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Short communication

Carotenoid Content and Retinol Activity Equivalents (RAE) of Seven Varieties of Mas Cotek (Ficus deltoidea) Leaf Extracts

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Abstract

Ficus deltoidea, also known as mistletoe fig or *Mas cotek* in Malay is believed to have health benefits due to the presence of bioactive compounds such as flavonoids and carotenoids. However, there are limited studies that show evidence of carotenoid compounds in *Ficus deltoidea* leaf extracts, especially in Malaysia. The aim of this study is to determine the carotenoid content of seven varieties of *Ficus deltoidea* leaf extracts and its retinol activity equivalent (RAE). Carotenoid content was identified by using High Performance Liquid Chromatography (HPLC) and its RAE was determined by using a formula where 12µg of β-carotene is equivalent to 1 RAE. The carotenoids detected in seven varieties of *Ficus deltoidea* were lutein and β-carotene. The highest carotenoid content detected in *Ficus deltoidea* leaf extract was β-carotene which ranged from 14.25 ± 8.84 ug/ul (*Ficus deltoidea* var. *deltoidea*) to 1612.00 ± 17.68 ug/ul (*Ficus deltoidea* var. *angustifolia*), while lutein content ranged from 23.00 ± 0.71 ug/ul (*Ficus deltoidea* var. *deltoidea*) to 62.25 ± 0.35 ug/ul (*Ficus deltoidea* var. *bilobata*). The RAE found ranged from 171.00 ± 106.07 (var. *deltoidea)* up to 18726.00 ± 1080.87 *(*var. *angustifolia).* The data obtained from this study indicated that *Ficus deltoidea* leaves of different varieties had significantly different carotenoid contents. Thus, there is potential for *Ficus deltoidea* to be used as an alternative natural carotenoid source for food and pharmaceutical industries.

Keywords*: β-carotene ;Carotenoid; Ficus deltoidea, , high performance liquid chromatography, lutein, , retinol activity equivalent.*

1. Introduction

Ficus deltoidea, commonly known as *Mas Cotek* in Malay, is a herbal plant found in Malaysia, Africa, Indonesia, and southern Philippines. They also have other local names like *Serapat Angin* in Terengganu, *Telinga Beruk* and *Telinga Gajah* in Kelantan and *Telinga Kera* in north-west Malaysia [1]. According to Berg and Corner (2005), *Ficus deltoidea* is categorised as a pan tropical genus; or identified as a terrestrial and hemi-epiphytic tree ,shrub or climber with an exclusive inflorescence (syconium, fig) from the Moraceae or mulberry family. They are found in lowlands and montane forests (ephiphytic and terrestrial) at altitudes of up to 1,500 metres to 2,500 metres or in thickets and shrubs on sandy soil, or rocks and as terrestrial or epilithic shrubs on seashore [2]. It is one of many ficus species planted in various parts of the world as a houseplant or an indoor or outdoor attractive flowering shrub [3]. Besides that, *Ficus deltoidea* is also planted for use in the medicinal industry.

Ficus deltoidea consists of over 800 species and one of about 40 genera of the mulberry family [4]. Previous study reported that they were 15 known varieties of *Ficus deltoideae* and eight of the varieties crop up in Sabah and Sarawak [5]. While, other study reported that they were seven varieties of *Ficus deltoidea* which were var. *deltoidea*, var. *angustifolia*, var. *trengganuensis*, var. *bilobata*, var. *intermedia*, var. *kunstleri* and var. *motleyana* [2].

Carotenoids are organic pigments within the tetraterpenoid group. They are useful for human health and are known to be bioactive. They belong to a group of natural pigments and act as antioxidants. Besides, they also hold many vital dietary functions, such as being converted into vitamin A. Many studies have reported that carotenoids can boost immune responses and lessen the risks of degenerative diseases, such as cancer and cardiovascular illnesses due to their abilities to quench singlet oxygen atoms and react with free radicals [6]. Carotenoids also play a fundamental role in defining the quality parameters of fruits and vegetables [7]. According to previous study on carotenoids content in Mas cotek leaves, the carotenoid detected were α-carotene 379.35 + 0.06 µg/g (dry weight) and β-carotene 874.62 + 0.04 µg/g (dry weight) [8]. However, the varieties of *Ficus deltoidea* used were not mentioned.

U.S. Institute of Medicine (IOM) introduced "retinol activity equivalent" (RAE) to replace "retinol equivalent" (RE) used by FAO/WHO. This ammendment was made to take into account new research on the vitamin A activity (bioefficacy) of carotenoids. Several different units have been applied to expressvitamin A activity over the years. To explain vitamin A activity, International units (IU) were first used and are still used for Nutrition Labeling (FDA, 1993). In 1974, the RDA National Research Council (NRC), changed the unit for expressing vitamin A to retinol equivalents (RE). Vitamin A activity represented the equivalent weight of retinol. Considering conversion and absorption, the general consumption of β-carotene was only one-sixth of retinol, α-carotene, and cryptoxanthin had half the activity of β-carotene. The vitamin A activity of β-carotene was acknowledged as being half of what was previously believed in the latest dietary reference intakes (DRIs) for vitamin A released in 2001 [9]. The unit of expression was changed to µg retinol

activity equivalents (RAE). The equivalent of β-carotene to RAE is shown as follows: 1 IU = 0.3 µg retinol or 0.6 µg β-carotene; or 1.2 µg of other provitamin A carotenoids, 1 RE = 1µg retinol or 6µg β-carotene or 12µg of other provitamin A carotenoids and 1 RAE = 1 µg retinol or 12 µg β-carotene or 24µg of other provitamin A carotenoids.

In this study, *Ficus deltoidea* leaves were extracted to determine its carotenoid content and the RAE amount was quantified. Since there was a limited study of carotenoid compounds in these herbal leaves, especially in the varities found in Malaysia, this new data of carotenoid content and its RAE was recorded for further study. This study would benefit future studies in carotenoid research .

2. Materials and Methods

2.1. Sample preparation

Ficus deltoidea leaves were obtained from Agriculture and Biotechnology plantations at Besut Campus, Universiti Sultan Zainal Abidin, in Besut, Terenganu. The morphology of *Ficus deltoidea* leaves (shape, length, and width) used in this study was recorded. Leaf samples were washed, freeze-dried and ground to reduce size prior to analysis.

2.2. Morphology of *Ficus deltoidea* Leaves

The same leaves used to extract carotenoids were used for the qualitative measurement of length and width. Ten samples were observed from one plant for each variety per observation and a total of three plants were used. This resulted in three replicates of each *Ficus deltoidea* variety. The smallest and the biggest values of the length and width of the leaves were observed and recorded.

2.3. Sample extraction

The extraction procedure described by Rashidi Othman [10] was followed, with slight modifications. For each sample, 1g of dried *Ficus deltoidea* leaves powder was weighed and 50 ml of acetone and methanol mixture (7:3, v/v) was mixed to allow for efficient solvent penetration. The solution was allowed to stand overnight in the dark at room temperature. The next day, the samples were vortexed and centrifuged for 5 min at 9500 rpm in a refrigerated centrifuge and the supernatant was transferred into another centrifuge tube. This procedure was repeated three times or until the sample became colourless. The samples were done in triplicates. Subsequently, 10 ml of petroleum ether was added to the supernatant with distilled water until it reached 50 ml. The solutions were then allowed to separate and the upper layers of petroleum ether containing carotenoids were collected. Vials or test tubes were then capped and sealed with a parafilm to exclude oxygen and were immediately stored at –20 °C for further analysis.

2.4. Saponification

Saponification was done, as described by Rashidi Othman [10], with again slight modifications. This step was done to eliminate chlorophyll in *Ficus deltoidea* leaves. Carotenoid extracts which was dried by using a rotary evaporator after the extraction process was then subjected to certain steps. 20 µl of ethyl acetate was added into the samples, followed by 380 µl acetonitrile: distilled water (9:1;v/v). Then, 400 µl methanolic potassium hydroxide solution (10 %; w/v) was added. Base carotenoids were then extractedwith the addition of 2 ml hexane with 0.1 % butylated hydroxytoluene (BHT), followed by 10 % of sodium chloride (NaCl). Finally, the extracts were washed with distilled water, while the upper layer was collected and dried and re-suspended in ethyl acetate for High Performance Liquid Chromatography (HPLC) analysis.

2.5. High Performance Liquid Chromatography (HPLC) Analysis

HPLC analysis was conducted, as described by Rashidi Othman [10], to quantify the carotenoid content in each treatment. The HPLC analysis of carotenoids was performed by using Agilent model 2100 series, which comprised a micro vacuum degasser, thermostat column compartment, and a diode array detector with an auto sampler injector and binary pump. The column used was a HPLC column with the following specifications; ZORBAX Eclipse XDB-C18, analytical 4.6 × 150 nm (5 micron) end capped 5 µm. The solvents used were (A) acetonitrile: water (9: 1 v/v) and (B) ethyl acetate. The solvent gradient used was developed as follows: 0-40 % solvent B (0-20mins), 40-60 % solvent B (20-25 mins) , 60-100 % solvent B (25-25.1mins), 100 % solvent B (25.1-35 mins), and 100-0 % solvent B (35-35.1mins) at a flow rate of 1.0 ml min^{-1}. The temperature of the column was maintained at 20°C. The injection volume was 10 µl. Carotenoid standards of β-carotene and lutein was obtained commercially from Sigma-Aldrich. Detection for carotenoid peaks was in the range of 350 to 430 nm. Individual carotenoid concentration were calculated by comparing their relative proportions, as reflected by the integrated HPLC peak areas.

2.6. Calculation of Retionol Activity Equivalent (RAE)

RAE was determined by using the equation of 1 RAE = 1 µg retinol or 12 µg β-carotene or 24 µg of other provitamin A carotenoids.

2.7. Statistical Analysis

The results obtained in the present study are represented as mean values of three individual replicates ± standard deviation (s.d.). One-way analysis of variance was performed with a statistics software programme, IBM SPSS Statistic version 20.0. The significant differences between the mean values were determined by using the Duncan's multiple range test at a significance level of $p<0.05$.

3. Results and Discussion

3.1. Morphology of *Ficus deltoidea* leaves

Morphology of seven varieties *Ficus deltoidea* were observed and recorded in Fig. 1 to Fig. 7. They were var. *motleyana* with elongated shape ranging from 4.8 - 9.2 cm long and 1.8 - 3.5 cm wide; var. *angustifolia* were sphatulate or lanceolate obovate in shape and measured between 3.0 to 4.6 cm long and 1.2 to 1.5 cm wide; var. *bilobata* were recorded asheart shaped and ranged between 1.5 to 4.0 cm in length and 1.5 to 3.0 cm in width; var. *borneensis* showed a cone-shape with rounded ends while the length was between 3.5 to 8.4 cm and the width ranged between 2.2 to 6.5 cm; var. *deltoidea* showed deltoid leaf shape and were 3.3 to 4.5 cm long and 2.4 to 3.0 cm wide; var. *kunstleri* were obtriangular to obovate-shape with 5.0 to 11.3 cm long and 6.5 to 10.7 cm wide; while var. *trengganuensis* were found to have elliptic to rounded obovateshape and ranged from 3.3 to 6.0 cm long and 2.2 to 3.5 cm wide.

Kochummen and Rusea [5] stated that leaf shape of *Ficus deltoidea* are vastly varied which included deltoid, elliptic, obovate, spathulate or rhomboid shapes. While, according to Berg and Corner [2], the shape of *Ficus deltoideae* leaves lamina tend to be oblong, elliptic, obtriangular, oblanceolate, spathulate, linear, and suborbicular. According to other study, the longest leaves of

Ficus deltoidea that were recorded were of var. motleyana (11.5 to–17.0 cm) found in Sarawak and the shortest were those of var. *deltoidea* (3.0 to 3.6 cm) which were samples recorded from Kelantan, Terengganu and Pahang. The widest leaves belonged to the var. *kunstleri* (6.5–to 8.0 cm) samples from Kelantan, Perak, and Pahang and the narrowest leaves belonged to var. *angustifolia* (1.0 to–2.0 cm) from Perak, Pahang, and Terengganu [11].

In this study the longest leaf was found in *Ficus deltoidea* var *kunstleri* (11.3 cm), while the shortest leaf recorded was found in *Ficus deltoidea* var *bilobata* (1.5 cm). The widest leaf recorded was *Ficus deltoidea* var *kunstleri* (10.7 cm) and the narrowest leaf was found among *Ficus deltoidea* var *angustifolia.*

Scientifically, variations in the leaf morphology, such as shape dimension, venation, presence and distribution of waxy glands, and length of petiole have beenquite confusing due to such broad varieties resulting in major obstacles for researchers to classify the species. However, the morphologyr in each study would prove helpful for other researchers in terms of proper documentation for further study of the samples.

Fig.1: Ficus deltoidea var. motleyana

Description
Shape leaves: Elongated
Leaf length (cm) : 4.8 cm - 9.2 cm
Leaf width (cm): 1.8 cm - 3.5 cm

Fig. 2: Ficus deltoidea var. angustifolia

Description
Shape leaves: Sphatulate or lanceolate obovate
Leaf length (cm) : 3.0 cm- 4.6 cm
Leaf width (cm): 1.2 cm – 1.5 cm

Fig. 3: Ficus deltoidea var. bilobata

Description
Shape leaves: Heart shaped
Leaf length (cm) : 1.5 cm – 4.0 cm
Leaf width (cm): 1.5cm – 3.0 cm

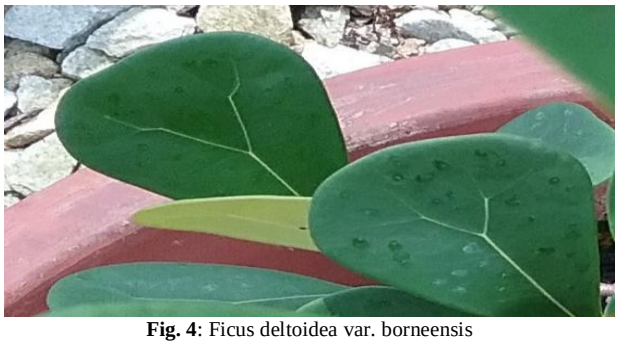

Fig. 4: Ficus deltoidea var. borneensis

Description
Shape leaves: Cone shape with rounded end
Leaf length (cm) : 3.5 cm – 8.4 cm
Leaf width (cm): 2.2 cm – 6.5 cm

Fig. 5: Ficus deltoidea var. deltoidea

Description
Shape leaves: Deltoid
Leaf length (cm) : 3.3 cm – 4.5 cm
Leaf width (cm): 2.4 cm – 3.0 cm

Fig. 6: Ficus deltoidea var. kustleri

Description
Shape leaves: Obtriangular to obovate
Leaf length (cm) : 5.0 cm – 11.3 cm
Leaf width (cm): 6.5 cm – 10.7 cm

Fig. 7: Ficus deltoidea var. trengganuensis

Description
Shape leaves: Elliptic to rounded obovate
Leaf length (cm) : 3.3 cm – 6.0 cm
Leaf width (cm): 2.2 cm – 3.5 cm

3.2. Carotenoid content and Retinol Activity Equivalent (RAE) of *Ficus deltoidea* leaves

In this study, two types of individual carotenoid extracted from *Ficus deltoidea* leaf was recorded in Table 1, which were lutein and β-carotene. To assure the correct determination of carotenoids, the spectrum of carotenoids detected in each sample was observed based on the retention time (RT) and UV-VIS spectrum recorded by the standard. Figure 8 shows an example of a chromatogram for β-carotene detection of *Mas cotek Ficus Deltoidea* var. *motleyana*. β-carotene compound was detected based on the retention time at 21.607 min and λ = 430 nm; while the lutein compound was detected based on the retention time at 8.366 min and λ = 430 nm. Commonly, the peak shape for lutein and β-carotene was different. For lutein compound, the spectral characteristics were recorded at λ=421, 445, 474 and for β-carotene compound, the spectral characteristics were recorded at λ=425, 450, 477.

Based on the results in Table 1, β-carotene found in *Ficus deltoidea* var. *angustifolia* had the highest value, with an amount of 1612.00 ± 17.68 ug/ul, while the lowest content of β-carotene was found in var. *deltoidea* with the amount of 14.25 ± 8.84 ug/ul. For lutein compound, var. *bilobata* showed the highest content of 62.25 ± 0.35 ug/ul, while the lowest lutein compound was found in var. *deltoidea* 23.00 ± 0.71 ug/ul. There were no significant differences between β-carotene content in *Ficus deltoidea* var *angustifolia, terengganuensis, and motleyana*, as well as in var *deltoideae* and *Borneonsis*. The results showed a significant difference between each group (a, b, ab, c) at ($p<0.05$).

The varied content of individual carotenoid (lutein and β-Carotene) present in *Ficus deltoidea* leaves may be due to some factors. According to previous study, factors such as climate changes and farming practices including the type of fertiliser used, type of soil, water (irrigation) supply, and postharvest handling will contribute to the accumulation of bioactive compounds in plants. Besides that, there were some other factors that may have given the varied amount of carotenoidcompounds, such as genetics, exposure to sunlight, rainfall, locality, type of soil, topography, season and maturity, and type of fertiliser used [12].

In a study conducted by Vera et al. reported that when acerola fruits were harvested during rainy season , carotenoid content in the fruits was found to be higher as compared to other seasons. Soil fertility increases during the rainy season. Soil fertility is defined as the capacity of the soil to provide plants with nutrients, water, and oxygen [13]. In another study conducted by Markus et al. on red peppers, during periods of long sunshine, low rainfall, and high temperatures, it was found that carotenoid content was lower in fruits as compared to those harvested in seasons with short sunshine periods, high rainfall, and low temperatures [14]. The location of pumpkins from different states in Malaysia [15] also showed a significant difference in carotenoid content while also being affected by seasons and storage times [6]. All factors mentioned above could significantly affect the biosynthesis, and the metabolism of carotenoids in plants [16].

From the current study, *Ficus deltoidea* leaf samples were collected from the Agriculture and Biotechnology plantations at the Tembila Campus, in Besut. During sampling, the climate was recorded at 38 °C – 40 °C with very limited rainfall. However, the plants were watered by farmers twice a day; once in the morning (at 9.00 am to 10.00 am) and once in the afternoon (at 4.00 pm to 5.00 pm). The soil used for planting all the *Ficus deltoidea's* plants was peat soil with NPK fertilizer. Even though the climate, soil, fertiliser, and watering time and quantity were provided almost equally to each plant, the carotenoid content was different between samples due to its varieties, where the genetics that build their metabolic pathway may significantly reflect the carotenoid accumulation in the different varieties of *Ficus deltoidea* plants.

Table 2 shows the retinol activity equivalent (RAE) for the seven varieties of *Ficus deltoidea* leaves. *Ficus deltoidea* var. *angustifolia* showed the highest reading as compared to the other varieties. This is because retinol activities equivalents are dependent on the β- Carotene compound value. 1 RAE is the same as 1μg retinol or 12 μg β-carotene or 24 μg of other provitamin A carotenoids. The vitamin A activity of β-carotene was acknowledged as being half of what was previously believed as reported in the latest DRI for vitamin A as stated by IOM [9].

Table 3 shows the amount of dietary retinol (μg) when expressed as retinol equivalents (μg RE) or retinol activity equivalents (μg RAE). According to the Panel on Micronutrients, Subcommittees on the Upper Reference Levels of Nutrients and of Interpretation and Use of Dietary Reference Intakes [17], Vitamin A is a necessary fat-soluble nutrient for eye health, immune function, embryonic development, cell differentiation, and growth hormone production. Other than that, low vitamin A intake may cause blindness and increased morbidity and mortality. In low-income districts, especially in south Asia and sub-sahara Africa, vitamin A deficiency stays a major public-health issue in rising countries [18]. Furthermore, vitamin A shortage is now believed to be a moderate public-health problem in China. However, recent studies showed that vitamin A status has been reclaimed in the past decade among Chinese children and pregnant women [19, 20]. Gudas et al. reported that one of retinol functions in humans is to integrate the epithelial cells throughout the body via retinoic acid molecules which regulate the expression of various genes that encode enzymes, extracellular matrix proteins, structural proteins, and retinol binding proteins and receptors [21]. While other researcher recorded that retinoic acid plays an important role in embryonic development, particularly in the development of the spinal cord and vertebrae, limbs, heart, eyes, and ears [22]. Besides, it is also required to maintain differentiation of the cornea and conjunctiva, preventing xerophthalmia, as well as for photoreceptor rod and cone cells in the retina [23].

Vitamin A is a standard term for retinol and provitamin A carotenoids (α-carotene, β-carotene, and β-cryptoxanthin). People acquire dietary vitamin A either as retinol from animal foods or as provitamin A carotenoids from vegetables and fruits [23, 24, 25]. Based on RNI recommendations for Malaysians, an adult should obtain 600 μg of Vitamin A for men and 500 μg of Vitamin A for women [27]. Preformed vitamin A such as retinol and its derivatives could be obtained from animal sources, while from plant sources, carotenoids with provitamin A activity exists as α- and β-carotenes and β-cryptoxanthin [28].

The main sources of pro-vitamin A are from dark-leafy vegetables, oranges, and yellow sweet potatoes, carrots, mangoes squash, and red palm oil containing generous provitamin A activity [29]. However, sources of carotenoids from green leafy plants were limited due to restraints in bioavailability. Based on findings, bioavailability of carotenoids from green leafy plant depends on the food matrix and the food processing methods. Heat treatment during food processing denatured the carotenoid content, and thus decreased its content value [30].

Table 1: Carotenoid content of *Ficus deltoidea*.

Mas cotek *Ficus deltoidea* Varieties	Amount of Carotenoid Content (ug/ul)	
	β- Carotene	Lutein
var. *deltoidea*	$14.25^{a} \pm 8.84$	$23.00^{a} \pm 0.71$
var. *kunstleri*	$486.00^{c} \pm 13.44$	$50.00^{ab} \pm 38.89$
var. *angustifolia*	$1612.00^{d} \pm 17.68$	$35.75^{ab} \pm 0.35$
va .*borneensis*	$27.50^{a} \pm 5.66$	$34.25^{ab} \pm 0.35$
var. *trengganuensis*	$61.50^{b} \pm 3.54$	$159.75^{c} \pm 11.67$
var. *motleyana*	$64.00^{b} \pm 2.12$	$46.00^{ab} \pm 0.00$
var. *bilobata*	$55.75^{b} \pm 1.77$	$62.25^{b} \pm 0.35$

Data expressed as the mean ± standard deviation (n = 7) of triplicates of seven samples.[a,b,c,d,e] Variation in the letters between samples in the same columns indicated significant differences at 5% level ($P<0.05$) utilising One-Way Anova.

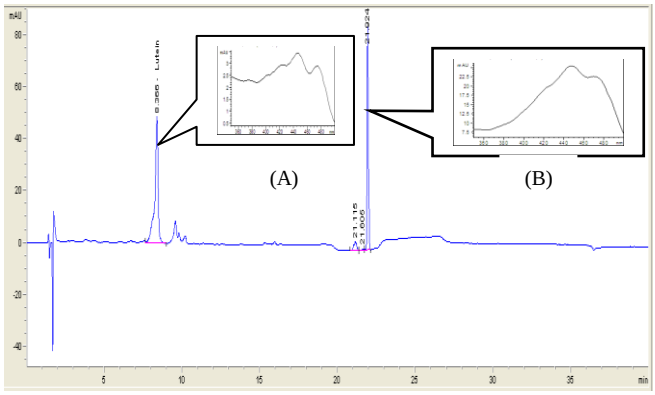

Fig. 8: High Performance Liquid Chromatography chromatogram of carotenoid detected in *Ficus Deltoidea* var. *motleyana* based on variety. The identity of individual carotenoids was confirmed by the retention time recorded by standard and their spectral characteristics. (A) Spectral characteristics (λ=421, 445, 474) of lutein at retention time of 8.366min and (B) Spectral characteristics of β –carotene (λ=425,450,477) at retention time (RT) of 21.607min

Table 4 shows the Vitamin A intake requirements and daily recommendations as advised by the Institute of Medicine (IOM), the WHO/FAO and the European Food Safety Authority (EFSA). The former equivalent of vitamin A intake is suggested by FAO/WHO (1988) as RE (retinol equivalent) which is equal to retinol + (β-carotene/6) + (α-carotene/12) + (β-cryptoxanthin/12), (FAO and WHO, 1988) with respect to the retinol activity, while, the present equivalent brought in by IOM as RAE (retinol activity equivalent) which is equal to retinol + (β-carotene/12) + (α-carotene/ 24) + (β-cryptoxanthin/24) [31, 32, 33].
Several countries such as the United States, Canada, and Australia have assigned new dietary reference intakes (DRIs) of vitamin A as RAE, where it recommended 900 µg for men and 700 µg for women [17, 34]. According to Cheng. Chinese DRIs suggested 800 µg for men and 700 µg for women [35]. For this research, total retinol activity equivalents (RAE) that were gained by green leaves like *Ficus deltoidea* was about 1.19 µg/µl up to 130.04 µg/µl. That means *Ficus deltoidea* eaves rich with provitamin A activities.

Table 2: Retinol Activity Equivalents of *Mas cotek Ficus deltoidea* leaves.

Ficus deltoidea Varieties	Retinol Activity Equivalents (RAE) (µg/µl)
var. *deltoidea*	$1.19^{a} \pm 0.74$
var. *kunstleri*	$40.50^{d} \pm 1.12$
var. *angustifolia*	$130.04^{e} \pm 7.51$
var .*borneensis*	$2.29^{a} \pm 0.47$
var. *trengganuensis*	$5.13^{c} \pm 0.29$
var. *motleyana*	$5.33^{c} \pm 0.18$
var. *bilobata*	$4.50^{b} \pm 0.27$

Data expressed as the mean ± standard deviation (n = 7) of triplicates of seven samples. ^{a,b,c,d,e} Variation in the letters between samples in the same columns indicated significant differences at 5% level (P<0.05) utilising One-Way Anova.

Table 3: Amount of dietary retinol (µg) when expressed as retinol equivalents (µg RE) or retinol activity equivalents (µg RAE).

	RE	**RAE**
Preformed retinol	1	1
Provitamin A		
All-trans β-carotene	6	12
α-Carotene	12	24
B-Cryptoxanthin	12	24

(Source from FAO&WHO, 1967) [35].

Table 4: Vitamin A intake requirements and daily recommendations as advised by the Institute of Medicine (IOM), the WHO/FAO and the European Food Safety Authority (EFSA)[9].

	IOM,2001		WHO/FAO, 2004		EFSA,2015	
	EAR (RAE)	RDA(RAE)	MR (RE)	RNI (RE)	AR(RE)	PRI (RE)
Children 1-6years	210-275	300-400	200	400-450	205-245	250-300
Women, > 18 years	500	700	270	500	490	650
Men, > 18 years	625	900	300	600	570	750
Pregnant women	550	770	370	800	540	700
Lacting women	900	1300	450	850	1020	1300

EAR, Estimated Average Requirement; RAE, retinol activity equivalents; MR, Mean Requirement; RE, retinol equivalents; RNI, Recommended Nutrient Intake; AR, Average Requirement; PRI, Population Reference Intake. (Sources fromPublic Health Nutrition: 2011, 1903–1906).

4. Conclusion

The results of this study showed that different varieties of *Ficus deltoidea* had different amounts of individual carotenoid compounds and Retinal Activity Equivalents. *Ficus deltoidea* var. *angustifolia* showed the highest amount of beta carotene compound which was about 1612.00 + 17.68 µg / µl ; while var. *bilobata* showed the highest amount of lutein compound about 62.25 ± 0.35 µg/ µl due to many factors such as climate changes, fertilizer used, rainy seasons, and post-harvest handling. Even though the topic of carotenoids is discussed often and vigorously among researchers worldwide there are few publications in the field, the data for carotenoid compounds for the herbs found in Malaysia, specifically for *Mas cotek* (*Ficus deltoidea)* are still inadequate. This study provided new data for carotenoid content and RAE value found in seven varieties of *F.deltoidea* leaves which contributed to the extension of carotenoid study in these herbs.

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