Carotenoid Content and Composition in 20 Medicinal Plant Species of Traditional Malay Midwifery Postnatal Bath

Rashidi Othman*, Siti Farah Aliya Abdul Halim, Farah Ayuni Mohd Hatta and Mohd Aizat Jamaludin

International Institute for Halal Research and Training (INHART), Herbarium Unit, Department of Landscape Architecture, Kulliyyah of Architecture and Environmental Design, International Islamic University Malaysia, 53100 Kuala Lumpur, Malaysia

Abstract: Today in Malay community, midwifery traditional knowledge of herbal medicine has disappeared and extinct. The facts are Malay midwives are becoming rare and the more crucial is medicinal plants are over-harvested. The aim of this research is to identify and investigate the active pharmaceutical ingredients content in 20 selected species used in the Malay traditional bath. There is a solid need to analyse the potential of these natural bioactive compounds, particularly carotenoids to be fully utilised and commercialised especially in halal market and health advantages. Through High performance liquid chromatography (HPLC) analysis, all 20 species were found to have at least four individual carotenoid pigments with a relatively high concentration of lutein and β-carotene and lower concentrations of zeaxanthin. *Strobilanthes crispus* (Pecah Kaca) leaf was detected to have the highest total carotenoid content (1546.80±283.45 µg/g DW) while *Psidium guajava* (Jambu Batu) shoot has the lowest total carotenoid content (112.9±82.2 µg/g DW). The significant outcome of the research was a new findings of new natural bioactive compound sources as health promoting agents which covers not only the Shariah requirement, but also safety aspects. Moreover, it will preserve the traditional knowledge of Malay traditional bath practices.

Keywords: Malay midwifery, postnatal traditional bath, carotenoids, antioxidant, active pharmaceutical ingredients, natural bioactive compounds.

INTRODUCTION

'Bidan kampung' is a well-known individual among Malay communities whom involved in delivering babies and providing a broad range of services related to women. Their services have been recognised for hundreds of years [1] and formally recorded since 1960s [2]. Many journals at that time focused on various elements such as on spiritual care, the work of bidan kampung on pregnant women during pregnancy and confinement, and sociocultural aspect [2,3]. In Malay traditional bath, the treatments were conducted with focused on revitalising the body conditions and also the mind of the mothers [4]. Usually, bidan kampung will use various kinds of plants that is believed to give therapeutic effects. The usage of these medicinal plants is mostly during the confinement period. The documentation of traditional knowledge of medicinal plants is still an ongoing research in Malaysia [5]. Midwifery is the practice of assisting a woman through childbirth using natural procedures. It was practiced primarily among traditional peoples with limited access to biomedicine. However, today in Malay community, midwifery continues to play an important role and still being practiced as an alternative to biomedicine in providing health care to women and children [5]. In both traditional and modern cultural settings, midwifery is involved with providing health care, during pregnancy, childbirth and postnatal care to both mother and newborn. Most midwifery species are wild, but many important species are native to certain location or area [2,3]. Due to rapid changes in socio-economic, environmental and cultural beliefs in Malaysia, the use of ethno-botanical plant species as herbal medicine is in transformation**,** and the impact on societies of traditional medicines and modern medical systems has varied, but the facts are traditional knowledge of herbal medicine is disappearing, traditional healers are becoming rare and less respected as well as medicinal plants are over-harvested [4,5]. Therefore the conservation of medicinal plants and traditional medicinal knowledge must run in parallel because these three factors are important and interrelated in order to prevent this knowledge from become extinct and disappear.

Scientist believed that the therapeutic effects given by these plants are due to the presence of bioactive compound such as carotenoids, flavonoids, terpenoids, alkaloids, tannins and glycosides [6,7]. These compounds served as the source of drugs in the modern world. Carotenoid is an antioxidant that helps to prevent cancer and other chronic diseases [8,9,10].

*Address correspondence to this author at the International Institute for Halal Research and Training (INHART), Herbarium Unit, Department of Landscape Architecture, Kulliyyah of Architecture and Environmental Design, International Islamic University Malaysia, 53100 Kuala Lumpur, Malaysia; Tel: +6012 6644772; Fax: +603 61964864; E-mail: rashidi@iium.edu.my

ISSN: 2223-3806 / E-ISSN: 1927-5951/17

Only few researches were found related to the carotenoid content in medicinal plants used by Malay traditional midwives, however, rarely focused on plants for traditional post-natal bath. The research reports on these folklore medicinal plants were limited. In this study, it was aimed to determine the carotenoid content and to establish the scientific basis for the therapeutic actions of these medicinal plants for better understanding and at the same time preserved the traditional knowledge.

MATERIALS AND METHODS

Sample Preparation

Fresh leaves samples of 20 selected medicinal plants were collected from local Malay midwives house gardens in Sungai Bakap, Penang. These samples were freeze dried for 72 hours and ground into fine powder and stored at -20°C until further analysis.

Extraction of Carotenoids

The extraction procedure essentially follows the methods described by Othman [11]. Each powdered sample weighed 1.0 g was rehydrated with distilled water and extracted with a mixture of acetone and methanol (7:3) at room temperature until colorless. The crude extracted was then centrifuged for 5 min at 10 000 g and stored at 4ºC in the dark prior to analysis. To extract carotenoids, an equal volume of hexane and distilled water was added to the combined supernatants. The solution was then allowed to separate and the upper layer containing the carotenoids was collected. The combined upper phase was then dried to completion under a gentle stream of oxygen-free nitrogen.

Spectrophotometric Determination of Total Carotenoid Content

Total carotenoid concentration was determined by spectrophotometry as described by Othman [11]. The dried carotenoid was resuspended in 300 µl of ethyl acetate and for determination of total carotenoid, 50 µl of the redissolved sample was then diluted with 950 µl chloroform for spectrophotometric analysis. Carotenoid containing solutions were measured at three different wavelengths, λ: 480 nm, 648 nm and 666 nm using Varian Cary 50 UV-Vis spectrophotometer. The Wellburn Equation [12] in chloroform was applied to obtain the total carotenoid content as described below:

$C_a = 10.91A_{666} – 1.2A_{648}$

$C_b = 16.36A_{648} – 4.57A_{666}$

$C_{x+c} = (1000A_{480} – 1.42C_a – 46.09C_b)/202$ (µg/ml)

HPLC Analysis of Carotenoids

The HPLC analysis of carotenoids were performed on an Agilent model 1200 series comprised of a quarternary pump with autosampler injector, micro-degassers, column compartment equipped with thermostat and a diode array detector. The column used was a ZORBAX Eclipse XDB-C_{18} end capped 5 µm, 4.6x150 mm reverse phase column (Agilent Technologies, USA). The eluents used were (A) acetonitrile:water (9:1 v/v) and (B) ethyl acetate. The column separation was allowed via a series of gradient such as follows: 0-40% solvent B (0-20 min), 40-60% solvent B (20-25 min), 60-100% solvent B (25-25.1 min), 100% solvent B (25.1-35 min) and 100-0% solvent B (35-35.1 min) at a flow rate of 1.0 mL min^{-1}. The column would be allowed to re-equilibrate in 100% A for 10 min prior to the next injection. The temperature of the column was maintained at 20°C. The injection volume is 10 µL each. Detection of individual carotenoids was made at the wavelengths of maximum absorption of the carotenoids in the mobile phase: neoxanthin (438 nm), violaxanthin (441 nm), lutein (447 nm), zeaxanthin (452 nm), β-carotene (454 nm), β-cryptoxanthin (450 nm) and α-carotene (456 nm). Compounds were identified by co-chromatography with standards and by elucidation of their spectral characteristics using a photo-diode array detector. Detection for carotenoid peaks was in the range of 350 to 550 nm. Individual carotenoid concentrations were calculated by comparing their relative proportions, as reflected by integrated HPLC peak areas, to total carotenoid content determined by spectrophotometry. The total and individual carotenoid concentration would be expressed in terms of milligram per 1.0 g dry weight of freeze-dried matter (µg/g DW).

RESULTS AND DISCUSSION

20 selected medicinal plants that were used in the traditional post-natal bath were evaluated for xanthophylls (lutein, neoxanthin, violaxanthin and zeaxanthin) and carotenes (α- and β-carotene) levels. Carotenoids are part of the chlorophyll components, however, the chlorophylls content were not the major subject of this research thus it was not included in the calculation. All procedures were conducted under minimum of light exposed to minimise the photo-isomerisation and photo-oxidation of the carotenoids as they hold highly conjugated double bonds in their

structure [13]. Saponification was conducted to remove the chlorophyll pigments and unwanted ester-carotenoids concurrently. Results are expressed in µg/g DW (mean±SD, n=3). The values are highly significant different ($p < 0.0001$, one way ANOVA, Tukey's post-hoc test).

Amongst 20 samples evaluated, *pecah kaca* leaf has shown significantly high level of total carotenoid (1546.80±283.45 µg/g DW) followed by *inai* and *sirih* which contained 1392.03±38.9 µg/g DW and 1391.03±11.2 µg/g DW respectively. Meanwhile, *pucuk daun jambu batu* showed the least amount of carotenoid content (112.89±82.2 µg/g DW) among those 20 samples. The accumulation of carotenoids in leaf could be explained by the biological variability, maturity at harvest and geographic site of the different species of plants [14, 15]. Carotenoid analysis was

Table 1: Total and Individual Carotenoid Content (µg/g DW) and Composition of 20 Selected Medicinal Plants Used in Malay Traditional Post-Natal Bath

Species	Total Carotenoid (µg/g DW)	Lutein (µg/g DW)	β-Carotene (µg/g DW)
Pucuk Daun Jambu Batu *Psidium guajava*	112.89±82.2	116.74±1.44	20.48±1.12
Daun Durian Belanda *Annona muricata*	349.28±108.9	155.92±2.55	60.33±5.95
Pandan *Pandanus amaryllifolius*	539.66±75.2	165.83±4.04	40.54±0.57
Daun Manggis *Garcinia mangostana*	585.67±189.2	150.64±4.59	32.72±2.42
Gendang Rusa *Gendarussa vulgaris*	591.83±200.7	156.16±1.55	18.18±2.76
Daun Lemuni Hitam *Vitex negundo*	645.04±148.3	142.37±7.39	57.87±16.73
Daun Serai Wangi *Andropogon nardus*	692.48±32.5	139.16±4.14	69.54±9.25
Daun Kunyit *Curcuma longa*	1043.8±162.7	124.9±2.09	55.65±5.34
Daun Bunga Tahi Ayam *Lantana camara*	1160.70±98	163.76±10.02	78.66±10.22
Daun Lengkuas *Alpinia galangal*	1241.93±44.9	161.51±4.64	72.49±2.57
Daun Seringan *Flemingia macrophylla*	1290.03±11.5	167.13±31.92	90.31±22.98
Daun Mengkudu *Morinda citrifolia*	1329.1±44.7	168.69±8.86	92.68±13.24
Cenderai *Microcos tomentosa*	1355.74±6.21	161.87±6.45	63.94±7.55
Daun Nilam *Pogostemon cablin*	1373.34±53.7	180.08±2.62	89.10±1.35
Daun Durian *Durio zibethinus*	1376.72±55.8	154.5±6.83	115.03±12.49
Sirih *Piper betle*	1391.03±11.2	157.84±7.24	133.85±19.86
Inai *Lawsonia inermis*	1392.03±38.9	156.54±6.06	65.42±6.55
Daun Pisang Kelat *Musa paradisiaca*	1403.56±4.83	145.86±12.15	134.36±23.63
Daun Lempoyang *Zingiber aromaticum*	1410.24±14.5	137.67±10.58	88.59±19.71
Kaca *Strobilanthes crispus*	1546.80±283.45	143.46±37.49	80.88±14.36

performed by using HPLC system and two major peaks were detected in all 20 samples: Lutein and β-Carotene. As shown in Table **1**, highest Lutein level was detected in *nilam* (180.08±2.62 µg/g DW) and highest β-carotene level was detected in *pisang kelat* leaves (134.36±23.63 µg/g DW). Other group of carotenoids; neoxanthin, violaxanthin and zeaxanthin were not detected during HPLC analysis.

Different carotenoids exhibit different beneficial effects Othman [11]. Many previous researches were carried out to evaluate the carotenoid concentrations that were obtained from fruits, vegetables and edible leaves [10,13,16,17]. It is widely known for its antioxidant, anti-cancer, macula protection, vitamin A precursor and other proven benefits [14,18,19,20,21]. In Malay traditional post-natal bath, several medicinal plants was selected and boiled up to get the water extraction [4]. Usually the midwives will use the leaves of the plants and the common selections are *inai, sirih, pandan, serai wangi, kunyit, lengkuas, pisang kelat, bunga tahi ayam, lemuni hitam* and *durian belanda*. The assortment of these plants are differed among midwives as it is depends on the availability of the plants around them.

CONCLUSION

There were few journals reported about carotenoid content in medicinal plants, especially that were being used in the Malay traditional post-natal bath. Most of the researches are focused on one type of species or more towards the practices itself. All in all, the concentration of total carotenoid and the distribution of individual carotenoids may not represent the high levels of total carotenoids as the accumulation of individual carotenoid pigments may vary within each grouping. There is still very limited information on selected medicinal plants analysed in this study. Nevertheless, the result obtained were part of the effort on providing scientific evidence in conjunction with the preservation of Malay traditional knowledge, mainly on traditional post-natal bath.

ACKNOWLEDGEMENT

This work was supported by Ministry of Higher Education (MOHE) and International Islamic University Malaysia (IIUM) under Research Grant RIGS16-396-0560

REFERENCES

[1] Skeat WW. Malay Magic - an introduction to the folklore and popular religion of the Malay Peninsula. Macmillan and Co., Limited, UK, 1900.

[2] Ali A, Howden-Chapman, P. Maternity services and the role of the traditional birth attendant, bidan kampung, in rural Malaysia. J Public Health Manag Pract 2007; 13(3): 278-286. https://doi.org/10.1097/01.PHH.0000267686.08282.3c

[3] Karim WJ. Malay midwives and witches. Soc Sci Med 1984; 18(2): 159-166. https://doi.org/10.1016/0277-9536(84)90036-4

[4] Hasan Z, Mazlan NA, Yaacob, M. Tumbuhan ubatan untuk kesejahteraan wanita. Institut Penyelidikan dan Kemajuan Pertanian Malaysia (MARDI) dan NRH Publications Sdn. Bhd., Malaysia 2005.

[5] Ramli AG, Shamsaini S, Jaafar L, Fariza, F. Traditional and Complementary Medicine Practice Guideline on Malay Massage. Traditional and Complementary Medicine Division, Ministry of Health, Malaysia 2009.

[6] Ajayi I, Ajibade O, Oderinde, R. Preliminary phytochemical analysis of some plant seeds. Res J Chem Sci 2011; 1(3): 3-7.

[7] Sati SC, Sati N, Rawat U, Sati, OP. Medicinal plants as a source of antioxidants. Res J Phytochem 2010; 4(4): 213-224. https://doi.org/10.3923/rjphyto.2010.213.224

[8] Othman R, Kammona S, Jaswir I, Jamal, P. Characterisation of carotenoid content in diverse local sweet potato (*Ipomoea batatas*) flesh tubers. Int J Pharm Pharm Sci 2015; 7(2): 347-351.

[9] Rodriguez-Amaya DB. Latin American food sources of carotenoids. Arch Latinoam Nutr, 1999; 49: 74S-84S.

[10] Sangeetha RK, Baskaran, V. Carotenoid composition and retinol equivalent in plants of nutritional and medicinal importance: Efficacy of β-carotene from Chenopodium album in retinol-deficient rats. Food Chem 2010; 119(4):1584-1590. https://doi.org/10.1016/j.foodchem.2009.09.047

[11] Othman R. Biochemistry and genetics of carotenoid composition in potato tubers. 2009; Ph.D. Dissertation. Lincoln University, New Zealand.

[12] Wellburn AR. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol, 1994; 144: 301-313. https://doi.org/10.1016/S0176-1617(11)81192-2

[13] Fatimah MZ, Norazian MH, Rashidi, O. Identification of carotenoid composition in selected "ulam" or traditional vegetables in Malaysia. Int Food Res J 2012; 19(2): 527-530.

[14] Wong JY, Matanjun P, Ooi YBH, Chia, KF. Characterization of phenolic compounds, carotenoids, vitamins and antioxidant activities of selected Malaysian wild edible plants. Int J Food Sci Nutr 2013; 64: 1-11. https://doi.org/10.3109/09637486.2013.763910

[15] Znidarcic D, Ban D, Sircelj, H. Carotenoid and chlorophyll composition of commonly consumed leafy vegetables in Mediterranean countries. Food Chem 2011; 129: 1164-1168. https://doi.org/10.1016/j.foodchem.2011.05.097

[16] Gutierrez RMP, Mitchell S, Solis, RV. Psidium guajava: a review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol 2008; 117(1): 1-27. https://doi.org/10.1016/j.jep.2008.01.025

[17] Hirschberg J. Production of high-value compounds: carotenoids and vitamin E. Curr Opin Biotechnol 1999; 10:186-191. https://doi.org/10.1016/S0958-1669(99)80033-0

[18] Cunningham FX, Gantt, E. Genes and enzymes of carotenoid biosynthesis in plants. Ann Rev Plant Physiol Plant Mol Biol 1998; 49: 557-583. https://doi.org/10.1146/annurev.arplant.49.1.557

[19] Rodriguez-Amaya DB. Important Food Sources of Provitamins A. Carotenoids and food preparation: The retention of provitamin A carotenoids in prepared, processed, and stored foods. OMNI Press, Washington, 1997.

[20] Khoo HE, Prasad KN, Kong KW, Jiang Y, Ismail, A. Carotenoids and their isomers: Color pigments in fruits and vegetables. Molecules 2011; 16(2): 1710-1738. https://doi.org/10.3390/molecules16021710

[21] Morosinotto T, Caffarri S, Dall'Osto L, Bassi, R. Mechanistic aspects of the xanthophyll dynamics in higher plant thylakoids. Physiol Plant 2003; 119: 347-354. https://doi.org/10.1034/j.1399-3054.2003.00213.x

Received on 24-05-2017 Accepted on 09-10-2017 Published on 06-11-2017

DOI: https://doi.org/10.6000/1927-5951.2017.07.04.6