2), 387–398.