THE FLOWER OF *RADERMACHERA IGNEA* (KURZ) STEENIS, A NEW SOURCE OF ZEAXANTHIN

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Abstract

The flower of *Radermachera ignea* (Kurz) Steenis or "Peep Thong", the emblem of Suranaree University of Technology, was selected for the study as a potential new source of antioxidant. The main antioxidant component was isolated from the ethyl acetate extract of the flower and its chemical structure was determined by spectroscopic data as zeaxanthin (β, β-carotene-3,3'-diol), one of the two significant carotenoids present in the marcula lutea of the retina of human eyes. The antioxidant study confirmed the activity of the isolated compound with the IC_{50} of 1.13 mol antioxidant/mol DPPH. This is the first report to identify the bioactive constituent in this plant.

Keywords: ***Radermachera ignea*** **(Kurz) Steenis, Peep Thong, antioxidant, carotenoids**

Introduction

Oxidative damage process has been indicated as a major cause of several degenerative diseases including cancer, cardiovascular disease, diabetes, Alzheimer's disease, and Parkinson's disease (Ames *et al*., 1993; Banerjee *et al*., 2005). Antioxidants have been found to play an important role to prevent and inhibit such process (Ames *et al*., 1993).

In the search for a new source of antioxidants, the flower of *Radermachera ignea* (Kurz) Steenis or commonly known as "Peep Thong", the emblem of Suranaree University of Technology, was selected to be the subject of interest because of its bright orange color, which normally suggests the presence of natural substances with antioxidant activity. *R. ignea* (Kurz) Steenis belongs to the family Bignoniaceae. The tree is evergreen or partly deciduous with 6-15 m high and typically scattered in several areas of Southeast Asian region.

Neither the data about the chemical constituent nor the antioxidant activity of this plant has been previously reported in the literature. Therefore, this is the first paper to describe the isolation and characterization of the bioactive compound from the flower of *R. ignea* (Kurz) Steenis.

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Materials and Methods

Plant Materials

The flower of *R. ignea* (kurz) Steenis was collected directly from the trees around the campus of Suranaree University of Technology, Nakhon Ratchasima, Thailand. The plant specimen was authenticated by the Forest Herbarium, the Office of Forest and Plant Conservation Research, National Park, Wildlife andPlant Conservation Department, Bangkok, Thailand.

General Experimental Procedure

Commercial grade solvents were distilled prior to use. Silica gel 60 (0.063-0.200 μM) and silica gel 60 (0.040-0.060 μM) for flash column chromatography were from Merck. Unless otherwise stated, all analytical grade solvents were from Carlo Erba. DPPH (1,1-Diphenyl-2-picryl-hydrazyl) and L-ascorbic acid were from Fluka and (+)-catechin was from Sigma.

NMR spectra were measured by Varian Inova 600 MHz, Bruker DRX 600 MHz, and DRX 500 MHz. All NMR spectra were recorded in $CDCl_3$ (δ in ppm, J in Hz) with tetramethylsilane (TMS) as an internal standard. Mass spectra were measured using APCI-MS techniques by Agilent, LC-MS Series 1100. Infrared spectra were taken as KBr pallet on Perkin-Elmer, FT-IR Spectrophotometer Model Spectrum GX. UV spectra were obtained in ethanol (abs.) on CARY Varian-1E.

Extraction and Isolation Procedures

The fresh flowers were cleaned, dried in the oven at 40°C, and ground into small pieces before used. Dried flowers of *R. ignea* (463 g) were extracted with 90% methanol (3.0 L) in a soxhlet extractor for 24h. The solvent was removed by rotary evaporation yielding methanol crude extract. The obtained methanol crude extract was extracted consecutively with hexane followed by ethyl acetate to yield ethyl acetate extract. The subsequent chromatographic separations were performed to provide the antioxidant component as illustrated in Figure 1.

Antioxidant Study

The antioxidant activity was determined from the scavenging activity against DPPH radicals. Antioxidant TLC-screening assay (Hostettmann, 1999; Cuendet, 1997) was used for directing the isolation process. The radical scavenging activity was evaluated by spraying the developed TLC plates with 0.2 mM methanolic solution of DPPH. The quantitative antioxidant activity analysis was measured as the IC_{50} values by using a modified version of the method described by Jiménez-Escrig *et al.* (2000) using DMSO as the solvent to confirm the activity of the isolated compound. (+)-Catechin and ascorbic acid were used as the antioxidant standards.

Results and Discussion

Isolation of the Main Antioxidant Component from the Flower of *R. Ignea* (Kurz) Steenis.

The main antioxidant component was isolated by series of chromato-graphic separations guided by the antioxidant TLC-screening assay (Figure 1). The ethyl acetate extract was isolated by flash column chromatography using hexane-EtOAc (1:1) as the mobile phase. The fraction containing the main antioxidant was concentrated and purified by crystallization in EtOAc to yield 0.0660 g of a reddish orange powder (0.0143% based on the weight of dried plant material).

Characterization of the Main Antioxidant Component in the Flower of *R. Ignea* (Kurz) Steenis.

The LC/APCI-MS spectrum showed the molecular ion peak $[M+H]^+$ at m/z 569.4, corresponding to the chemical formula of $C_{40}H_{56}O_2$. The FT-IR spectrum showed the absorption peaks at ν 3435 cm^{-1} (O–H stretching), 2921 cm^{-1} (C–H stretching), 1628 cm^{-1} (C=C stretching), 1384 cm^{-1} (CH_3 bending), 1049 cm^{-1} (C–O stretching), 958 cm^{-1} and 688 cm^{-1} (C=C–H out of plane bending). In the UV spectrum, the observed λ_{max} were at 450 and 477 nm, which are the characteristic absorption peaks of carotenoids (Sajilata e*t al.*, 2008).

The ^{1}H and ^{13}C NMR spectral data are presented in Table 1. Twenty carbon peaks in the ^{13}C spectrum indicated molecular symmetry. Correlation between protons and carbons were assigned according to HSQC and HMBC spectra. The two equivalent carbons bearing hydroxyl groups showed a ^{13}C NMR peak at δ 65.1. The ten methyl groups showed the ^{13}C NMR signals at δ 30.3, 28.7, 21.6, 12.8, and 12.8, which were correlated to the ^{1}H NMR signals at δ 1.07(s), 1.07(s), 1.74(s), 1.97(s), and 1.97(s), respectively. The NMR data are consistent with those of zeaxanthin (β, β-carotene-3,3'-diol; Figure 2) as previously

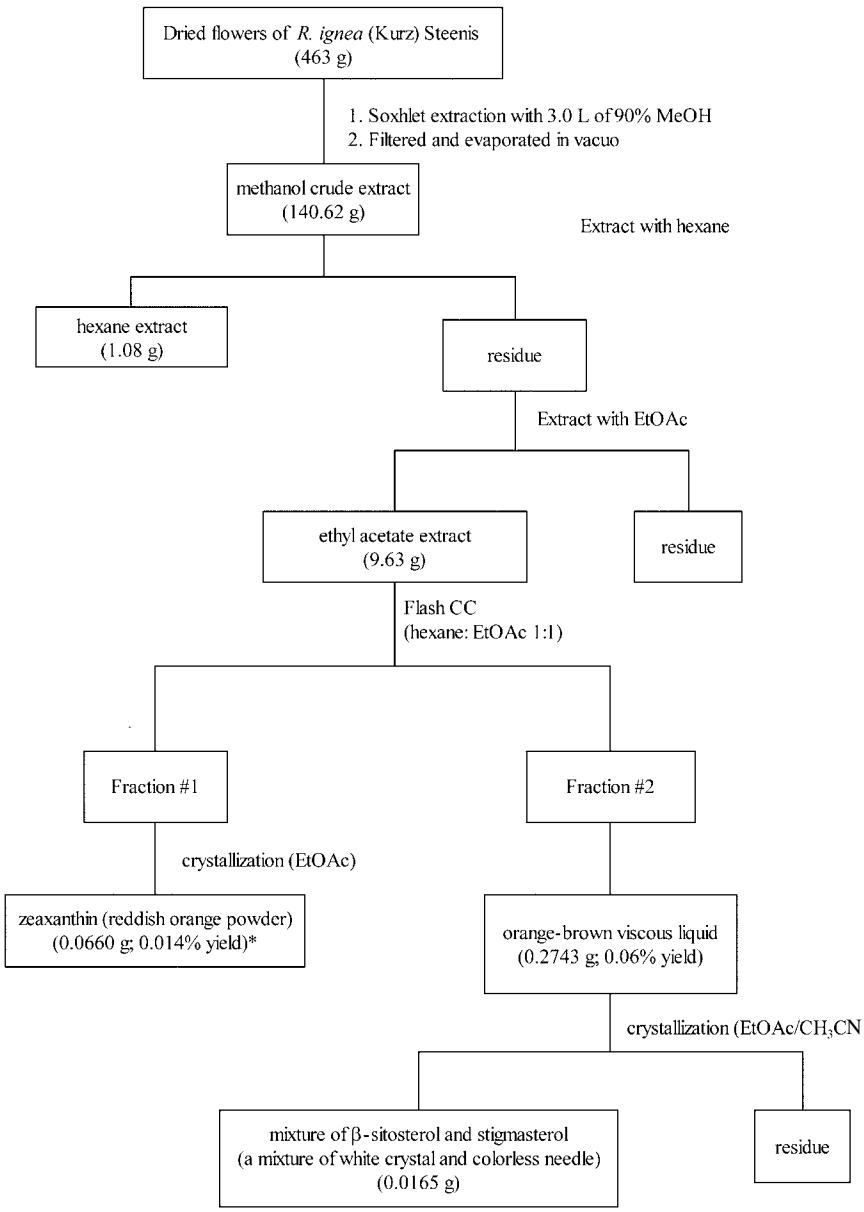

Figure 1. Extraction diagram of the flower of *R. ignea* (Kurz) Steenis
* based on the weight of the dried flowers

Table 1. ^{1}H and ^{13}C NMR data[a] of zeaxanthin isolated from the flower of *R. ignea* (Kurz) Steenis

Position		^{1}H NMR (J = Hz)	^{13}C NMR
1, 1’	C	- (q)	37.1
2, 2’	CH_2	1.77dd(12, 2.4), 1.48t(12)	48.4
3, 3’	CH	4.00	65.1
4, 4’	CH_2	2.39dd(16.8, 5.4) 2.05dd(16.8,10.2)	42.6
5, 5’	C	- (q)	126.2
6, 6’	C	- (q)	137.8
7,7’	CH	6.10d(16.8)	125.6
8, 8’	CH	6.13d(15.6)	138.5
9, 9’	C	- (q)	135.7
10, 10’	CH	6.16d(10.8)	131.3
11, 11’	CH	6.65dd(13.2, 10.8)	124.9
12, 12’	CH	6.63d(15)	137.6
13, 13’	C	- (q)	136.5
14, 14’	CH	6.25brd(9)	132.6
15, 15’	CH	6.64d(15)	130.10
16, 16’	CH_3	1.07s	30.3
17, 17’	CH_3	1.07s	28.7
18, 18’	CH_3	1.74s	21.6
19, 19’	CH_3	1.97s	12.8
20, 20’	CH_3	1.97s	12.8

[a] *The NMR spectra were measured in $CDCl_3$ using TMS as the internal references.*

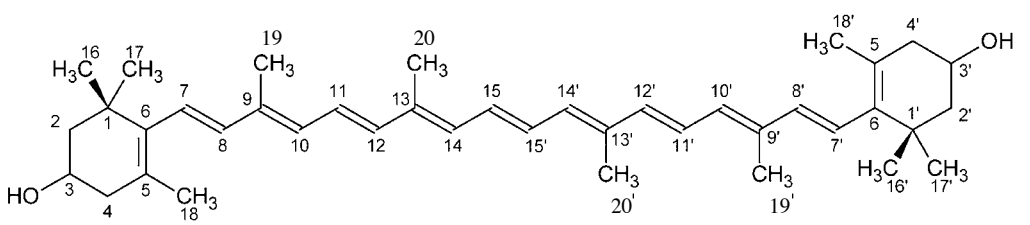

Figure 2. Chemical structure of zeaxanthin (β,β-carotene-3,3’-diol)

reported by Moss (1976) and Eisenreich (2002).

In addition to zeaxanthin, the isolation yielded a mixture of two steroidal compounds. Their structures were characterized based on the NMR and LC/APCI-MS data as β-sitosterol and stigmasterol.

Antioxidant Study

The result of the antioxidant study is presented in Table 2. The testing confirmed the antioxidant activity of the isolated zeaxanthin from the flower of *R. ignea* (Kurz) Steenis. The isolated compound exhibited the antioxidant activity with the IC_{50} of 1.13 mol antioxidant/ mol DPPH, which was about the same level as (+)-catechin; IC_{50} 1.10 mol /mol DPPH, and was much stronger than ascorbic acid; IC_{50} 304.30 mol /mol DPPH under the same condition.

The Content of Zeaxanthin in the Flower of *R. Ignea* (Kurz) Steenis.

The main antioxidant isolated from the flower of *R. ignea* (Kurz) Steenis was identified as zeaxanthin in its free (native) form. It has been widely known that zeaxanthin along with lutein are the only two carotenoids presented in the marcula lutea of the retina of human eyes (Roberts *et al.*, 2009). Several studies suggested that zexanthin may help to protect against the development of aged-related marcula degeneration (AMD) and aged-related cataract formation (Roberts *et al.*, 2009; Weller and Breithaupt, 2003). Like other carotenoids, zeaxanthin cannot be produced in human body; therefore, its presence must be obtained primarily from dietary intake. Although lutein is abundant in green leafy vegetables and fruits, only trace amount of zeaxanthin is present in common diet (Lam and But, 1999, Humphries and Khachik, 2003).

One of the known rich sources of zeaxanthin is Gou Qi Zi or goji berry or wolfberry *(Lycium barabarum)*, which is commonly used in Chinese cooking and traditional herbal medicine for preventing and treating of visual problems. The amount of the total zeaxanthin in wolfberries obtained by hydrolysis of its dipalmitate derivative was ranging from 2.4 to 82.4 mg/100 g of the dried berries while the free zeaxanthin amount was reported at 1.22 mg/100 g of the dried berries (Lam and But, 1999; Weller and Breithaupt, 2003). Comparing to the flower of *R. ignea* (Kurz) Steenis, the zeaxanthin obtained was 0.0143% or 14.3 mg/100 g of the dried plant material, all of which was in the free zeaxanthin form. Typically, a commercial production process of zeaxanthin supplement products requires a saponification step, where ester derivatives of zeaxanthin are hydrolyzed to their native form using strong bases such as KOH. The rather high amount of zeaxanthin of its native form in the flower of *R. ignea* (Kurz) Steenis suggested that the plant could be a promising alternative source for this antioxidant. The isolation of zeaxanthin directly from the plant would be an interesting shortcut in obtaining the pure compound without going through the expensive saponification and further purification steps.

Table 2. Antioxidant activity study of zeaxanthin isolated from the flower of *R. ignea* (Kurz) Steenis.

Compound	IC_{50} (mol antioxidant/mol DPPH)
zeaxanthin (isolated compound)	1.13
(+)-Catechin	1.10
Ascorbic acid	304.30

Conclusions

Zeaxanthin was identified as the main antioxidant in the flower of *R. ignea* (Kurz) Steenis and presumably responsible for its bright orange color. The results from the study suggested that this local plant could potentially be a promising new source of zeaxanthin. Our future investigation will focus on developing the effective isolation and purification methods to maximize the yield of this carotenoid antioxidant from various local plants of this type.

Acknowledgments

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