FORMULATION OF ORAL GEL CONTAINING BACCAUREA ANGULATA FRUIT EXTRACT FOR WOUND HEALING OF TOOTH EXTRACTION

BY

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ABSTRACT

This study investigated the potential of different gelling agents to be developed as dental gel formulation of *Baccaurea angulata* fruit extract as well as the effect of *B. angulata* fruit extract towards wound healing at tooth extractions sites. Since wound healing process is interrupted in diabetic patient, a well characterised and optimized *B. angulata* dental gel is believed to have the potential in promoting the healing process due to its high antioxidant content. Rheological properties of 24 different gelling agents were screened, then the best gelling agents were further evaluated for their spreadability, texture profile analysis, and their release profiles using Franz cell diffusion to identify best formulation for *in vivo* study. In *in vivo* study, the dental gels were applied in the alveolar socket after tooth extraction in diabetic rats. Diabetic was induced using Streptozotocin, and their left mandibular first molar teeth were extracted. Rats were divided into 6 groups (diabetic rats applied with plain gel, alveolex, 3%, 5%, and 10% Baccaurea angulata extract respectively). Group 6 were non diabetic rats applied with plain gel. The detailed rheological profiles of each of the 24 different gels were successfully documented. Guar gum, konjac gum and carbopol were found to be among the best candidates for *B. angulata* dental gel. The Franz cell study had found that 4% carbopol gel had the best release profile in finite an infinite dosing. Regarding *in vivo* study, it was found that *B. angulata* significantly promoted tooth extraction wound healing in diabetic rats comparable to diabetic group treated with alveolex and non-diabetic rats at early phase of the wound. However the positive effect were not obvious at day 7, while at day 18, groups treated with *B. angulata* had better mean scores in most wound healing parameters (e.g. presence of fibroblast, collagen fibres and epithelization) although not statistically significant different. *B. angulata* dental gel was found to have the best positive effects on wound healing. As a conclusion, *B. angulata* fruit extract promotes tooth extraction wound healing in diabetic rats during some stages of the wound healing process. This study also explored the rheological properties of different gelling polymers thus contributing towards their application in pharmaceutical technology and industry. Besides, the potential of *B. angulata* wound healing activity will open up new possibilities in promoting the wound healing in diabetic patient.

خلاصة البحث

درس هذا البحث إمكانيات عوامل التبلور المختلفة التي سيتم تطويرها كهلام للأسنان والمصنع من مستخلص فاكهة الباكوريا أنغولاتا، وكذلك تمت دراسة تأثير مستخلصات الباكوريا انغولاتا على التئام الجروح في مواقع استخراج الأسنان. عملية التئام الجروح في مرضى السكري مضطربة، ولذلك يعتقد أن استعمال الباكوريا أنغولاتا كهلام للأسنان لديه القدرة على تعزيز عملية الشفاء بسبب المحتوى العالي للمضادات للأكسدة. تم فحص الخصائص الريولوجية لـ24 عاملا مختلفا للتبلور، ثم تم تقييم أفضل عوامل التبلور لزيادة انتشارها، وتحليل الملمس الشخصي، وملامح التفريغ باستخدام طريقة فرانز لنشر الخلايا لتحديد أفضل صياغة دوائية للدراسة في الجسم الحي. في الدراسة في الجسم الحي، تم وضع المواد الهلامية في المقبس السنخي بعد استخراج الأسنان في الفئران المصابة بالسكري. تم استحداث مرض السكري باستخدام عقار الستريبتوزوتوسين، وتم استخراج أسنان الفئران المولية في الفك السفلي في الجهة اليسرى. تم تقسيم الفئران إلى 6 مجموعات (الفئران المصابة السكري تم اعطاؤها الهلام العادي، والألفيوليكس، و ٪5 ، و3%، و10٪ من مستخلصات الباكورا أنغولاتا، على التوالي). أعطيت المجموعة 6 ذي الفئران العادية بهلام عادي. تم توثيق الملامح الريولوجية المفصلة لـ 24 مادة الهلامية مختلفة بنجاح. كان صمغ الغوار، صمغ الكونجاك و الكاربوبول أفضل المرشحين لهلام الباكوريا أنغولاتا للأسنان. وجدت دراسة الخلية بطريقة فرانز أن هلام الكاربوبول 4% كان له أفضل تفريغ في جرعات لانهائية محدودة. فيما يتعلق بالدراسة في الجسم الحي، وجد أن الباكوريا أنغولاتا قد عززت بشكل كبير التئام جروح الاستخراج في الجرذان المصابة بالسكري، مماثلة لمجموعة السكري المعطاة الفلفيكس والجرذان الغير مصابة بالسكري في المرحلة المبكرة من الجرح. ومع ذلك فإن التأثير الإيجابي لم يكن واضحا في اليوم السابع، بينما في اليوم 18، كانت المجموعات التي تعاملت مع باكوريا أنغولاتا أفضل من الدرجات في معظم معلمات التئام الجروح (مثل وجود الخلايا الليفية، ألياف الكولاجين والظهارة) على الرغم من أنها قد لا تكون ذات دلالة إحصائية مختلفة. تم العثور على أن هلام الفاكهة كان أفضل على البكتيريا في التئام الجروح. ونتيجة لذلك، فإن مستخلصات الباكوريا عززت استخراج الأسنان التئام الجروح في الفئران السكري خلال بعض مراحل عملية التئام الجروح. واستكشفت هذه الدراسة أيضا الخصائص الريولوجية لمختلف البوليمرات التبلور وبالتالي المساهمة في تطبيقها في مجال التكنولوجيا الصيدلانية والصناعة. الى جانب ذلك، فإن إمكانات الباكوريا أنغولاتا في التئام الجروح تفتح إمكانيات جديدة في تعزيز التئام الجروح في مريض السكري.

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 Pharmaceutical Sciences (Pharmaceutical Technology)

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DECLARATION

I hereby declare that this thesis 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.

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CHAPTER ONE

INTRODUCTION

1.1 RESEARCH BACKGROUND

Topical medications are effective to treat certain diseases because it can be applied directly at pathological sites, thus can give faster therapeutic effects to the specific sites. Topical medications include gels, ointments or creams. The topical preparation itself needs to be optimised in term of its physical appearance, pH, skin irritation possibilities, and spreadability to be a good and effective drug delivering agents (Misal et al., 2012). Gels are defined by United States Pharmacopeia (USP) as semisolids consisting either of suspensions of small inorganic particles or of organic molecules interpenetrated by a liquid. Gel is a component of two medium, in which the polymers were crosslinked with three dimension network of polymers and liquids to form a rigid and infinite network structure that can be flexible and immobilize the continuous phase of liquid within (Kaur et al., 2013).

Polymers are used as the main ingredients that contribute to the gelling properties of the gels. Different gel polymers exhibit different properties and they are normally chosen based on the application sites and the intended characteristics. On patient side, topical application can give advantage in term of improving the compliance to the medication, providing continuous and stable drug delivery to the specific sites, and minimising systemic effect since the dose needed to be given is lower compared to the oral dosage forms (Das et al., 2013). The concept of topical gels is that the active compound incorporated in the gel comes into contact on the skin and permeates through the micro pores at the stratum corneum. To be specific, the pathways can be through hair follicles, sweat ducts, and across the stratum corneum

interspaces between the hair follicles, sebaceous glands, apocrine glands, eccrine, and nails (Bhowmik et al., 2012; Kaur et al., 2016). Physicochemical profiles of gels include the concept of rheology, spreadability, and texture profiles. These profiles play important part in the production and application of topical formulations.

Regarding physicochemical evaluation, a few physicochemical parameters are normally tested, including pH, spreadability, consistency, homogeneity, drug uniformity, skin irritation test and *in vitro* release kinetics. Previous researcher had reported that the viscosity of major commercial herbal gels to be in the range of 4700-4800 cP and the spreading diameter in the spreadability test were found to be 38-55 mm after 1 min duration (Misal et al., 2012).

The active ingredient that will be incorporated into the topical gel in this study is *Baccaurea angulata* fruit extract, which contains high antioxidant and anti-inflammatory properties and most importantly, it is from local sources which is *B. angulata* (known as Belimbing Dayak as local Malaysian name). This research can give a big impact in helping diabetes mellitus patient not to suffer from wound infections after having a certain operations including tooth extraction. The relevance is even more since the prevalence of diabetes mellitus patients among Malaysian is high, which is about 3.2 million people (Malaysia OHDMOH, 2015). This study can contribute to the development of halal topical wound healing products that is cheaper compared to other imported commercial products, thus contributing to more efficient health spending in government system.

1.2 PROBLEM STATEMENT

Diabetes mellitus can cause delay in wound healing. Oral healthcare is one of the major concerns among diabetic patients in which the process of tooth extraction is very risky since it may easily lead to wound infections. Oral environment is full of bacterial and there are many possibilities that infection can override and spoil the wound healing process after the tooth extraction procedure. It is very important to speed up the wound healing process especially among patients with diabetes mellitus to prevent any unwanted infections.

B. angulata fruit had been used by ancestors to treat wound healing. However, there is no commercial product in the market using this fruit extracts as medication, and no studies to prove the wound healing activity of this fruit. Since diabetic patient is prone to wound infections, tooth extractions are not usually recommended. However in certain cases, there are situations that tooth extraction need to be done to prevent further pain, such as the removal of wisdom tooth which overlaps with the gums/ other teeth. Therefore, this study aims to test the wound healing potential of *B. angulata* fruit extract in diabetic model.

Gels are amongst the simple and effective topical formulations. However, before proceeding to *in vivo* study, proper characterisation needs to be done in order to select the best gelling polymers. Several gelling polymers are available commercially but their suitability to formulate dental gel had not been widely investigated. To be suitable for dental applications, the gel should remain viscous even in high shear stress condition, i.e. the condition of mouth movement. The gel have to be consistent enough to flow as pseudoplastic (flow behaviour < 1), and remains as a stable viscous gel even when exposed to high shear stress (refers to rough dental movements). This is important in order to prolong the residence time at the alveolar socket, thus releasing

larger amount of *B. angulata* extract. Therefore, we aim to screen and characterise the potential gelling agents to be developed as *B. angulata* dental gel. After the suitable gelling polymer is selected, it is used to prepare dental gel formulation containing *B. angulata* fruit extract and the potential of this gel to promote wound healing at the tooth extraction is investigated in diabetic rats.

1.3 RESEARCH HYPOTHESIS

Dental gel containing *B. angulata* fruit extract can promote wound healing at the tooth extraction site in the diabetic rats.

1.4 RESEARCH OBJECTIVES

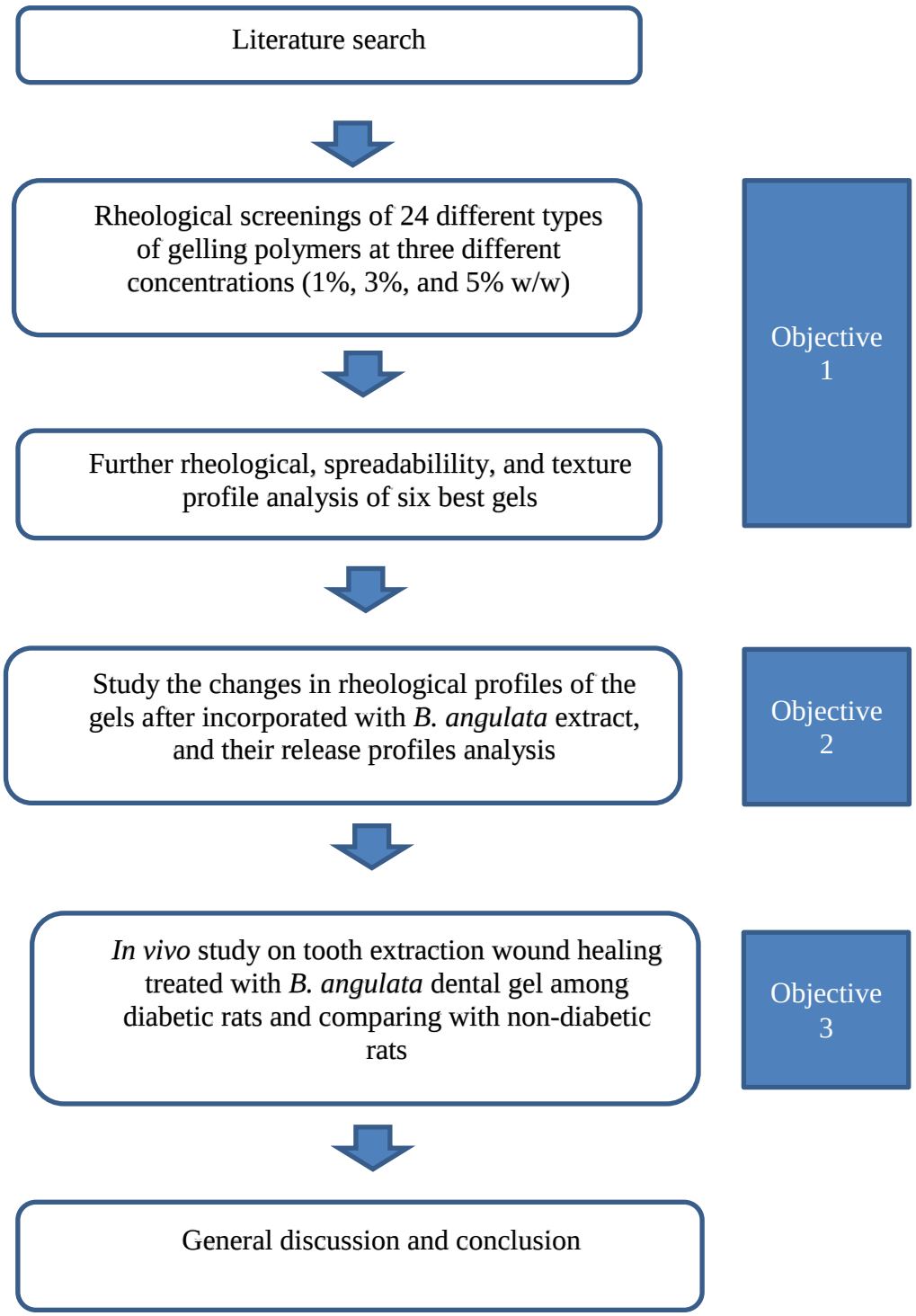
1.4.1 General Objective

To formulate dental gel containing *B. angulata* fruit extract and evaluate its wound healing effect on tooth extraction wound in diabetic rats.

1.4.2 Specific Objectives

1) To screen different gelling polymers for their suitability to prepare the dental gel.
2) To formulate and characterise the dental gel containing *B. angulata* fruit extract.
3) To evaluate the effect of dental gel containing *B. angulata* fruit extract on wound healing at the tooth extraction sites in diabetic and non-diabetic rats.

1.5 STUDY PLAN



CHAPTER TWO

LITERATURE REVIEW

2.1 BACCAUREA ANGULATA

Antioxidant compounds can be easily found within the food that human takes. Fruits and vegetables are rich in flavonoids/polyphenols such as quercetin and other important minerals and vitamins such as ascorbic acid (Lima et al., 2014). One of the most popular herbal fruit that had been taken by ancestors as traditional remedies in Malaysia is *B. angulata* (Belimbing Dayak). This fruit is widely found in the inner part of forest in Borneo and Indonesia (Mikail et al., 2016). Studies found high content of antioxidants in *B. angulata*, suggesting a positive effect towards arteriosclerosis and cardiovascular disease (Mikail et al., 2014).

The freeze-dried whole fruit of *B. angulata* contains approximately 19.63 ± 0.04% of moisture, 4.74 ± 0.25% ash, 1.33± 0.05% protein, 0.11 ± 0.03% total fat, 74.12 ± 0.0% carbohydrate, 0.07 ± 0.06% crude fiber, 302.79 ± 0.00 kcal/100g gross energy, 6.3% w/w total dietary fiber, and 0.210W_A water activity Freeze dried whole fruit of *B. angulata* had a good amount of total phenolic content (TPC) which is 7.91 ± 0.05 mg GAE/g. The unit is in term of gallic acid equivalent because gallic acid is one of the major phenolic compound in plant total flavonoid content is 12.74 ± 0.24 mg QE/g, and total anthocyanin content of 0.42 ± 0.28 mg c-3-gE/100 g (Darina et al., 2013).

The shape of *B. angulata* fruit is like a star fruit (*Averhhoa carambola*), from which the name “belimbing” was derived (Darina et al., 2013). The ripe fruit is bright red in color. The fruit is pointed at the end, and rounded at the base. There are about 1 – 4 cloves of berry which is white in color and sticky One previous study had

investigated the characteristic of *B. angulata* in which it was found that the skin of the fruit contained a high amount of antioxidants with significant correlation with its total flavonoid and phenolic content. The antioxidants properties and phytochemical composition of *B. angulata* fruit have substantially proved its potential benefits as effective anti-atherosclerotic, anti-inflammatory, and anti-hypercholesterolemia agent (Mikail et al., 2014)

Apart from that, another study had shown that *B. angulata* had cardio protective effects due to its ability to suppress inflammatory response (Ahmed et al., 2015) . The fruit contains high amount of antioxidant and flavonols in the fruit, berries and whole fruit. Dried fruit of *B. angulata* is also a good source of energy, carbohydrate, and fibres (Jauhari et al., 2013). The total phenolic content in freeze-dried whole fruit (FDWF) is 7.91 mg GAE/g (gallic acid equivalent per gram), higher than the freeze-dried berried (FDB) which had the value of 4.78 mg GAE/g. The result is in gallic acid equivalent because gallic acid is known as one of the major phenolic compounds in plants (Fernandes et al., 2016).

2.2 RHEOLOGY OF TOPICAL DRUG FORMULATIONS

2.2.1 Rheology profile of the gels

An effective topical dosage form should enable the drug to remain at the targeted site of action for an adequate time at an effective concentration (Malathi et al., 2014). Advantages of local drug delivery in the context of dental treatment includes that drugs can reach the specific sites in adequate amount, improve patient compliance, and improve pharmacokinetics (Raghavendra Rao et al., 2009). An ideal dental dosage form should be sufficiently mobile to ease the delivery of the dosage form to the tooth socket. At the same time, it should be adequately viscous in order to remain at the

application site (Jones et al., 2000). Current topical or dental gels were developed based on the most common gelling agents that are expected to be suitable as polymers for gels. However, there is lack of comparison for candidate gelling polymers especially in term of rheological, spreadability, and texture profile analysis. A few studies characterised individual gelling agents and successfully described their rheological profiles such as gelling agent of keratin hydrogel (Sharma et al., 2017), bleached shellac (Phaechamud et al., 2016) and gelatins (Ersch et al., 2016).

Rheology plays an important part in determining the flow behaviour of the dosage forms. There is specific range of rheology for specific applications. For instance, an oral jelly for geriatrics needs an extent of viscosity, and specific range of flowability in order for the dosage form to be accepted well by patients. In contrast, a topical cream had to be more viscous in order for the drug to maintain at the skin surface for longer time. This is important for the permeation of the active compound from the dosage forms into the skin layers. Otherwise it will be a waste should the topical application only last for a few minutes and easily wiped away by physical movement. Topical application is well known for its poor permeability and slow penetration into the skin. Therefore the rheology profile of the topical dosage form is important to maximize the contact period between the medication and the skin. This can be accomplished by modifying the nature of the vehicle (Hussain et al., 2012).

Rheology is the study of the flow of matter in term of liquid states, but the concept also covers the properties of soft solids or solids. Newtonian and non-Newtonian fluids are the concepts that fall under rheology. Semi-solid preparations for creams, ointments, gels and lotions are different, and their characterisation will depend on a few parameters such as rheological properties, volatiles and water (Chang et al., 2013). In some circumstances, pH will affect the drug's stability and the

physicochemical properties of the formulation will be changed according to the pH, especially when using carbopol as gelling polymer. Carbopol needs to be neutralized to pH of around 7 in order to achieve optimum viscosity, elasticity and yield stress (Giuseppe et al., 2015).

Topical medication is a favourable drug delivery route for wound healing due to its benefits of avoiding first pass metabolism, patient compliance (Bartosova et al., 2012) and direct delivery of the active ingredient to the target site. However, there are some limitations of this route such as low permeability across stratum corneum or membranes. Gel formulation is one of the preferred vehicle for topical drug delivery (Ueda et al., 2009).

Generally, viscosity is the main parameters to be discussed in rheological characterisation. Higher viscosity is associated with reduced fluid mobility within the semi-solid dispersion compared to normal non-Newtonian fluid (Lauterbach et al., 2014). Rheological concept can be explained in term of modelling, whether Herschel Bulkley or Ostwald de-Waele model. Gels that exhibited yield stress will fits well to Herschel Bulkley modelling (Equation 1), while those without yield stress will fits well to Ostwald de-Waele modelling equation (Equation 2).

$$\tau = \tau_0 + \mathrm{K(y)^n} \qquad \text{(Equation 2.1)}$$

where τ is shear stress Pa, τ_0 is yield stress (mpa), *K* is consistency factor, *y* is shear rate s^{-1}, and n is flowability index (Markis et al., 2014).

$$\tau = K(y)^n \qquad \text{(Equation 2.2)}$$

where τ is shear stress, *K* is consistency factor (Pas^n), *y* is shear rate, and n is flow behavior index (Fonseca et al., 2009). The numerical value of of consistency factor *K*

value relates to its concentration, consistency, and how pseudoplastic it is along the flow behavior curve (Qiao et al., 2016). Flow behavior index (*n*) illustrates the type of fluid, in which if $n < 1$ the fluid exhibited pseudoplastic behavior. If $n = 1$, the fluid is Newtonian and if $n > 1$, the fluid is dilatant (Binsi et al., 2009; Ibrahim et al., 2010; Lewis, 1990). Low value of *n* illustrates better pseudoplasticity of the gels (Wang et al., 2016). Salad dressing is one of the examples that demanded finite yield stress (shear stress) in order to starts flowing. The specific Herschel Bulkley profile is important to determine salad dressing's ability to adhere to the vegetable surfaces. The regression value, denoted by R^2 indicates the flow behavior linearity, whether they fits well to the rheological modelling. $R^2 > 0.98$ indicates high and acceptable regression that firs well to the modelling (Ibrahim et al., 2016)

2.2.2 Gelling polymers

Gelling agents can be of various subtypes such as carrageenans, cellulose derivatives, and others.

2.2.2.1 Carrageenans derivatives

Carrageenan can be classified into many types, including κ , ε, λ, μ ,and ι, depending on their solubiity in potassium chloride . The classification is based on their percentage of sulphate group in which varies from 22 to 35% (Necas et al., 2013). Carrageenans are composed of anhydrogalactose units linked by glycosidic unions, as well as galactose (Coviello et al., 2007; Jiao et al., 2011; Li et al., 2014). They are natural polysaccharides derived from edible seaweed (Chondrus crispus species i.e. carrageenan moss). Carrageenan consist of alternate d-galactose units and 3,6-anhydro-galactose (3,6-AG) units interconnected by α-1,3 and β-1,4-glycosidic