AGARWOOD EXTRACT LOADED POLY(VINYL ALCOHOL) (PVA) ELECTROSPUN FIBRE AS HALAL BIOMATERIAL FOR WOUND HEALING

BY

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ABSTRACT

Electrospun nanofibers have been extensively studied for wound healing application due to their remarkable properties. The incorporation of plant extract in electrospun nanofibers has contributed to the development of bioactive dressings enabling the effective and efficient wound repair. In this work, the fabrication of such nanofibers was undertaken following halal built-in concept. Poly (vinyl alcohol) (PVA) fibre mats containing *Aquilaria malaccensis* leaf extract (ALEX) [5, 10 and 15 %(w/w)] were fabricated by electrospinning for wound healing application. Prior to the fabrication of ALEX-loaded PVA nanofibers, the phytochemical constituents of both derivatized and non-derivatized ALEX were identified using gas chromatography - mass spectrometry (GC-MS). The anti-bacterial activity of ALEX against *Escherichia coli, Vibrio vulnificus, Bacillus subtilis* and *Staphylococcus aureus* was evaluated by modified Kirby Bauer disc diffusion method. The anti-inflammatory activity of ALEX was performed via in-vitro lipoxygenase assay. GC-MS analysis of ALEX confirmed the occurrence of a total 127 compounds from the derivatized sample and only 22 compounds in the non-derivatized sample. ALEX showed comparable anti-bacterial activity with zone of inhibition of 10.2 - 21.7 mm for gram positive bacteria and 10.7 - 19.7 mm for gram negative bacteria. Minimum inhibitory concentration (MIC) values of ALEX against these bacteria ranged from 5.625 mg/ml to 0.352 mg/ml. ALEX also showed high lipoxygenase inhibitory activity with an IC_{50} of 21.365 μg/ml in comparison to the positive control, nordihydroguaiaretic acid (NDGA) with an IC_{50} of 6.383 μg/ml. These results supported the use of ALEX as an active ingredient in the fibre mats. The nanofibers were uniform, beadless and randomly oriented with average diameters ranged between 195.27 – 281.20 nm. The presence of ALEX in the PVA nanofibers were evaluated by Attenuated total reflectance-Fourier transform infrared microscopy (ATR-FTIR) and differential scanning calorimetry (DSC). Next, the mechanical properties, swelling degree and weight loss of nanofiber mats were also determined. ALEX was rapidly released from the ALEX-loaded PVA nanofibers in the first 12 hours followed by gradual release afterwards. The released rate was dependent on ALEX content in the PVA nanofibers. Swelling degree and porosity of the nanofibers were found to be between 241.66 – 305.86% and 64.53 – 30.81%, respectively. Meanwhile, the tensile stress and maximum elongation at break for all electrospun nanofiber mats were in the range of 8.56 – 2.68 MPa and 205.94 – 166.31%, respectively. The nanofiber mats inhibited growth of *Escherichia coli, Vibrio vulnificus, Bacillus subtilis* and *Staphylococcus aureus* with zone of inhibition of 7.5 - 15.0 mm for gram positive bacteria and 6.1 - 11.7 mm for gram negative bacteria. ALEX-loaded PVA nanofibers also showed potent anti-inflammatory activity against lipoxygenase with percentage of inhibition between 80.887 – 86.977%. Taken together, the results of this study suggest that ALEX-loaded PVA nanofibers have the desired properties of bioactive wound dressing and could open up new horizon in the fabrication of wound dressing through its *halal* built-in concept.

خُلاصة البحث

تمت هذه الدراسة على تصنيع حصير الألياف من كحول الفينيل المتعدد (PVA) والذي يحتوي على خلاصة أوراق العود الهندي (ALEX) بالنسب التالية 5%، و10% و15٪، وذلك عن طريق الغزل الكهربائي لتطبيق التئام الجروح. قبل تصنيع ألياف PVA النانوية المحملة بـ ALEX، تم تحديد المكونات الكيميائية النباتية لكل من ALEX المشتق وغير المشتق باستخدام طريقة تحليل الاستشراب الغازي ومطيافية الكتلة (GC–MS). تم تقييم نشاط ALEX المضاد للبكتيريا على بكتيرا الإشريكية القولونية وفايبريو فولنيفيكوس والعصوية الرقيقة والبكتيريا الكروية العنقودية الذهبية وذلك بواسطة الطريقة المعدلة للاقراص الناشرة (طريقة كيربي- باير). تم تجربة نشاط ALEX المضاد للالتهابات بواسطة اختبار الإنزيم المؤكسد للدهون. أكد تحليل (GC–MS) الذي تم إجراؤه على العود الهندي وجود 127 مركبًا من العينة المشتقة و 22 مركبًا فقط في العينة غير المشتقة. كما أظهر ALEX نشاطًا ملحوظًا لكونه مضادًا للبكتيريا مع منطقة تثبيط يبلغ قطرها 10.2 – 21.7 ملم للبكتيريا إيجابية الجرام و 10.7 – 19.7 ملم للبكتيريا سالبة الجرام. تراوحت قيم التركيز المثبط الأدنى (MIC) الخاصة بـALEX ضد هذه البكتيريا بين 5.625 مجم/مل إلى 0.352 مجم/مل. وأظهر ALEX كذلك نشاطًا مثبطًا عاليًا في الإنزيمات المؤكسدة للدهون بقيمة IC_{50} تبلغ 21.365 ميكروغرام/مل. دعمت هذه النتائج استخدام ALEX كمكون نشط في حصائر الألياف. كانت الألياف النانوية متجانسة وخرزية الشكل وموجهة عشوائياً بمتوسط قطر يتراوح بين 195.27 – 281.20 نانومتر. تم تقييم وجود ALEX في ألياف PVA النانوية من خلال الانعكاس الكلي المخفض بالاقتران مع التحليل الطيفي بالأشعة تحت الحمراء (ATR–FTIR) ومسعر المسح التباينى الحراري (DSC). بعد ذلك، تم تحديد الخواص الميكانيكية ودرجة الانتفاخ ونسبة فقدان الوزن لحصائر الألياف النانوية. تم إخراج ALEX بسرعة من ألياف PVA النانوية المحملة بـALEX في أول 12 ساعة، وقد زاد تدريجيًا بعد ذلك. اعتمد معدل ALEX الذي تم إصداره على محتوى ALEX في ألياف PVA النانوية. هذه النتيجة كانت كذلك بناءً على درجة الانتفاج والمسامية للألياف النانوية حيث تراوحت نتائجهم بين 241.66 – 305.86٪ و 64.53 – 30.81٪ على التوالي. وفي الوقت نفسه، تراوح إجهاد الشد والحد الأقصى من الاستطالة عند الكسر لجميع حصائر ألياف النانو المغزولة كهربائيًا في نطاق 2.68– 8.56 ميجا باسكال و166.31–205.94٪، على التوالي. كبحت الحصائر النانوية نمو الإشريكية القولونية وفايبريو فولنيفيكوس والعصوية الرقيقة والبكتيريا الكروية العنقودية الذهبية مع منطقة تثبيط 7.5 – 15.0 مم للبكتيريا إيجابية الجرام و 6.1 – 11.7 ملم للبكتيريا سالبة الجرام. كما أظهرت ألياف PVA النانوية المحملة بـ ALEX نشاطًا قويًا مضادًا للالتهابات ضد إنزيمات الأكسدة الدهنية بنسبة تثبيط تتراوح بين 80.887 – 86.977٪. وبعد النظر إلى جميع النتائج ، بإمكاننا القول أن نتائج هذه الدراسة تشير إلى أن ألياف PVA النانوية المحملة بـ ALEX تمتلك الخصائص المطلوبة لتضميد الجروح النشطة حيويًا ويمكن أن تفتح أفاقًا جديدة في تصنيع ضماد جروح مدمج وحلال من خلال المفهوم الشرعي للحلال.

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 Science (Halal Industry Science)

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Hamzah bin Mohd Salleh
Dean, Kulliyyah of INHART

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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LIST OF ABBREVIATIONS

A	*Aquilaria*
ALEX	*Aquilaria malaccensis* leaf extract
ANOVA	Analysis of variance
ASTM	American Society for Testing and Materials
ATR	Attenuated total reflectance
C	Celsius
CA	Cellulose acetate
CFU	Colony forming unit
CHC	Consumer health care
CITES	Convention on International Trade in Endangered Species
cm	centimetre
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DSC	Differential scanning calorimetry
ECM	Extracellular matrix
e.g.	For example
et al.	(*et alia)*: and others
FTIR	Fourier-transform infrared spectroscopy
GCMS	Gas chromatography – mass spectrometry
GMP	Good manufacturing practice
i.e.	That is
kg	kilogram
LOX	Lipoxygenase
NDGA	Nordihydroguaiaretic acid
nm	nanometre
mg	milligram
MIC	Minimum inhibitory concentration
mL	millilitre
Pa	Pascal
PCT	Patent cooperation treaty
PEO	Poly(ethylene oxide)
PIC/S	Pharmaceutical Inspection Co-operation Scheme
PLGA	Poly(lactic-co-glycolic acid)
PLLA	Poly(lactic acid)
PVA	Poly(vinyl alcohol)
PVP	Poly(vinyl pyrrolidone)
V	Volt
SEM	Scanning electron microscopy
SF	Silk fibroin
spp.	Species

INTRODUCTION

1.1 BACKGROUND OF STUDY

Electrospinning is a simple but powerful method used in the fabrication of continuous ultrafine fibres at the range of nanometres to submicron scales with well-defined morphologies (Bhattacharjee & Rutledge, 2011). These electrospun fibres have shown outstanding potential in many applications from electrical to biomedical technology. In the past decades, this technology has shown significant advances in biomedical field particularly in enzyme immobilization, tissue engineering and drug delivery due to its high surface area to volume ratio, superior mechanical properties and ability in mimicking the architecture of the native dermal extracellular matrix (ECM). It has been widely used as an alternative to conventional wound healing applications by manipulating different types of polymeric nanomaterial as fibre mats (which act as a scaffold) in improving the cell adhesion, migration, proliferation (Mutlu et al., 2017). This method has shown significantly better results compared to the conventional dressings owing to their unique properties.

Recently, there has been growing interest in the incorporation of plant extracts with polymers by using electrospinning technique for the purpose of wound dressings (Zhang et al., 2017). These plants have shown promising potential in pharmacological practices due to its naturally occurring compound that exhibits good antioxidant, antimicrobial and anti-inflammatory effects for the effective healing of wound. It is halal in nature as it is non-hazardous to health, does not contain *najs* or involve any processing which against the *Shariah* guidelines. A number of fibre mats have been successfully produced by utilizing polymer along with different composite

incorporations such as extract from the fruit hull of mangosteen (Ruktanonchai et al., 2017), soursop leaves extract (Aruan et al., 2017) and bitter melon (*Momordica charantia*) fruit extract (Alippilakkotte et al., 2017).

Meanwhile, ethnopharmacological practices have shown significant evidences on the ability of agarwood as a source of material for treatment of numerous pharmacological activities including anticancer, antipyretic and antioxidant (Sattayasai et al., 2012; Hashim et al., 2014). To the best of our knowledge, to date there is no report on the development of poly(vinyl alcohol) (PVA) incorporated with extract from agarwood leaf. Hence, the objective of this study is to use poly(vinyl alcohol) (PVA) as a matrix material to incorporate *Aquilaria malaccensis* leaf extract (ALEX) by electrospinning technique to fabricate fibre mat that is potential for use as halal functional biomaterial particularly in wound treatment.

1.2 PROBLEM STATEMENT

The rapid growth of nanotechnology has spurred the development of nanofibrous scaffolds due to the diversity of the electrospinnable materials and the preparation methods which makes electrospun scaffold as an attractive material for variety of biomedical applications. Numerous types of organic polymers, including both natural and synthetic polymers, have been successfully electrospun with plant derived-natural products. Plants are also halal except those that are poisonous, intoxicating or hazardous to health. Agarwood species is well-known for its aromatic fragrance originated from the resin embedded heartwood. It has shown good

pharmacological effects which may be helpful in the treatment of acute and malignant wound.

As the conventional wound dressings, e.g. woven gauze only provides common protection and recuperation without delivering any extraordinary properties, nanofibrous dressings (fibre mats) incorporated with agarwood extracts is a better alternative to the conventional dressing.

Apart from that, wound dressings are also commonly associated with the use of gelatine, human placenta and others (Ab Manan et al., 2016; Al-Teinaz et al., 2020), which is one of the long-standing issues for Muslim consumers in regard to pharmaceutical ingredients. As such, this study intends to integrate the agarwood leaf ethanolic extract with PVA in producing a biologically active wound dressing.

Agarwood leaf is commonly discarded as waste during the cultivation of the dark fragrant resinous wood waste due to its lesser market value in comparison to the resin and woody parts of the tree. The utilization of discarded leaves would be an environmentally friendly step as it promotes zero waste lifestyle while providing a sustainable resource when developed into useful products. This will initiate further exploitation of the halal and safe abundant resources of A. malaccensis leaf as a functional biomaterial for wound healing. Ultimately, this may help in the development of halal pharmaceutical products through halal built-in concept whereby the halal requirements are integrated into every aspect of the production from the beginning until delivery of finished product to its point of sale.

Halal is not merely an Islamic concept, but rather a universal concept where it covers all spectrum in the development of products. It is not limited to the

ingredients but also include the safety and hygiene, meaning that it suits for both Muslims and non-Muslims.

1.3 IMPORTANCE OF STUDY

Modern dressings biomaterial made up of electrospun nanofibers (also known as fibre mats), act as good substitutes to conventional fibrous dressing material such as gauze due to their high surface area to volume ratio and biologically active compounds that aid wound healing process. The electrospun nanofibrous dressing also able to mimic the structural and mechanical characteristics of dermal extracellular matrix (ECM). In particular, the successful fabrication of PVA-loaded biologically active materials such as agarwood extract can open up new door for the development of effective dressing material incorporating natural products for acute and chronic wounds. The use of agarwood leaf also fulfils the sustainable development goals through waste reduction initiatives where the discarded materials are resources for another useful product. This sustainable development goals concept is aligned with Islamic teaching which is to avoid wastage by conserving and recovering all resources. It is our responsibility as a Muslim to utilizes bounties bestowed by Allah in a proper manner. Indeed, Allah (SWT) says in Quran:

وَهُوَ ٱلَّذِى جَعَلَكُمْ خَلَٰٓئِفَ ٱلْأَرْضِ وَرَفَعَ بَعْضَكُمْ فَوْقَ بَعْضٍ دَرَجَٰتٍ لِّيَبْلُوَكُمْ فِى مَآ ءَاتَىٰكُمْ ۗ إِنَّ رَبَّكَ سَرِيعُ ٱلْعِقَابِ وَإِنَّهُۥ لَغَفُورٌ رَّحِيمٌ

*"**And it is He who has made you successors upon the earth** and has raised some of you above others in degrees [of rank] that He may try you through what He has given you. Indeed, your Lord is swift in penalty; but indeed, He is Forgiving and Merciful."* (Al-Quran 6:165)

1.4 OBJECTIVES

The aim of the research is to fabricate electrospun PVA-based fibre mats incorporated with *A. malaccensis* leaf ethanolic extract (ALEX).

The specific objectives in this study are:

a) To investigate antibacterial and anti-inflammatory activities of ALEX for incorporation into electrospun PVA fibre mats.
b) To analyse the physicochemical properties and mechanical behaviour of extract loaded PVA fibre mats at different concentrations.
c) To validate antibacterial and anti-inflammatory activities of ALEX-loaded PVA fibre mats.

1.5 SCOPE OF STUDY

The study involves the antibacterial and anti-inflammatory test of *Aquilaria malaccensis* leaf extract (ALEX) crude extracts prior to electrospinning. Subsequently, electrospinning of ALEX loaded PVA nanofibers, characterization of electrospun PVA-ALEX composite nanofibers to determine the fibrous morphology and size of the of the fibre mats by using Scanning Electron Microscope (SEM). FTIR analysis was conducted to determine the existing functional groups in PVA nanofibers, ALEX solution and PVA-ALEX composite nanofibers. The physicochemical properties and mechanical behaviour of the fibrous materials was also evaluated to validate the flexibility and durability of the fibre mats for the preparation of an effective wound dressing material. Apart from that, a validation test for antibacterial and anti-inflammatory test for as-loaded extract electrospun

fibre mats were performed to ensure that the fibre mats possess the properties that are essential for wound healing management. This work, however does not include the study of the fabricated fibre mats on skin or skin equivalent.

1.6 THESIS OUTLINE

The thesis is divided into five chapters: Chapter 1 presents a brief explanation about the development of electrospun fibre mats from PVA and agarwood extract leaves as loaded material. Chapter 2 is the literature review of past research which is related to the scope of this study. Chapter 3 describes the methodology of the study for the preparation of fibre mats, physicochemical and mechanical behaviour assessments along with biological studies. Chapter 4 contains results and discussions. Finally, the conclusions and recommendations are discussed in Chapter 5.

CHAPTER TWO

LITERATURE REVIEW

2.1 ELECTROSPINNING

2.1.1 Introduction

Electrospinning is a dry spinning process which utilizes electrostatic forces to draw long polymer fibres in nanometre scale from a liquid or melt polymer solution (Reneker et al., 2007). During electrospinning, a continuous jet strands are ejected from the needle tip due to high voltage electric field applied to the polymer solution which induced an electric charge on the surface of the liquid, leading to the acceleration of the polymer fibres toward the oppositely charged ground collector. As the intensity of electric field increases, the polymer droplets at the tip of capillary tube elongates and developed into a conical shape known as "Taylor Cone". When the electric field magnitude sufficiently great to overcome the surface tension of the liquid, an electrified polymer jet is ejected from the apex of the Taylor Cone (Doshi & Reneker, 1995).

Since this fibre jet is induced with an electric charge, its trajectory can be manipulated by an electric field. After the charged jet travels through the atmosphere, the solvent evaporates and solid polymer fibres are deposited on a grounded collector as a mesh or scaffold (Bhardwaj & Kundu, 2010). The fibre will be continuously whipped and elongated as long as there is no disruption on the feed of the electrospinning jet.

The electrospinning process can be affected by several parameters which are classified broadly into three categories namely solution parameters, process parameters

and ambient parameters (Z. Li & Wang, 2013). Each of the parameter significantly affects the fibre morphologies and diameters. As such, optimization of these parameters is essential in obtaining smooth nanofibers while reducing undesirable properties such as formation of beads-on-string fibres. Fong et al., (1999) reported on the formation of beads as by products on the electrospun fibres; which is related to the instability of the jet polymer solutions that occur due to changes in the parameter.

2.1.2 History of Electrospinning

Electrospinning (or 'electrostatic spinning') technique has received more attention from researchers in recent years due to its versatility and potential for applications in diverse fields. It has a notable history which began in 1902 when John Francis Cooley became the first person who patented the remarkable discovery on electrospinning which become an important step for upcoming applications. In 1914, John Zeleny had reported on behavior of fluid droplets at the end of metal capillaries and a mathematical model has been developed to examine the behavior of fluids under electrostatic forces. Between 1931 and 1944, Anton Formhals has issued at least 22 series of patents which focused on electrospinning apparatus after numbers of attempt in modification and improvement of the device (Tucker et al, 2012).

In 1938, N.D. Rozenblum and I.V. Petryanov-Sokolov successfully manufactured filter materials known as 'Petryanov filters' which was generated from electrospun fibres (Heikkil, 2008). Between 1964 and 1969, Sir Geoffrey Ingram Taylor developed a theory underpinning the electrospinning phenomena through mathematical modelling of the conical shape fluid knowns as Taylor cone that originates from the formation of fluid droplet when it was ejected at the tip of the capillary under the effect

of an electric field. Later Doshi & Reneker (1995) published their paper that popularized the term electrospinning through demonstration of electrospun nanofibers and its applications. Since then, there has been an increase in the number of publications and technologies pertaining to electrospinning applications.

2.1.3 Description of electrospinning apparatus

The typical set up of electrospinning apparatus consists of four primary components which are listed in Table 2.1 below.

Table 2.1 Apparatus for electrospinning

Apparatus	Function
i. High voltage DC power supply	Induced electric charge into a polymer solution
ii. Spinneret or pipette tip	Concentrate the drawing of polymer solution
iii. Syringe pump	Control the flow rate of polymer solution
iv. Grounded collector plate	Deposition of solid polymer fibres as mats

There are two experimental setups commonly used for electrospinning which are horizontal and vertical as illustrated in Figure 2.1 (a,b). In the typical horizontal setup, the syringe is positioned parallel to the floor and the grounded collector plate is placed perpendicular to the floor across the needle of the syringe. Meanwhile, for the typical vertical electrospinning setup, the syringe pump is settled above the collector that is located on the floor. The difference in the setup might affect the shape of the polymer droplet and the Taylors Cone due to gravitational force and electrostatic repulsion.