THE ANTIBACTERIAL AND ANTI-ADHESION EFFECTS OF DATE FRUIT EXTRACTS (*Phoenix dactylifera*) AGAINST BACTERIA CAUSING GASTROENTERITIS

NURUL FARHANA BINTI MOHD ISHAK

UNIVERSITI SAINS ISLAM MALAYSIA

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NURUL FARHANA BINTI MOHD ISHAK
(Matric No: 4150141)

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AUTHOR DECLARATION

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged

Date :

Signature

Name :Nurul Farhana binti Mohd Ishak
Matric No : 4150141
Address: [redacted], Selangor

BIODATA OF AUTHOR

Nurul Farhana binti Mohd Ishak was born on 6th May 1986. She is currently residing at [redacted] Ehsan. She previously was a student of Universiti Teknologi Mara (UiTM) and obtained bachelor's degree of Science (Biology) and obtained her master's degree in environmental science from Universiti Putra Malaysia (UPM). She is at present a PhD student of Universiti Sains Islam Malaysia (USIM), under the supervision of Dr. Wan Shahida Wan Sulaiman, Dr. Natasya Abdullah and Dr. Zetty Nadia Mohd Zain.

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ABSTRAK

Gastroenteritis akut dilaporkan menyebabkan 5 bilion kes di seluruh dunia dan ia membawa kepada hampir 1.5 juta kematian setahun. Walaupun kebanyakannya disebabkan oleh jangkitan virus, jangkitan bakteria lebih parah dan sesetengahnya mungkin memerlukan rawatan antibiotik. Kebelakangan ini, minat pengguna dalam pengunaan makanan sunnah sebagai rawatan alternatif telah meningkat seperti Habbatus sauda, kurma, susu kambing, madu, dan buah ara. Kajian terdahulu telah melaporkan kurma mempunyai antioksidan, anti-mutagenik, anti-radang, dan aktiviti antimikrobial, namun tiada kajian yang menumpukan pada bakteria yang menyebabkan gastroenteritis. Kajian ini bertujuan untuk mengkaji kesan antibakteria buah kurma (*Phoenix dactylifera*) dan mekanisme terlibat dengan bakteria klinikal penting yang menyebabkan gastroenteritis. Kajian *in-vitro* dijalankan dengan menggunakan ekstrak tiga jenis kurma yang berbeza iaitu Ajwa, Medjool dan Mariami. Kurma itu diekstrak dengan menggunakan air sejuk, air panas dan metanol dan diuji untuk aktiviti antibakteria mereka menggunakan asai resapan telaga terhadap *Staphylococcus aureus, Salmonella enterica* serotype *Typhi, Salmonella enterica* serovar Typhimurium*, Shigella flexneri, Escherichia coli* dan *Vibrio cholerae*. Kepekatan rencatan minima (MIC) dan kepekatan bakterisidal minima (MBC) bagi ekstrak kurma juga ditentukan. Aktiviti anti-pelekatan diperiksa menggunakan model sel Caco-2. Sebatian aktif iaitu flavonoid dan fenolik telah diasingkan dari kurma dan diuji untuk kesan antibakteria mereka. Mekanisme ini kemudiannya ditentukan menggunakan mikroskop elektron. Semua tiga jenis kurma menunjukkan aktiviti antibakteria yang baik dengan ekstrak metanol kurma Ajwa menunjukkan kesan antibakteria tertinggi dengan zon perencatan 25-27.33mm terhadap *S. Typhi, V. cholerae,* dan *E. coli*. Mereka juga menunjukkan aktiviti anti-lekatan yang baik dengan ekstrak air panas dari kurmaAjwa mempamerkan aktiviti anti-lekatan yang tertinggi berbanding dengan Medjool dan Mariami dengan hampir 95.6% aktiviti anti-pelekatan terhadap semua bakteria yang diuji. Analisis fitokimia menunjukkan bahawa kurma Ajwa mengandungi kandungan fenolik dan flavonoid yang lebih tinggi berbanding dengan kurma Medjool dan Mariami (p<0.05). Flavonoid yang dipencilkan menunjukkan aktiviti antibakteria terhadap *S. Typhimurium, S. flexneri* dan *V. cholerae* dengan zon perencatan di antara 13-26mm dan nilai MIC antara 3.125mg/ml hingga 6.25mg/ml. Nilai MBC flavonoid yang dipencilkan hanya boleh ditentukan untuk *V. cholerae* dengan nilai MBC 3.125mg/ml. Flavonoid yang dipencilkan juga menunjukkan 47% aktiviti anti-pelekatan. Mikroskopi elektron mendedahkan bahawa kedua-dua ekstrak kurma dan flavonoid yang dipencilkan dapat menyebabkan kerosakan ultra-struktur pada dinding sel bakteria seperti pembentukan lepuh, kecutan dan lisis sel-sel. Kajian ini menunjukkan bahawa buah kurma mempunyai ciri-ciri antibakteria dengan keupayaan membunuh bakteria dan menghalang lekatan bakteria yang menyebabkan gastroenteritis. Sebatian flavonoid pada ekstrak buah kurma adalah bertanggungjawab terhadap aktiviti antibakteria dengan keupayaan untuk menyebabkan kerosakan ultra-struktur pada dinding sel bakteria mengakibatkan kematian sel. Sifat antibakteria buah kurma Ajwa mungkin menunjukkan pengambilan buah kurma Ajwa sebagai makanan berfungsi atau rawatan alternatif pelengkap untuk mencegah / merawat gastroenteritis bakteria.

ABSTRACT

Acute gastroenteritis was reported to lead to five billion cases worldwide and nearly 1.5 million deaths per year. Although this condition is predominantly caused by viral infections, bacterial gastroenteritis is more severe, while some conditions might require antibiotic treatment. In recent years, there has been an increasing interest among consumers' regarding prophetic food, such as black seed, dates, goat milk, honey, and figs as alternative treatments. Although previous studies recorded the antioxidant, anti-mutagenic, anti-inflammatory, and antimicrobial activities, there was no research focusing on the bacteria gastroenteritis. This study aims to investigate the antibacterial effects of date fruit (*Phoenix dactylifera*) extract and the mechanisms involved against clinically important bacterial gastroenteritis. *In-vitro* studies were conducted using the extract of three different types of dates, including Ajwa, Medjool, and Mariami. The dates were extracted using cold aqueous, hot aqueous, and methanol, followed by the testing of antibacterial activities using well diffusion assay method against *Staphylococcus aureus*, *Salmonella enterica* serotype *Typhi, Salmonella enterica* serovar Typhimurium, *Shigella flexneri*, *Escherichia coli*, and *Vibrio cholerae*. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract of the date were also determined, followed by the examination of anti-adhesion activity using Caco-2 cells model. Active compounds i.e. flavonoid and phenolic were isolated from the dates and tested for their antibacterial effect. The antibacterial mechanism of date extracts and active isolated fractions was later determined using electron microscopy. As a result, all three types of dates showed good antibacterial activity, while the methanol extract of Ajwa dates exhibited the strongest antibacterial effect with the inhibition zone of 25 - 27.33 mm against *S. Typhi*, *V. cholerae*, and *E. coli* (p<0.05). The dates also showed good anti-adhesion activity up to 95.6% against all tested bacteria, with hot aqueous extract of Ajwa dates showing the highest anti-adhesion activity compared to Medjool and Mariami dates. Phytochemical analysis indicated that the Ajwa dates contained significantly higher phenolic and flavonoid content compared to Medjool and Mariami dates (p<0.05), while isolated flavonoids (e.g.; luteolin, catechin, lutein, kaempferol, and cyanidin) in Ajwa dates showed antibacterial activity against *S. Typhimurium*, *S. flexneri*, and *V. cholerae* with the inhibition zone ranging from 13 – 26 mm and MIC values ranging from 3.125 mg/ml to 6.25 mg/ml. The MBC value of flavonoids could only be determined for *V. cholerae* with an MBC value of 3.125 mg/ml. Moreover, the isolated flavonoids showed 47% of anti-adhesion activity. It was found from the electron microscopy that both date extract and isolated flavonoids could cause ultra-structural damages to the bacterial cell wall, blisters on the cells , shrunken of the cells, and lysis of the cells. Luteolin, catechin, lutein, kaempferol, and cyanidin in Ajwa dates extract had a role in their antibacterial activity and they could cause ultra-structural damage to the bacterial cell wall, which results in cell death. This study showed that date fruits exhibited antibacterial properties which able to kill bacteria and prevent bacterial adhesion that can leads to gastroenteritis. The finding of this study suggests that the Ajwa date fruits can be used as a functional food or complementary alternative treatment to prevent/treat bacterial gastroenteritis.

الملخص

تم تسجيل خمسة مليارات حالة اصابة حادة بالتهاب المعدة والأمعاء في جميع أنحاء العالم ومليون ونصف حالة وفاة سنوياً. على الرغم من ان هذه الحالات ناتجة عن اصابة فايروسية الا ان التهاب المعدة والامعاء البكتيري يعد اكثر حدة ، وان بعض الحالات قد تتطلب العلاج بالمضادات الحيوية. في السنوات الأخيرة كان هناك اهتمام متزايد بالأطعمة المتعلقة بالغذاء النبوي مثل الحبة السوداء ، التمور ، حليب الماعز ، العسل والتين كعلاجات بديلة والتي سجلت تأثيراتها المضادة للأكسدة والمضادة للطفرة والمضادة للالتهابات والمايكروبات ، الا انه لا يوجد دراسة حول تأثيرها كمضاد لالتهابات الامعاء والمعدة. تهدف الدراسة الحالية التحري عن تأثيرات مستخلص التمر (*Phoenix dactylifera)* المضادة للبكتريا والآليات المستخدمة ضد التهاب المعدة والامعاء البكتيري. اجريت دراسات خارج الجسم الحي *In vitro* اذ استخدمت ثلاثة أنواع من التمور ، عجوة ومجدول ومريامي ، تم استخلاص التمور باستخدام مستخلص مائي بارد ومستخلص مائي حار ومستخلص ميثانولي ثم اختبرت فعاليته المضادة لبكتريا *Staphylococcus aureus, Salmonella enterica serotype Typhi, ` enterica serovar Typhimurium, Shigella flexneri, Escherichia coli, and Vibrio cholera* باستخدام طريقة الانتشار بالحفر. تم تحديد الحد الأدنى للتركيز المثبط *(*MIC*)* للبكتريا والحد الأدنى للتركيز القاتل للبكتريا *(*MBC*)* بواسطة مستخلص التمر ، تبعه فحص الفعالية المضادة للالتصاق باستخدام Caco-2 cells model . تم عزل المركبات الفعالة من التمور كالفلافونيدات والفينولات واختبارها كمضادات للبكتريا. تم تحديد ميكانيكية عمل هذه المركبات لاحقاً باستخدام المجهر الالكتروني. اوضحت النتائج ان الانواع الثلاثة من التمور كانت فعاليتها جيدة كمضاد للبكتريا وأظهر المستخلص الميثانولي للتمر أقوى تأثير مضاد للبكتريا وبمنطقة تثبيط تراوحت بين 25-27.33 ملم ضد بكتريا *S. Typhi, V. cholerae* و *E. coli*. وأوضحت التمور أيضاً فعالية جيدة مضادة للالتصاق وصلت الى 95.6% ضد جميع انواع البكتريا المختبرة وكان استخدام المستخلص المائي الحار لتمر عجوة له فعالية أعلى مضادة للالتصاق مقارنة بالانواع الاخرى من التمور المستخدمة ،مجدول ومريامي، اشار التحليل الكيميائي للنباتات ان تمر عجوة احتوى كميات عالية معنوياً من الفينولات والفلافونويدات مقارنة بالتمور مجدول ومريامي وكانت الفلافونويدات المعزولة لها تأثير مضاد لبكتريا *S. Typhimurium, S. flexneri, and V. cholerae* وبمنطقة تثبيط تتراوح بين 13-26 ملم وكانت قيمة الحد الادنى للتركيز المثبط متراوحة بين 3.125-6.25 ملغم/مللتر. تم تحديد الحد الادنى القاتل فقط لبكتريا *V. cholerae* والبالغ 3.125 ملغم/مللتر من قبل الفلافونويدات المعزولة. علاوة على ذلك ، اظهرت الفلافونويدات المعزولة فعالية مضادة للالتصاق وبنسبة 47%. وجد من خلال المجهر الالكتروني ان كلا من مستخلص التمر والفلافونويدات المعزولة منه سببت تحطيم للجدار الخلوي للبكتريا ، وظهور بثور على الخلايا وانكماش في الخلايا ، وتحلل الخلايا. سجلت الدراسة الحالية ان لثمار التمر خصائص مضادة للبكتريا وله القابلية على قتل البكتريا ومنع التصاق البكتريا المؤدي الى التهاب المعدة والامعاء. وان مركبات الفلافونويد الموجودة في مستخلص التمر كان لها دور في تلك الفعالية المضادة للبكتريا والتي أدت الى تحطيم الجدار الخلوي للبكتريا وبالتالي موت الخلية.

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LIST OF ABBREVIATION AND UNIT OF MEASUREMENTS

Abbreviations

CAM	Complementary alternative treatment
PBUH	Peace be Upon Him
WHO	World Health Organization
EM	Electron microscopy
TEM	Transmission electron microscopy
SEM	Scanning electron microscopy
MIC	Minimum Inhibitory concentration
MBC	Minimum Bactericidal Concentration
Caco-2	Human epithelial colorectal adenocarcinoma
UHPLC-ESI_QTOF-MS/MS	Ultra-high-performance liquid chromatography coupled with electrospray ionization-quadrupole-time of flight-mass spectrometry
TPC	Total Phenolic Content
TFC	Total Flavonoid content
TTC	Total Tannin Content
P. dactylifera	*Phoenix dactylifera*
S, aureus	*Staphylococcus aureus*
S. Typhi	*Salmonella enterica serotype Typhi*
S. Typhimurium	*Salmonella enterica serovar Typhimurium*
E. coli	*Escherichia coli*
V. cholerae	*Vibrio cholerae*
S. flexneri	*Shigella flexneri*
S. pyogene	*Streptococcus pyogenes*
P. aeruginosa	*Pseudomonas aeruginosa*
B. subtilis,	*Bacillus subtilis*
ETEC	*Enterotoxigenic Escherichia coli*
EPEC	*Enteropathogenic Escherichia coli*
EAEC	*Enteroaggregative Escherichia coli*