



**UNIVERSITI PUTRA MALAYSIA**

***MICROBIAL PROFILE AND ANTIMICROBIAL PROPERTIES OF  
EDIBLE BIRD NEST***

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**MICROBIAL PROFILE AND ANTIMICROBIAL PROPERTIES OF  
EDIBLE BIRD NEST**

By

**SHAZREENA BINTI ZUBER**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
Malaysia, in fulfilment of the Requirements for the Degree of  
Master of Science**

**November 2018**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

## MICROBIAL PROFILE AND ANTIMICROBIAL PROPERTIES OF EDIBLE BIRD NEST

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November 2018

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Edible-birdnest (EBN) is the hardened salivary secretion of swiftlets (*Aerodramus* sp.) that has been reported to have numerous nutritional benefits. The consumption and trade of EBN can be traced as far back since the Tang dynasty and considered to be one of the expensive animal by-product consumed by humans. In recent years, various scientific studies have been carried out to explain the medicinal properties of EBN. However, there is a limited study on the microbiological diversity of EBN and the antimicrobial properties of EBN. In this study, the microbial quality of 45 EBN comprising of raw unclean and raw clean EBN from two different premises were analyzed, namely premise A (GMP-certified premise) and premise B (non GMP-certified premise). Dilution of EBN samples were made and microbial enumeration was done using 3M™ Petrifilm. The results revealed that the Total Aerobic Count (TAC) of raw clean EBN in both premises and Total Coliform Count (TCC) in all EBN in premise B except for 8 weeks old EBN exceeded limit stated in Malaysia Standard (MS 2334:2011; SIRIM/DVS 3:2015). A total of 255 bacteria and 201 fungi species were isolated from EBN and identified using biochemical test and API kit, the most common bacteria encountered in both premises were *Staphylococcus* sp., *Corynebacterium* sp. and *Actinomyces* sp. While in premise B, *Aeromonas* sp. and *Ochrobactrum anthropi* were also found to be prevalent. The difference in occurrence of bacteria in EBNs in both premises at all stages were found to be non-significant ( $P < 0.05$ ). As for fungi, *Fonsecaea pedrosoi* were the most encountered fungi in premise A. While in premise B, *Penicillin* sp. and *Candida* sp. were the most frequently isolated in that premise. The most common fungi encountered in both premises were *Aspergillus niger* and *Mucor* sp. Differences in occurrence of the fungi were only significant in the raw unclean EBN and non-significant in the rest of the stages. The bacteria and fungi mostly found in this study were environmental microorganism. It could be concluded that although one of the premise

practices Good Manufacturing Practice (GMP), however the results indicates that the cleaning process of both premises are similar. The source of contamination could be caused by mishandling, cleaning, processing step and equipment.

Another study was carried out to compare the prevalence of bacteria from 30 swiftlet faeces in bird ranch located at three different geographical areas (oil palm area, paddy field area, town area). A total of 174 bacteria species were isolated and identified with the most encountered bacteria from the paddy field area were *Escherichia coli* (35.7%), followed by *Proteus* sp. and *Vibrio* sp. (17.8%). While in the town area, *E. coli* (20%) and *Proteus mirabilis* (10%) were the most frequently isolated bacteria, and in oil palm plantation, the most common bacteria were *Enterobacter cloacae* (17%) and *Serratia marcescens* (13%). As for Gram-positive bacteria, *Staphylococcus* sp. (70%), *Streptococcus* sp. (42.8%), and *Actinomyces* sp. (50%) were found to be most common bacteria in faeces from oil palm plantation, paddy field and town area respectively. The prevalence of bacteria in all three locations were found to be non-significant ( $P < 0.05$ ). In conclusion, the similarity in prevalence of bacteria in faeces in all three areas were because the isolates were part of normal faecal and environmental flora and relatively similar eating habits of the swiftlets which is dependent on two main insect orders, Diptera and Hymenoptera that were mainly found in the edible-nest swiftlets in various locations around Malaysia.

The therapeutic qualities of EBN were also screened, and in this study the antibacterial and antifungal activity of 15 pure EBN extracts were screened using the disk and agar well diffusion methods. Despite many reports confirming EBN possessed bioactive component, unfortunately, all 15 pure EBN extract tested showed no inhibitory effects against selected bacteria which included *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Rhodococcus equi*, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Pasteurella multocida*. Following that, no antifungal activity was observed against fungal strains *A. niger*, *Aspergillus flavus*, *Malassezia* sp., *Candida albicans* and *Mucor* sp. The absence of antimicrobial activity could be caused by the loss of the bioactive component responsible for the antimicrobial properties in EBN possibly due to the cleaning, drying, extraction process and long period of EBN storage.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
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## PROFIL MIKROB DAN SIFAT ANTIMIKROB SARANG BURUNG WALIT

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Sarang burung walit (SBW) merupakan rembesan air liur yang mengeras yang dihasilkan oleh burung walit (*Aerodramus* sp.) dimana pelbagai kebaikan nutrisi yang dimilikinya telah dilaporkan. Pengambilan dan perdagangan SBW boleh dijejaki seawal dinasti Tang dan dianggap sebagai salah satu hasil produk haiwan termahal yang dimakan manusia. Dalam beberapa tahun kebelakangan ini, pelbagai kajian saintifik telah dijalankan untuk menerangkan sifat ubatan SBW. Namun, kajian tentang kepelbagaian mikrobial dan sifat antimikrobial terhadap SBW adalah terhad. Di dalam kajian ini, kualiti mikrobial 45 SBW merangkumi SBW mentah kotor dan mentah bersih dari dua buah premis telah ditafsir, iaitu premis A (premis berlesen GMP) dan premis B (premis tiada lesen GMP). Pencairan sampel EBN telah dibuat dan pengiraan mikrobial telah dilakukan menggunakan 3M™ Petrifilm. Hasil kajian mendapati jumlah kiraan aerobik untuk SBW yang mentah bersih di kedua-dua buah premis dan jumlah kiraan koliform di kesemua SBW di premis B kecuali SBW minggu ke 8 telah melepasi had dibenarkan Standard Malaysia (MS 2334:2011; SIRIM/DVS 3:2015). Sejumlah 255 spesis bakteria dan 201 spesis kulat telah berjaya dipencilkan dan dikenalpasti dari SBW menggunakan ujian biokemikal dan API kit. Bacteria yang umum di kedua-dua buah premis adalah *Staphylococcus* sp., *Corynebacterium* sp., dan *Actinomyces* sp.

Manakala di premis B, *Aeromonas* sp. dan *Ochrobactrum anthropi*, juga didapati prevalen. Perbezaan kekerapan dalam pencilan SBW di kedua-dua buah premis di setiap peringkat didapati tidak signifikan ( $P < 0.05$ ). Bagi kulat, *Fonsecaea pedrosoi* sering ditemui di premis A. Manakala di premis B, *Penicillin* sp. dan *Candida* sp. paling banyak dipencilkan di premis tersebut. Kulat yang paling umum ditemui di kedua-dua premis adalah *Aspergillus niger* dan *Mucor* sp. Perbezaan kekerapan hanya signifikan terhadap SBW yang

kotor dan tidak signifikan pada peringkat yang lain. Bakteria dan kulat yang ditemui dalam kajian ini adalah sebahagian dari organisma persekitaran. Kesimpulan yang dapat dibuat adalah walaupun salah satu daripada premis mengamalkan Good Manufacturing Practice (GMP), namun hasil kajian menunjukkan proses pembersihan di kedua-dua buah premis adalah sama. Punca kontaminasi mungkin datang dari pengendalian yang salah, proses pembersihan, pemprosesan dan alatan.

Kajian seterusnya dibuat untuk membandingkan prevalen bakteria dari 30 tinja burung walit di rumah burung walit yang terletak di tiga lokasi yang berbeza mengikut geografi (kawasan kelapa sawit, sawah padi, bandar). Jumlah 174 spesis bakteria telah berjaya dipencilkan dan bakteria yang sering ditemui di sawah padi adalah *E. coli* (35.7%), *Proteus* sp. dan *Vibrio* sp. (17.8%). Manakala di kawasan bandar, *Escherichia coli* (20%) dan *Proteus mirabilis* (10%) adalah bakteria yang paling kerap dipencilkan, dan di kawasan kelapa sawit, bakteria yang paling umum adalah *Enterobacter cloacae* (17%) dan *Serratia marcescens* (13%). Untuk bakteria Gram-positif, *Staphylococcus* sp. (70%), *Streptococcus* sp. (42.8%), dan *Actinomyces* sp. (50%) paling banyak didapati di semua tinja burung walit dari kawasan kelapa sawit, sawah padi dan bandar mengikut urutan. Prevalen bakteria di ketiga-tiga lokasi didapati tidak signifikan ( $P < 0.05$ ). Kesimpulannya, persamaan prevalen bakteria di dalam tinja di ketiga-tiga kawasan adalah disebabkan pencilan didapati adalah sebahagian dari flora tinja dan persekitaran normal dan tabiat pemakanan yang sama burung walit bergantung kepada dua jenis order serangga, Diptera dan Hymenoptera yang sering ditemui pada burung walit di kebanyakan lokasi dalam Malaysia.

Sifat terapeutik SBW juga disaring. Di dalam kajian ini aktiviti antibakterial dan antikulat akan 15 ekstrak tulen SBW telah dijelaskan menggunakan kaedah difusi disk agar dan difusi telaga agar. Walaupun banyak laporan yang mengesahkan SBW mempunyai komponen bioaktif, namun, kesemua 15 ekstrak tulen SBW tidak menunjukkan sebarang rancangan terhadap bakteria terpilih iaitu *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*, *Rhodococcus equi*, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* dan *Pasteurella multocida*. Berikutan itu, tiada aktiviti antikulat dapat dilihat pada kulat *Aspergillus niger*, *Aspergillus flavus*, *Malassezia* sp., *Candida albicans* dan *Mucor* sp. Ketiadaan sifat antimikrobia mungkin disebabkan oleh kehilangan komponen bioaktif yang terdapat dalam SBW ketika prosedur pembersihan, pengeringan, pengekstrakan dan tempoh penyimpanan SBW yang lama.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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## LIST OF ABBREVIATIONS

API	Analytical Profile Index
CFU	Colony forming unit
ETP	Economic Transformation Programme
EBN	Edible-birdnest
FDA	Food and drug administration
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis and Critical Control Points
IARC	International Agency for Research on Cancer
MDCK	Madin-Darby Canine Kidney
MHA	Mueller Hinton Agar
MRVP	Methyl Red Voges Proskauer
MS	Malaysian Standard
NANA	N-Acetylneuraminic acid
OTA	Ochratoxin A
SIM	Sulphide Indole Motility
TAC	Total Aerobic Count
TCC	Total Coliform Count
TSI	Triple Sugar Iron
TYMC	Total Yeast and Mould Count
WHO	World Health Organization

## CHAPTER 1

### INTRODUCTION

Edible-birdnest (EBN) is produced by edible-nest swiftlet's salivary secretions of their sublingual salivary glands. The nest serves as a shelter for swiftlets to breed and roost (Koon & Cranbrook, 2002). Since ancient times, the Chinese have consumed EBN as tonic food which is famously known as "bird's nest soup". The nests are cooked in a double boiler with sugar to produce the gastronomic delicacy (Hobbs, 2004). The consumption of this delicacy can be traced back since the Tang Dynasty (618-907 AD) due to their extensive medicinal properties and well-being effects (Marcone, 2005).

For centuries, this natural product has been also used for treatment of many acute and chronic respiratory disorders (Hobbs, 2004). The interest of scientific community in this natural product is growing, which aims to understand the biological roles and the bioactive compounds of the EBN (Amin et al., 2016). In some research, EBN has been found to possess interesting properties such as wound healing in corneal keratocytes (Zainal Abidin et al., 2011) and human adipose-derived stem cells (Roh et al., 2012), chondro-protective effects on human chondrocytes (Chua et al., 2013), anti-viral (Guo et al., 2006), anti-cancer (Hou et al., 2010), bone strengthening (Aswir & Wan Nazaimoon, 2010) and a lot more which increases the value of this precious bioproduct.

Increasing price of EBN has led swiftlet's entrepreneurs to run the business more seriously (Alif et al., 2016). Furthermore, this industry has encouraged and provided many other downstream business opportunities (Koon, 2011). Hence, many entrepreneurs around Southeast Asia take the opportunity to work in this industry on a large scale, including Malaysia (Alif et al., 2016). Continuous demand of EBN by the Chinese community from China, Taiwan, Singapore and North America has amplified the local EBN industry as well as the establishment of swiftlet house farming (Seow et al., 2016).

Production of EBN is an emerging industry in Malaysia. This outstanding growth has made Malaysia as the second largest country to produce EBN next to Indonesia with a total production of 228-tonne metrics of EBN in 2016 (Isa, 2016). In that same year, the industry was reported to be worth RM 1.2 billion (Akmal et al., 2017). However, the overall value of Malaysia's EBN is generally unstable and is mainly determined by the quality of raw EBN (Looi & Omar, 2016). Due to its high demand and high economic value, it is important to have a standardised criteria and quality control from the relevant authorities to ensure the EBN products from Malaysia are safe for consumption (Koon, 2011).

Although many researches have been done on the study of EBN, the microbiological quality and the anti-microbial quality of EBN is yet to be confirmed. As Malaysia is one of the biggest exporters of EBN, extensive research on EBN is important to produce high quality EBN and to gain knowledge of its medicinal properties which in turn, this valuable information could help EBN industry in our country to sustainably grow.

### **1.1 Hypothesis of the study**

The study was carried out with the following hypothesis:

- 1) EBN that is processed in premise that practices Good Manufacturing Practice (GMP) were expected to have better microbiological quality and lower microbial load compared to non-GMP certified premise during two months of storage,
- 2) Prevalence of bacterial normal flora of swiftlet's faeces at different geographical areas are expected to be different,
- 3) Pure EBN extract were expected to possess antibacterial and antifungal properties due to the bioactive component it possesses.

### **1.2 Objectives of the study**

The objectives of the study were:

- 1) to assess the microbial quality of EBN from two premises,
- 2) to identify and compare the bacterial prevalence of swiftlet's faeces collected from three different geographical locations,
- 3) to assess the antimicrobial properties of EBN against selected pathogenic bacteria and fungi.

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