

**EVALUATION OF METABOLITES IN HUMAN
URINE POST CONSUMPTION OF *Phoenix
dactylifera* L. USING MULTI-PLATFORM
METABOLOMIC APPROACH**

NURUL ELMA BINTI SABRI

UNIVERSITI SAINS ISLAM MALAYSIA

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CONSUMPTION OF *Phoenix dactylifera* L. USING MULTI-
PLATFORM METABOLOMIC APPROACH**

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AUTHOR DECLARATION

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

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ABSTRAK

Isi kurma (*Phoenix dactylifera* L.) telah dilaporkan boleh memberi kesan baik terhadap badan manusia. Oleh itu, kajian ke atas perubahan metabolit air kencing manusia selepas pemakanan kurma Ajwa atau kurma Nabi telah dijalankan. Pada awalnya, sebatian kimia dalam kurma Ajwa diekstrak menggunakan reka bentuk tiga campuran dan dikenal pasti dengan menggunakan GCMS. Sebatian utama yang telah dijumpai ialah 2, 3-dihidro-3, 5-dihidro-6-metil-4H-piran-4-one; β -sitosterol; asid kaprilik dan asid kaprik dilaporkan berfungsi sebagai antioksidan, antitumor, antikulat dan antikeradangan, masing-masing. Setelah itu, kajian metabolomik telah dijalankan ke atas 10 orang sukarelawan yang tidak mempunyai sejarah penyakit kronik. Semua sukarelawan diminta untuk memakan 7 biji isi kurma Ajwa dengan pengambilan diet yang dikawal sepanjang tempoh kajian untuk memperolehi maklumat tambahan. Air kencing dari semua sukarelawan telah diambil dua kali pada hari 1 (7am dan 7pm), empat kali pada hari 2 (0j, 4j, 8j, 12j), dua kali pada hari 3 (24j dan 36j) dan dua kali pada hari 4 (48j dan 60j) selepas pengambilan kurma Ajwa. Perubahan metabolit dalam air kencing sampel telah dikaji dengan menggunakan $^1\text{H-NMR}$, GCMS and LC-QToF-MS. Penyiasatan ke atas data $^1\text{H-NMR}$ mendapati buah kurma memberi kesan yang sangat baik kepada badan manusia. Ini kerana, selepas memakan 7 biji isi kurma Ajwa, keamatan puncak metabolit penentuukur untuk tekanan (alanin; sitrat, dan hipurat) didapati berkurang selepas pemakanan kurma Ajwa, tetapi meningkat semula kepada tahap asalnya (0j) selepas 24 jam. Begitu juga keamatan puncak kreatinin (penentuukur fungsi renal) yang tersingkir dalam 4j, 8j, dan 12j selepas memakan isi kurma Ajwa dan kembali meningkat selepas 24j. Plot-plot *Scores* dan *Loadings* (PCA) ke atas data $^1\text{H-NMR}$ didapati melalui kitaran lengkap selama 24 jam kesan pemakanan kurma terhadap badan manusia, sekaligus menunjukkan kesan kurma dalam badan manusia adalah selama 24 jam. Oleh itu, kajian ini diteruskan dengan analisis GCMS dan LC-QToF-MS untuk mengkaji sebatian yang hadir dalam 24 jam pemakanan kurma. Tambahan lagi, sampel air kencing (0j hingga 24j) bagi seorang daripada sepuluh sukarelawan telah dipilih untuk dianalisis berdasarkan profil $^1\text{H-NMR}$. Data LC-QToF-MS menunjukkan fitokimia termasuk alkaloid, sterol, fenol dan flavonoid kelihatan berubah selepas mengambil kurma. Manakala data GCMS pula menunjukkan bahawa asid lemak seperti asid palmitik dan asid stearik di dalam air kencing bertambah selepas pengambilan kurma kerana kurma yang dimakan mengandungi asid-asid lemak. Penyiasatan lanjut mengenai metabolit dalam air kencing menggunakan NMR dan MS dapat mengesahkan kesan kebaikan kurma Ajwa terhadap kesihatan manusia. Hasil penemuan dalam kajian ini mengesahkan bahawa metabolomik adalah cara yang berkesan yang dapat digunakan untuk menilai metabolisma sebatian kimia terhadap kesihatan manusia.

ABSTRACT

Dates flesh (*Phoenix dactylifera* L.) has been reported can give beneficial effects on human body. Therefore, a study on metabolites changes in human urine after consumption of Ajwa dates or *kurma Nabi* has been performed. Initially, chemical compounds in Ajwa date were extracted using three mixture design and were identified using GCMS. The main compounds were found are 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-pyran-4-one; β -sitosterol; caprylic acid and capric acid which have been reported having pharmacological functions as antioxidants, anticancer, antifungal and anti-inflammatory, respectively. Then, metabolomics study was conducted on 10 volunteers with no history of chronic disease. All volunteers were required to consume 7 Ajwa date flesh with control diets during the experiments (4 days). Urine samples were taken twice on Day 1 (7am and 7pm), four times on Day 2 (0h, 4h, 8h, 12h), twice on Day 3 (24h and 36h) and twice on Day 4 (48h and 60h) after consumption of Ajwa date. Metabolites changes in urine samples were studied using $^1\text{H-NMR}$, GCMS and LC-QToF-MS. Investigation on $^1\text{H-NMR}$ data found that date fruits give beneficial effect on human body where the intensity of the stress biomarker metabolites (alanine; citrate and hippurate) were significantly decreased after the consumption of Ajwa dates, but were elevated back to previous level (0h) after 24 hours. The intensity of creatinine (renal function biomarker) was significantly eliminated in 4h, 8h and 12h after consuming Ajwa dates and significantly increased after 24 hours consuming Ajwa dates. Scores and Loadings plots (PCA) on $^1\text{H-NMR}$ data has illustrated a complete 24h cycle of the effects of Ajwa dates intake, which indicates the effect of consuming Ajwa dates on human body is within 24 hours. Furthermore, urine sample (0h to 24h) from one of ten volunteers was selected for further analysis using LC-QToF-MS technique. The results revealed that some phytochemicals such as alkaloids; sterols; phenols and flavonoids in urine samples were changed after taken date fruits. Meanwhile GCMS data on acid, alkaline and neutral extraction on urine samples showed that fatty acids such as palmitic acid and stearic acid in urine increased after consumption of date fruits where the dates containing fatty acids. Further investigation on the urinary metabolites using NMR-based and MS-based had confirmed the beneficial effect of Ajwa dates in human health. Findings in this study have confirmed that metabolomics is an effective tool that can be applied to evaluate the metabolism of chemical compounds for human health.

المخلص

تم دراسة علي لب التمر الذي أثبتت دراسة تأثيره المفيد علي جسم الإنسان لذلك في هذي الدراسة تم دراسة علي الايض التمر في البول البشري بعد تناول التمر العجزة او الكرمة في البداية تم استخراج المركبات الكيميائية من العجزة باستخدام ثلاثة تصميقات من خليط من المذيبات و تم تحديدها باستخدام GCMS تم العثور علي مركبات الرئيسية مثل 3,2- دي هيدرو 5,3 دي هيدروكسي ميثيل بيران, بيتا- سيتوستيرول. حمض كابريك و حامض كابريك التي تم كشف عن وجود وظائف دوائية في هذي المركبات مثل مضادات الأكسدة و مضادات للسرطان علي توالي. بعد ذلك تم دراسة ايض التمر في البول علي 10 أشخاص متطوعين و كانوا بصحة جيدة خلال تناول 7 تمرات مع عجوة مع نظام التحكم الغذائية للحصول علي نتائج جيدة. في اليوم الأول و اليوم الثاني و حتي اليوم السابع 0,4,8,12 ساعة تم الكشف عن الايض التمر في البول باستخدام , CGMS , $^1\text{H-NMR}$ LC-QToF-MS قد وجد في تحليل $^1\text{H-NMR}$ ان تمر العجوة يعطي تأثير مفيدا علي الجسم الإنسان حيث انخفضت كثافة المركبات التي تسبب الإجهاد لجسم مثل الانين و سترات و هيبيورات بشكل ملحوظ بعد حوالي 24 ساعة. كانت شدة الكرياتينين في تناقص كبير خلال 4 الي 12 ساعة في البول . أوضحت التحليل الاحصائي PCA علي بيانات $^1\text{H-NMR}$ ان الجسم يعود إلي وضعه الطبيعي بعد 24 ساعة من تناول تمر العجزة . بعد ذلك تم اختيار عينة بول واحدة من عشر متطوعين لتحليلها باستخدام تقنية LC-QToF-MS و أظهرت النتائج أن بعض المواد الكيميائية النباتية مثل القلويدات الجامدة تم تغيير الي الفينولات و الفلافونويد بعد تناول تمر . و في نفس الوقت أظهرت بيانات GCMS الخاصة بالحامض ان حامض الستريك قد زاد في البول بعد تناول التمر ذلك لانه التمر يحتوي علي احماض دهنية و لكن بكميات قليل. يمكن أن يؤكد التحقيق في المستقبلات البولية باستخدام NMR المستندة إلى MS و MS على الأثر المفيد لتمر عجوة في صحة الإنسان. تؤكد النتائج في هذه الدراسة أن الأيض هو أداة فعالة يمكن أن تكون بارعة من أجل مزيد من اكتشاف عملية التمثيل الغذائي للمركبات الكيميائية وعلاقتها بصحة الإنسان

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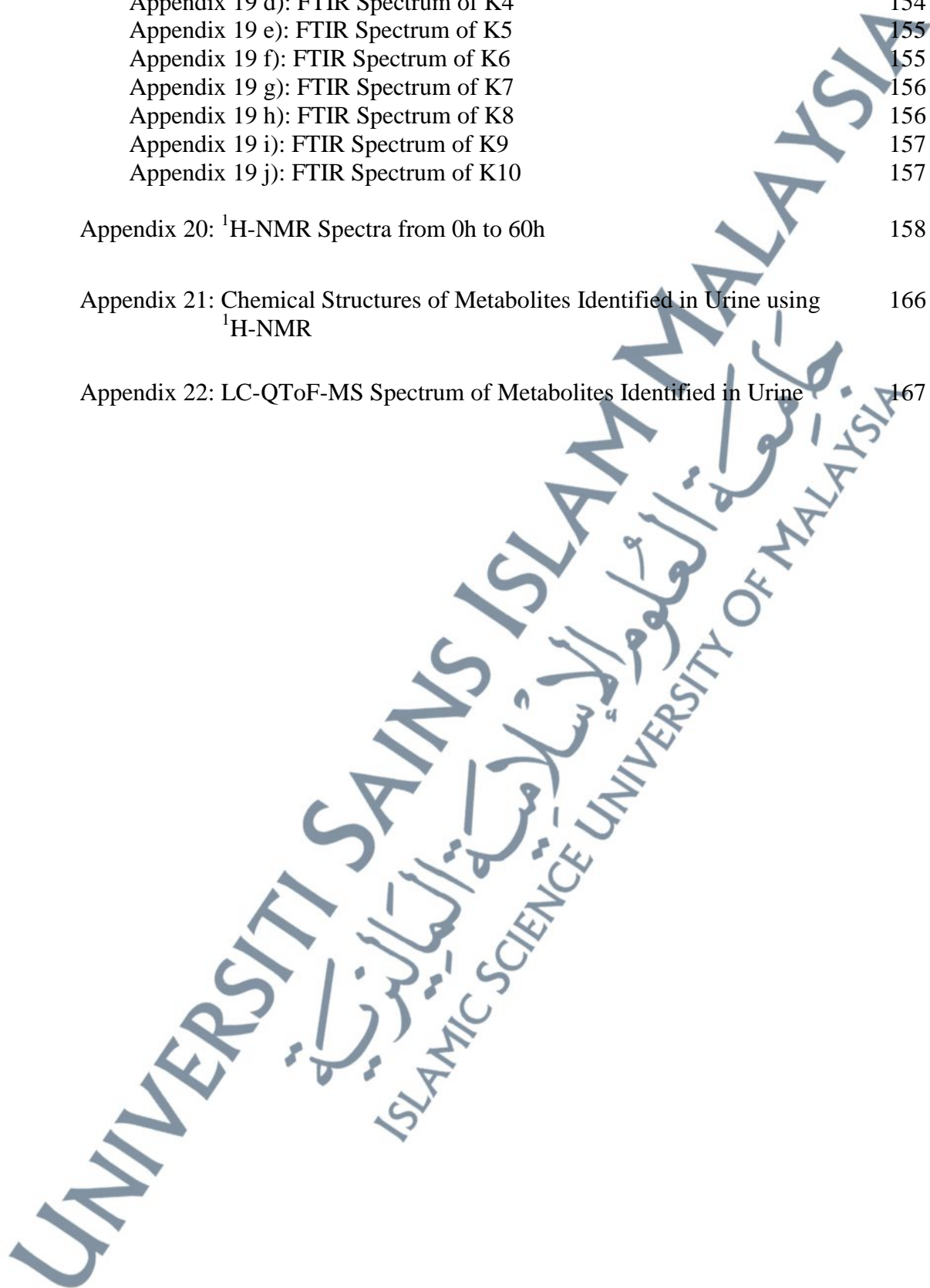
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LIST OF UNIT OF MEASUREMENTS

Maximum particle size	μm
Volume in milliliter	mL
Volume in microliter	μL
Temperature in celcius	$^{\circ}\text{C}$
Weight in gram	g
Length in meter	m

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LIST OF ABBREVIATIONS

¹ H-NMR	¹ H-Nuclear Magnetic Resonance
GCMS	Gas Chromatography Mass Spectrometry
LC-QTOF-MS	Liquid Chromatography Quadrupole Time of Flight Mass Spectrometry
nc	No change
PCA	Principal Components Analysis
HMDB	Human Metabolome Database
ATP	Adenosine triphosphate
NAD	Nicotinamide adenine dinucleotide

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