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DEVELOPMENT OF TOPICAL ANTIMICROBIAL GEL CONTAINING BEEHIVE EXTRACT

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MASTER OF SCIENCE
IN PHARMACEUTICAL SCIENCES

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TANYA SONTHIKOON

A THESIS SUBMITTED TO THE GRADUATE SCHOOL IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PHARMACEUTICAL SCIENCES

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ABSTRACT

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This study aimed to determine the antimicrobial activity of beehive extract and developed into topical gel. The beehives were extracted with 3 different solvents: distilled water (W), 50% ethanol (WE) and 95% ethanol (E), then each filtrate was evaporated by vacuum rotary evaporator to obtain concentrated crude extracts. These extracts were tested for their antimicrobial activity against five bacterial strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, Methicillin Resistance *Staphylococcus aureus* (MRSA) and β-hemolytic *Streptococcus* group A (GAS), then their MIC and MBC values were evaluated. The results revealed that the W, WE and E extracts were amounted to 16.25, 14.42 and 8.67% yield respectively. Whereas all of the extracts were found to be effective against *P. aeruginosa*, *S. aureus*, MRSA and GAS. The W extract exhibited the highest antimicrobial activity against all of strains with MIC of 31.25-62.50 mg/ml and MBC of 62.50 mg/ml, but all of the extracts ineffective against *E. coli*. Then the gel bases using different gelling agents such as Carbopol,

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Hydroxyethyl cellulose (HEC) and Hydroxypropyl methylcellulose (HPMC were formulated and evaluated for their physical) properties: appearance, texture, pH, spreadability and stability. The formula containing HEC was selected to incorporate with the W extract which showed dark brown color, smooth texture, good spreadability on the skin and good stability. The W gel exhibited a promising antimicrobial activity in both before and after stability test and also expressed no skin irritation.

ชื่อเรื่องวิทยาบิพบ**ร**์

การพัฒนาผลิตภัณฑ์เจลใช้เฉพาะที่เพื่อต้านจุลินทรีย์ซึ่งมีส่วนผสมของ

สารสกัดจากรังผึ้ง

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บทคัดย่อ

F 47206 งานวิจัยนี้ได้ศึกษาฤทธิ์ในการยับยั้งแบคทีเรียของสารสกัดจากรังผึ้ง โดยนำรังผึ้งมาสกัด ด้วยตัวทำละลายที่ต่างกัน 3 ชนิด คือ น้ำ (W), 50% เอทานอล (WE) และ 95% เอทานอล (E) สารสกัดที่ผ่านการกรองแล้ว ถูกนำไประเหยแห้งด้วยเครื่องระเหยแบบหมุน ได้เป็นสารสกัดรังผึ้ง เข้มข้น สารสกัดดังกล่าวถูกนำไปทดสอบฤทธิ์ในการยับยั้งเชื้อแบคทีเรียก่อโรค 5 สายพันธุ์ ได้แก่ Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 25923, Methicillin Resistance Staphylococcus aureus (MRSA) ແລະ β-hemolytic Streptococcus group A (GAS) โดยหาค่า MIC และ MBC ผลการวิจัยพบว่าการสกัดรังผึ้งได้ % yield ของสารสกัด W, WE และ E เท่ากับ 16.25, 14.42 และ 8.67 ตามลำคับ ส่วนฤทธิ์ในการ ยับยั้งเชื้อแบคทีเรียนั้น พบว่าสารสกัดทั้งสามชนิดสามารถยับยั้งเชื้อแบคทีเรีย P. aeruginosa, S. aureus, MRSA และ GAS ได้ สารสกัดรังผึ้ง W มีฤทธิ์ยับยั้งและฆ่าเชื้อแบคทีเรียทั้ง 4 สาย พันธุ์ได้ดีที่สุด โดยมีค่า MIC อยู่ในช่วง 31.25-62.50 มก./มล. และ MBC เท่ากับ 62.50 มก./มล. แต่สารสกัดทั้งสามชนิดไม่สามารถยับยั้งเชื้อ $E.\ coli$ ได้ สำหรับการตั้งตำรับเจลพื้นนั้นทำโดยใช้ สารก่อเจลชนิดต่างๆ ได้แก่ Carbopol, Hydroxyethylcellulose (HEC) และ Hydroxypropyl

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methylcellulose (HPMC) แล้วประเมินคุณสมบัติทางกายภาพของตำรับ เช่น ลักษณะเนื้อเจล, pH, การซึมซาบรวมทั้งการทดสอบความคงสภาพทางกายภาพ และตำรับเจลพื้นที่ใช้ HEC เป็นสาร ก่อเจล ถูกเลือกมาผสมกับสารสกัดรังผึ้ง W ได้เป็นเจลรังผึ้ง W ซึ่งมีสีน้ำตาลเข้ม เนื้อเจลเนียน ซึมซาบเร็ว และยังคงมีฤทธิ์ในการยับยั้งเชื้อแบคทีเรียได้ดี ทั้งก่อนและหลังจากการทดสอบความ คงสภาพ และไม่ทำให้เกิดการระคายเคืองต่อผิวหนังด้วย

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ABBREVIATIONS AND SYMBOLS

B. alvei

Bacillus alvei

GAS

Beta-Hemolytic Streptococci group A

Ca

Calcium

C. albicans

Candida albicans

C. pseudotropicalis

Candida pseudotropicalis

C. stellatoidea

Candida stellatoidea

C. tropicalis

Candida tropicalis

CFU/ml

Colony forming units/milliliter

°C

Degree celcius

E. faecalis

Enterococcus faecalis

E. coli

Escherichia coli

et al

Et alibi, and others

GC

Gas Chromatography

g

Gram

H/C

Heating-cooling

HPLC

High Performance Liquid Chromatography

Hr

Hour

HCl

Hydrochloric acid

HEC

Hydroxyethylcellulose

HPMC

Hydroxypropyl methylcellulose

i.e.

Id est, that is to say

kg

Kilogram

TEA

Triethanolamine

Mg

Magnesium

MRSA

Methicillin Resistant Staphylococcus aureus

μl

Microliter

μm

Micrometre

μg

Microgram

mg

Miligram

ml

Milliliter

mm

Millimetre

MBC

Minimum Bactericidal Concentration

MIC

Minimum Inhibitory Concentration

nm

Nanometer

pН

Negative logarithm of hydrogen ion concentration

N

Normality

NMR

Nuclear Magnetic Resonance

No.

Number

P

Phosphorus

PDII

Primary Dermal Irritation Index

PII

Primary Irritation Index

p-value

Probability value

P. mirabilis

Proteus mirabilis

P. aeruginosa

Pseudomonas aeruginosa

P. pyocyanea

Pseudomonas pyocyanea

NaOH

Sodium hydroxide

S. aureus

Staphylococcus aureus

TLC

Thin layer chromatography

THB

Todd Hewitte Broth

TSA

Tryptic Soy Agar

TSB

Tryptic Soy Broth

W/V

Weight by volume