DETERMINATION OF HYDROXYCITRIC ACID (HCA) CONTENT IN ROSELLE (*Hibiscus sabdariffa* L.) THROUGH EXTRACTION OF POTASSIUM HYDROXYCITRATE AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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AUTHOR DECLARATION

بسم الله الرحمن الر حيم

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

PENENTUAN KANDUNGAN HIDROKSISITRIK ASID (HCA) DALAM ROSEL (*Hibiscus sabdariffa* L.) MELALUI PENGEKSTRAKAN KALIUM HIDROKSISITRAT DAN KROMATOGRAFI CECAIR BERPRESTASI TINGGI (HPLC)

HALIMATON SAADIAH BINTI OTHMAN

JANUARI 2012

Rosel (*Hibiscus sabdariffa* L.) dari famili Malvaceae merupakan spesies tanaman yang masih baru di Malaysia. Rosel adalah kaya dengan vitamin C dan antosianin tetapi penemuan terkini menunjukkan rosel turut mengandungi asid hidroksisitrik (HCA). Kajian ini dijalankan untuk mengekstrak HCA di dalam bentuk kalium hidroksisitrat dan menentukan kandungan kalium hidroksisitrat di dalam aksesi rosel melalui dua kaedah perbandingan pengekstrakan HCA iaitu kaedah pengekstrakan asal dan kaedah pengekstrakan termodifikasi yang menggunakan arang teraktif. Sebelas sampel digunakan dalam kajian ini iaitu roselle Aksesi 2, 3, 6, 12 dan 21 dan mutan mereka masing-masing HS0275-30-4-4-1-1-1, HS0275-30-3-3-1-1-1, HS03100-29-2-1-17-15-1, HS03100-29-7-1-6-3-1, HS1250-18-18-1-1-1-1 dan HS1250-1-18-1-1-1-1 manakala asam keping (*Garcinia atroviridis*) dan *G. cambogia* yang mana diketahui mengandungi HCA digunakan sebagai kawalan. HCA diekstrak daripada kaliks roselle kering menggunakan metanol dan HCA yang telah diekstrak kemudiannya distabilkan di dalam bentuk kalium hidroksisitrat, suatu bentuk yang kedua-duanya stabil dan aktif secara biologi. Kalium hidroksisitrat disahkan melalui analisis HPLC menggunakan kalium hidroksisitrat piawai. Keputusan menunjukkan ekstrak dari kedua-dua kaedah pengekstrakan memberikan perbezaan dari segi warna. Kaedah pengekstrakan asal menunjukkan warna ekstrak dari julat kuning air, coklat terang dan coklat. Kaedah pengekstrakan termodifikasi menunjukkan warna ekstrak dari putih ke putih kekuningan. Kaedah pengekstrakan asal menunjukkan Acc. 21 dengan kandungan hasil ekstrak tertinggi dengan 17.8%. Hasil ekstrak dari keadah pengekstrakan termodofikasi adalah sedikit berkurangan akibat dari terhapusnya kesemua kompaun terlarut air oleh arang terakif, walaubagaimanapun menunjukkan kedua-dua Acc. 21 dan HS03100-29-7-1-6-3-1 dengan 6%. pH bagi larutan ekstrak 5% dari kedua- dua kaedah pengekstrakan menunjukkan nilai pH berjulat 7.0-8.6 yang kesemuanya beralkali. Ekstrak dari dua kaedah pengekstrakan menunjukkan bahawa sangat larut dalam air dan akues methanol manakala ianya larut dalam asid hidroklorik. Ekstrak adalah tidak melarut dalam benzene, klorofom dan metanol. Kesemua ciri fiziko-kimia adalah menepati ciri-ciri seperti kawalan yang digunakan. Hasil HPLC mengesahkan bahawa kalium hidroksisitrat adalah wujud di dalam kesemua sampel roselle dan kawalan. Masa tahanan (MT) bagi kalium hidroksisitrat adalah di sekitar 4.500 min. Bagi kaedah pengekstrakan asal, kepekatan kalium hidroksisitrat adalah paling tinggi dalam Acc. 6 dengan 82.1mg/100g atau 0.082%. Manakala dalam kaedah pengekstrakan termodifikasi, kepekatan kalium hidroksisitrat tertinggi adalah dalam HS1250-1-18-1-1-1-1 dengan 42.5mg/100g atau 0.043%. Kajian ini membuktikan bahawa roselle di UKM mengandungi HCA dan menerusi kaedah pengekstrakan HCA yang besesuaian, matlamat untuk pembangunan produk berasaskan kepada HCA daripada roselle boleh dilaksanakan dan dijayakan.

ABSTRACT

DETERMINATION OF HYDROXYCITRIC ACID (HCA) CONTENT IN ROSELLE (*Hibiscus sabdariffa* L.) THROUGH EXTRACTION OF POTASSIUM HYDROXYCITRATE AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

HALIMATON SAADIAH BINTI OTHMAN

JANUARY 2012

Roselle (*Hibiscus sabdariffa* L.) from family Malvaceae is relatively a new crop in Malaysia. Roselle is rich with vitamin C and anthocyanins but recent discovery showed that roselle also contains hydroxyctric acid (HCA). This study was conducted to extract HCA in the form of potassium hydroxycitrate and to determine the concentration of potassium hydroxycitrate in roselle accessions through two comparative HCA extraction methods i.e the original extraction method and modified extraction method which used activated charcoal. Eleven samples were used in this study which were roselle Accession 2, 3, 6, 12 and 21 and their respective mutants HS0275-30-4-4-1-1-1, HS0275-30-3-3-1-1-1, HS03100-29-2-1-17-15-1, HS03100-29-7-1-6-3-1, HS1250-18-18-1-1-1-1 and HS1250-1-18-1-1-1-1 while asam keping (*Garcinia atroviridis*) and *G. cambogia* which were known to contain HCA were used as controls. HCA was extracted from dried roselle calyces using methanol and HCA extracted was then stabilised in the form of potassium hydroxycitrate, a from that is both stable and biologically active. Potassium hydroxycitrate was confirmed through HPLC analysis using standard potassium hydroxycitrate. Results showed that the extracts from both extraction methods gave differences in term of colour. Original extraction method gave extracts colours ranged from beige, pale brown and brown. Modified extraction method gave extract colours from white to offwhite. Original extraction method gave Acc. 21 with the highest yield of extract with 17.8%. The extract yield in modified HCA extraction method was slightly decreased due to the elimination of all water soluble compounds by activated charcoal, however showing Acc. 21 and HS03100-29-7-1-6-3-1 both with 6.0%. pH of 5% extract solution from both extraction methods showed pH value ranged around 7.0-8.6 which were all alkaline. Extracts from both extraction methods showed that they were highly soluble in water and aqueous methanol while soluble in hydrochloric acid. The extracts were insoluble in benzene, chloroform and methanol. All their physico-chemical characteristics complies all the characteristics the same to the controls used. HPLC results proved that potassium hydroxycitrate were present in all of roselle samples and controls. The retention time of potassium hydroxycitrate was around 4.500 min. For original extraction method, potassium hydroxycitrate concentration was the highest in Acc. 6 with 82.1mg/100g or 0.082%. While in modified extraction method, the highest potassium hydroxycitrate concentration was in HS1250-1-18-1-1-1-1 with 42.5mg/100g or 0.043%. This study demonstrates that the roselle in UKM have content of HCA and through appropriate HCA extraction method, the aim for product development of HCA-based products derived from roselle can be proceeded and succeeded.

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LIST OF SYMBOLS

%	percentage
ha	hectare
cm	centimetre
mm	milimetre
kg	kilogram
lbs	pound
min	minute
mg	milligram
γ	gamma
°C	degree Celcius
µM	micromole
µm	micrometre
µL	microlitre
g	gram
m	metre
ml	millilitre
nm	nanometre
M	mole
mm^2	millimetre square
lb/in^2	pound per inch square

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LIST OF ABBREVIATIONS

Acc.	Accession
UKM	Universiti Kebangsaan Malaysia
WHO	World Health Organisation
USD	United States Dollar
ATP	Adenosine triphosphate
HCA	Hydroxycitric acid
HPLC	High performance liquid chromatography
LD	Lethal dose
R_f	Retention factor
K_i	Affinity constant
GC	Gas chromatography
GLC	Gas-liquid chromatography