PROXIMATE ANALYSIS AND SAFETY PROFILE OF FARMED EDIBLE BIRD’S NEST IN MALAYSIA AND ITS EFFECT ON CANCER CELLS

TAN SIN NEE

FPSK(m) 2019 17
PROXIMATE ANALYSIS AND SAFETY PROFILE OF FARMED EDIBLE
BIRD’S NEST IN MALAYSIA AND ITS EFFECT ON CANCER CELLS

By

TAN SIN NEE

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

PROXIMATE ANALYSIS AND SAFETY PROFILE OF FARMED EDIBLE BIRD’S NEST IN MALAYSIA AND ITS EFFECT ON CANCER CELLS

By

TAN SIN NEE

November 2018

Chairman: Associate Professor Christopher Lim Thiam Seong
Faculty: Medicine and Health Sciences

Edible bird’s nest (EBN), a solidified swiftlet’s saliva, is the most valuable animal products consumed by human in Asia. EBN was documented to contain high nutritional values and various medicinal properties. Good nutritional profile containing high protein, carbohydrate, fat, and trace elements found in EBN have been well established from previous studies done in different countries in Malaysia, Indonesia and Thailand. However, the previous proximate analysis studies of Malaysia EBN were not representative from all the regions in Malaysia. In recent years, many safety issues which included high nitrate and nitrite contents, presence of heavy metal, fungal infection and cancer cell stimulation in vitro were also found to be associated with EBN. Although the EBN are known to be dirtier during haze period as the saliva from the swiftlet are contaminated from air pollution, there has been no prior study to assess the effect on haze towards the quality of EBN. Hence, this study was carried out to determine the proximate analysis, safety profile during normal and haze period, and its effect on cancer cells of farmed EBN in Malaysia.

Seven raw cleaned EBN samples were sourced from 6 different regions in Malaysia. Proximate analysis and were performed by using official AOCA methods according to the Malaysia Standard MS 2509:2012. This study showed a consistency of high protein (53.03%-56.37%) and carbohydrate (27.97%-31.68%) with acceptable level of moisture (10.8-14.04%) and ash (2.22%-3.38%). Besides that, a good safety profile was obtained with low nitrite and nitrate level, undetectable heavy metals and no significant growth for pathogenic microorganism. However, this study had found above tolerance level of mould in EBN. Haze contaminated EBN exhibited no significant differences in terms of nutritional, heavy metal and microorganism profiles. The presence of epidermal growth factor (EGF) like peptides was postulated as the important key of cancer cell stimulation. Chicken EGF Enzyme-linked immunosorbent assay (ELISA) was used for quantification of EGF content. In this study, EGF (30.7 pg/mL and 74.5 pg/mL) were detected in crude EBN 01 (Rompin) and EBN 02 (Sibu), respectively but not in all digested EBN samples.
and even after post 10 times concentrated EBN extract. However, both the results were below quantification level.

Microculture tetrazolium assay (MTT) was carried out for the assessment of growth stimulation by different concentration of human EGF (hEGF) in comparison to EBN in 4 different cancer cell lines: MCF-7, Caco-2, HCT116, and A549 for 24, 48, and 72 h. Cancer cell growth was significantly increased after treatment with hEGF. However no significant cell growth was observed after treatment with EBN.

In conclusion, EBN in Malaysia has good consistency of nutritional profile, free from heavy metals, within tolerance level of nitrate and nitrite, and also microorganism profile except mould content. Furthermore, in vitro study indicated that EBN is not associated with cancer cell growth stimulation.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

ANALISIS PROKSIMAT DAN PROFIL KESELAMATAN SARANG BURUNG WALIT MALAYSIA DAN KESAN TERHADAP SEL KANSER

Oleh

TAN SIN NEE

November 2018

Pengerusi : Profesor Madya Christopher Lim Thiam Seong
Falkuti : Perubatan dan Sains Kesihatan

Sarang burung walit (EBN), iaitu air liur burung walit merupakan salah satu produk haiwan yang paling mahal di seluruh dunia. EBN dianggap sebagai bahan makanan mewah dan berprestij yang mengandungi kandungan nutrisi yang tinggi dan pelbagai khasiat perubatan. Pelbagai kajian dari Malaysia, Indonesia dan Thailand telah membuktikan bahawa sarang burung merupakan bahan makanan yang mengandungi kandungan nutrisi yang tinggi termasuklah protein, karbohidrat, lemak dan unsur elemen. Walaubagaimanapun, kajian analisis proksimat EBN Malaysia sebelum ini tidak merangkumi semua kawasan di Malaysia. Sejak kebelakangan ini, pelbagai isu keselamatan telah dikaitkan dengan EBN seperti kandungan nitrit dan nitrat yang tinggi, pencemaran logam, pencemaran kulat atau bakteria serta perangsangan sel kanser dapat dikesan melalui \textit{in vitro}. Selain itu, kehilatan EBN Malaysia adalah lebih kotor semasa musim jerebu disebabkan pencemaran udara, tetapi, tiada kajian sebelum ini yang menilai kesan pencemaran jerebu terhadap kualiti EBN. Oleh demikian, kajian ini adalah bertujuan untuk menentukan analisis proksimat, profil keselamatan dalam udara normal dan semasa pencemaran jerebu, dan kesan EBN Malaysia terhadap sel kanser.

Tujuh EBN mentah yang telah dibersihkan telah dikumpulkan dari 6 kawasan berbeza di Malaysia. Analisa proksimal dijalankan menggunakan kaedah rasmi AOCA mengikut piawai Malaysia (MS) 2509:2012. Keputusan mendapati bahawa EBN Malaysia mengandungi tahap protein dan karbohidrat yang konsisten (53.03% - 56.37% dan 27.97%-31.68%) serta tahap kelembapan dan abu yang boleh diterima (10.8-14.04% dan 2.22%-3.38%). Selain itu, EBN Malaysia juga mempunyai profil keselamatan yang bagus, dengan kandungan nitrit dan nitrat yang rendah, tiada kandungan logam yang dikesan, dan tanpa pembiaakan mikroorganisma yang boleh menyebabkan penyakit. Walaubagaimanapun, kajian ini telah menemukan kandungan kulat yang melebihi tahap yang boleh diterima. EBN yang terdedah kepada jerebu juga tidak menunjukkan perbezaan dari segi nutrisi, kandungan logam, serta profil mikroorganisma. Kandungan peptida merupai faktor pertumbuhan epidermal dianggapkan sebagai factor penting yang berfungsi untuk merangsang sel kanser. ELISA (enzyme-linked
immunosorbent assay) telah digunakan untuk menentukan kuantiti kandungan EGF dalam EBN. Menerusi kajian ini, EGF sebanyak 30.7 pg/mL dan 74.5 pg/mL telah dikesan dalam EBN 01 (Rompin) dan EBN 02 (Sibu) yang mentah. Tetapi, EGF tidak dapat dikesan dalam EBN yang telah diproses atau dalam EBN yang telah diproses dan selepas menjalani proses kepekatan 10 kali dari kepekatan asal. Namun begitu, kedua-dua kandungan ini adalah dibawah tahap yang boleh dikuantiti.

Microculture tetrazolium (MTT) digunakan untuk menilai kesan rangsangan terhadap pertumbuhan sel kanser selepas dirawat dengan EBN berbanding kepada EGF manusia (hEGF) yang berlainan kepekatan untuk 4 sel kanser termasuklah MCF-7, Caco-2, HCT116, dan A549 selama 24, 48 dan 72 jam. Pertumbuhan sel kanser meningkat dengan ketara setelah dirawat dengan hEGF. Walau bagaimanapun, tiada pertumbuhan sel kanser ketara yang disaksikan setelah dirawat dengan EBN.

Kesimpulannya, EBN dari Malaysia adalah berkandungan nutrisi yang tinggi, bebas dari logam, dan mempunyai tahap nitrit, nitrat, dan profil mikroorganisma yang boleh diterima. Tambahan lagi, kajian in vitro juga menunjukkan bahawa EBN tidak dikaitkan dengan perangsangan sel kanser.
ACKNOWLEDGEMENTS

Firstly, I would like to express my deepest gratitude to my supervisor, Associate Prof. Dr. Christopher Lim Thiam Seong for giving me with this great opportunity to work on this research project. I would like to express my appreciation toward him for his precious time to facilitate me by providing me his valuable advices, suggestions and constant guidance in writing this thesis. His continuous guidance and encouragement without failed had made the completion of this thesis possible. I am very thankful for his patience, dedication, intellectual support, trust and have confidence on me in completing this thesis. Without his support, I might not be able to complete my work and thesis.

I would also like to extend my sincere gratitude and appreciation to my co-supervisor, Prof. Dr. Johnson Stanslas, who had guided me throughout my laboratory experience and thesis writing. His guidance, support, and valuable feedback had helped me a lot in handling the investigation of this research work. His criticism had stimulates me to push myself further to produce a quality thesis. It has been a real honour and good learning experience working in your laboratory. I am also thankful for his time in reading and giving feedback to improve my thesis. I could not have wished for a better and helpful co-supervisor.

During the course of this master of sciences programme, I have met and worked with a great numbers of people who had contributed in all sort of ways to my research thesis. I would like to take this opportunity to convey my gratitude to them by special mention in my humble acknowledgement. My heartfelt thanks go to my research mate, Careena Shobana for facilitating in EBN preparation and her constant support, Dr Dahiru Sani and Teh Yuan Han for helping out with my ELISA dilemma, Michelle Tan Siying for teaching me in cell culture work, and Ibrahim Sulaiman for assisting me in my statistical analysis. I would like to express my sincere gratitude again to them in making the completion of my research thesis possible.

Last but not least, I would like to express my deepest appreciation toward my loving father, Tan Zee Chow; mother, See Sio Ke; elder brothers, Tan Kah Ong and Tan Kah Shin; and younger sisters Tan Sin Shi and Tan Sin Yi, for their everlasting support, concern and unconditional love throughout the years of my study. Therefore, I dedicated this thesis to my beloved family members.
I certify that a Thesis Examination Committee has met on 21 November 2018 to conduct the final examination of Tan Sin Nee on her thesis entitled "Proximate Analysis and Safety Profile of Farmed Edible Bird's Nest in Malaysia and its Effect on Cancer Cells" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

**Rajesh a/l Ramasamy, PhD**  
Associate Professor  
Faculty of Medicine and Health Sciences  
Universiti Putra Malaysia  
(Chairman)

**Chong Gun Hean, PhD**  
Associate Professor  
Faculty of Food Science and Technology  
Universiti Putra Malaysia  
(Internal Examiner)

**Aman Shah bin Abdul Majid, PhD**  
Associate Professor  
Quest International University Perak  
Malaysia  
(External Examiner)

[Signature]

**RUŞLI HAJI ABDULLAH, PhD**  
Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 31 January 2019
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

**Dr Christopher Lim Thiam Seong, Mb BCH BAO (QUB, UK), MRCP (UK), FAMS (S’pore), FRCP (Edinburgh)**
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

**Johnson Stanslas, BSc (Hons), MSc, PhD**
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

---

**ROBIAH BINTI YUNUS, PhD**
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:
Declaration by graduate student

I hereby confirm that:
• this thesis is my original work;
• quotations, illustrations and citations have been duly referenced;
• this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
• intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
• written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
• there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _______________________                              Date: __________________

Name and Matric No.: Tan Sin Nee (GS41415)
Declaration by Members of Supervisory Committee

This is to confirm that:
• the research conducted and the writing of this thesis was under our supervision;
• supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature : _________________________
Name of Chairman of Supervisory Committee : _________________________

Signature : _________________________
Name of Member of Supervisory Committee : _________________________
# TABLE OF CONTENTS

| Section                                      | Page |
|----------------------------------------------|------|
| ABSTRACT                                    | i    |
| ABSTRAK                                      | iii  |
| ACKNOWLEDGEMENTS                             | v    |
| APPROVAL                                     | vi   |
| DECLARATION                                  | viii |
| LIST OF TABLES                               | xiii |
| LIST OF FIGURES                              | xiv  |
| LIST OF ABBREVIATIONS                        | xv   |

## CHAPTER

### 1 INTRODUCTION

1.1 Overview  
1.2 Hypothesis  
1.3 Objectives  
1.4 Specific objectives

### 2 LITERATURE REVIEW

2.1 Edible nest swiftlet
2.2 Swiftlet ranching
2.3 Edible bird nest
   2.3.1 Medicinal Properties
      2.3.1.1 Anti-inflammatory and antioxidant activity
      2.3.1.2 Anti-aging properties
      2.3.1.3 Cell proliferation
      2.3.1.4 Anti-influenza virus
      2.3.1.5 Neuroprotective properties
      2.3.1.6 Bone and cartilage regeneration
      2.3.1.7 Cytoprotective properties
      2.3.1.8 Sex hormones
      2.3.1.9 Gastric protection
      2.3.2 Nutritional Values
      2.3.3 Safety Issues
         2.3.3.1 Nitrate and nitrite
         2.3.3.2 Heavy metal
         2.3.3.3 Effects EBN on cancer cells
2.4 Overview of cancer
   2.4.1 Factors influencing cancer growth
3 PROXIMATE ANALYSIS, EPIDERMAL GROWTH FACTOR AND SAFETY PROFILES OF EDIBLE BIRD’S NEST

3.1 Introduction 20
3.2 Materials and methods
  3.2.1 Material
    3.2.1.1 EBN samples 21
    3.2.1.2 EBN coding 22
    3.2.1.3 Chemical/Reagents 22
  3.2.2 Methodology
    3.2.2.1 Analysis methods 23
    3.2.2.2 EBN Preparation 23
    3.2.2.3 Sample (extract) Digestion 23
    3.2.2.4 Sample Concentration 24
    3.2.2.5 ELISA for EGF 24
3.3 Statistical analysis 24
3.4 Results
  3.4.1 Protein 25
  3.4.2 Carbohydrate 25
  3.4.3 Moisture 25
  3.4.4 Ash 25
  3.4.5 Crude fat 25
3.5 Safety profile
  3.5.1 Nitrate and nitrite 25
  3.5.2 Heavy metal 26
  3.5.3 Microbiology profile 26
3.6 Comparing EBN collected during haze period 26
3.7 EBN ELISA EGF result 26
3.8 Discussion 32
3.9 Conclusion 35

4 EFFECT OF EBN ON THE GROWTH OF CANCER CELLS

4.1 Introduction 36
4.2 Materials and methodology
  4.2.1 Materials
    4.2.1.1 EBN preparation 36
    4.2.1.2 Cell lines 37
    4.2.1.3 Chemicals and Reagents 37
    4.2.1.4 Tissue culture materials 37
  4.2.2 Methods
    4.2.2.1 Preparation of Test Compound 37
    4.2.2.2 General cell culture procedure 37
    4.2.2.3 Plating 38
    4.2.2.4 Microculture Tetrazolium (MTT) 38
    4.2.2.5 MTT cell viability assay 39
4.3 Statistical analysis 39
4.4 Results 40
4.5 Discussion 45
4.6 Conclusion 46
5 SUMMARY, CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH
5.1 Summary 47
5.2 Conclusion 48
5.3 Recommendation for Future Research 48

REFERENCES 49
APPENDICES 58
BIODATA OF STUDENT 59
LIST OF PUBLICATIONS 60
# LIST OF TABLES

| Table | Page |
|-------|------|
| 3.0   | 22   |
| 3.1   | 27   |
| 3.2   | 28   |
| 3.3   | 29   |
| 3.4   | 30   |
| 3.5   | 31   |

3.0 EBN coding according to the region

3.1 Proximate composition of the different samples of EBN collected from different regions in Malaysia

3.2 Nitrite and nitrate content of the different samples of EBN collected from different regions in Malaysia

3.3 Heavy metals content of the different samples of EBN collected from different regions in Malaysia

3.4 Microbiology profile of the different samples of EBN collected from different regions in Malaysia

3.5 Chicken EGF ELISA quantification result of seven crude and digested EBN samples from different regions in Malaysia and post ultra-centrifugal filter of the digested EBN from three selected regions (EBN 01, 02 and 07)
## LIST OF FIGURES

| Figure | Description                                                                 | Page |
|--------|-----------------------------------------------------------------------------|------|
| 3.1    | Seven raw cleaned EBNs that collected from different regions in Malaysia.    | 21   |
| 3.2    | The physical appearance of raw cleaned EBNs collected from Jerantut with API < 100 and Port Klang during the haze period with unhealthy API 100 - 200. | 22   |
| 4.1    | Caco-2 cell viability post treatment with different concentrations of hEGF and EBN at 24, 48 and 72 h | 41   |
| 4.2    | MCF-7 cell viability post treatment with different concentrations of hEGF and EBN at 24, 48 and 72 h | 42   |
| 4.3    | HCT116 cell viability post treatment with different concentrations of hEGF and EBN at 24, 48 and 72 h | 43   |
| 4.4    | A549 cell viability post treatment with different concentrations of hEGF and EBN at 24, 48 and 72 h | 44   |
| 4.5    | Protein blast of human EGF and avian EGF                                   | 46   |
**LIST OF ABBREVIATIONS**

| Abbreviation | Full Form |
|--------------|-----------|
| AKT          | Serine/threonine kinase |
| ANOVA        | Analysis of variance |
| AOCA         | Association of Official Analytical Chemistry |
| API          | Air pollution Index |
| AREG         | Amphiregulin |
| ATCC         | American Tissue Culture Collection |
| BLAST        | Basic Local Alignment Search Tool |
| BTC          | Betacellulin |
| CFU          | Colony-forming units |
| CO₂          | Carbon dioxide |
| DMSO         | Dimethylsulfoxide |
| DNA          | Deoxyribonucleic acid |
| EBN          | Edible bird’s nest |
| EDTA         | Ethylenediaminetetraacetic acid |
| EGF          | Epidermal growth factor |
| EGFR         | Epidermal growth factor receptor |
| ELISA        | Enzyme-linked immunosorbent assay |
| EPGN         | Epigen |
| EREG         | Epiregulin |
| ERK          | Extracellular signal-regulated kinase |
| FBS          | Fetal bovine serum |
| FDA          | Food and Drug Authority |
| FIMS         | Flow Injection Mercury System |
| GalNAc       | N-Acetyl-D-Galactosamine |
| GMP          | Good Manufacturing Practice |
| hADSCs       | Human adipose-derived stem cells |
| HBEGF        | Heparin-binding EGF-like growth factor |
| hEGF         | Human epidermal growth factor |
| HFD          | High fat diet |
| H₂O₂         | Hydrogen peroxide |
| ICP-MS       | Inductively coupled plasma-mass spectrometry |
| IG           | Immunoglobulin |
| IL           | Interleukin |
| ISO          | International Organization for Standardization |
| LF           | Lactoferrin |
| LPS          | Lipopolysaccharide |
| MAPK         | Mitogen-activated protein kinase |
| MDCK         | Madin-Darby canine kidney |
| MTT          | Microculture tetrazolium |
| NANA         | N-Acetyl-neuraminic acid |
| NHFs         | Normal human fibroblasts |
| NO           | Nitrite oxide |
| NS1          | Non-structural protein 1 |
| OA           | Osteoarthritis |
| OVF          | Ovotransferrin |
| PAGE         | Polyacrylamide gel electrophoresis |
| PBS          | Phosphate buffered saline |
| PDT          | Population doubling time |
| Abbreviation | Full Form |
|--------------|-----------|
| PPB          | Parts-per billion |
| PPM          | Parts-per million |
| ROS          | Radical oxygen species |
| RPMI         | Roswell Park Memorial Institute |
| SD           | Standard deviation |
| SDS          | Sodium dodecyl sulphate |
| SIRIM        | Standards and Industrial Research Institute of Malaysia |
| SPSS         | Statistical Package for Social Sciences |
| STAT         | Signal transducer and activator of transcription |
| TGF-α        | Transforming growth factor-alpha |
| TNF-α        | Tumour necrosis factor alpha |
| VEGF         | Vascular endothelial growth factor |
| WHO          | World Health Organization |
CHAPTER 1

INTRODUCTION

1.1 Overview

Edible Bird’s Nest (EBN), a solidified saliva made from the birds of Aerodramus genus family, total of four different species of swiftlets. There are Aerodramus fuciphagus, Aerodramus maximus, Aerodramus germani and Aerodramus unicolor. EBN has existed many centuries ago and can be traced back as early as the Tang (618–907 AD) and Sung (960–1279 AD) dynasties whereby they were used as main ingredients in traditional Chinese medicine and Chinese cuisine (Lim and Cranbrook, 2002). EBN was prepared by using double boiler method with additional rock sugar to produce the gastronomic delicacy, well known as “bird's nest soup”. Since ancient times, EBN has been also regarded as a luxurious and prestigious food item which contains high nutritional values and various medicinal properties (Oda et al., 1998). For the past 500 years, EBN has been traded in Malaysia and it was documented that trading occurred between the Malay Archipelagos and China since the 16th century (Sankaran, 2001).

High nutritional and medicinal values of EBN and the dangers that might be encountered during the nest collection, EBN is one of the world's most valuable animal product which consumed by mankind with average selling price of $2500 per kilogram. The Malaysian government recognised the EBN industry has huge economic potential. Malaysia had exported beyond 100 metric tons of EBN with the value of RM 500 million in 2009. EBN trading is expected to contribute more than RM 5.2 billion to the Gross National Income in 2020 (Rabu and Mohd, 2015). Because of the continuous increase demand of the EBN to avoid overharvesting practice, it has led to a large decline in cave yields EBN (Sankaran, 2001). In Malaysia, with the present rate of cave EBN harvesting, cave swiftlet species may be wipe out in 10-15 years time. Malaysia wildlife and National Park Department has reported that drastic decline in nest production as the wild swiftlet population in the Niah Cave has dropped from 1.7 millions in 1935 to 200000 in 1996 and 65000 in 2002 due to uncontrolled and overharvesting (Lim, 2006). The decreased of cave nest production had bloomed the development of EBN farming in many South East Asia countries (Nugroho and Whendrato, 1996). The most famous natural cave swiftlet nesting sites in Malaysia are located in Sarawak and Sabah, and other areas are mainly EBN farming located in Peninsular Malaysia are Sitiawan, Teluk Intan, Kota Bharu, Kuala Terengganu, Bukit Mertajam, Nibong Tebal, Kuantan, Muar, Segamat and many other old townships.

EBN contains high protein, carbohydrate, and trace elements (Marcone, 2005). The proximate analysis of EBN from different countries for example, Malaysia (Huda et al., 2008; Norhayati et al., 2010; Lee et al., 2015), Indonesia (Marcone, 2005) and Thailand (Saengkrajang et al., 2013) has been well established. A useful glycoprotein found in EBN is sialic acid, about 7.1% - 9% (Wang et al., 2006) which may have benefit on neurological development (Palmano et al., 2015). Other than nutritional profile which has been well studied, many researchers have identified some of its impressive medicinal properties include potent anti-inflammatory activity by
suppressing the production of tumour necrosis factor alpha (TNF-α) (Aswir and Wan, 2011), inhibition of influenza virus infection (Haghani et al., 2016), alleviation of asthma (Matsukawa et al, 2011), chemoprotective properties from cyclophosphamide (Zhao et al., 2016), neuroprotective effect (Careena et al., 2018), EGF like peptide which promote cell proliferation (Kong et al., 1987), and estradiol hormones (Ma and Liu, 2012) which may be beneficial in women health by improving menstrual dysfunctions such as amenorrhea and irregular menstruation (Brendan, 2016).

EGF was found to be present in EBN traced back in 1987 (Kong et al., 1987). Human EGF is known to have the ability to stimulate cell growth and proliferation. Total of seven ligands regulation were identified: EGF, transforming growth factor-alpha (TGFA), heparin-binding EGF-like growth factor (HBEGF), betacellulin (BTC), amphiregulin (AREG), epiregulin (EREG), and epigen (EPGN) and four ErbB (HER) family receptor tyrosine kinases: EGFR/ErbB1, ErbB2, ErbB3 and ErbB4 (Wee and Wang, 2017). EGF acts as a mitogenic factor that plays a growth stimulating role in various epidermal and epithelial tissues (Kumar, Abbas, and Aster, 2015). The activation of EGF will lead to the initiation of a cascade of downstream signaling pathways, such as signal transducer and activator of transcription (STAT), mitogen-activated protein kinase (MAPK), and the modulation of calcium channels. Hence, EBN is also deemed to have rejuvenating and anti-aging properties. However, the receptor for EGF (EGFR) has been found highly expressed in various solid tumours and the dysregulation of EGF is also associated with the growth and progression of many cancers. Previous paper had shown that different source and type of EBN stimulates colon cancer cells growth in vitro (Aswir and Wan, 2011). This raises an important issue whether the presence of EGF in EBN may stimulate cancer cell growth after consumption.

Besides the presence of EGF and possible of cancer cell stimulation issue, there are many others safety issues arise from Malaysia EBN in recent years for example, high nitrate and nitrite contents (Paydar et al., 2013; Quek et al., 2015), presence of heavy metal (Chen et al., 2014; Lee et al., 2015), pathological microorganism and fungal infection (Chen et al., 2015). Moreover, Malaysia experiences severe haze episode every years due to open burning from nearby countries. From observation by the expert, the EBN collected from haze affected area with high Air Pollution Index (API) appeared to be dirty and blackish colour. However, there was no previous study has looks into the effect of haze toward the nutritional and safety profile of EBN. Therefore, this paper is to establish the nutritional and safety profile in term of heavy metal, microbiology and nitrate and nitrite contents of EBN, to assess the effect of haze on the quality of EBN, to quantify the EGF in EBN from different regions in Malaysia and to determine the possible growth effect on cancer cells by EGF found in EBN.
1.2 Hypothesis

Despite many studies regarding proximate analysis and medicinal properties of EBN but there were still many safety issues arise from EBN like high nitrate and nitrite contents, presence of heavy metal, microorganism and fungal infection. Those safety issues were not thoroughly investigated before. The EBN issues which arised may potentially harmful to human. Hence, this study was carried out to ensure that EBN is safe for human consumption in term of nitrate and nitrite, heavy metal, microorganism profile and carcinogenic risk.

The null hypotheses in this study were

- EBN has no good nutritional value or safety profile for human consumption.
- There are no significant differences in term of safety and nutritional profile found in EBN collected from different regions in Malaysia.
- There are no significant differences in term of safety and nutritional profile for the haze polluted EBN.
- EBN does not contain EGF.
- EBN does not stimulate human cancer cell growth.

1.3 General objective

The general objective of this study was to determine the nutritional, epidermal growth factor, safety and microorganism profile of EBN sourced from various regions in Peninsula and East Malaysia and the potential of cancer cell growth stimulation by EBN in vitro.

1.4 Specific objective

The specific objectives as follow:

1) To determine the nutritional values and epidermal growth factor level of EBN from different regions in Malaysia
2) To assess the toxicity and safety profile of EBN harvested in normal and haze conditions.
3) To investigate the in vitro effect of EBN on various cancer cell lines.
REFERENCES

Ai, G.H., Shao, X.W., Meng, M.M.D., Song, L., Qiu, J., Wu, Y., Zhou, J., Cheng, J., & Tong, X. (2017). Epidermal growth factor promotes proliferation and maintains multipotency of continuous cultured adipose stem cells via activating STAT signal pathway in vitro. *Medicine (Baltimore).* 96 (30): 7607.

Anand, P., Kunnnumakkara, A.B., Sundaram, C., Harikumar, K.B., Tharakan, S.T., Lai, O.S., Sung, B., & Aggarwal, B.B. (2008). Cancer is a preventable disease that requires major lifestyle changes. *Pharmaceutical Research.* 25 (9): 2097-116.

Ani, M., & Boedi, M. (2004). Habitat & productivity of edible-nest swiflets: lesson learned from Indonesia. In: Proceedings Edible-Nest Swiflets Management in Asia. 59-6.

AOAC, (2005). AOAC Official Methods of Analysis, 17th ed. Association of Official Analytical Chemistry, Washington, DC.

Argueso, P., Tisdale, A., Mandel, U., Letko, E., Foster, C.S., & Gipson, I. (2003). The cell-layer and cell-type-specific distribution of GalNac-transferases in the ocular surface epithelia is altered during keratinisation. *Investigation Ophthalmology and Visual Science.* 44, 86-92.

Aswir, A.R., & Wan Nazaimoon, W.M. (2011). Effect of edible bird’s nest on cell proliferation and tumor necrosis factor-alpha (TNF-a) release in vitro. *International Food Research Journal.* 18 (3) 1073–1077.

Azman, O., Siti, H.Y., & Norazlinda, A.R. (2004). Conservation of swiflets in Peninsular Malaysia. In: Proceedings Edible-Nest Swiflets Management in Asia, 28-34.

BirdLife International. (2016). Aerodramus fuciphagus. The IUCN Red List of Threatened Species 2016: e.T60847684A95163737. Available from [http://dx.doi.org/10.2305/IUCN.UK.2016-3](http://dx.doi.org/10.2305/IUCN.UK.2016-3)

Birtwistle, M.R., Rauch, J., Kiyatkin, A., Aksamitiene, E., Dobrzyński, M., Hoek, J.B., Kolch, W., Ogunnaike, B.A., & Kholodenko, B.N. (2012). Emergence of bimodal cell population responses from the interplay between analog single-cell signaling and protein expression noise. *BMC Systems Biology.* 6:109.

Brendan, V.I. (2016). Estrogen and the Menstrual Cycle in Humans. *Embryo Project Encyclopedia.* ISSN: 1940-5030. [http://embryo.asu.edu/handle/10776/11344](http://embryo.asu.edu/handle/10776/11344)

But, P.P.H., Jiang, R.W., & Shaw, P.C. (2013). Edible bird’s nests—How do the red ones get red? *J Ethnopharm.* 145(1): 378–380.

Bynny, R.L., Orth, D.N., Cohen, S., & Doyne, E.S. (1974). Epidermal growth factor: Effect of androgens and adrenergic agents. *Endocrinology.* 95, 776-782.
Caroline, A., Diana, N.T., & Phillip, S.L. (2016). Skin rejuvenation using cosmetic products containing growth factors, cytokines, and matrikines: a review of the literature. *Clin Cosmet Investig Dermatol*. 9: 411–419.

Chen, J.X.J., Lim, P.K.C, Wong, S.F., & Mak, J.W. (2014). Determination of the presence and levels of heavy metals and other elements in raw and commercial edible bird nests. *Malaysian Journal of Nutrition*. 20(3): 377-391.

Chen, J.X.J., Wong, S.F., Lim, P.K.C., & Mak, J.W. (2015). Culture and molecular identification of fungal contaminants in edible bird nests. *Food Additives & Contaminants: Part A*. 32:12, 2138-2147.

Chua, K.H., Lee, T.H., Nagandran, K., Yahaya, N.H., Lee, C.T., Tan, E.T.T., & Aziz, R.A. (2013). Edible Bird's nest extract as a chondro-protective agent for human chondrocytes isolated from osteoarthritic knee: in vitro study. *BMC Complement Alternative Med*. 13:19.

Cohen, S. (1962). Isolation of a mouse submaxillary gland protein accelerating incisor eruption and eyelid opening in new-born animal. *J. biol. Chem.* 237(5), 1555-1562.

Corsinovi, L., Biasi, F., Poli, G., Leonarduzzi, G., & Isaia, G. (2011). Dietary lipids and their oxidized products in Alzheimer's disease. *Molecular Nutrition and Food Research*. 55(2): S161–S172.

Cranbrook, Earl, Goh, W. L., Lim, C. K., & Rahman, M. A. (2013). The Species of White-nest Swiftlets (Apodidae, Collocaliini) of Malaysia and the Origins of House-farm Birds. *Forktail*. 29: 78–90.

Dunn, L.B., Damesyn, M., Moore, A.A., Reuben, D.B., & Greendale, G.A. (1997). Does estrogen prevent skin aging? Results from the First National Health and Nutrition Examination Survey (NHANES I). *Arch Dermatol*. 133(3):339-42.

FAO/WHO/UNU Expert Consultation. (2007). Protein and Amino Acid Requirements in Human Nutrition. Technical Report Series 935.

Farhana, S., Hafidzi, M.N., & Burhanuddin, M. (2016). Diversity of the Insects in the Diet of Edible Nest Swiftlets in Oil Palm Plantations. *Journal of Biodiversity and Environmental Sciences*. 8. 39-48.

Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D.M., Forman, D., & Bray, F. (2015). Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer*. 136(5): 359-86.

Fox, A. Microbiology and immunology Online. Columbia. (2016). Chapter 11. Bacteriology: Enterobacteriaceae, Vibrio, Campylobacter and helicobacter. Available from: [http://www.microbiologybook.org/fox/enterobact.htm](http://www.microbiologybook.org/fox/enterobact.htm)

Fujita MS. (2012). Sustainable use of Rich Bird Diversity in “Biomass Society”. Newsletter Southeast Asian Studies Kyoto University. No 66 Autumn. 19-21.
Gausset, Q. (2004). Chronicle of a Foreseeable Tragedy: Birds’ Nests Management in the Niah Caves (Sarawak). Human Ecology. 32(4): 487-507.

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 Funct. 8(5): 2046-2052.

Giovanni, D. P., & Franco, S. (2013). Obesity as a Major Risk Factor for Cancer. J Obes. 291546.11.

Goh, D.L.M., Chua, K.Y., Chew, F.T., Seow, T.K., Ou, K.L., Yi, F.C., & Lee, B.W. (2001). Immunochemical characterization of edible bird's nest allergens. The Journal of Allergy and Clinical Immunology. 107(6), 1082–1088.

Goumans, M.J., Valdimarsdottir, G., Itoh, S., Lebrin, F., Larsson, J., Mummery, C., Karlsson, S., & Dijke, P. (2003). Activin receptor-like kinase (ALK) 1 is an antagonistic mediator of lateral TGFbeta/ALK5 signaling. Mol Cell. 12:817–828.

Goyer, R., & Golub, M. (2003). Paper human health effects of metals. US EPA. 1-29.

Guo, C.T., Takahashi, T., Bukawa, W., Takahashi, N., Yagi, H., & Kato, K. (2006). Edible bird’s nest extract inhibits influenza virus infection. Antiviral Research. 70, 140–146.

Haghani, A., Mehrbod, P., Safi, N., Aminuddin, N.A., Bahadoran, A., Omar, A.R., & Ideris, A. (2016). In vitro and in vivo mechanism of immunomodulatory and antiviral activity of Edible Bird’s Nest (EBN) against influenza A virus (IAV) infection. J Ethnopharmacol. 185: 327–340.

Hardwicke, J., Schmaljohann, D., Boyce, D., & Thomas, D. (2008). Epidermal growth factor therapy and wound healing – past, present and future perspectives. Surgeon. 6(3):172–177.

Hou, Z., Imam, M.U., Ismail, M., Ismail, N., Yida, Z., Ideris, A., Sarega, N., & Mahmud, R. (2015). Effects of edible bird's nest on hippocampal and cortical neurodegeneration in ovariectomized rats. Food Function. 6(5): 1701-1711.

Hou, Z., Imam, M.U., Ismail, M., Azmi, N.H., Ismail, N., Ideris, A., & Mahmud, R. (2015). Lactoferrin and ovotransferrin contribute toward antioxidative effects of Edible Bird’s Nest against hydrogen peroxide-induced oxidative stress inhuman SH-SY5Y cells. Biosci Biotechnol Biochem. 79(10): 1570-8.

Hu, Q., Li, G., Yao, H., He, S., Li, H., & Liu, S. (2016). Edible bird's nest enhances antioxidant capacity and increases lifespan in Drosophila Melanogaster. Cell Mol Biol (Noisy-le-grand). 30; 62 (4):116-22.

Huda, N.M.Z., Zuki, A.B.Z., Azhar, K., Goh, Y.M., Suhaime, Y.M., Awang, H., A.J., & Zairi, M.S. (2008). Proximate, elemental and fatty acid analysis of preprocessed edible birds’ nest (Aerodramus fuciphagus): a comparison between regions and type of nest. Journal of Food Technology. 6 (1) 39–44.
Ismail, M.F., Sabri, N.A., & Tajuddin, S.N. (2014). A study on contaminated of nitrite in edible bird’s nest (SWIFTLETS). http://www.dvs.gov.my/dvs/resources/user_1/DVS%20pdf/Aneka%20Haiwan/poster%20papers/8_Ismail_UTM.pdf.

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew. B.B., & Beeregowda, N.K. (2014). Toxicity, mechanism and health effects of some heavy metals. Interdiscip Toxicol. 7(2): 60–72. DOI: https://doi.org/10.2478/intox-2014-0009

Jin, K.O., & Elisabete, W. (2014). Infection and Cancer: Global Distribution and Burden of Diseases. Annals of Global Health. 80(5), 384-392.

Kaplan, R.N., Riba, R.D., Zacharoulis, S., Bramley, A.H., Vincent, L., Costa, C., MacDonald, D.D., Jin, D.K., Shido, K., Kerns, S.A., Zhu, Z., Hicklin, D., Wu, Y., Port, J.L., Altorki, N., Port, E.R., Ruggero, D., Smelkov, S.V., Jensen, K.K., Rafii, S., & Lyden, D. (2005). VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature. 438:820–827.

Kathan, R.H., & Weeks, D.I. (1969). Structure studies of Collocalia mucoid: Carbohydrate and amino acid composition. Archives of Biochemistry and Biophysics. 134(2), 572–576.

Kobayashi, M., Lee, H., Nakayama, J., & Fukuda, M. (2009). Roles of gastric mucin-type Oglycans in the pathogenesis of Helicobacter pylori infection. Glycobiology. 19(5), 453–461.

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

Kong, Y.C., Keung, W.M., Yip, T.T., Ko, K.M., Tsao, S.W., & Ng, M.H. (1987). Evidence that epidermal growth factor is present in swiftlet’s (Collocalia) nest. Comparative Biochemistry and Physiology, Part B. Comparative Biochemistry. 87(2): 221–226.

Kuhan, H., & Lee, J. (2005). Swiftlet Farming - The Complete Introductory Guide to Swiftlet Farming, Penang: Struan Inc. Sdn. Bhd.

Kumar, V., Abbas, A., & Aster, J. (2013). Robbins basic pathology. Philadelphia: Elsevier/Saunders. 179.

Kumar, V., Abbas, A., & Aster, J. (2015). Robbins & Cotran Pathology Basis of Disease. Philadelphia: Elsevier/Saunders. 19.

Langham, N. (1980). Breeding biology of the edible-nest swiftlet Aerodramus fuciphagus. Int J Avian Sci. 122(4): 447–461.

Lau, S.M., & Melville, D.S. (1994). International Trade in Swiftlet Nests (with Special Reference to Hong Kong). TRAFFIC International, Cambridge.
Lee, T.H., Waseem, A.W., Tan E.T.T., Nur, A.A., Yong, L.L., & Ramlan, A.A. (2015). Investigations into the physicochemical, biochemical and antibacterial properties of Edible Bird’s Nest. *Journal of Chemical and Pharmaceutical Research*. 7(7):228-247.

Li, H., & Fan, X. (2014). Analysis of Sialic Acids in Chinese Conventional Foods by HPLC-FLD. *Open Journal of Preventive Medicine*. 4: 2, 57-63.

Lim, C. K., & Cranbrook, Earl. (2002). Swiftlets of Borneo - Builders of edible nests. Sabah, Malaysia: Natural History Publication (Borneo) SDN, B.H.D.

Lim, C. K., & Cranbrook, Earl. (2014). Swiftlets of Borneo: Builders of Edible Nests, 2nd ed. Natural History Publications (Borneo), Kota Kinabalu.

Lim, C.K., & Oswald, B.K. (2004). Swiftlets management in Sarawak; conserving wild colonies Malaysia: In: Proceedings Edible-Nest Swiftlets Management in Asia, 35-46.

Lim, C.T.S. (2006). Make millions from swiftlet farming: A definitive guide. Malaysia: Truewealth Sdn Bhd; 6p.

Lin, J. R., Zhou, H., & Lai, X. P. (2006). Review of research on edible bird's nest. *Journal of Chinese Medicinal Materials*. 29(1), 85–90.

Looi Q.H., & Omar A.R. (2016). Swiftlets and edible bird’s nest industry in Asia. *Pertanika J Scholarly Res Rev*. 2(1): 32–48.

Lu, Y., Han, D. B., Wang, J. Y., Wang, D. R., He, R. Y., & Han, L. X. (1995). Study on the main ingredients of the three species of edible swift's nest of Yunnan province. *Zoological Research*. 16(4), 385–391.

Lyden, D., Hattori, K., Dias, S., Costa, C., Blaikie, P., Butros, L., Chadburn, A., Heissig, B., Marks, W., Witte, L., Wu, Y., Hicklin, D., Zhu, Z., Hackett, N.R., Crystal, R.G., Moore, M.A., Hajjar, K.A., Manova, K., Benecza, R., & Rafii, S. (2001). Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. *Nat Med*. 7:1194–1201.

Ma, F.C., & Liu, D.C. (2012). Extraction and determination of hormones in the edible bird’s nest. *Asian Journal of Chemistry*. 24 (1) 117–120.

Malaysia National Cancer Registry Report 2007-2011. (2016). Malaysia cancer statistics, data and figure, 13-17.

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

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. *Biosci Biotechnol Biochem*, 75(3): 590-592.
Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods.* 65(1-2): 55-63.

MS 2273:2010. (2010). Good animal husbandry practice-edible-birdnest swiftlet ranching and its premises. Department of Standards Malaysia.

MS 2333:2010. (2010). Good Manufacturing Practice (GMP) for Processing Raw-Unclean and Raw-Clean Edible-Birdnest (EBN). Department of Standards Malaysia.

MS2334:2011. (2011). Edible-Birdnest - Specification. Department of Standards Malaysia.

Ng, M.H., Chan, K.H., & Kong, Y.C. (1986). Potentiation of mitogenic response by extracts of the swiftlet’s (Collocalia) nest. *Biochemistry International.* 13(3), 521–531.

Nick, I., Markevich, Hoek, J.B., & Kholodenko, B.N. (2004). Signaling switches and bistability arising from multisite phosphorylation in protein kinase cascades. *Journal of cell biology.* 164 (3): 353.

Norhayati, M.K., Azman, O., & Wan Nazaimoon, W.M. (2010). Preliminary study of the nutritional content of Malaysian edible bird’s nest. *Malaysian Journal of Nutrition.* 16 (3) 389–396.

Noriah, R., & Saiful, M.N.A. (2012). Food Safety Governance: Standard Operating Procedure on Controlling of Nitrite Level, Handling and Processing of Edible Bird’s Nest. *Australian Journal of Basic and Applied Sciences.* 6(11): 301-305.

Nugroho, E., & Whendrato, I. (1996). The farming of edible-nest swiftlets in Indonesia. In: Technical Workshop on Conservation Priorities and Actions for Edible-nest Swiftlets, Surabaya, Indonesia.

Oda, M., Ohta, S., Suga, T., & Aoki, T. (1998). Study on food components: the structure of N-linked Asialo carbohydrate from the edible bird’s nest built by Collocalia fuciphaga. *Journal of Agricultural and Food Chemistry.* 46, 3047–3053.

Palmano, K., Rowan, A., Guillermo, R., Guan, J., & Jarrow, P. M. (2015). The Role of Gangliosides in Neurodevelopment. *Nutrients.* 7(5): 3891–3913.

Paydar, M., Wong, Y.L., Wong, W.F., Hamdi, O.A.A., Kadir, N.A., & Looi, C.Y. (2013). Prevalence of nitrite and nitrate contents and its effect on edible bird nest’s color. *J Food Sci.* 78 (12), 1940-1947.

Pehlivan, E., Ozkan, A.M., Dinc, S., & Parlayici, S. (2009). Adsorption of Cu\textsubscript{2+} and Pb\textsubscript{2+} ion on dolomite powder. *Journal of Hazardous Materials.* 167:1-3, 1044–1049.
Perez, R., Crombet, T., Leon, J., & Moreno, E. (2013). A view on EGFR-targeted therapies from the oncogene-addiction perspective. *Front. Pharmacol.* DOI: https://doi.org/10.3389/fphar.2013.00053

Powlson, D.S., Addiscott, T.M., Benjamin, N., Cassman, K.G., Kok, T.M., Van, G.H., Hirondel, J.L., Avery, A.A., & Van, K.C. (2008). When does nitrate become a risk for humans? *J Environ Qual.* 37:291–5.

Quek, M. C., Chin, N. L., Yusof, Y. A., Tan, S. W., & Law, C. L. (2015). Preliminary nitrite, nitrate and colour analysis of Malaysian edible bird’s nest. *Information Processing in Agriculture.* 2(1), 1-5.

Rabu, M.R., & Mohd, N.M.S. Malaysia’s Edible Bird Nest Industry. Taipei, Taiwan: Food and Fertilizer Technology Center Agricultural Policy Platform; (2015). Available from: http://ap.fftc.agnet.org/ap_db.php?id=414

Ramji, M.F.S., Koon, L.C., & Rahman, M.A. (2013). Roosting and nest-building behaviour of the white-nest swiftlet *Aerodramus fuciphagus* (Thunberg) (Aves: Apodidae) in farmed colonies. *Raffles Bull Zool.* 29: 225–235.

Roh, K.B., Lee, J., Kim, Y.S., Park, J., Kim, J.H., Lee, J., & Park, D. (2011). Mechanisms of edible bird's nest extract-induced proliferation of human adipose-derived stem cells. *Evidence-Based Complementary and Alternative Medicine.* 2012: 797520.

Saengkrajang, W., Matan, Na., & Matan, Ni. (2013). Nutritional composition of the farmed edible bird’s nest (Collocalia fuciphaga) in Thailand. *Journal of Food Composition and Analysis.* 31, 41-45.

Sankaran, R. (2001). The status and conservation of the Edible-nest Swiftlet (*Collocalia fuciphaga*) in the Andaman and Nicobar Islands. *Biological Conservation.* 97, 283–294.

Shobana, C., Sani, D., Tan, S.N., Lim, C.W., Hasan, S., Mohtarrudin, N., Kirby, B., Ideris, A., Basri, H., Stanslas, J., & Christopher, T.S.Lim. (2018). Effect of Edible Bird Nest Extract On Lipopolysaccharide-Induced Impairment Of Learning And Memory In Wistar Rats. *Evidence-based Complementary and Alternative Medicine.* 7p.

Singh, P., & Chauhan, M. (2013). Influence of environmental factors on the growth of building deteriorating fungi: *Aspergillus Flavus* and *Penicillium chrysogenum*. *Int J Pharm Sci Res.* 4(1): 425-429.

Stimpson, C.M. (2013). A 48 000 year record of swiftlets (Aves: Apodidae) in Northwestern Borneo: morphometric identifications and palaeoenvironmental implications. *Palaeogeogr, Palaeoclimatol, Palaeocel.* 374: 132–143.

Stoscheck, C. M., & King, L.E.Jr. (1986). Role of epidermal growth factor in carcinogenesis. *Cancer Res.* 46:1030–1037.
Thomas, R., Gathoye, A.M., & Lambert, L. (1976). A complex control circuit. Regulation of immunity in temperate bacteriophages. *Eur. J. Biochem.* 71:211–227.

Thun, M., Peto, R., Lopez, A., Monaco, J., Henley, J., Heath, C., & Doll, R. (1997). *Alcohol consumption and mortality among middle-aged and elderly US adults.* *N Engl J Med.* 337: 1705–1714.

Tong, T. J., & Liu, D. Y. (1985). Isolation and identification of glycopeptide in edible bird's nest. *Science Bulletin.* 12, 949–952.

Torre, L.A., Bray, F., Siegel, R.L., Ferlay, J., Lortet, T.J., & Jemal, A. (2015). Global cancer statistics, 2012. *CA Cancer J Clin.* 65(2):87-108.

Tung, C.H., Pan, J.Q., Chang, H.M., & Chou, S.H. (2008). Authentic Determination of Bird’s Nests by saccharides profile. *J Food and Drug Analysis.* 16 (4): 86-91.

Vainio, H., Bianchini, F. 2002. International Agency for Research on Cancer. Weight control and physical activity. *IARC Handbook of Cancer Prevention.* 6: 1–315.

Venturi, S., & Venturi, M. (2009). Iodine in evolution of salivary glands and in oral health. *Nutritional and health.* 20 (2): 119-134

Vimala, B., Hussain, H., & Nazaimoon, W.M.W. (2012). Effects of edible bird's nest on tumour necrosis factor-alpha secretion, nitric oxide production and cell viability of lipopolysaccharide-stimulated RAW 264.7 macrophages. *Food and Agricultural Immunology.* 23(4): 303-14.

Virupintu, S., Thirakhupt, K., Pradatsundarasar, A.O., & Poonswad, P. (2002). Nest-site characteristics of the edible-nest swiftlet *Aerodramus fuciphagus* (Thunberg, 1812) at Si-Ha Islands, Phattalung Province, Thailand. *Nat Hist J Chulalongkorn Univ.* 2(2): 3135.

Walker, F., Abramowitz, L., Benabderrahmanc, D., Duval, X., Descatoire, V., Hénin, D., Lehy, T., & Aparicio, T. (2009). Growth factor receptor expression in anal squamous lesions: modifications associated with oncogenic human papillomavirus and human immunodeficiency virus. *Human Pathology.* 40 (11): 1517–27.

Wan, K. Z., Liau, L.L., & Chua, K.H. (2016). Bird’s nest extract promotes human stem cells proliferation. Paper presented at the meeting of Stem Cells in Drug Discovery 2016, Cambridge, United Kingdom. April 2016.

Wang, C.C. (1921). The composition of Chinese edible birds' nests and the nature of their proteins. *Journal of Biological Chemistry.* 49(2), 429–439.

Wang, H., Naghavi, M., Allen, C., Barber, R.M., Bhutta, Z.A., & Carter, A. (2015). Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet.* 388 (10053): 1459–1544.
Wang, H., Ni, K.Y., & Wang, Y. (2006). Determination of sialic acid in edible bird’s nest. *Chin J Pharm Anal.* 26(9): 1251–1253.

Wee, P., & Wang, Z.X. (2017). Epidermal Growth Factor Receptor Cell Proliferation Signaling Pathways. *Cancers (Basel).* 9(5): 52. DOI: 10.3390/cancers9050052

WHO (1985a) Guidelines for the study of dietary intake of chemical contaminants. Geneva, World Health Organization (WHO Offset Publication No. 87).

Wong, R. S. (2013). Edible bird's nest: food or medicine? *Chinese Journal of Integrative Medicine.* 19(9), 643–649.

World Cancer Report, 2014. (2014). World Health Organization, Chapter 1.1-6.7.

Yagi, H., Yasukawa, N., Yu, S.Y., Guo, C.T., Takahashi, T., Bukawa, W., Takahashi, N., Yagi, H., & Kato, K. (2008). The expression of sialylated high-antennary N-glycans in edible bird’s nest. *Carbohydr Res.* 343(8):1373-7.

Yang, M., Cheung, S.H., Li, S.C., & Cheung, H.Y. (2014). Establishment of a holistic and scientific protocol for the authentication and quality assurance of edible bird’s nest. *Food Chemistry.* 151, 271-278.

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. *BMJ Complement Alternative Med.* 14:391.

Yida, Z., Imam, M.U., Ismail, M., Hou, Z.P., Abdullah, M.A., Ideris, A., & Ismail, N. (2015). Edible Bird’s Nest attenuates high fat diet-induced oxidative stress and inflammation via regulation of hepatic antioxidant and inflammatory genes. *BMJ Complement Altern Med.* 15: 310.

Yida, Z., Imam, M.U., & Ismail, M. (2014). In vitro bioaccessibility and antioxidant properties of edible birds nest following simulated human gastro-intestinal digestion. *BMJ Complement Altern Med.* 14: 468.

Zainal, A.F., Chua, K.H., Ng, S.L., Mohd, R.E.S., Lee, T.H., & Norzana, A.G. (2011). Effects of edible bird’s nest (EBN) on cultured rabbit corneal keratocytes. *BMJ Complement Altern Med.* 11 (1): 94.

Zetterquist, W., Pederoletti, C., Lundberg, J.O., & Alving, K. (1999). Salivary contribution to exhaled nitric oxide. *Eur Respir J.* 13:327–33.

Zhang, X., Meng, J., & Wang, Z.Y. (2017). A switch role of Src in the biphasic EGF signaling of ER-negative breast cancer cells. *PLoS One.* 7:e41613

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 immune-suppression induced by cyclophosphamide. *Drug Des Devel Ther.* 10: 371-381.
BIODATA OF STUDENT

This student was born in Kuala Lumpur on 1987. She had obtained her degree of Doctor of Medicine 2006-2011 from Universiti Putra Malaysia. After that, she served her housemanship in Hospital Teluk Intan and subsequently transferred to Hospital Serdang as Medical officer after 2 years. She took up her Master in Science in Pharmacology & Toxicology under Universiti Putra Malaysia back in 2014. Along the master programme, she had presented a few posters during the conference and managed to publish a paper in Evidence-Based Complementary and Alternative Medicine. While for her career path, she had passed her external paper by Membership of the Royal Colleges of Physicians, London UK in 2016. Currently, she works as a gazetted physician in Hospital Tengku Ampuan Afzan, Kuantan. She is interested in the field of complementary medicine, cancer research and palliative care medicine.
LIST OF PUBLICATIONS

Publication

Shobana, C., Sani, D., Tan, S.N., Lim, C.W., Hasan, S., Mohtarrudin, N., Kirby, B., Ideris, A., Basri, H., Stanslas, J., & Christopher, T.S.Lim. (2018). Effect of Edible Bird Nest Extract On Lipopolysaccharide-Induced Impairment of Learning and Memory In Wistar Rats. Evidence-Based Complementary and Alternative Medicine. Article ID 9318789. p7.

Tan, S.N., & Christopher, T.S.Lim. (2019). Cryptococcal meningitis in an immunocompetent swiftlet rancher – first case report. Malaysia Journal of Medicine & Health Sciences. 15(1): 82-84.

Poster

Tan, S.N., Shobana, C., Sani, D., Lim, C.W., Ideris, A., Stanslas, J., Christopher, T.S.Lim. 2014. Safety Profile and Nutritional Content of Raw Cleaned Edible Bird’s Nest. Poster presentation in 2nd Edible Birdnest Industry Conference 2014, 25th-26th November, Marriott Hotel, Putrajaya. Malaysia. Article published by Department Of Veterinary Services. http://www.dvs.gov.my/dvs/resources/user_1/DVS%20pdf/Aneka%20Haiwan/poster%20papers/14_Tan_UPM.pdf.

Tan, S.N., Shobana, C., Sani, D., Lim, C.W., Ideris, A., Stanslas, J., Christopher, T.S.Lim. 2016. The potential proliferation of cancer cell by Edible birdnest in Malaysia. Poster presentation in 3rd Edible Birdnest Industry Conference 2016, 11th-12th October, Marriott Hotel, Putrajaya. Malaysia.

Tan, S.N., Shobana, C., Sani, D., Lim, C.W., Ideris, A., Stanslas, J., Christopher, T.S.Lim. 2016. Quantification of epidermal growth factor in Edible birdnest from different regions in Malaysia. Poster presentation in 3rd Edible Birdnest Industry Conference 2016, 11th-12th October, Marriott Hotel, Putrajaya. Malaysia.
UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION: _____________________

TITLE OF THESIS / PROJECT REPORT:

PROXIMATE ANALYSIS AND SAFETY PROFILE OF FARMED EDIBLE BIRD'S NEST IN MALAYSIA AND ITS EFFECT ON CANCER CELLS

NAME OF STUDENT: TAN SIN NEE

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as:

*Please tick (✓)

☐ CONFIDENTIAL (Contain confidential information under Official Secret Act 1972).

☐ RESTRICTED (Contains restricted information as specified by the organization/institution where research was done).

☐ OPEN ACCESS I agree that my thesis/project report to be published as hard copy or online open access.

This thesis is submitted for:

☐ PATENT Embargo from __________ until __________

(date) (date)

Approved by:

(Signature of Student) _____________________ (Signature of Chairman of Supervisory Committee)
New IC No/ Passport No.: __________________ Name:

Date: __________________ Date: __________________

[Note: If the thesis is CONFIDENTIAL or RESTRICTED, please attach with the letter from the organization/institution with period and reasons for confidentially or restricted.]