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Carotenoid composition in twenty *ulam* species as potential halal active pharmaceutical ingredients

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Abstract

Ulam is a traditional Southeast Asian vegetable that has been consumed and practised by old folks for decades. Several studies have found that *ulam* has numerous benefits, including being high in natural antioxidants, antimicrobials, vitamins, and minerals and being used as an early illness preventive method. Even yet, many *ulam* have yet to be identified and documented. This study aimed to identify the carotenoid content and composition in a variety of *ulam* species as a potential halal active pharmaceutical ingredient. The individual carotenoid composition was examined in twenty *ulam* species with three groups of distinct parts: leaves, fruits, and rhizomes. Using HPLC analysis, neoxanthin, lutein, and β-carotene were the primary carotenoids discovered in the overall species. The results presented 12 species with 4 carotenoid pigments, 4 species with 3 carotenoid pigments, 2 species with 2 carotenoid pigments and 2 species with 1 carotenoid pigment. *Mentha arvensis* (51.38±0.24 µg/g DW) has substantially greater neoxanthin. In contrast, *Manihot esculenta* (1179±9.90 µg/g DW), (457±2.12 µg/g DW), and (2434.5±5.66 µg/g DW) had significantly higher β-carotene, lutein, and violaxanthin, respectively. Naturally available carotenoid sources as prospective components for halal pharmaceutical products will be discovered as a result of this research. These proven findings have also given communities the confidence to include *ulam* in their daily diets because it is a natural source with exceptional biological qualities.

1. Introduction

Muslim consumers are notoriously cautious when selecting halal foods to consume regularly. However, the selection of halal pharmaceutical products has still been compromised (Mukhtar and Butt, 2012; Husin *et al.*, 2015). It is suggested that identical requirements be met when ingesting meals and pharmaceutical products, as both must be halal. However, some consumers have no other options in some situations involving pharmaceutical products. Their lack of knowledge about the halal status of the ingredients in their prescriptions leads them to take those products to be cured (Husin *et al.*, 2015). As a result, creating halal pharmaceuticals from halal sources may inform and educate Muslim communities about the availability of these products to fulfil their healthcare needs (Mukhtar and Butt, 2012). This growth and integration of the halal pharmaceutical business into the global market benefit Muslim and non-Muslim consumers (Bashir, 2020).

Ulam or traditional salad produced from fresh leaves is a type of vegetable that has been consumed and practised by ancient people for centuries because of its flavour, which adds variety and flavour to cuisine while also providing health benefits. Communities, particularly in Southeast Asia, eat it either steamed or uncooked (You *et al.*, 2018). These traditional Malay vegetables are made up of approximately 120 species from diverse families and genotypes in Malaysia. Leaves, branches, fruits, flowers, tubers, and rhizomes are the edible elements of the *ulam*. Unfortunately, there is a scarcity of published information about the nutritional value of

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ulam in Malaysia (Othman *et al*., 2017). Since fruits and vegetables are considered the richest sources of provitamin A carotenoid in the human diet, accounting for 70-90% of carotenoid ingested, *ulam* constitutes only a small part of it (Othman *et al.,* 2017).

According to Aruna and Baskaran (2010), eating fruits and vegetables high in carotenoids reduces the risk of cancer, cardiovascular disease, eye cataract formation, and age-related macular degeneration (ARMD). In the Philippines, these edible plants have been recognised by the Department of Health. They discovered that edible plants like *Allium sativum* (Garlic), *Clinopodium douglasii* (Mint), *Momordica charantia* (Bitter melon), *Peperomia pellucida* (Silver bush), and others had therapeutic properties and the potential to be used as pharmaceutical products that can benefit entire communities (Boy *et al*., 2018). Therefore, this research has been conducted to explore carotenoid composition in tenty *ulam* species as potential halal active pharmaceutical ingredients.

2. Materials and methods

2.1 Samples collection

Raw materials of twenty species of *ulam* have been purchased from the wet market at Selayang, Selangor, Malaysia. Before further processing, the samples were cleaned, chopped into small pieces, and stored in the freezer at -20°C before being dried using a freeze drier (FDU-1100, EYELA, Tokyo Rikakikai Co., Japan), at a temperature of -50°C and a vacuum level at 6.5 Pa for five days. The samples were then ground into a fine powder with a heavy-duty mixer (LB20E, Laboratory Blender). To minimise enzymatic reactions, the ground samples were kept in an airtight container at a temperature of -20°C (Othman, 2009).

2.2 Carotenoid extraction process

The carotenoid extraction process fundamentally follows the methods defined by Othman (2009) with a certain amendment. A total of 1 g of every grounded plant sample was prepared in triplicate, added into a 50 mL tube and rehydrated by adding 5 mL of distilled water, respectively. Then 15 mL of solvent; acetone: methanol mixture (7:3 v/v) was added to each tube. The samples were then stored overnight in the freezer at a temperature of -20°C. The next day, the sample was added with 5 mL hexane and stored back in the freezer for 15 mins before being centrifuged for 3 mins at 8500 rpm, 20°C (Thermo Scientific Sorvall Biofuge Primo R Centrifuge, US). The upper layer of samples has been collected and stored in a 2 mL Eppendorf tube. The step of adding 5 mL of hexane is repeated until the upper layer of the sample becomes colourless (typically two or three times). Next, the combined upper layer was inserted into a 15 mL tube and dried under a gentle stream of oxygen-free nitrogen until thickening (typically 2 mL left). The samples were then washed up to 10 mL with distilled water and centrifuged for 3 mins at 8500 rpm, 20°C. Finally, the upper layer of the sample oil was collected and inserted into a 2 mL Eppendorf tube and dried under a gentle stream of oxygen-free nitrogen. Then, 300 µL of ethyl acetate was added to the dried sample extracts and centrifuged for 3 mins at 8500 rpm, 20°C before 200 µL of the extract was inserted into the HPLC vial.

2.3 HPLC analysis

The HPLC analysis of carotenoid content extracted from *ulam* has been done by using HPLC Agilent 1200 series (Agilent Technologies, USA), which encompassed micro degassers, quaternary pump with autosampler injector, column compartment with thermostat and diode array detector following the procedure described by Mohd Hatta and Othman (2020). Individual carotenoids of each sample will be detected at wavelengths of 400-500 nm: neoxanthin (438 nm), violaxanthin (441 nm), lutein (447 nm), β-carotene (452 nm) and zeaxanthin (454 nm). Their compounds will be perceived by co-chromatography with standards and by an explanation of the spectral characteristics of the compounds via a photodiode array detector. The result of individual carotenoid concentrations will be indicated in microgram per gram dry weight of freeze-dried matter (µg/g DW).

3. Results and discussion

Twenty *ulam* species, *Piper sarmentosum* (Kaduk), *Momordica charantia* (Peria katak), *Coriandrum sativum* (Ketumbar), *Althernanthera sissoo* (Bayam brazil), *Lactuca sativa* (Salad), *Ocimum basilicum* (Selasih), *Psophocarpus tetragonolobus* (Kacang botor), *Mentha arvensis* (Pudina), *Manihot esculenta* (Ubi kayu), *Cosmos caudatus* (Ulam raja), *Parkia speciose* (Petai), *Cucumis sativus* (Timun), *Anacardium occidentale* (Gajus), *Syzygium polyanthum* (Serai kayu), *Abelmoschus esculentus* (Bendi), *Solanum lycopersicum* (Tomato), *Archidendron bubalinum* (Kerdas), *Sauropus androgynous* (Cekur manis), *Archidendron pauciflorum* (Jering), and *Curcuma amada* (Temu pauh) were selected. All 20 species had been gathered in four groups depending on the accumulation of specific carotenoid pigments. Twelve species were found to have all four individual carotenoid pigments with *M. esculenta* (Pucuk ubi) was found to have the highest content of violaxanthin, lutein, and β-Carotene with (2434.5±5.66 µg/g DW), (457±2.12 µg/g DW) and (1179±9.90 µg/g DW) respectively (Table 1). The highest content of neoxanthin was found in *M. arvensis* (pudina)

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Table 1. Individual carotenoids content (μg/g DW) and composition of 20 *ulam* species

Common name	Scientific name	Neoxanthin (μg/g DW)	Violaxanthin (μg/g DW)	Lutein (μg/g DW)	β-Carotene (μg/g DW)
Cultivars with 4 carotenoid pigments					
Kaduk	*Piper sarmentosum*	29.06±0.10	739.75±34.29	113.25±1.06	222±12.02
Peria	*Momordica charantia*	4.21±0.11	423.75±10.96	41.75±0.35	182.25±6.01
Ketumbar	*Coriandrum sativum*	33.97±0.22	1474.5±31.82	132±2.83	266.5±7.78
Bayam Brazil	*Althernanthera sissoo*	31.61±0.28	943±107.48	108.25±4.60	137±23.33
Salad	*Lactuca sativa*	28.01±0.36	1467±39.60	182.5±0.71	615.5±4.95
Selasih	*Ocimum basilicum*	19.42±0.02	977±15.56	125.75±0.35	204.75±1.77
Kacang Botor	*Psophocarpus tetragonolobus*	9.10±0.54	358±2.83	55.75±1.77	185±14.14
Pudina	*Mentha arvensis*	51.38±0.24	1657±62.23	202.5±3.54	574.5±17.68
Pucuk Ubi	*Manihot esculenta*	36.89±0.19	2434.5±5.66	457±2.12	1179±9.90
Ulam Raja	*Cosmos caudatus*	30.39±0.16	1098.5±11.31	172±9.90	362±33.94
Petai	*Parkia speciosa*	4.25±0.08	487.75±0.35	51.25±0.35	84.5±1.41
Timun	*Cucumis sativus*	12.13±0.07	574.25±83.09	42.25±1.06	120±0.71
Cultivars with 3 carotenoid pigments					
Pucuk Gajus	*Anacardium occidentale*	10.30±0.18	ND	166±0.71	277±87.68
Serai Kayu	*Syzygium polyanthum*	5.43±0.12	ND	75±1.41	178.75±13.08
Bendi	*Abelmoschus esculentus*	8.77±0.03	446±4.95	30.25±0.35	ND
Tomato	*Solanum lycopersicum*	ND	107.69±0.93 Lycopene	26.25±1.06	97.75±1.06
Cultivars with 2 carotenoid pigments					
Kerdas	*Archidendron bubalinum*	ND	100.37±1.24	16.75±1.77	ND
Cekur Manis	*Sauropus androgynous*	ND	ND	310.07±25.5	52.85±3.16
Cultivars with 1 carotenoid pigment					
Jering	*Archidendron pauciflorum*	ND	ND	14.13±0.18	ND
Temu Pauh	*Curcuma amada*			443.53±1.80 Curcumin	

(51.38±0.24 μg/g DW). *Anacardium occidentale, S. polyanthum, A. esculentus,* and *S. lycopersicum* were perceived to have three out of four carotenoid pigments, and *A. bubalinum* and *S. androgynous were* found to have only two carotenoid pigments whereas, *A. pauciflorum* and *C. amada* had only one carotenoid pigment detected which is lutein and curcumin respectively. The carotenoid composition possessed by the *ulam* species is illustrated in Figure 1. This result established that carotenoid content and composition differ between *ulam* species, suggesting that different plant species will react differently towards the stability of individual carotenoids accumulated in plants. In addition to genotypic factors, environmental factors have a considerable effect on influencing the accumulation of individual carotenoids in plants. More research on *ulam* species produced under various environmental conditions is required to corroborate this concept.

The recognition of significant levels of individual carotenoids within all 20 species has driven the maximisation of carotenoid levels either as active pharmaceutical ingredients or for daily consumption. There are several studies on the advantages of these individual carotenoids. The research on photoprotective

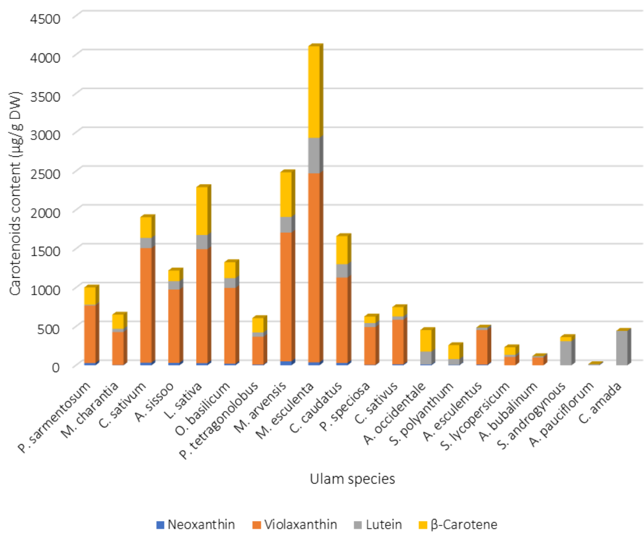

Figure 1. Carotenoids composition possessed by the *ulam* species.

effects of violaxanthin against ultraviolet B-induced damage in human dermal fibroblasts (HDFs) has verified that violaxanthin is one of the potential agents for anti-photoaging where this illness may lead to skin cancer if not treated (Kim *et al.,* 2019). It is beneficial in preventing skin irritation, neurological disorders, and eye

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disease (Mitra *et al.,* 2021). Significantly, lutein is necessary for eye health and useful for protection from age-related macular degeneration (AMD). This disease usually affects a human's central vision, resulting in severe loss of central vision (Nesterenko and Sink, 2003). β-Carotene is commonly found in fruits and vegetables, is an essential pigment that has various functions in preventing and treating numerous diseases such as gastric cancer and serves as a natural antioxidant and the immune system (Chen *et al.,* 2021). Next, neoxanthin is usually found in green leafy vegetables. From previous research in the United States, this pigment has been proven to reduce the risk of prostate cancer and also as a prostate cancer chemoprevention (Asai *et al*., 2004). Therefore, finding these carotenoid contents in *ulam* from this study is vital in identifying more sources of halal active pharmaceutical ingredients, ensuring the benefits can be utilised by all consumers, especially Muslim consumers.

The study by Chaiareekitwat *et al*. (2022) mentioned that the different parts of the plant have contributed to the differentiation of the carotenoid content. As for *M. esculenta*, carotenoid is detected highest at the shoot apex, which this part clarifies as *ulam*. Carotenoids in *ulam*, such as β-carotene, lutein, zeaxanthin, violaxanthin, and neoxanthin, are integrated into plastids and accumulate primarily in the thylakoid membranes of chloroplasts and yield senescence leaves (Othman *et al*., 2017). Chaiareekitwat *et al*. (2022) have shown that *M. esculenta* shoots contain 406 to 804 µg/g dry basis of β-carotene and 416.8 to 772.1 µg/g dry basis of lutein. Similarly, Ospina *et al*. (2021) found that this *ulam* consists of high β-carotene and lutein with 174 to 547 µg/g and 15 to 181 µg/g dry basis, respectively. Yaacob *et al*. (2018) reported that there is no violaxanthin detected in *M. esculenta* compared to this study in which 2434.5±5.66 µg/g DW violaxanthin has been detected in *M. esculenta.* This shows that there are several factors involved in giving those results.

Since carotenoid is one of the largest groups of metabolites produced by plants, there are complex regulatory mechanisms that control carotenoid accumulation in plants. This mechanism remains in the chloroplast of green plants and the chromoplast of fruits, roots, and flowers (Hermanns *et al*., 2020). Chlorophyll and carotenoid consist in the chloroplast as light-harvesting for biosynthesis in it. Thus, environmental conditions will affect carotenoid composition (Hermanns *et al*., 2020). In a study by Othman *et al*. (2017), the factors that affect carotenoid pigments in plant species respond to internal and external stresses as well as genetic and environmental effects. The factors such as growing season, locality and variety of stress remarkably will reduce or enhance carotenoid accumulation by plants. In addition, Colasuonno *et al*. (2017) and Landi *et al*. (2020) have shown that genotype and monochromatic light environment are significantly related to the amount of carotenoid accumulated by plants. A study by Colasuonno *et al*. (2017) also reported that parental genotypes and environmental interactions are directly involved in the biosynthesis of the carotenoid compound. Interestingly, Cruet-Burgos *et al*. (2020) have found several significant effects between carotenoids' traits and environmental conditions such as locality, time, and storage conditions. They found that samples that were kept in the freezer for more than a year showed carotenoid degradation.

The genome-wide association study (GWAS), which focused on recognizing the causal genes underlying significant interrelations, is needed to improve the understanding of the carotenoid pathway control to produce a high content of carotenoid in *ulam* for human benefit (Cruet-Burgos *et al*., 2020; Baseggio *et al*., 2020). The bioavailability of carotenoids in plants is a complicated issue because the content depends on various factors such as genes, type of cultivar, locality, association with their surrounding environments, and other conditions that affect the carotenoid pathway.

4. Conclusion

The study of carotenoid content and composition in *ulam* is vital for the further establishment of pharmaceutical industries and as a utility for the global market to discover innovative halal active pharmaceutical ingredients. The carotenoids' superior biological activity will fuel various commercial applications and halal market development. Thus, further investigations are needed to verify the potential of *ulam* as a halal active pharmaceutical ingredient. The findings regarding selected *ulam* with the highest carotenoid content and composition can be exposed using green technology approaches. Those results will be documented information that can benefit the halal pharmaceutical industry in Malaysia.

Conflict of interest

The authors declare no conflict of interest.

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