FORMULATION AND OPTIMISATION OF TOPICAL PREPARATION FROM MALAYSIAN HONEY FOR PREVENTION AND TREATMENT OF BACTERIAL INFECTION

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We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Doctor of Philosophy.

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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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ABSTRAK

Jangkitan bakteria merupakan masalah yang kerap berlaku pada luka. Rawatan dengan menggunakan antibiotik tidak sentiasa berkesan kerana wujudnya bakteria perintang antibiotik. Madu adalah sumber semulajadi yang menjadi alternatif untuk mencegah jangkitan bakteria. Walaupun telah dikenalpasti mengandungi kesan bio-aktif yang bermanfaat seperti antibakteria, penggunaan madu Malaysia untuk mencegah pertumbuhan bakteria yang sering dikaitkan dengan jangkitan luka masih belum dipastikan. Berdasarkan pada kesan hutan hujan tropika, madu di Malaysia mengandungi kandungan lembapan yang tinggi menyebabkan ianya tidak sesuai untuk terus digunakan sebagai alternatif tanpa penambahbaikan sifat reologinya. Dalam usaha untuk memanfaatkan madu Malaysia untuk rawatan luka, kajian ini berusaha untuk menyediakan madu Malaysia sebagai produk sapuan antibakteria dengan menambahbaik sifat reologi madu menggunakan gam xanthan dan gam guar sebagai agen pempolimeran secara berasingan. Melalui kajian antibakteria yang mendalam, kesan bakteriostatik dan bactericidal pada madu Malaysia yang di wakili oleh madu kelulut, tualang and akasia diuji pada bakteria yang sering menjangkiti luka. Dalam usaha untuk menambahkan kefahaman, faktor seperti kadar keasidan, unsur peroksida dan bukan peroksida yang menyumbang kepada kesan antibakteria pada madu telah dikenalpasti secara kualitatif. Metodologi permukaan sambutan (RSM) telah digunakan untuk mengoptimumkan kesan antibakteria dalam produk sapuan yang disediakan. Setelah dioptimumkan, sifat fizikokimia, keberkesanan antibakteria dan kestabilannya produk telah diuji. Berdasarkan pada hasil kajian yang diperoleh, madu kelulut, tualang dan akasia adalah agen yang boleh digunakan untuk mencegah pertumbuhan bakteria yang sering menjangkiti luka yang telah disahkan berdasarkan pada kesan bakteriostatik pada kepekatan 50% (w/v) dan ke bawah. Bagi kesan bactericidal pula, madu kelulut adalah satu-satunya madu yang menunjukkan kesan bactericidal terhadap semua bakteria yang diuji pada kepekatan 50% (w/v) dan ke bawah. Kesan bactericidal tidak terdapat pada madu tualang dan akasia apabila diuji terhadap beberapa spesis bakteria termasuk *Escherichia coli* and *Enterococcus faecalis*. Faktor utama kewujudan kesan antibakteria pada madu kelulut adalah berpunca dari faktor keasidan, diikuti dengan unsur peroksida dan bukan-peroksida. Berdasarkan pada kesan antibakteria yang tinggi, madu kelulut telah digunakan dalam penyediaan produk sapuan yang boleh diformulasi secara optimum pada pH 3.5, kepekatan madu 90% (w/v), dan kepekatan agen pempolimeran 1.5% (w/v). Gam xanthan merupakan agen pempolimeran yang lebih baik berbanding guar gum kerana produk sapuan yang disediakan dengan menggunakan gam xanthan menunjukkan zon pencegahan sebanyak 1.2 kali ganda lebih besar, membuktikan kesan antibakteria yang lebih tinggi. Hal ini telah diperkukuhkan lagi dengan kadar kelikatan yang stabil pada produk yang disediakan dengan gam xanthan sepanjang enam bulan ujian kestabilan. Manakala keputusan yang berbeza telah diperoleh untuk produk yang menggunakan gam guar dengan penyusutan kadar kelikatan sebanyak 3.6 kali ganda diantara bacaan awal (bulan 0) dan bacaan akhir (bulan ke-6). Sebagai penutup, diantara madu Malaysia, kelulut adalah agen yang sangat berkesan bagi mencegah pertumbuhan bakteria yang sering menjangkiti luka. Kajian ini telah berjaya menyediakan madu kelulut sebagai produk sapuan dengan menggunakan gam xanthan bagi menghasilkan produk yang stabil dan sifat fizikokimia yang mencukupi tanpa menjejaskan kesan antibakterianya.

ABSTRACT

The bacterial infection is the most common contamination on a wound. The use of antibiotics as a primary treatment is not always effective due to the emergence of antibiotic-resistance bacteria. Honey is one alternative plant by-product that can be used to prevent bacterial infection. While known to possess beneficial bioactive properties, including antibacterial, the effective use of Malaysian honey to prevent the growth of wound-associated bacteria is undetermined. Due to tropical rainforest, Malaysian honey owned high moisture content that unsuitable to be directly applied without improvement on its rheological properties. In an effort to make use of Malaysian honey in the treatment of the wound, this study aims to prepare the Malaysian honey as a topical antibacterial preparation by improving the rheological properties of the honey using xanthan gum and guar gum, which were independently used as the polymeric agents. Considering thorough antibacterial evaluation, the bacteriostatic and bactericidal effects against bacteria associated with wound infection of three Malaysian types of honey (kelulut, tualang, and acacia) were evaluated. In the effort to further understand the antibacterial properties of the honey, the factors of acidity, peroxide, and non-peroxide compounds that contribute to antibacterial properties of the honey were qualitatively identified. Response Surface Methodology (RSM) was used to optimise the antibacterial properties of the preparations. Once optimised, the optimal preparations were evaluated in terms of physicochemical properties, antibacterial efficacy, and stability. Based on the findings, kelulut, tualang, and acacia have been beneficial agents to prevent the growth of wound infection bacteria, supported by the existence of bacteriostatic effect against the bacteria at the concentration of 50% (w/v) and below. As for the bactericidal effect, kelulut was the only honey that exhibited bactericidal effect against all bacteria tested at the concentration of 50% (w/v) and below. The bactericidal effect was absent in tualang and acacia on certain bacteria, including *Escherichia coli* and *Enterococcus faecalis*. The antibacterial properties of kelulut were observed to be majorly due to acidity, followed by peroxide and non-peroxide compounds. Based on the potent antibacterial properties, kelulut was considered for the topical preparation which the optimal preparation was formulated at pH of 3.5, honey concentration of 90% (w/v), and polymeric agent of 1.5% (w/v). Xanthan gum was revealed as the recommended polymeric agent since the preparation using xanthan gum showed a larger inhibition zone by 1.2-fold compared to guar gum, indicating higher antibacterial properties. This was supported by the stable viscosity of the preparation using xanthan gum within six months of stability study, while a contradicting outcome was observed using guar gum with reduction in viscosity by 3.6-fold between initial (0 month) and final (6th month) measurement. In conclusion, among the Malaysian honey, kelulut was the most promising agent that can be used to inhibit the bacteria associated with wound infection. The study has successfully prepared the kelulut honey as a topical preparation using xanthan gum that resulted with stable and adequate physicochemical properties, without compromising on its antibacterial efficacy.

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LIST OF SYMBOLS

™	Trademark
µ	Micro
±	Plus minus
α	Alpha
<	Less than
>	More than
ºC	Celcius
%	Percent
-	Not related
(w/v)	Weight over volume
®	Registered sign
α_w	Water activity

LIST OF ABBREVIATIONS

A.	Apis/ Acinetobacter
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
B.	Bacillus
C	Carbon
CCD	Central Composite Design
CFU	Colony forming unit
CLSI	Clinical and Laboratory Standard Institute
CoA	Certificate of analysis
cm	Centimetre
DNA	Deoxyribonucleic acid
DoE	Design of Expert
E.	Enterococcus/Escherichia
Eucast	European Committee on Antimicrobial Susceptibility Testing
FDA	Food and Drug Association
g	Gram
GAE	Gallic acid equivalent
H	Hydrogen
h	Hour
HAI	Hospital acquired infection
H_2O_2	Hydrogen peroxide
HMF	Hydroxy methyl furfural
ICH	International Council of Harmonisation
IIUM	International Islamic University of Malaysia
IIUMMC	International Islamic University of Malaysia Medical Centre
IREC	Institute of Research and Ethical Committee
K.	Klebsiella
k	kilo
KBioCorp	Kedah Bio-resource Corporation
L	Litre
LCQTOF-MS	Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry

m	Mili
M	Molar
MARDI	Malaysia Agricultural Research and Development Institute
MBC	Minimum bactericidal concentration
MGO	Methylglyoxal
MGI	Malaysia Genome Institute
MIC	Minimum inhibitory concentration
min	Minute
mm	Milimeter
MRSA	Methicillin Resistant *Staphylococcus Aureus*
MTCC	Microbial Type Culture Collection
n	Nano
NA	Not avaliable
NaOH	Sodium hydroxide
NP	Non-peroxide
O	Oxygen
OH	Hydroxyl group
OFAT	One factor at a time
P.	Pseudomonas
PAA	Poly-acrylic acid
PGI	Percentage growth of inhibition
PNP	Peroxide - Non-peroxide
PVA	Poly-vinyl alcohol
R	Correlation coefficient
R^2	Determination coefficient
RH	Relative humidity
rpm	Rotation per minute
RSM	Response surface methodology
S	Siemens
S.	Staphylococcus/Streptococcus/Salmonella
T.	Trigona
t	Time
TEA	Tri-ethanolamine

TSA	Trypticase soy agar
U	Unit
UAE	Ultrasonic assisted extraction
UMF	Unique manuka factor
UMP	Universiti Malaysia Pahang
UNIPEQ	Food Quality and Safety Research and Development
USA	United State of America
UT	Untreated

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