PHYTOCHEMICAL PROFILES AND BIOACTIVITIES FROM *Hystrix brachyura* BEZOAR EXTRACTS USING MACERATION AND ULTRASONICATION

BY

FAIZAH ABDULLAH ASUHAIMI

A thesis submitted in fulfilment of the requirement for the degree of Master of Health Science

Kulliyyah of Allied Health Science
International Islamic University Malaysia

FEBRUARY 2021

ABSTRACT

Porcupine bezoar (PB) has been traditionally claimed to be able to cure various type of diseases. However, the effect of extraction method on its biological activity and phytochemical profile has never been studied. Hence, three different types of PB namely, blood date (PB1), powdery date (PB2) and grassy date (PB3) were initially procured then extracted using sonication method (30 minutes for 3x) and evaluated for their antioxidant potentials through determination of total phenolic content (TPC) and total flavonoid content (TFC). Moreover, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay was also carried out to confirm free-radical- scavenging effect of all PBs extracts. Based on the results obtained, PB3 was chosen for the next phase of analysis and subjected to both maceration (72h at room temperature in magnetic stirrer) and sonication extraction method (30 minutes for 3x). In this regard, the antioxidant activities of both PB3 aqueous extracts obtained through maceration (PB3M) and sonication (PB3S) extraction methods were further evaluated using dot-blot (in DPPH, ABTS and β-carotene solution), TPC, TFC and DPPH assays. The phytochemical analysis was performed by gas chromatography–mass spectrometry (GC-MS) and anticancer screening was performed on three different types of cancer cell lines viz. malignant melanoma cell line (A375), cervical carcinoma cell line (HeLa) and breast adenocarcinoma cell line (MCF7). Normal HDF cells were used as control. The TPC value of the PBs showed increasing order from PB3 (5.56 ± 0.29 µg GAE/5 mg dry extract) < PB2 (7.70 ± 0.14 µg GAE/5 mg dry extract) < PB1 (8.00 ± 1.19 µg GAE/5 mg dry extract). All three PBs were devoid of flavonoids with negative values (PB1, -26.29 µg QE/5 mg dry extract, PB2, -23.30 µg QE/5 mg dry extract, PB3, -4.10 µg QE/5 mg dry extract). Among all the extracts, PB3 exhibited good radical-scavenging activity with >50% inhibition on DPPH radical. The dot-blot assay for PB3M and PB3S showed similar result in all three types of solutions. PB3S showed higher TPC value (5.56 ± 0.29 µg GAE/5 mg dry extract) and lower IC_{50} of DPPH assay (75.81 ± 7.33 µg/mL) compared to PB3M (4.55 ± 0.04 µg GAE/5 mg dry extract and 458.82 ± 15.80 µg/mL respectively). Both extracts were devoid of flavonoids. GC-MS analysis revealed myristic acid, ursodeoxycholic acid, pentadecyl acrylate, 2-palmitoylglycerol and stearic acid as the main bioactive compounds in PB3M while ursodeoxycholic acid, 17α-Hydroxypregnenolone, pentadecyl acrylate, stearic acid and 1-Dodecanol were detected as the main bioactive compounds in PB3S. The anti-proliferation assay showed similar inhibition results on A375 and MCF7 cells for both the extracts (PB3M and PB3S) while on HeLa cell, PB3M (1.27 ± 0.07 µg/mL) was found to be the more potent than PB3S (1.93 ± 0.07 µg/mL) with lower IC_{50} value. As conclusion, PB3 demonstrated better antioxidant activities compared to PB1 and PB2. PB3S, the aqueous extract obtained through sonication method, exhibited good antioxidant activities, higher abundancy of phytochemicals and similar anti proliferative activity on cancer cell lines compared to that of PB3M, the aqueous extract obtained through the maceration method. Hence, PB extract obtained through sonication technique is expected to manifest better biological effect and could prove more beneficial. However, more in-depth studies are still warranted to further confirm PB extract's beneficial effects to treat different disorders.

خلاصة البحث

يُزعم تقليديًا أن بازهر النيص (PB) قادر على علاج أنواع مختلفة من الأمراض. ومع ذلك ، لم يتم دراسة تأثير طريقة الاستخراج على نشاطها البيولوجي وخصائصها الكيميائية النباتية. ومن ثم ، تم شراء ثلاثة أنواع مختلفة من PB وهي تاريخ الدم (PB1) والتاريخ المسحوق (PB2) والتاريخ العشبي (PB3) في البداية ثم تم استخلاصها باستخدام طريقة الصوتنة (30 دقيقة لمدة 3 مرات) وتقييم إمكانات مضادات الأكسدة الخاصة بها من خلال تحديد المجموع. المحتوى الفينولي (TPC) ومحتوى الفلافونويد الكلي (TFC). علاوة على ذلك ، تم إجراء اختبار diphenyl-1-picrylhydrazyl (DPPH-2،2) أيضًا لتأكيد تأثير مسح الجذور الحرة لجميع مستخلصات PBs. بناءً على النتائج التي تم الحصول عليها ، تم اختيار PB3 للمرحلة التالية من التحليل وتعرض لكل من النقع (72 ساعة في درجة حرارة الغرفة في محرك مغناطيسي) وطريقة الاستخلاص الصوتي (30 دقيقة لمدة 3 أضعاف. في هذا الصدد ، الأنشطة المضادة للأكسدة لكل من PB3 المائي تم إجراء تقييم إضافي للمستخلصات التي تم الحصول عليها من خلال (PB3M) وطرق الاستخراج الصوتي (PB3S) باستخدام نقطية (في محلول DPPH و ABTS و β-carotene) و TPC و TFC و DPPH. تم إجراء التحليل الكيميائي النباتي بواسطة كروماتوجرافيا الغاز - مطياف الكتلة تم إجراء فحص (GC-MS) ومضادات السرطان على ثلاثة أنواع مختلفة من خطوط الخلايا السرطانية وهي: خط خلايا سرطان الجلد الخبيث (A375) ، خط خلايا سرطان عنق الرحم (هيلا) وخط خلايا سرطان الغدة الثديية (MCF7). تم استخدام خلايا HDF الطبيعية كخلايا التحكم. أظهرت قيمة TPC لـ PBs ترتيبًا متزايدًا من PB3 (5.56 ± 0.29 ميكروغرام GAE / 5 مجم مستخلص جاف) <PB2 (7.70 ± 0.14 ميكروغرام GAE / 5 مجم مستخلص جاف) <PB1 (8.00 ± 1.19 ميكروغرام GAE / 5 مجم) مستخلص جاف) كانت جميع PBs الثلاثة خالية من f اللافونويدات ذات القيم السالبة (PB1 ، -26.29 ميكروغرام من QE / 5 مجم مستخلص جاف ، PB2 ، -23.30 ميكروغرام QE / 5 مجم مستخلص جاف ، PB3 ، -4.10 ميكروغرام QE / 5 مجم مستخلص جاف). من بين جميع المستخلصات ، أظهر PB3 نشاطًا جيدًا في إزالة الجذور مع> 50٪ تثبيط لجذر DPPH. أظهر اختبار النقطة النقطية لـ PB3M و PB3S نتيجة مماثلة في جميع أنواع الحلول الثلاثة. أظهر PB3S قيمة TPC أعلى (5.56 ± 0.29 ميكروغرام من مستخلص جاف من GAE / 5 مجم) و IC50 أقل لمقايسة DPPH (75.81 ± 7.33 ميكروغرام / مل) مقارنة بـ PB3M (4.55 ± 0.04 ميكروغرام GAE / 5 مجم مستخلص جاف و 458.82 ± 15.80 ميكروغرام) / مل على التوالي). كلا المستخلصين كانا خاليين من مركبات الفلافونويد. كشف تحليل GC-MS أن حمض الميريستيك ، وحمض أورسوديوكسيكوليك ، وأكريلات بنتاديسيل ، و 2-بالميتويل جلسرين وحمض دهني كمركبات رئيسية نشطة بيولوجيًا في PB3M ، بينما تم اكتشاف حمض أورسوديوكسيكوليك ، 17α-هيدروكسي برغنينولون ، بنتاديسيل أكريلات ، حمض دهني رئيسي و D-1. المركبات النشطة بيولوجيا في PB3S. أظهر اختبار مكافحة الانتشار نتائج تثبيط مماثلة على خلايا A375 و MCF7 لكلا المستخلصين (PB3M و PB3S) بينما وجد أن PB3M (1.27 ± 0.07 ميكروغرام / مل) أقوى من PB3S (1.93 ± 0.07 ميكروغرام) على خلية هيلا. / مل) بقيمة IC أقل. في الختام ، أظهر PB3 أنشطة مضادات الأكسدة أفضل مقارنة بـ PB1 و PB2. أظهر PB3S ، المستخلص المائي الذي تم الحصول عليه من خلال طريقة الصوتنة ، نشاطًا جيدًا مضادًا للأكسدة ، ووفرة أعلى من المواد الكيميائية النباتية ونشاطًا مماثلًا مضادًا للتكاثر على خطوط الخلايا السرطانية مقارنةً بمستخلص PB3M ، المستخلص المائي الذي تم الحصول عليه من خلال طريقة النقع. ومن ثم ، فمن المتوقع أن يظهر مستخلص PB الذي تم تجريده من خلال تقنية الصوتنة تأثيرًا بيولوجيًا أفضل ويمكن أن يكون أكثر فائدة. ومع ذلك ، لا يزال هناك ما يبرر إجراء المزيد من الدراسات المتعمقة لتأكيد التأثيرات المفيدة لمستخلص PB في علاج الاضطرابات المختلفة.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences

……..
Ridhwan Abdul Wahab
Supervisor

……..
Qamar Uddin Ahmed
Co-Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences

……..
Siti Zaiton Mat So'ad
Internal Examiner

……..
Sreenivasa Rao Sagineedu
External Examiner

This thesis was submitted to the Department of Biomedical Science and is accepted as a fulfilment of the requirement for the degree of Master of Health Sciences

……..
Hanani Ahmad Yusof @ Hanafii
Head, Department of Biomedical Science

This thesis was submitted to the Kulliyyah of Allied Health Sciences and is accepted as a fulfilment of the requirement for the degree of Master of Health Sciences

……..
Suzanah Abdul Rahman
Dean, Kulliyyah of Allied Health Sciences

DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Faizah Abdullah Asuhaimi

Signature ..... Date .....

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

DECLARATION OF COPYRIGHT AND AFFIRMATION OF FAIR USE OF UNPUBLISHED RESEARCH

PHYTOCHEMICAL PROFILES AND BIOACTIVITIES FROM *Hystrix brachyura* BEZOAR EXTRACTS USING MACERATION AND ULTRASONICATION

I declare that the copyright holders of this dissertation are jointly owned by the student and IIUM.

By signing this form, I acknowledged that I have read and understand the IIUM Intellectual Property Right and Commercialization policy.

Affirmed by Faizah Abdullah Asuhaimi

……..…….. ……..
Signature Date

I dedicate my master degree thesis to ALLAH (s.w.t.) the almighty, to my beloved mother, (Solihah Mat), my father (Abdullah Asuhaimi Mohd Zin), my husband (Abdullah Munir Mohd Aizaini) and my loving son (Abdullah Yusuf Abdullah Munir).

ACKNOWLEDGEMENT

Bismillahirahmanirrahim. First and foremost, praises and thanks to the God, Allah the Almighty, for His showers of blessings throughout my research work to complete the research successfully. Although, it has been tasking, His Mercies and Blessings on me ease the herculean task of completing this thesis.

My utmost pleasure to dedicate this work to my dear parents (Abbdullah Asuhaimi and Solihah) who encouraged me to pursue this study, always pray for me and keep on believing in me. May Allah reward them with highest rank of *Jannah*! I couldn't have make it without my siblings; Farhana, Farhanin, Fadhilah, Fauzun, Fahmi, Fitriyyah, Fikri and Faishal. Thank you for the advices, wise words and encouragement. I am thankful for my parents in laws (Mohd Aizaini and Siti Salwa) for their understanding and motivation. It's totally a bless for me for having such a lovely in laws family.

I would like to express my deep and sincere gratitude to my research supervisor, Assoc. Prof. Dr Ridhwan Abdul Wahab whose enduring disposition, kindness, promptitude, thoroughness and friendship have facilitated the successful completion of my work. My appreciation to my co-supervisor, Dr Qamar Uddin Ahmed, who teach me patiently with all the lab works, troubleshoots and writing. I am lucky to have both of them guiding me throughout this journey. Thank you again for not giving up on me.

Special thanks to my colleagues, Al'aina Yuhainis who always there when in need. I am touched with the kindness and endless encouragement from the beginning until I completed this thesis. Huge amount of gratitude to my labmates, Sr. Fatimah Opeyemi Roheem and Br. Faris Osman for the insightful ideas, suggestions, and discussions.

Last and but not least, once more, I want to thank my husband, Abdullah Munir for always being supportive and understanding. My son, Abdullah Yusuf who accompanied me throughout this journey in my womb up till now. I will always remember those sacrifices made along this four years of wonderful journey.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Porcupine bezoar (PB) is claimed as a prince of antidote due to the ability of curing various types of diseases such as plague, cholera, poisoning, cancer, diabetes, dengue, and many deadlier diseases. This bezoar had been used starting from the tenth century and still consumed as remedies up till now (Barroso, 2014; Figueroa, 2015; Tan et al., 2019). Porcupine bezoar is a phytobezoar (consist of plant materials) that undergoes calcification process of organic and inorganic materials and turned into a stone-like structure that can be found in the gastrointestinal tract of the porcupine (Barroso, 2014; Duffin, 2013; Yew et al., 2018). There are few types of PB that had been reported namely, grassy date, black date, powdery date, kernel date and blood date. The most common and easily obtained is the grassy date (Yew et al., 2018) The healing properties of the bezoar most probably due to the diet of the *H. brachyuran* that eat various kind of foods namely, forest fruits, tubers, rhizomes, bulb, shoots, young stems and herbs (Farida al., 2019).

Hystrix brachyura or Malayan porcupine is protected under Malaysian law by Wildlife Conservation Act due to the decrease in number of their population. Porcupine had been poached for their meat and medicinal purposes which is the bezoar (Lunde, Aplin, & Molur, 2017). As bezoar not easily formed in all porcupine, thus PB considered as rare material that been sell at very high price (Tan et al., 2019). Each PB have their own unique characteristics and medicinal value depending on the period it resides in the porcupine. The longer it stays, the bigger in size, the higher value of the

medicinal properties and price (Duffin, 2013). Furthermore, the most valuable PB are formed from porcupines living in the rainforest of Borneo which consume unpolluted herbs that have high medicinal value thus turning the bezoar into great medicinal value and price (Khan et al., 2017).

There are several techniques of how PB was consumed based on the ancient way and current practice. Generally, PB was infused either the whole stone or in fine grains into several type of fluid such as water, wine, liquor, and cordial water. The duration also varies from few minutes until several hours (Duffin, 2013; Tan et al., 2019). However, the effectiveness of each method of extraction was never been evaluated. Hence presence study investigates the difference between the traditional and modern method of extraction by assessing the antioxidant activities, phytochemical compounds and anticancer properties.

Antioxidant research of various samples from plants such as leaf, bark, flower and fruits increased over the years due to the ability of the antioxidant to reduce the risk of cardiovascular disease, degenerative disease, metabolic disorders and also cancer (Ahmed et al., 2015; Santos-Sánchez et al., 2019). The formation of these diseases are very much caused by oxidative stress, a condition of imbalance between the production of free radical and antioxidant defence (Lobo et al., 2010). The oxidative stress disrupts biological functions by damaging the biomolecules such as lipid, protein and nucleic acid thus leading to various undesirable pathological conditions (Khacha-ananda, Tragoolpua, & Chantawannakul, 2016). The alteration of the base in nucleic acid is the initiation of cancer formation as the process produce mutagenesis and carcinogenesis (Santos-Sánchez et al., 2019).

Cancer had been reported as the second leading cause of death worldwide (Siegel, Miller, & Jemal, 2018, 2019) and ranked as the fourth in Malaysia (National

Cancer Registry, 2018). Cancer is a disease involved with abnormal growth of cells that capable to invade surrounding normal cells and metastasize to different part of body (Ranjan et al., 2019). Despite of such advances in its early detection, and enhancements in treatment and deterrence, cancer remains a huge challenge in terms of morbidity and mortality (Sammar et al., 2019). Hence, cancer prevention through chemopreventive agents especially from phytochemicals that can be obtained by healthy diet is the best practice (Tungmunnithum et al., 2018). Appropriate lifestyle modifications such as exercise and balance healthy diet may prevent two-thirds of the cancer incidence (Ruiz-Casado et al., 2017; Zhang et al., 2015).

1.2 STATEMENT OF THE PROBLEM

Porcupine bezoar had been studied previously for its antioxidant capacity and anticancer effect however the data available is only preliminary. As porcupine bezoar had been used rampantly at high price and very scarce in source, the urge to find appropriate extraction techniques that can increase the yield of the bezoar is important. To date, no studies have reported comparing the effect of maceration and sonication methods on the biological activity and phytochemical profile of porcupine bezoar.

1.3 RESEARCH OBJECTIVES

The study aimed to achieve the following objectives:

1) To evaluate the antioxidant activities from different types of PB extracts using maceration and sonication methods.
2) To determine the correlation between phytochemical constituent's content and antioxidant activities of PB extracts.

3) To determine the anticancer activities of different extraction method of PB extracts.

1.4 RESEARCH HYPOTHESIS

1) Sonication extraction method have higher antioxidant activities than maceration extraction method.
2) Sonication extract have higher abundancy of phytochemical constituents than maceration extract.
3) Anticancer activities of sonication extract more potent than maceration extract.

1.5 SIGNIFICANCE OF THE STUDY

This study scientifically provides the evidence for the antioxidant activities, phytochemical analysis and anticancer screening of porcupine bezoar extracts in different extraction method viz. maceration technique and sonication technique. Besides, further research could be conducted in depth pertaining to the claims on the medicinal properties of the porcupine bezoar.

CHAPTER TWO

LITERATURE REVIEW

2.1 PORCUPINE BEZOAR

2.1.1 Bezoar

The word bezoar comes from Persian “pahnzehr” or the Arabic “badzehr,” both of which mean counter poison or antidote (Sanders, 2004). Bezoar is an accumulation of indigestible foreign material either undigested organic or inorganic material that conglomerate in the gastrointestinal tract, most commonly in the stomach. Bezoar also can be found from oesophagus to rectum (Azizulkarim et al., 2018; Duffin, 2013; Sanders, 2004). The substance that may present in the bezoar includes hair, foods, medication, mucus and milk (Sanders, 2004; Yew et al., 2018). Generally, the presence of bezoar is an illness that can happened to human and animals. This condition need treatment and surgical removal especially when symptoms of illness exist such as weight loss, vomiting, diarrhoea and depression (Khan et al., 2019; Sanders, 2004).

There are four major categories of bezoar viz. trichobezoar, lactobezoar, pharmacobezoar and phytobezoar. Trichobezoar is a concretion of hair that will gradually increase in size by years while lactobezoar consist of milk protein. Pharmacobezoar is concretion of medicines from various types and phytobezoar formed by indigestible fruits and vegetables high in fibers. Above all, phytobezoar is the most common approximately by 40% and highly recurrent (Eng & Kay, 2012; Yew et al., 2018). Porcupine bezoar is categorised under phytobezoar with intertwined fibers of poorly metabolized plants in the stomach of the porcupine (Yew et al., 2017). The colour of the bezoar described as green grass of blackish to green hue in the centre and

the structure almost similar to unripe date or elongated acorn with a peel like onion (Barroso, 2014). Under phytobezoar consist of a subset named diospyrobezoar that was formed due to the excessive comsumption of persimmon *(Diospyros kaki)* (Zhang, Yang, & Fan, 2008). The agglutination of tannins from the skin and the unripe flesh of persimmon reacted with dilute acid in the stomach thus forming a glue-like coagulum (Toledo et al., 2012). Bezoar does not easily form in all porcupine, instead only under certain condition and situation. Hence, bezoar considered as exotic and rare material that been sold at implausibly high price. In Malaysia, the price for 500g of PB is around RM300–RM1000 (Tan et al., 2019). Previously in Singapore during 2005, PB was sold for USD 7,000 per gram which is 16 times the price of gold within the same year (Chung et al., 2016).

Abu Mansur Muwaffak had mentioned in his materia medica the earliest documented usage of bezoar was on tenth century. Followed by Al-Biruni, a philosopher, encyclopaedist, mathematician, astronomer, and geographer that wrote regarding bezoar in his book, "Book of Stones" during eleventh century. At the beginning of the twelve century, the records of bezoar were written in Moses Maimonides's "Treatise on Poison and Their Antidotes" and during mid thirteenth century in Ahmad ibn yusuf Al Tifaschi's "Best Thoughts on the Best of Stones". This illustrates the consumption of bezoar starting from Middle East by the Arabic physicians and crusaders, spread to Persia and followed by Europe until it finally widespread throughout the world (Barroso, 2014; Figueroa, 2015). During 17^{th} century the bezoar was described as luxury medicinal item in South East Asian and Europe as it was given as gift to the higher social status of person due to its high medicinal value. It was worn as pendant or engraved inside a cup (Khan et al., 2019; Malcom, 1998).

Garcia da Orta, a Sephardic Jew from Elvas had recorded the first bezoar that was found in a stomach of a goat (called as "pazam" by the Persian) (Figueroa, 2015). The occurance of bezoar had been reported on various type of animals viz. ox, cattle, primate, goat, deer, sheep, antelope, tamarins and porcupine. As compared to bezoar from other animal, porcupine bezoar possess highest quality and the best medicinal value (Barroso, 2014).

Figure 2.1 An oriental bezoar from 16th century, mounted on Indo-Portuguese golden filigree pendant. Size: height 9.4 cm, diameter 6.1 cm. Távora Sequeira Pinto Collection (Oporto). Adapted from Barroso, 2014.

2.1.2 Hystrix brachyura

Malayan porcupine (*H. brachyuran)*, also called as "Landak Raya" or "Landak Borneo" by the locals belongs to family Hystricidae, order Rodentia that can be found in various

places around South East Asia (Azizulkarim et al., 2018; Farida et al., 2019). This mammal generally in black, equipped with a short dark brown or blackish quills at the front body and long white quills at the back with a black band (Chung et al., 2016). *H. brachyuran* can grow to adult with weight up to 8kg, head-body length 550-750 mm, tail length 60-110 mm and hind foot 80-100 mm (Lim, 2000). The female porcupine can breed twice a year with a range of 1-4 porcupette per birth and a gestation period ranging 100-110 days (Farida et al., 2019).

The Malayan porcupine is a nocturnal with ordinary sense of sight and strong sense of smell and hearing. The habitat is terrestrial and can be found in several types of forest. They live in hole of roots or tree barks and also a burrow which has a network that can lead to surrounding habitat. The porcupine is a herbivores that prefers forest fruits, tubers, rhizomes, bulb, shoots, young stems, the inside of stem, bark, roots and bamboo shoots (Farida et al., 2019). Porcupine also favour bitter-tasting part of the plant, i.e roots and branch that by nature in Malaysia, that kind of plant are the source of medicinal herbs. Whenever it gets sick, porcupine heals quickly as it has strong immunity against toxins and poisons by eating certain herbs guided by their survival instinct (Yew et al., 2017). The herbs will react with the toxins in the body by produces a type of chemical that aid for speedy healing. However, chemicals left in its body would slowly accumulate and form a stone-like substance, or also known as porcupine bezoar (Tan et al., 2019).

A report from The International Union for Conservation of Nature (IUCN) Red List of Threatened Species stated that *H. brachyuran* categorized under 'Least Concern', however the population of the porcupine is in decreasing trend. Among the reasons for the falling population was due to the loss of habitat and they were poached for food and medicinal purposes (Lunde et al., 2017). In order to protect this species,

Malaysian government had placed it under Wildlife Conservation Act for conservation program.

Figure 2.2 The Malayan porcupine. Adapted from Chung et al., 2016.

2.1.3 Porcupine bezoar as a traditional medicinal

From the ancient history, porcupine bezoar that been called as “prince of antidotes” had been claimed to cure various type of diseases such as poisoning, measles, plague, jaundice, malignant fever, severe febrile diseases, leprosy, scabies, itching, cholera and melancholy (Barroso, 2014; Duffin, 2013). Recently, from testimonies of some patients, porcupine bezoar was believed to cure and delay the prognosis of cancer, diabetes, dengue, typhoid, epilepsy, hepatitis and many deadlier diseases. In Malaysia, according to Chinese folklore, PB was believed to cure cancer and consumed as supplement for post-cancer therapy (Tan et al., 2019).

The method to consume porcupine bezoar varies depending on the type and severity of the illness. According to Duffin (2013), the porcupine bezoar was steeped

for a while in a water vessel by sinking the stone at the bottom and let it infused. Other way, it was infused in wine for cholera, soaked in any ordinary drink or placed in gold box and infused in liquor until it tastes bitter for plague, soaked fine grains of bezoar in cordial water or real wine for remedy of poisons. In term of duration, 15 minutes is sufficient of its first soaking in order to extract the virtue and bitter taste of the stone but the next use, doubled the period. Several hours of steeping also mentioned to treat 'severish distempers' and 'strengthening the stomach', however the stone must be removed before greenish tincture appeared in the solution. *H. brachyuran* bezoar was observed to have abortifacient if prescribed on pregnant lady hence for women having menstrual problem, it was advised just to hold in the hand to receive the benefit of the stone. As prevention of disease, porcupine bezoar was mounted in gold filigree cages and worn as pendant on the neck.

Current prescription suggested the patient to consume the porcupine bezoar in fine powder form (Yew et al., 2018). Healthy individuals suggested to take 100–200 mg per month while individual with illness was recommended to have 300–400 mg, 2 to 3 times per day. It was best consume in empty stomach, either eaten directly or added in water (Tan et al., 2019). To date, no research had been done on the effective dose for the bezoar consumption thus the recommended dosage was prescribed by the seller or the practitioner without any strong basis.

2.1.4 Characterization of H. brachyuran bezoar

H. brachyuran bezoar have several subtypes classified by their appearance and structure viz. grassy, powdery, black, kernel, and blood date (Yew et al., 2018). Another way, based on the structure. Malaysia market consist of three types of porcupine bezoar which are grass bezoar, semi- perfect composited bezoar and perfect composited bezoar.