DETERMINATION OF CAROTENOIDS CONTENT AND ANTIOXIDANT ACTIVITY FROM *Cucurbita maxima* Duch **(PUMPKIN)**

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Abstract

Fruits and vegetables are good sources of carotenoids, antioxidant phyotochemicals that mitigate the damaging effect of oxidative stress. In this research paper, the vegetable selected for the study is the fruit of *Cucurbita maxima* Duch (pumpkin). The study was conducted to estimate the concentration of total carotenoids and β-carotene contents by applying UV-visible Spectrophotometer at Laboratory of Chemistry Department, Yadanabon University. It was found to have the concentration of total carotenoids and β-carotene (2.84mg/100g) and (108μg/100mL). Moreover, the antioxidant activities in ethanol and water extracts were evaluated by using UV-7504 Spectrophotometer at University of Yangon. The antioxidant activities of these extracts were investigated based on their ability to scavenge (DPPH) stable free radical. The excellent IC_{50} values of ethanol and water extracts were found to be (11.37μg/mL) and (38.76μg/mL).

Key words- pumpkin, carotenoids, β-carotene, antioxidant activity

Introduction

Carotenoids are a group of phytochemicals that are responsible for different colours of the foods. They are recognized as playing an important role in the prevention of human diseases and maintaining good health. Carotenoids in vegetables are of the significant importance, besides other vitamins, minerals, flavonoids and phytochemicals, which have been reported to contribute to health. Carotenoids are widely known as pro-vitamin A, while there is an increasing interest in their role as antioxidants. Anti-cancer activity and other health benefits provided by beta-carotene include the protection against cardiovascular disease or cataract prevention. *Cucurbita maxima* Duch is an excellent source of beta-carotene and ascorbic acid which acts as antioxidant to neutralize harmful free radicals in the skin there by helping to prevent wrinkles, resists infection, to keep the skin moist and youthful. Fruits and vegetables are good sources of antioxidant phytochemicals that mitigate the damaging effect of oxidative stress. Oxidative stress is an important contributor to the risk of chronic diseases such as diabetes, heart diseases, osteoporosis and cancer. Dietary guidelines recommended increased consumption vegetables to combat the incidence of such diseases in human.

Aim and Objectives of the Present Research Work

The aim of the research work is determination of total carotenoids contents and antioxidant activity of fruit of pumpkin.

Objectives of the Present Research Work

- To analyze the presence of different phytochemicals constituents
- To estimate the total carotenoids content and beta-carotene content and
- To evaluate the antioxidant activity of *Cucurbita maxima* Duch fruit in ethanol and water extracts

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Botanical Description

Plant of Pumpkin

Fruit of Pumpkin

Kingdom : Plantae
Family : Cucurbitaceae
Scientific name : *Cucurbita maxima Duch*
Common name : Pumpkin
English name : Red Gourd
Local name : Pha-yone-thee
Medicinal uses : Erthropoietic protoporphyria, Breast cancer, eye health Age-related muscular degeneration

Nutrition Benefits of Pumpkin

Table Nutritive Value Occur in Per 100g of Fresh Pumpkin

Principle	Nutrient Value	Percentage of RDA
Energy	26 Kcal	1 %
Carbohydrates	6.5 g	5 %
Protein	1.0 g	2 %
Total fat	0.1 g	0.5 %
Cholesterol	0 mg	0 %
Dietary fiber	0.5 mg	2 %
Folates	16 mg	4 %
Niacin	0.60 mg	4 %
Pantothenic acid	0.298 mg	6 %
Pyridoxine	0.061 mg	5 %
Riboflavin	0.110 mg	8.5 %
Thiamin	0.50 mg	4 %
Vitamin A	7384 IU	246 %
Vitamin E	9.0 mg	15 %
Vitamin C	1.06 mg	7 %
Vitamin K	1.1 mg	1 %
Sodium	1 mg	0.5 %
Potassium	340 mg	7 %
Calcium	21 mg	2 %
Copper	0.127 mg	14 %
Iron	0.80 mg	10 %
Magnesium	12 mg	3 %
Manganese	0.125 mg	0.5 %
Phosphorus	44 mg	5 %
Selenium	0.3 mg	<0.5 %

Zinc	0.32 mg	3 %

MATERIALS AND METHODS

Sample Preparation

The fruit of pumpkin (*Cucurbita maxima* Duch) used for this investigation was collected from the Shan-kalay-kyun village, Amarapua Township, Mandalay Region. The fresh and air dried samples were used for the respective determinations.

Instrument and Material Used

Jenway-6300 UV spectrophotometer and UV–visible spectrophotometer (UV-7504) were used for the determination of carotenoids and beta-carotene contents and for the measurement of antioxidant activity. Commercial grade reagents and solvent were used.

Phytochemical Tests for *Cucurbita maxima* Duch.

Preliminary phytochemical tests of the fruit of *Cucurbita maxima* Duch (pumpkin) were done.

Determination of Carotenoids Cotent by UV spectrophotometer

Using UV spectrophotometer, the maximum wavelength was detected. The absorbance of carotenoid was measured at 460 nm (λ_{max}). The amount of total carotenoids (mg/100 g) in pumpkin was calculated using the following equation:

$$\text{Amount of carotenoid in sample} = \frac{\text{Abs at 460 nm} \times \text{V} \times 10000}{(\text{A}_{1\text{cm}}^{1\%} \times \text{sample used})}$$

V = 10ml (total volume of sample)

$A_{1cm}^{1\%}$ = absorption coefficient) 2500 for total carotenoids)

Determination of β- Carotene Content by UV spectrophotometer

The content of β-carotene in the petroleum- ether extract was determined spectrophotometrically. The absorbency was measured at the wavelength of 440 nm (λ_{max}). The concentration of β-carotene (g/100 mL) was calculated using the response factors as follow:

Beta-carotene (μg/100mL) = $\frac{A}{EL}$

A= absorbance L= path length (1cm)

E= coefficient of absorbance (2560 100mL$g^{-1}cm^{-1}$)

Determination of Antioxidant Activity of *C.maxima* Fruit Extract by DPPH Method

Plant Extraction

Air dried powder fruit material 5g was successively extracted with ethanol and water at 60ºC and 80ºC. All the extracts were subjected for evaluation of antioxidant activity.

Screening of Antioxidant Activity of Crude Extracts by DPPH Assay

DPPH$^{\bullet}$ (2, 2-diphenyl -1-picry-hydrazyl) radical scavenging assay was chosen the antioxidant activity of plant materials. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\% \text{ Inhibition} = A_{Control} - A_{Sample}$$

$A_{Control}$ = absorbance of control solution

A_{Sample} = absorbance of tested sample solution

IC_{50} value was calculated by linear regressive excel program.

RESULTS AND DISCUSSION

The phytochemicals of the pumpkin were studied by preliminary phytochemical tests and the results were tabulated in the given table.

Table Phytochemical Tests of *Cucurbita maxima* Duch (pumpkin)

No	Test	Reagents	Observation	Remark
1	Alkaloid	Dragendroff's	Orange ppt	+
2	Flavonoid	Hydrochloric acid and magnesium turning	Pink colour	+
3	Phenolic compound	10%$FeCl_3$ solution	Purplish colour	+
4	Steroid/triterpene	Acetic anhydride and conc:H_2SO_4 solution	Red color at lower layer no green colour	+ (triterpene)
6	Glycoside	10% lead acetate	White ppt	+
7	Carbohydrate	Benedict's solution	Brown coloration	+
8	Protein	NaOH and $CuSO_4$ solution	Pink coloration	+
9	Starch	2% Na and 1% gelatin solution	Blue coloration	+
10	Tannin	Iodine solution	No red colour	–
11	Resin	Acetone	No brownish turbidity	–
12	Saponin	Water	Frothiness	+
13	Lipophilic group	0.5% M KOH solution	Deep colour	+
14	Amino acid	Ninhydrin	Purple colour	+

(+) = presence of constituents, (–) = absence of constituents

Table % RSA (Radical Scavenging Activity) and IC_{50} Values of Crude Extracts of Pumpkin

Tested sample	% RSA (mean ±SD) in different concentration (µg/mL)						IC50 (µg/mL)
	2.5	5	10	20	40	80	
Pumpkin EtOH I	32.36 ±0.79	44.91 ±0.91	48.79 ±0.24	57.80 ± 0.75	61.82 ±0.64	74.50 ±0.52	11.37
Pumpkin water I	22.52 ±0.43	24.19 ±0.52	27.58 ±0.64	32.85 ±0.42	47.05 ±0.79	51.35 ±0.91	67.76
Pumpkin EtOH II	35.48 ±0.32	38.81 ±0.73	42.34 ±0.84	44.49 ±2.19	48.72 ±0.12	51.28 ±0.32	60.15
Pumpkin water II	31.88 ±0.24	34.72 ±0.72	40.47 ±0.12	42.41 ±0.21	50.52 ±0.36	53.64 ±0.55	38.76

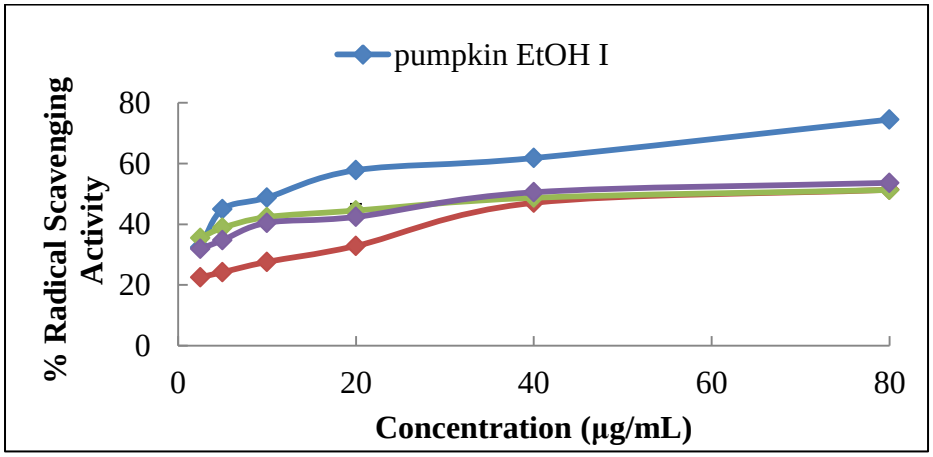

Figure Radical Scavenging Activities of Different Concentrations of Crude Extracts of Pumpkin

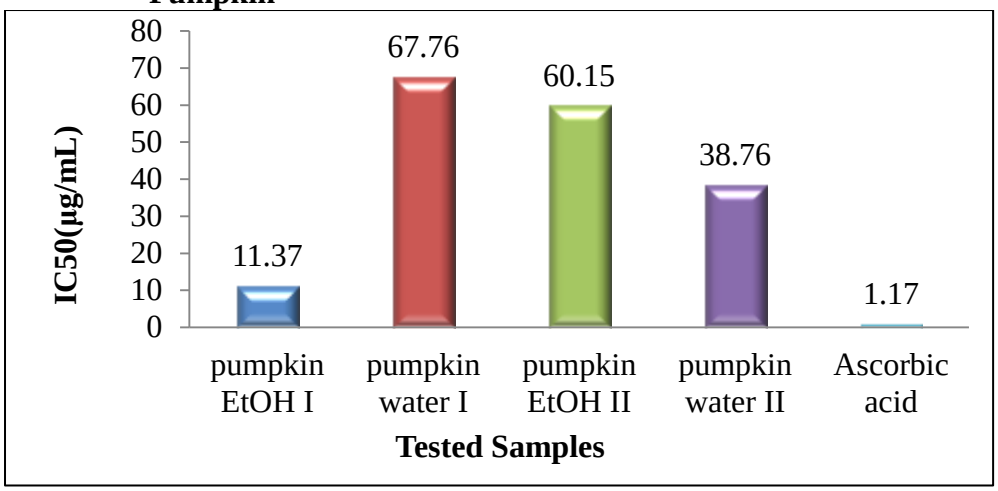

Figure a Bar Graph of IC_{50} Values of Tested Samples

In accordance with the preliminary phytochemical tests, phytochemicals such as alkaloid, flavonoid, terpenoid, polyphenol, glycoside, carbohydrate, protein, saponin, lipophilic group and amino acid were present in the *Cucurbita maxima* Duch (pumpkin). These may support the pumpkin to have protective and disease preventive properties.

In the determination of total carotenoids and beta-carotene contents, the total carotenoids 2.84mg/100g and beta-carotene 108μg/100mL were observed, respectively. From the present finding, the pumpkin contains high amount carotenoids and beta-carotene which made pumpkin to provide anti-cancer activity and other health benefits including the protection of cardiovascular diseases or cataract prevention.

From the experimental data, it was found that the ethanol extract of pumpkin at (80℃) shows low IC_{50} (11.37μg/mL) while water extract at (60℃) shows low IC_{50} (38.76μg/mL). IC_{50} is the concentration of extract required to produce 50% free radical scavenging activity. This value states that the amount of concentration of extract required producing 50% free radical scavenging activity. Hence, IC_{50} value is inversely related to the free radical scavenging activity. Here results clearly show that ethanol I has excellent DPPH free radical scavenging activity. Thus, pumpkin is a good source of antioxidant activity that helps to prevent wrinkles, resist infection, to slow the process of aging and to keep the skin moist and youthful.

Conclusion

In this research, the composition of phytochemical groups, the proximate contents of total carotenoids and beta-carotene and the antioxidant activity of the C. maxima Duch (pumpkin) were examined. From the preliminary phytochemicals screening, the positive tests of alkaloid, flavonoid, polyphenol, terpenoid, carbohydrate, protein, starch, lipophilic group, saponin and amino acid were observed.

According to the experimental results, the total carotenoids content (2.84mg/100g) and β-carotene content (108μg/100g) were detected. The present of high contents of total carotenoids and β-carotene support that the pumpkin is a good source of provitamin A and antioxidants. The high amount of crotenoids and beta-carotene contents present in pumpkin which help in the prevention of lung cancer, wrinkles, heart stroke, and for keeping immune system of the body.

Moreover, in the investigation of radical scavenging activity, the observed IC_{50} (60.15μg/mL) value of ethanol II (60℃) is higher than IC_{50} (11.37μg/mL) value of ethanol I (80℃) while the IC_{50} (38.76μg/mL) value of water II (60℃) is lower than IC_{50} (67.76μg/mL) value of water I (80℃). IC_{50} value is inversely related to the free radical scavenging activity. It can be concluded that water extract (60℃) and ethanol extract (80℃) possess maximum free radical scavenging activities. Thus, C. maxima Duch (pumpkin) have fairly antioxidant activity. C. maxima Duch (pumpkin) contains relatively high amount of carotenoids and antioxidant activity that are essential for reduce cancer diseases, heart stroke, keeping the immune system and to support eye health, fertility, delay aging and body degeneration. Hence, the study concluded that pumpkin is a healthy diet for human and should consume as a staple vegetable.

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Online Materials

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