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CAROTENOIDS, RETINOL ACTIVITY EQUIVALENTS (RAE), AND POLYPHENOLS-RICH PROFILING IN TRADITIONAL HERBS CONSUMED BY LOCAL FOLKS

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Active ingredients responsible for food colorant and aromatic features play a critical role in the quality of traditional herbs. Even though traditional herbs are popular among local consumers, scientific findings on their medicinal properties, specifically carotenoids and polyphenols activities are still lacking. This study evaluates the composition of carotenoids and polyphenol-rich in fifteen traditional herbs namely *Allium tuberosum, Anacardium occidentale, Cosmos caudatus, Morinda citrifolia, Murraya koenigii, Oenanthe javanica, Polygonum minus, Kaempferia galanga, Pandanus amaryllifolius, Premna foetida, Curcuma longa, Manihot esculenta, Cymbopogon citratus, Senna alata* and *Lawsonia inermis* which are consumed by the local folk. The leaves and stem were extracted using liquid-liquid extraction (carotenoids) and alkaline extraction (sodium hydroxide with 2M). For polyphenols, the crude extract supernatant was re-extracted with different solvents (petroleum ether, ethyl acetate and butanol). These fractional extracts were analysed qualitatively and quantitatively using High-Performance Liquid Chromatography (HPLC). The results revealed five types of carotenoids: neoxanthin, violaxanthin, lutein, zeaxanthin, and β-carotene. These carotenoid profiles varied in concentration and composition in different species. Total carotenoid content ranged from 2.0 to 600 µg/g DW, with *P. foetida* leaves displaying the highest content (6646.16 ± 12.43 µg/g DW). The total vitamin A activity (retinol equivalent, RE) of *O. javanica* and *P. minus* species was also included. Meanwhile, for polyphenol analysis, most phenolic acids were detected in ethyl acetate extract compared to ethanol and hexane, which is dominated by trans-p-coumaric acid, followed by vanillic acid and caffeic acid. These traditional herbs may be promoted as active pharmaceutical ingredients to improve human health.

Keywords: Traditional herbs, carotenoids, polyphenols-rich, retinol activity equivalent, food security

INTRODUCTION

Approximately 80% of the global population relies on forest plants as a source of therapeutic plants for health (Dossou-Yovo et al. 2017). Traditional herbs have been consumed and used by ancient people for centuries because of their nice and interesting flavour, which adds variety and flavour to cuisine; in Southeast Asia, it can be eaten fresh or cooked (You et al. 2018). Even though traditional herbs are popular among local consumers, the availability of scientific findings on the evaluation of their medicinal properties, specifically carotenoids and polyphenols activities, are still lacking (Othman et al. 2017). Vitamin A deficiency (VAD) has affected 19.1 million pregnant women (Laillou et al. 2013), while the insufficient amount of retinol available has resulted in affected rhodopsin synthesis and night blindness (WHO/FAO 2004). VAD can lead to many health consequences, with children, pregnant, and lactating women known to be the prominent groups suffering from VAD in many low-income countries. Countries such as Indonesia, Malaysia, Philippines, Thailand, and Vietnam recommend additional amounts of vitamin A during pregnancy and lactation, with considerable differences in recommendations (Tee et al. 2023). In Malaysia, vitamin A

nutritional needs vary; infants (375–400 µg), children (400–500 µg), adolescents (600 µg), adults (600 µg), elderly 600) pregnancy (800 µg), and lactation (850 µg) (Tee et al. 2023). Despite great development in socioeconomic status throughout the 52 years of independence, intestinal parasitic infections and malnutrition are still public health problems in Malaysia, particularly among Aboriginal children in rural areas. Little is known about the vitamin A status of Aboriginal schoolchildren in rural Malaysia (Al-Mekhlafi et al. 2010). Vitamin A deficiency (VAD) remains a major cause of preventable childhood blindness and mortality in developing countries. Irreversible bilateral optic neuropathies secondary to VAD are uncommon and could be attributed to the chronicity of the disease (Cheah et al. 2022).

Vitamin A plays a key role in the correct functioning of multiple physiological functions. The human organism can metabolise natural forms of vitamin A (retinol and derivatives) and provitamin A (carotenoid) from vegetables into biologically active forms that interact with multiple molecular targets (Carazo et al. 2021). Carotenoids are yellow to orange-colored organic pigments found in several fruits and vegetables and are known for their antioxidant activities. Some of carotenoids are β-carotene, α-carotene, lutein, lycopene, and cryptoxanthin. For example, β-Carotene is mostly ingested through red and orange vegetables (Carazo et al. 2021), while β-cryptoxanthin is most commonly obtained from various citrus fruits and juices (Olmedilla-Alonso et al. 2020). The retinol equivalent (RE) values in each sample are primarily linked to their individual concentrations of β-carotene. It was observed that 21 of the analysed *ulam* species exhibited superior RE activity compared to a widely recognised source of pro-vitamin A (orange-fleshed carrots) (Mohd Zaifuddin et al. 2014). According to Mohd Zaifuddin et al. (2014), *beluntas* accumulated substantially the highest total carotenoid (4897.43 ± 15.51 µg/g DW), followed by *cekur manis* and *pegaga* with 4267.84 ± 0.45 µg/g DW and 4223.15 ± 17.63 µg/g DW, respectively.

Fruit and beverage crops are richer sources of phenolic acids, where they have been studied in depth; however, phenolic acids from vegetables remain largely overlooked (Rashmi & Negi 2020). Vegetables as a source of phenolic acids in the daily diet can help ameliorate the adverse effects of certain lifestyle diseases. Phenolics are secondary metabolites with a chemical structure comprising one or more aromatic rings attached to hydroxyl groups (Chaturvedi et al. 2022). Various bioactive compounds, such as phenolics and flavonoids, were identified in the plant extracts exhibited significant antioxidant activities (Jain 2023). Phenolic acids may make up about one-third of the phenolic compounds in the human diet. These substances possess powerful antioxidant activity that may help protect the body from free radicals, exert antioxidant and anti-inflammatory actions (Tresserra-Rimbau et al. 2014) that can potentially help in the prevention of cardiovascular diseases and various cancers (Goleniowski et al. 2013), protect against oxidative damage diseases; and exhibit antimicrobial, antimutagenic, hypoglycemic and anti-platelet aggregating activities (Saxena et al. 2012). Vanillic acid, gallic acid, caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid and m-coumaric acid are the most frequently found in green leafy vegetables (Khanam et al. 2012). Among the hydroxybenzoic acids, protocatechuic acid is the major phenolic acid widely distributed in vegetables, whereas 5-caffeoylquinic acid is the most prominent hydroxycinnamic acids found in many vegetables (Rashmi & Negi 2020). The solubility of phenolic compounds varies with other compounds in plant tissues depending on the type of solvent, the degree of polymerisation, and their interaction (Nasrollahi et al. 2022).

Despite their popularity, there is still a lack of scientific reports on their medicinal properties, particularly those related to the activities of pro-vitamin A carotenoids. Leaves, branches, fruits, flowers, tubers, and rhizomes are the edible elements of the traditional herbs. Furthermore, the combination of such compounds bounces an important view on the quality of traditional herbs. As such, the identification and characterisation of chemical compounds in terms of their biological activity on the spectrum, potency, toxicity, and safety are deemed crucial. Therefore, this study aims to explore carotenoids, retinol activity equivalents (RAE), and polyphenols-rich profiling in traditional herbs consumed by local folks.

Therefore, three objectives are highlighted, firstly to identify carotenoid content and composition in traditional herbs, secondly, to investigate the total vitamin A activity in terms of retinol equivalent and thirdly, to identify polyphenol-rich properties content.

MATERIALS AND METHODS

Sample preparation

All parts of 15 traditional herbs samples (Table 1) were collected and freeze-dried for 72 hours, after which the samples were ground into a fine powder and kept at −20 °C until further analysis as detailed by Othman (2009).

Extraction of carotenoids

The extraction procedure essentially follows the methods described by Othman (2009), with some modifications. Each 1.0 g powdered sample was rehydrated with distilled water and extracted with a mixture of acetone and methanol (7:3) at room temperature until colorless. The crude extract was then centrifuged for 5 min at 10,000 g and stored at 4 °C in the dark before 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.

Determination of total carotenoid content

Total carotenoid concentration was determined by spectrophotometry as described by Mohd Zaifuddin et al. (2014). 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 (Wellburn 1994) in chloroform was applied to obtain the total carotenoid content as described below:

$$Ca = 10.91A666 - 1.2A648$$
$$Cb = 16.36A648 - 4.57A666$$
$$Cx + c = (1000A480 - 1.42Ca - 46.09Cb)/202 \ (\mu g/mL)$$

Determination of retinol equivalent of carotenoid

The vitamin A activity exhibited by the detectable pro-vitamin A carotenoid will be converted into retinol equivalent units, whereby 12 mg of beta carotene is equal to 1 mg retinol activity equivalent or RAE.

1 µg retinol = 1 RE
1 µg β-carotene = 0.167 µg RE, (1/6 µg)
1 µg other pro-vitamin A carotenoids = 0.084 µg RE, (1/12 µg)

Saponification

Samples were saponified with a mixture of acetonitrile and water (9:1) and methanolic potassium hydroxide solution (10% w/v). Base carotenoids were then extracted by addition of 2 mL hexane with 0.1% butylated hydroxytoluene (BHT), followed by addition of 10% NaCl until phase separation was achieved. The extracts were washed with distilled water, dried under a gentle stream of oxygen-free nitrogen and resuspended in ethyl acetate for spectrophotometry and HPLC analysis, as described in detail by Othman (2009).

HPLC analysis

The HPLC analysis of saponified carotenoids were performed on an Agilent model 1200 series (Agilent Technologies, USA) comprised of a quaternary pump with autosampler injector, micro-degassers, column compartment equipped with thermostat and a diode array detector. The column used was a ZORBAX Eclipse XDB-C18 end capped 5 µm, 4.6 × 150 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, 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 minutes 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), and β-carotene (454 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 microgram per 1.0 g dry weight of freeze-dried matter (µg/g DW).

Alkaline extraction

To prepare alkaline extraction, in 100 mL NaOH (2 M) solution, soaking of 10 g of freeze-dried powdered material was done. Heat was applied to the sample at a temperature of 60°C for 12 hours in an oven, after that, it was cooled to a temperature of 20°C. Then, hydrochloric acid (HCl) was used to treat the alkaline extract to reach pH 2 so that precipitation of hemicellulose would occur. Then, the final leaves extracts were re-extracted with hexane, ethyl acetate, and ethanol extracts as described by Bertin et al. (2003) with some minor modifications by employing a funnel separator. Next, the extracts were evaporated to dryness by using a rotary evaporator at a temperature of 45°C. The crude extract was then resuspended by using 5 mL of methanol and then kept at −25 °C for subsequent analysis.

Quantification of phenolic acids content with High-Performance Liquid Chromatography (HPLC)

LC rapid resolution apparatus Agilent 1200 series (Agilent Technologies, Palo Alto, CA, USA) was used for phenolic acid HPLC analysis as described by Ramya et al. (2023). The setup comprised of auto-injection system-based binary pump, thermostat-controlled column area, micro vacuum degassing chambers, and a diode array detector (DAD). The column used was a Zorbax SB-C_{18} column (Eclipse 100 × 2.1 mm, 1.8 µm) with a diode array detector. The temperature of the column was set at 25°C. The injection volume was 20 µl, and the flow rate was set at 0.5 mL/min. Phenolic acids standards; caffeic acid, trans-p-coumaric acid, 4-hydroxybenzoic acid, and vanillic acid were purchased from Sigma-Aldrich.

Table 1 List of traditional herbs used by the old folks

Botanical name		Local name	Plant part
Traditional herbs	*Allium tuberosum*	Kucai	Leaves
	Anacardium occidentale	Gajus	Leaves
	Cosmos caudatus	Ulam raja	Leaves
	Morinda citrifolia	Mengkudu	Leaves
	Murraya koenigii	Daun kari	Leaves
	Oenanthe javanica	Selom	Leaves
	Polygonum minus	Kesum	Leaves
	Kaempferia galanga	Daun cekur	Leaves
	Pandanus amaryllifolius	Daun pandan	Leaves
	Premna foetida	Bebuas	Leaves
	Curcuma longa	Daun kunyit	Leaves
	Manihot esculenta	Pucuk ubi kayu	Leaves
	Cymbopogon citratus	Serai	Stem
	Senna alata	Gelenggang	Leaves
	Lawsonia inermis	Daun inai	Leaves

RESULTS

Analysis of carotenoids contents on traditional herbs

Fifteen traditional herbs that have been consumed daily in Malaysian diet that popular among Malay community representing diverse genetic backgrounds and growth habits were selected for this study (Table 1). As a result, from the 15 traditional vegetables and medicinal plant species, the carotenoid content and composition range can be divided into four groups as detailed in Table 2. There was a positive relationship between total carotenoid content and types of carotenoid pigment. *Bebuas* (*Premna foetida*) was found to have the highest total carotenoid content (646.16 ± 12.43 µg/g DW), substantially higher than all other plant species tested. In contrast, the lowest total carotenoid concentration was found in *mengkudu* leaves (*Morinda citrifolia*) (2.67 ± 0.56 µg/g DW).

Carotenoid analysis performed by HPLC system detected at least five major carotenoid peaks: neoxanthin, violaxanthin, lutein, zeaxanthin, and β-carotene. As shown in Table 2, neoxanthin, violaxanthin and zeaxanthin were found highest in *Premna foetida* (bebuas); lutein was highest in *Cosmos caudatus* (ulam raja), whereas β-carotene was detected highest in *Polygonum minus* (kesum). All 15 herbs were grouped into distinct groups based on the accumulation of specific carotenoid pigments (Table 2). Six herbs were found to have four types of carotenoids, whereas two herbs were found to have three types of carotenoids, and six herbs were detected to have two types of carotenoids and only one species has one type of carotenoid.

Table 2 shows the comparison of the total retinol equivalent, which portrays that it has met the Recommended Dietary Allowance (RDA) for vitamin A of 1000 retinol equivalents per day (Herbers 2003). Only two species (*selom* and *kesum*) were found to have accumulated β-carotene between 0.52 and 0.58 retinol equivalents per g DW of samples.

In general, the highest carotenoid concentrations, either in total or individual

Table 2 A relative distribution of carotenoid types in 15 traditional herbs

	Traditional herbs	Total carotenoid (µg/g DW)	Neoxanthin (µg/g DW)	Violaxanthin (µg/g DW)	Lutein (µg/g DW)	Zeaxanthin (µg/g DW)	β-Carotene (µg/g DW)	RE
Herbs with 4 types of carotenoids	Cekur	55.14±4.83	34.5±0.03	1.03±0.01	0.18±0.03	19.43±0.96	nd	nd
	Selom	135.12±12.72	119.5±11.4	10.96±1.31	1.5±0.50	nd	3.16±0.12	0.526
	Gelenggang	511.04±16.35	120.78±13.2	3.69±0.01	1.8±0.12	384.77±13.22	nd	nd
	Daun pandan	370.08±15.55	81.09±2.70	8.44±0.81	2.48±0.04	278.07±10.97	nd	nd
	Bebuas	646.16±12.43	198.4±12.65	13.96±1.71	3.18±0.31	430.62±12.68	nd	nd
	Daun kunyit	397.98±12.73	83.79±2.78	13.16±1.41	2.7±0.32	298.33±12.70	nd	nd
Herbs with 3 types of carotenoids	Daun kari	401.25±11.80	28.90±0.11	nd	1.74±0.03	370.612±10.89	nd	nd
	Pucuk ubi	8.65±1.94	2.49±0.01	nd	2.05±0.01	4.11±0.25	nd	nd
Herbs with 2 types of carotenoids	Serai	16.36±0.30	nd	nd	2.54±0.55	13.82±0.28	nd	nd
	Kesum	7.63±0.11	nd	nd	4.11±0.11	nd	3.52±0.02	0.586
	Gajus	34.37±0.10	nd	nd	2.55±0.02	31.82±0.02	nd	nd
	Kucai	2.8±0.01	nd	nd	0.06±0.11	2.74±0.16	nd	nd
	Daun inai	45.49±0.38	nd	nd	2.58±0.11	42.91±2.32	nd	nd
	Daun mengkudu	2.67±0.56	nd	nd	0.38±0.11	2.29±0.16	nd	nd
Herb with only 1 carotenoid	Ulam raja	11.09±0.25	nd	nd	11.09±0.25	nd	nd	nd

*nd – non-detectable

carotenoids, were detected in *P. foetida* (*bebuas*). It can be concluded that the total carotenoid is strongly associated with the concentration of individual carotenoid pigments, especially neoxanthin and zeaxanthin. However, the relative distributions of individual carotenoids within each grouping did not necessarily correlate to the levels of total carotenoids.

Analysis of phenolic acids contents on traditional herbs

The sequential extraction of individual phenolic acids, such as caffeic acid, trans-p-coumaric acid, vanillic acid, and 4-hydroxybenzoic acid from different extracts (hexane, ethyl acetate and ethanol) from 15 traditional herbs are shown in Table 3. Based on the HPLC analysis, most of the phenolic acids were detected in ethyl acetate extract compared to ethanol and hexane, which is dominated by trans-p-coumaric acid, followed by vanillic acid and caffeic acid. For ethyl acetate extract, the highest phenolic acid content detected was trans-p-coumaric acid in *kunyit* (414.97 µg/g DW), followed by *cekur* (63.56 µg/g DW) and *gajus* (36.09 µg/g DW). Meanwhile, vanillic acid was highly detected in *selom* (46.54 µg/g DW), followed by *gelenggang* (10.02 µg/g DW) and *serai* (3.59 µg/g DW). On the other hand, the caffeic acid content of *bebuas* and *daun inai* showed almost identical level at 13.24 µg/g DW and 13.67 µg/g DW, respectively.

In comparison with ethyl acetate and hexane extracts, 4-hydroxybenzoic acid was detected only in ethanol extract. The high value of 4-hydroxybenzoic was detected in *daun inai* (6.30 µg/g DW), followed by *daun kari* (4.59 µg/g DW) and *daun kunyit* (2.91 µg/g DW). Meanwhile, caffeic acid was highly detected in kucai (4.44 µg/g DW), followed by *mengkudu* (2.80 µg/g DW) and *bebuas*

Table 3 Phenolic acids content in different extracts (hexane, ethyl acetate and ethanol) in 15 traditional herbs

	Traditional herbs	Caffeic acid (µg/g DW)	trans-p-Coumaric acid (µg/g DW)	Vanilic acid (µg/g DW)	4-Hydroxybenzoic acid 5-(µg/g DW)
Ethyl acetate extract	Serai	nd	nd	3.59	nd
	Kesum	nd	4.60	nd	nd
	Selom	nd	3.95	46.54	nd
	Gajus	nd	36.09	nd	nd
	Gelenggang	nd	11.54	10.02	nd
	Daun kari	nd	3.67	nd	nd
	Daun pandan	nd	9.25	nd	nd
	Bebuas	13.24	nd	nd	nd
	Ulam raja	nd	29.31	nd	nd
	Daun kunyit	nd	414.97	nd	nd
	Kucai	nd	13.41	nd	nd
	Daun inai	13.67	35.23	nd	nd
	Mengkudu	nd	15.67	nd	nd
	Pucuk ubi	nd	7.37	nd	nd
	Daun cekur	nd	63.56	nd	nd
Ethanol extract	Serai	nd	nd	nd	1.30
	Kesum	nd	nd	nd	nd
	Selom	nd	nd	nd	nd
	Gajus	nd	nd	nd	nd
	Gelenggang	nd	nd	nd	nd
	Daun kari	nd	nd	nd	4.59
	Daun pandan	nd	nd	nd	nd
	Bebuas	2.53	nd	nd	nd
	Ulam raja	nd	nd	nd	nd
	Daun kunyit	nd	nd	nd	2.91

countinued

Table 3 countinued

	Traditional herbs	Caffeic acid (µg/g DW)	trans-p-Coumaric acid (µg/g DW)	Vanilic acid (µg/g DW)	4-Hydroxybenzoic acid 5-(µg/g DW)
	Kucai	4.44	nd	nd	nd
	Daun inai	nd	nd	nd	6.30
	Mengkudu	2.80	nd	nd	nd
	Pucuk ubi	nd	nd	nd	nd
	Daun cekur	nd	nd	nd	nd
Hexane extract	Serai	1.19	nd	nd	nd
	Kesum	nd	nd	nd	nd
	Selom	nd	nd	nd	nd
	Gajus	nd	nd	nd	nd
	Gelenggang	nd	nd	nd	nd
	Daun kari	nd	nd	nd	nd
	Daun pandan	2.09	nd	nd	nd
	Bebuas	nd	nd	nd	nd
	Ulam raja	nd	nd	nd	nd
	Daun kunyit	4.10	nd	nd	nd
	Kucai	nd	nd	nd	nd
	Daun inai	nd	nd	nd	nd
	Mengkudu	0.94	nd	nd	nd
	Pucuk ubi	nd	nd	nd	nd
	Daun cekur	nd	nd	nd	nd

*nd = non-detectable

(2.53 µg/g DW). Last but not least, the hexane extract showed the lowest detection of phenolic acid in this study, as only caffeic acid was detected.

DISCUSSION

Previous studies on several traditional herbs as detailed in Table 4 established that carotenoids profiles were predominantly neoxanthin, violaxanthin, lutein, β-carotene, and zeaxanthin, whereas the carotenoid profiles in traditional herbs in this study were dominated by neoxanthin, violaxanthin, lutein, zeaxanthin, and β-carotene. This result suggested that different plant species will react differently towards the stability of individual carotenoids accumulated in plants. Furthermore, the environmental conditions and response can also influence the presence of specific carotenoid compounds and their concentration in the *ulam* species (Mat Ali et al. 2023). There are two possibilities to explain these carotenoid profiles instability:

i. The conversion of other carotenoids such as violaxanthin, neoxanthin to zeaxanthin from the β-carotene and α-carotene branch point is due to irradiance stress condition from high-light exposure. As a result, zeaxanthin concentration will increase. This reaction will restrict the supply of precursors for abscisic acid (ABA) biosynthesis and the plant responds by increasing carotenogenic metabolic flux to compensate for this restriction (Ruban et al. 1994, Farber et al. 1997 & Othman 2009). In agreement with the Polle et al. (2001), it is also reported that zeaxanthin can successfully replace lutein and violaxanthin under irradiance stress conditions.
ii. The presence and absence of zeaxanthin is in response to changes in pH. Acidity will trigger the de-epoxidation reaction by the conversion of violaxanthin and other precursors of ABA to zeaxanthin, whereas alkaline conditions will induce lutein or the supply of precursors for ABA biosynthesis which will lead to the conversion of zeaxanthin to violaxanthin, neoxanthin or other precursors for ABA biosynthesis through epoxidation reaction (Morosinotto et al. 2003 & Othman 2009). Overall, this clearly demonstrated that the

Table 4 Comparison of individual carotenoid compounds from previous studies of traditional herb species

Traditional herbs	Carotenoid compounds	Reference
Daun selom	Lutein, β-carotene	Fatimah et al. (2012)
	β-carotene	Rodriguez-Amaya (1997)
Mengkudu	Neoxanthin, violaxanthin, lutein and β-carotene	Fatimah et al. (2012)
	Lutein, β-carotene	Speek et al. (1988)
Ulam raja	Lutein, β-carotene	Fatimah et al. (2012)
	Lutein and zeaxanthin	Liu et al. (2007)
	Neoxanthin, violaxanthin, lutein and β-carotene	Mat Ali et al. (2023)
Gajus	Lutein, β-carotene	Fatimah et al. (2012)
	Lutein and zeaxanthin	Liu et al. (2007)
	Neoxanthin, lutein and β-carotene	Mat Ali et al. (2023)
Pucuk ubi	Neoxanthin, violaxanthin, lutein and β-carotene	Mat Ali et al. (2023)

environmental conditions can strongly influence the total and individual pigment content of carotenoids in plants, which can significantly affect the quality and nutritional value of *ulam* and medicinal plant species. Therefore, in addition to genotypic factors, environmental factors also play an important role in determining the accumulation of individual carotenoids in plants, especially in *ulam* and medicinal plant species. Clearly, further studies utilising *ulam* and medicinal plant species grown under different environmental conditions are necessary to confirm this hypothesis.

Habitual *ulam* intake was associated with lower waist circumference, better Mini-Mental State Examination (MMSE) scores, less anger, less tension and positive total mood disturbance after adjustment for gender, age, energy intake, as well as total fruits and vegetables (non-*ulam*) consumption (You et al. 2020). According to You et al. (2018), *O. javanica* (*selom*) and *A. occidentale* (*pucuk gajus*) were ranked in the top three highest total phenolic content (TPC) among all the selected *ulam*, whereas *O. javanica* (*selom*) and *M. koenigii* (*daun kari*) are beneficial in improving cognitive status and mood. On top of that, consumption of plant products rich in polyphenols is correlated with reduced risk of heart and cardiovascular diseases (Bhat et al. 2013).

Rashmi & Negi (2020) reported that vegetables contain phenolic acids in variable amounts depending on their maturity, exposure to biotic and abiotic stress, and varietal variation. It may support the finding of this study where the lower number of phenolic acids detected may be influenced by the plant maturity as well as environmental conditions. Interestingly, there is lack of study in comparing the individual phenolic acids compared to total phenolic content especially for Malaysian traditional herbs. Most of the previous research focusing on total phenolic content as compared to individual phenolic acids, various type of ulam (You et al. 2018), *Amaranthus gangeticus* (*bayam merah*) (Sarker & Oba 2020), green leaves such as *kucai, kangkong, ubi keledek* (Bhat et al. 2013), *Solanum melongena* (eggplant) (Mohd Zulkhairi et al. 2020), *Piper betle* (*sirih*), and *C. longa* (*kunyit*) (Sumazian et al. 2010) and *Sauropus androgynus* (*cekur manis*) (Arif 2020).

Based on the findings of the National Health and Morbidity Survey 2018, which included 6,795 pre-elderly and elderly participants, the proportion of individuals consuming an adequate amount of vegetables (defined as at least three servings per day) was 11.4% for the pre-elderly and 10.9% for the elderly (Institute for Public Health 2019). The results of this study may establish traditional herbs with notable potential for incorporation into everyday meals to combat or alleviate issues linked to the negative effects of certain lifestyle diseases. Ideally, it will offer scientific insights into the advantages of consuming *ulam* to promote a nutritious diet for enhancing and sustaining health and wellness.

CONCLUSION

The quantified total carotenoid content across all samples ranges from 2.0 to 600 µg/g DW, with *bebuas* leaves exhibiting the highest content (6646.16 ± 12.43 µg/g DW). Furthermore, this study includes an assessment of the total vitamin A activity (expressed as retinol equivalent, RE) for *selom* and *kesum* species. The results also indicate that phenolic acids, notably 4-hydroxybenzoic acid, caffeic acid, vanillic acid, and *trans-p*-coumaric acid, are predominant. These discoveries deepen comprehension of the significance of incorporating these traditional herbs into daily diets to enhance human nutrition and health. Hence, it is likely that these traditional herbs can be promoted as fortified food items to broaden their availability in the international market. Therefore, the evaluation of the health benefits of carotenoids and phenolic acids from vegetables requires further study.

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