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Unlocking the Antioxidant Potential of Malaysian Traditional Salad (Ulam) as a *Halal* Natural Carotenoid Source

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Excessive free radicals in human bodies are a leading cause of skin ageing and serious illnesses. To combat this issue, there is an increased interest in investigating dietary sources that are rich in antioxidants. The traditional Malaysian salad, known as *ulam*, has been used for decades by the locals for its potentials healing properties. However, there is a lack of scientific evidence regarding the antioxidant properties and carotenoid content of these ulam species, despite their high availability and economic feasibility. Therefore, this study aimed to investigate the potential of 40 different ulam species as sources of carotenoids and antioxidants. Our findings revealed that ulam is a rich, *halal* source of carotenoids and possesses significant antioxidant properties. Carotenoid levels differ among ulam species, with some exhibiting higher levels than others. The results showed that ulam can be recognised as a *halal* natural source of carotenoids for the food, cosmetic, and pharmaceutical industries. Ulam availability and economic feasibility make it a sustainable option for *halal* antioxidant supplementation. The findings of this study will benefit manufacturers, scientists, consumers, and others, with the potential to unlock the antioxidant potential of ulam and draw further attention to its nutritional and therapeutic benefits.

Keywords: Ulam; carotenoids; antioxidants; nutrition; *halal*

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Free radicals are molecules produced through cell metabolism and can naturally form in the body, playing a significant role in many normal cellular processes. Exposure to ionising radiation and toxic environments results in the production of free radicals in the body because of the death of cells caused by ionising radiation [1-3]. Environmental pollution, like heavy metal contamination and a high-oxygen atmosphere, can cause an increase in free radicals in the environment and stimulate cells in the body to generate free radicals [4, 5]. This occurs because free radicals contain oxygen elements that are commonly produced in living tissues and are known as reactive oxygen species (ROS) [6]. While ROS can be beneficial in moderate or low amounts, they can be harmful in high concentrations, leading to oxidative stress. Excessive concentrations of free radicals in bodies may destroy other molecules and cells, including lipids, proteins, DNA, and cell membranes, which are implicated in several human appearance changes such as pigmentation and the skin ageing process, as well as serious diseases such as cancer, cardiovascular disease, and diabetes [7-10].

Antioxidants, or free radical scavengers, are chemical compounds that act as protective and neutralizing agents to prevent free radicals from causing harm. The body produces endogenous antioxidants, but in a small amount that functions to neutralise free radicals [11, 12]. Since this amount is not sufficient, the body needs to depend on external antioxidant sources. External antioxidant sources can be derived either from natural sources such as fruits, vegetables, herbs, spices, and grains or from artificial or synthetic sources. Some of the natural sources are also available as supplements, such as beta-carotene and vitamins A, C, and E [13]. Consuming fruits and vegetables is the simplest method of obtaining antioxidants, as they contain essential nutrients for growth and maintenance, along with bioactive compounds that can help slow down the aging process and prevent diseases [14-16].

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Unfortunately, global industries predominantly use synthetic antioxidants because they are easy to obtain, inexpensive, stable during processing, and effective in suppressing oxidation [17]. The advantages of synthetic antioxidants are emphasised because of the lack of research on natural antioxidants, despite their potential to be safer than synthetic ones [18, 19]. Synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), pyrogallol (PY), and tert-butyl hydroquinone (TBHQ) are well-known and extensively used in various industries, such as food additives, cosmetics, and pharmaceuticals, including several healing products [20]. In the global era, consumers are increasingly aware of what they consume and use. Due to the potential cancer risks associated with long-term use of synthetic antioxidants, consumers are shifting their interest from synthetic to natural antioxidants [16, 21].

Malaysia is rich in traditional salads, with more than 120 species recognised within Asia. The old folks have consumed this vegetable, either raw or cooked, for decades. This traditional salad is also known as ulam. This diet has been practiced because they believe these ulam have several health benefits and can cure diseases [22]. In addition, ulam also consists of several medicinal functions such as antioxidant, antimicrobial, anti-inflammatory, neuroprotective, and others. It is also high in polyphenols, carotenoids, vitamins, and minerals [22, 23]. Studies by Islam et al. [23] revealed that differences in the composition of ulam contribute to the differences in antioxidant activities. Furthermore, some ulam is also recognised to contain several carotenoid compositions that function as the key to metabolism for human nutrition and health.

This study explores traditional Malaysian salad ulam as a potential natural source of carotenoids with antioxidant properties. Carotenoids are one of the most essential micronutrients and are an essential precursor of vitamin A that has been proven to ensure appropriate eye functions, and proper metabolic processes, and reduce the risk of several chronic illnesses [24, 25]. It has an essential antioxidant function in plants, which acts to deactivate the singlet oxygen that forms during photosynthesis [6, 26]. Fundamentally, carotenoids are produced by living organisms that undergo photosynthesis, especially plants, and come from non-photosynthetic organisms such as fungi. Carotenoids produced by fruits and leafy vegetables can be identified by their colour ranging from yellow-orange-red. For example, tomatoes are rich in lycopene, and turmeric is rich in curcumin [26].

The research focuses on the antioxidant potential of ulam, known for its diverse mix of herbs and vegetables. By investigating ulam as a *halal* carotenoid source, the study aims to highlight the health benefits of this traditional food item. This research contributes to the understanding of how traditional foods can serve as valuable sources of bioactive compounds that promote health and well-being. The novelty also lies in the cultural significance of ulam within Malaysian cuisine. By highlighting ulam as a *halal* carotenoid source, the study bridges the gap between traditional culinary practices and modern nutritional science. This integration not only promotes the preservation of cultural heritage but also emphasises the importance of incorporating traditional foods into contemporary dietary patterns for enhanced health outcomes.

Ulam can be recognised as a *halal* natural source of carotenoids due to the benefits it offers, which comply with the regulations and standards set by authorities. According to the Malaysian Standard MS 1500:2019 [27], *Halal* Food – General Requirement which governs the production, preparation, handling, and storage of *halal* food, under item 4.5.1.2, it is generally accepted that all plant-based products and derivatives are *halal*, except for those that are harmful or pose a health risk. Similarly, in the pharmaceutical sector, the Malaysian Standard MS 2424:2012 [28] delineates comprehensive guidelines to produce *halal* pharmaceutical products. One important requirement specified in this standard is that the plants used in manufacturing must be non-prohibited species according to regulatory and national laws. In the realm of *halal* food, it is crucial to verify that all ingredients, including those derived from plants, adhere to Islamic dietary laws [29, 30]. The restriction of specific plant species in *halal* products is determined by Islamic dietary laws, which distinguish between what is allowed (halal) and what is prohibited (haram) for consumption [31]. Regulatory bodies overseeing *halal* food production typically maintain a list of plant species that are forbidden in *halal* products. This list can be used by producers as a guide to ensure compliance with *halal* standards. By aligning with both *halal* standards and national regulations, producers can guarantee that their products meet the required criteria for quality, safety, and genuineness [32-34]. Therefore, this study will propose alternatives for *halal* natural carotenoids, focusing on the antioxidant potential of ulam.

EXPERIMENTAL

Analysis of Carotenoid Composition

A total of 40 samples of selected ulam were collected from the wet market in Selayang, Selangor. Samples were then washed, chopped, and labelled before being inserted into the freeze-dryer and run for 72 hours to remove water content. Afterward, samples were ground into powder, kept in an airtight container, and placed in a dark room. Next, the extraction of carotenoid pigments was done by using the alkaline method. Each sample was weighed with 1.0 g of dried weight, added to a sodium hydroxide solution, followed by an ethyl acetate solution, and left overnight for 24 hours at a temperature of -20 °C. Subsequently, the extracted pigment was collected, kept in an Eppendorf tube, and stored at -20 °C prior to further analysis (Figure 1) [35].

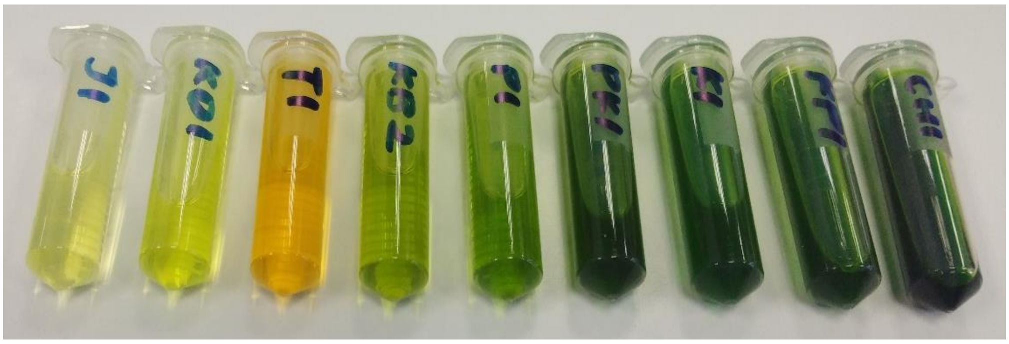


Figure 1. The extracted carotenoid pigments from ulam were kept in an Eppendorf tube and stored at -20°C prior to further processing.

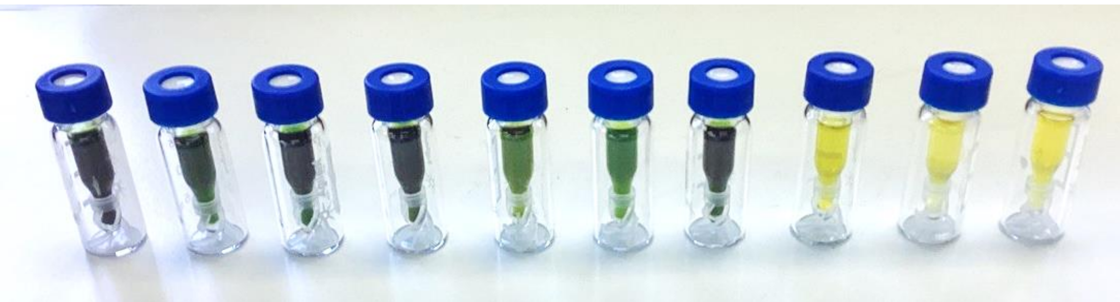


Figure 2. The extracted carotenoid samples were prepared in HPLC vials.

The analysis of carotenoid content and composition was conducted using high-performance liquid chromatography (HPLC). 300 µL of the extracted carotenoid sample was inserted into a vial and prepared in three replicates (Figure 2). Each replicate of the sample took 40 minutes to be analysed by HPLC [36].

DPPH Free Radical Scavenging Assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) from Sigma-Aldrich was used to test free radical scavenging in the top three ulam samples. Three extracts from the ulam with the highest carotenoid content were chosen for this assay. The extract was dissolved in methanol (MeOH) to produce a stock solution with a concentration of 1 µg/µL. Approximately 100 µL of MeOH was added using a multipipettor (8 tips) into a 96-well microplate from well B1 to B6, continually until the last row (H1-H6). Next, 200 µL of the prepared sample extract was pipetted into wells A1 to A6. All samples in the first row (A1 to A6) were then transferred to the well in row B and mixed properly using a pipette 4–5 times before being two-fold diluted until row H. Then, 100 µL of MeOH was added to A4 to A6 until the last row of H4 to H6, respectively. 100 µL of DPPH methanolic solution was added to A1 to A3 until the last row of H1 to H3, respectively (Figure 3). The final concentrations of the sample were 500, 250, 125, 62.5, 31.3, 15.6, 7.8, and 3.9 µg/mL. Finally, the microplate was covered with a lid and kept in the dark at room temperature for 40 minutes. After that, absorbance was read at 517 nm of wavelength using a Varioskan™ LUX multimode microplate reader (Thermo Scientific™). The result obtained was calculated and expressed as the percentage of DPPH free radical scavenging activity using the formula stated below [37]:

$$1 - \frac{\text{Absorbance sample}}{\text{Absorbance DPPH}} \times 100 = \%\ \text{Inhibition}$$

*Absorbance sample = Absorbance (sample + DPPH) – Absorbance (sample+ MeOH)

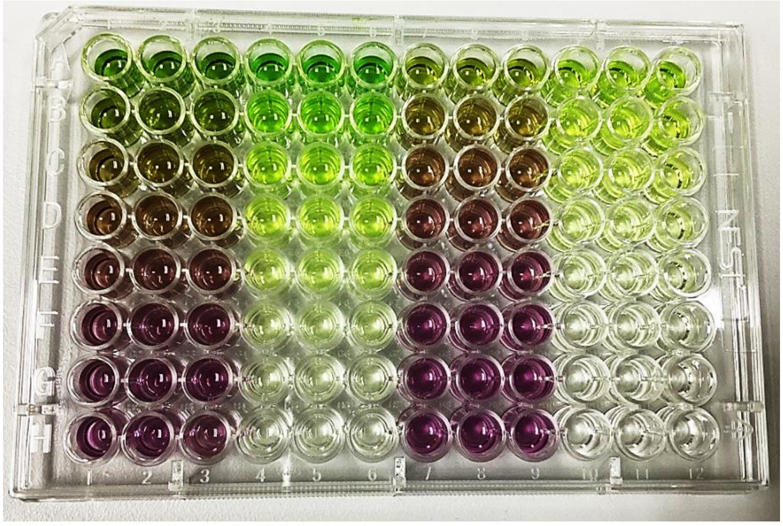


Figure 3. The extracted samples were prepared for antioxidant DPPH analysis.

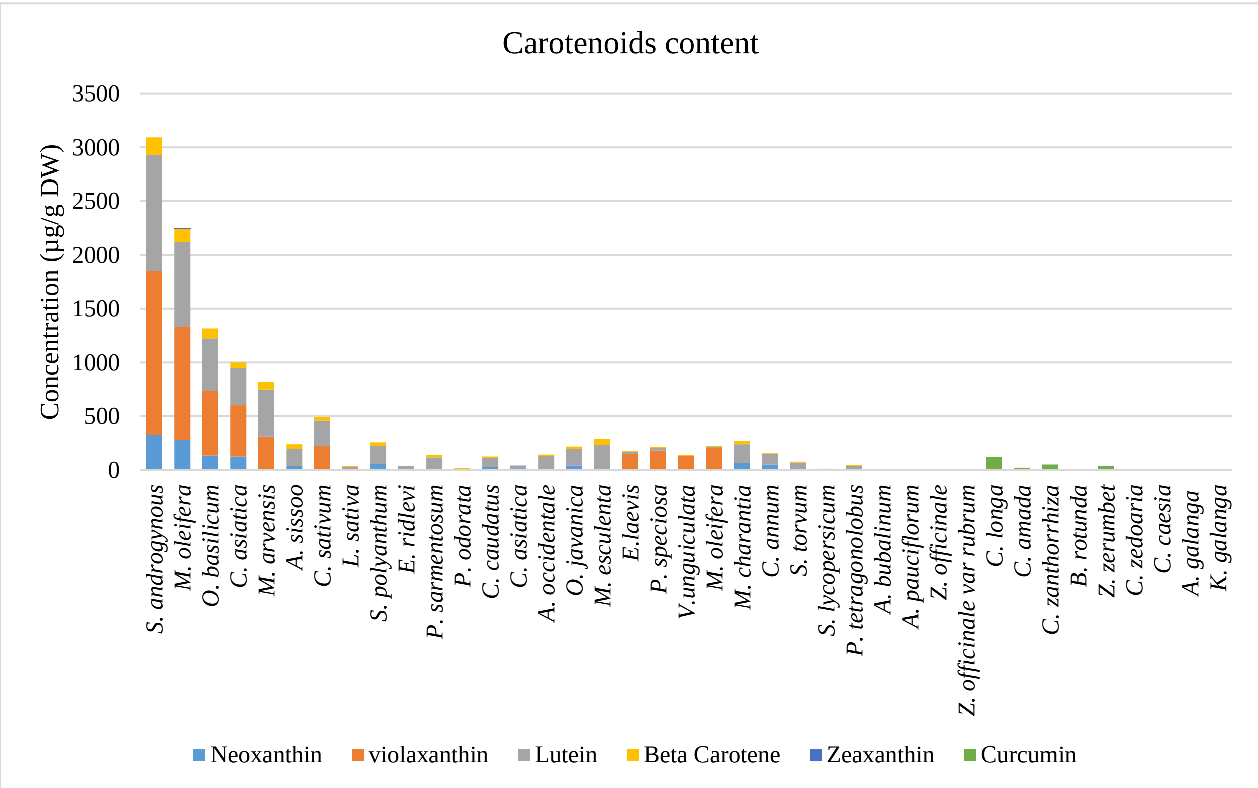


Figure 4. Carotenoids content of 40 ulam species

RESULTS AND DISCUSSION

Analysis of Carotenoid Composition

The HPLC analysis results showed that the total carotenoid content in all samples ranged between 1.14±0.02 and 1078.30±3.60 μg/g DW (Figure 4). The three samples with the highest lutein, β-carotene, and violaxanthin were *Sauropus androgynus* (Cekur manis) (1078.30±3.60, 160.17±3.85, and 1525.30±13.38 μg/g DW), *Moringa oleifera* (Kelor leaves) (793.26± 8.97, 122.92±8.30, and1048.60±11.58 μg/g DW), and *Ocimum basilicum* (Selasih) (493.03±8.32, 90.68± 0.93, and 598.55±24.95 μg/g DW) (Figure 5). Other carotenoids such as neoxanthin, zeaxanthin, and curcumin were also detected in several samples analysed, but with lower content. The highest amount of carotenoids in these three samples is supported by previous reports. *S. androgynus* was identified as having the highest total carotenoid content in the study by Othman et al. [38] and Andarwulan et al. [39]. In addition, Platel and Srinivasan [40] reported that carotenoid content in *S. androgynus* depends on the maturity of the leaves. As for *M. oleifera*, higher β-carotene was found in the dried leaves than in its foliage, fruits, and flowers [41, 42]. While other carotenoids such as lutein, violaxanthin, and zeaxanthin were also detected, but in lesser amounts [43]. The study by Dumbrava et al. [44] showed that *O. basilicum* extracted with two different methods had the highest carotenoid composition compared to other vegetables tested. This result was supported by Filip [45], who reported that *O. basilicum* contains high concentrations of carotenoids, with β-carotene being the main compound detected. A previous study also detected high concentrations of violaxanthin and β-carotene in *O. basilicum*, with levels of 977 ± 15.56 and 204.75 ± 1.77 µg/g DW, respectively [46]. These studies also noted that β-carotene offers more benefits than vitamin A alone and is recognised as a potent antioxidant. Consequently, the three samples with the highest carotenoid content were selected for further antioxidant assays.

Antioxidant Activity

The Varioskan™ LUX multimode microplate reader (Thermo Scientific™) at wavelength 517 nm was used to analyse the antioxidant activity of the three ulam samples. The inhibition percentage for ascorbic acid standard, *S. androgynus* (Cekur manis), *M. oleifera* (Kelor leaves), and *O. basilicum* (Selasih) is demonstrated in Figure 7. From the graph, it showed that *S. androgynus* has exhibited a higher DPPH scavenging activity with 98% inhibition at a concentration of 500 µg/mL compared to *M. oleifera* and *O. basilicum*, with 91% and 89% inhibition at a concentration of 500 µg/mL, respectively.

The scavenging activity of *S. androgynus* is comparable to the findings of Kuttinath et al. [47], which detected more than 70% DPPH inhibition at a concentration of 100 µg/mL. Other studies have also noted that this ulam contains significant amounts of phenolic compounds, carotenoids, antioxidants, vitamins, and minerals that contribute to its therapeutic properties [48]. Kumar et al. [49] reported excellent scavenging activity of *M. oleifera* with 95.52% inhibition at a concentration of 100 µg/mL, while Siti Mahirah et al. [50] demonstrated a DPPH scavenging activity of 92.60% for *O. basilicum* at the same concentration. Naidu et al. [51] found 73.75% DPPH inhibition for the same ulam species, highlighting *O. basilicum*'s high β-carotene content, which contributes to its potent antioxidant capacity. Several studies have also reported positive scavenging activities of *S. androgynus*, *M. oleifera*, and *O. basilicum*, highlighting their potential as powerful antioxidant agents [38, 52, 53, 54]. However, variations in inhibition percentages may arise due to factors such as plant maturity, temperature, pH, storage duration, solvent type, and extraction method [54, 55].

The findings of this research support the exceptional qualities of *S. androgynus*, *M. oleifera*, and O. *basilicum* as plants with diverse applications in traditional ethnic cuisines and ethnomedicinal traditions [56-58]. Given their high antioxidant levels, these ulam species have the potential to protect cells from oxidative damage and serve as precursors to vitamin A.

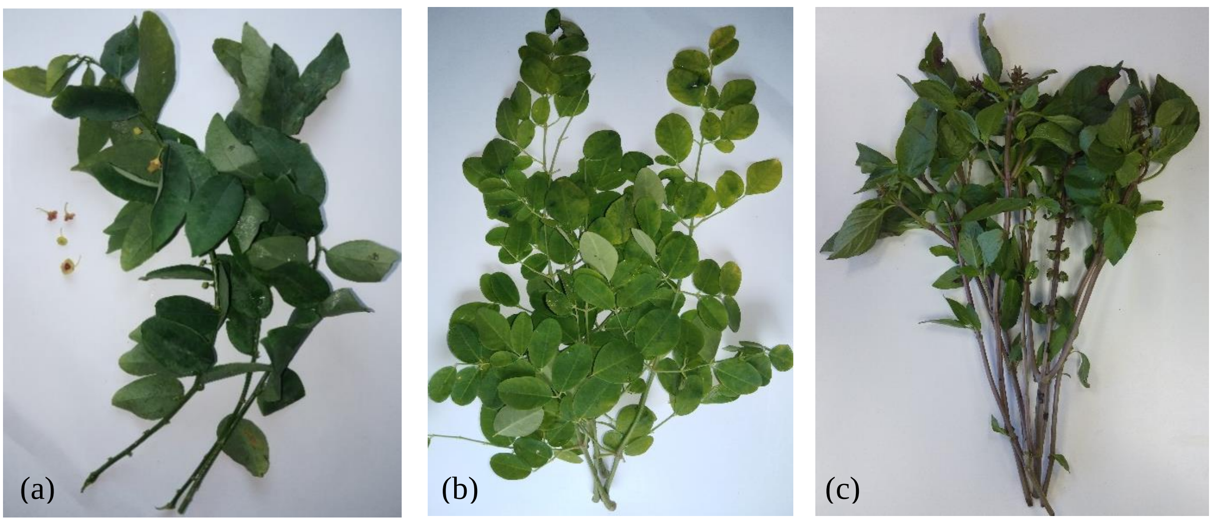


Figure 5. Three ulam species with the highest carotenoids content; a) *S. androgynus* (Cekur manis), b) *M. oleifera* (Kelor leaves), c) *O. basilicum* (Selasih).

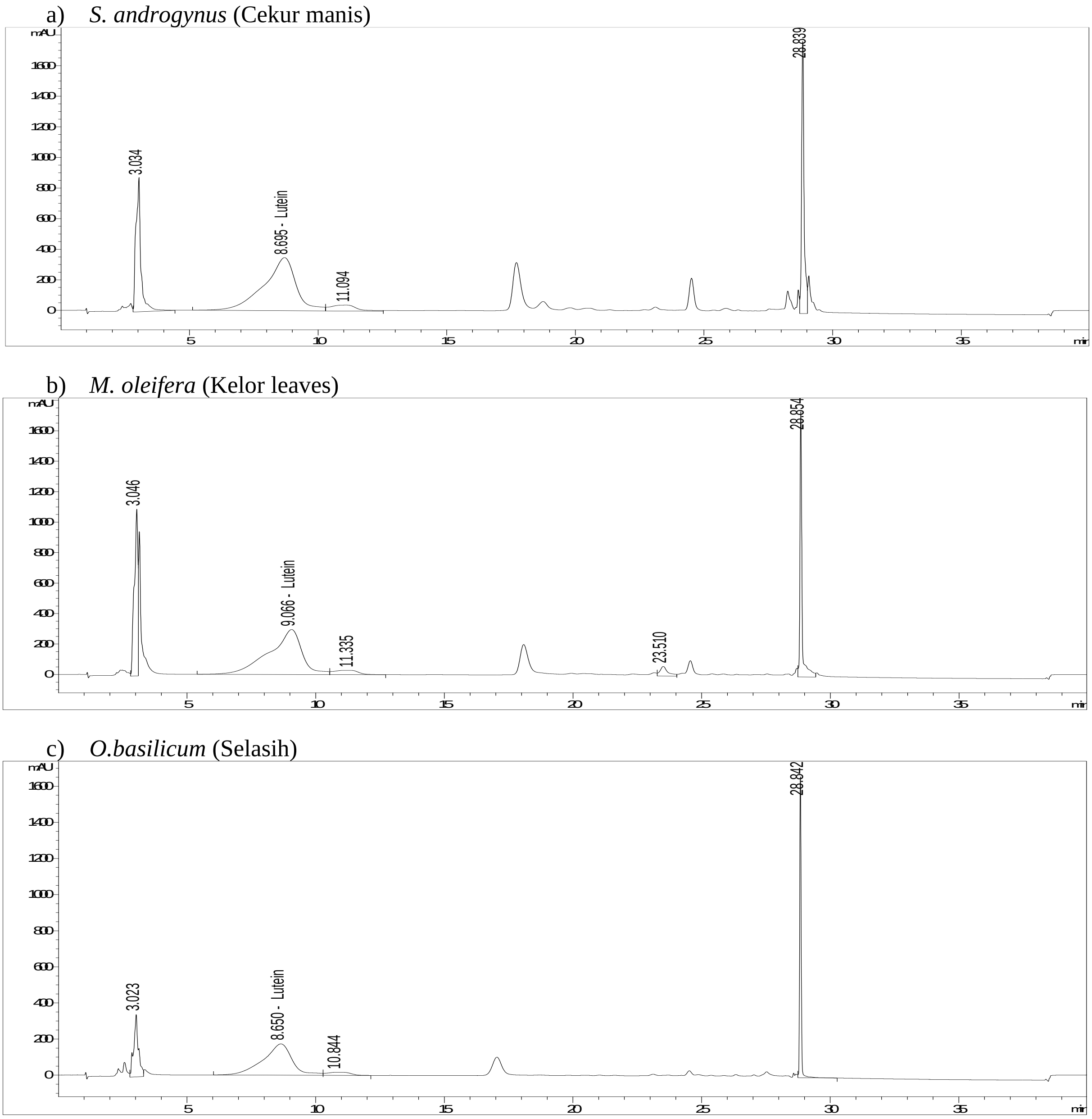
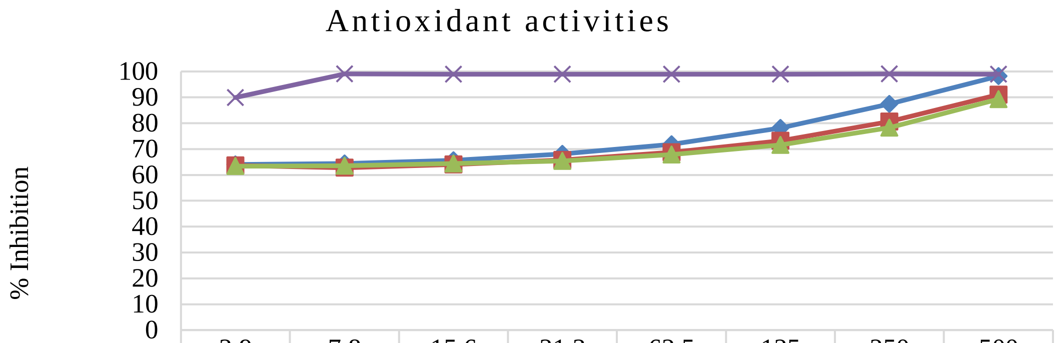


Figure 6. HPLC chromatogram of three ulam species showing the highest content and composition of carotenoids: lutein, β-carotene, and violaxanthin.

Carotenoids, renowned for their potent antioxidant properties, help promote overall well-being by combating harmful free radicals and reducing oxidative stress [59]. Beyond dietary significance, ulam shows promise for future medicinal applications based on emerging research [60-63].

Furthermore, numerous studies have identified a variety of phytochemicals in ulam beyond antioxidants, suggesting their potential medicinal properties [64-66]. Indigenous communities have long utilized ulam for its medicinal properties in treating common ailments [67, 68], and current research, including this study, aims to scientifically validate these traditional uses. Continued exploration of ulam's phytochemical composition may reveal new bioactive compounds with therapeutic potential against various diseases, potentially leading to the development of novel medicines [57, 69, 70]. Therefore, as highlighted in this study, the presence of antioxidants in ulam not only enhances dietary diversity but also presents a range of bioactive compounds with potential medicinal uses, deserving further exploration and research [71]. Studying the carotenoid content and composition of ulam is crucial for advancing the pharmaceutical industry and the global market by discovering alternative *halal* active pharmaceutical ingredients [46]. Findings on ulam with the highest carotenoid content can be further explored using green technology, benefiting Malaysia's *halal* pharmaceutical sector.



	3.9	7.8	15.6	31.3	62.5	125	250	500
Cekur manis	64.032	64.375	65.588	68.165	71.799	78.138	87.474	98.195
Kelor	63.624	62.760	64.003	65.787	68.691	73.144	80.582	91.068
Selasih	63.368	63.509	64.4	65.365	67.8250	71.575	78.306	89.306
Ascorbic acid	89.918	99.027	98.983	98.976	98.979	98.995	99.04	98.981

Figure 7. Antioxidant activities (inhibition percentage) of *S. androgynus* (Cekur manis), *M. oleifera* (Kelor leaves), and *O. basilicum* (Selasih) as well as the ascorbic acid standard.

CONCLUSION

Given the potential links between synthetic antioxidants and cancer, there is a growing shift in the food, cosmetics, and pharmaceutical industries towards natural and safe antioxidant sources derived from plants, renowned for their potent antioxidant properties. Moreover, with the exception of those prohibited by Islamic authorities, all plant species, their products, and byproducts are considered *halal*. This research aims to identify optimal sources of natural antioxidants. *Sauropus androgynus*, *Moringa oleifera*, and *Ocimum basilicum* are highlighted as promising natural antioxidant sources due to their high carotenoid content. The robust biological activity of carotenoids supports diverse commercial applications and contributes to the growth of the *halal* market. Further investigations are required to substantiate the potential of ulam as a *halal* active pharmaceutical ingredient. Consequently, this discovery is expected to benefit society and generate interest among producers, researchers, consumers, and other stakeholders committed to advancing human health.

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