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Investigation of the Antioxidant Activity of *Cydonia cathayensis* Hemsl. (Chinsaw-ga) Fruit

San San Oo

Abstract

This paper presents the determination of the antioxidant activity of chinsaw-ga fruit (*Cydonia cathayensis* Hemsl.). Free radicals can cause oxidative damage to biomolecules of living systems. Antioxidants which scavenge free radicals can prevent the oxidation of biomolecules. Firstly, rapid screening of antioxidant activity of 95% ethanol, ethyl acetate and watery extracts of *Cydonia cathayensis* fruit were carried out by dot-blot and DPPH staining method. After achieving the results which showed radical scavenging activity, antioxidant activity of these fruit extracts were determined by DPPH assay using UV-Vis spectrophotometric method. In this assay, ascorbic acid (vitamin C) was used as a standard. Antioxidant activity is expressed as 50% inhibition concentration (IC_{50}) values. The lower IC_{50} value indicates the greater antioxidant acitvity. IC_{50} values for ethyl acetate, ethanol and watery extracts of *Cydonia cathayensis* fruit were found to be 32.51, 20.51 and 34.12 μg/mL respectively. Ethanol extract showed the most potent antioxidant activity.

Key words: Antioxidant activity, *Cydonia cathayensis*, DPPH assay, radical scavenging activity, IC_{50} value

Introduction

Oxidative stress, the consequence of an imbalance of pro-oxidants and anti-oxidants in the organism, is a key phenomenon in chronic diseases. Majority of the diseases and disorders are mainly linked to oxidative stress due to free radicals. Free radicals and reactive oxygen species are produced naturally through mitochondrial oxidative metabolism and are present in many environmental pollutants (Gutteridge, 1995).

In living systems, free radicals can cause oxidative damage to DNA, lipids and proteins and may lead to certain diseases such as arthritis, diabetes, coronary heart disease, cancer, and aging etc. Antioxidants, which scavenge free radicals, can prevent the oxidation of biomolecules. High fruit consumption has been associated with lower incidence and motality rates of coronany heart disease and cancer. In addition to the classical nutrient

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antioxidants (*e.g.*, vitamin C, E and β-carotene), fruits contain polyphenolic antioxidants, which may play an important role in the overall antioxidant activity of fruits (Ma, *et. al.*, 2003).

1, 1 - Diphenyl - 2 - picrylhydrazyl (DPPH) assay is one of the methods for the determination of antioxidant capicity. The DPPH radical is one of the few stable organic nitrogen radicals, which bears a deep purple colour. Thiş radical is widely used to investigate the antioxidant capicity of several natural compounds such as phenolic compounds, anthocyanins or crude extracts of plants. DPPH radical is scavenged by antioxidants through the donation of hydrogen, forming the reduced DPPH-H. The colour changes from purple to yellow after reduction. This colour changes can be measured by its decrease of absorbance at wavelength 517 nm using UV-Vis spectrophotometer (Huang *et. al.*, 2004).

With the aim of investigation of antioxidants of plant origin, current research was conducted on a deciduous shrub, chinsaw-ga (*Cydonia cathayensis* Hemsl.) which belongs to Rosaceae family. Ethanol, ethyl acetate and watery extracts of air-dried chinsaw-ga fruit sampling from Kutkai Township, Northern Shan State of Myanmar were used.

The common name of *Cydonia cathayensis* is Chinese quince. It is also called chinsaw-ga in Myanmar. It is distributed to East and South East Asia. In Myanmar, one of the 4 species, chinsaw-ga (*Cydonia cathayenis* Hemsl.) is cultivated in hill forest of Kachin, Shan State and Mandalay Division on account of its fruits (Kress *et. al.*, 2003).

The fruit is antiemetic, antirheumatic, antispasmodic and digestive. The fruits of *C. cathayensis* are also fragrant, juicy and very sour in taste. It can be eaten in the raw state or after cooking. It can be used for making jam, pickle and curry with meat. Dried fruit can be used to get sour taste in soup with or without meat.

Materials and Methods

The fruits of chinsaw-ga (*Cydonia cathayensis* Hemsl.) were collected from the Kutkai Township, Northern Shan State of Myanmar. Unpeeled fruits of *C. cathayensis* fruit samples were thoroughly washed, cut into slices, air-dried, ground into powder and finally stored in air-tight containers for further works. The 1,1-diphenyl-2-picryl hydrazyl (DPPH) reagent from Sigma-Aldrich, Germany, silica gel 60 F_{254} precoated

aluminium sheets (TLC plates) from Merck Ltd. Japan and other chemicals from B.D.H chemical Co., Ltd., England were used. The apparatus used include rotatory evaporator (BÜCHI Rotavapor R-205, CH-9230 Flawil, Switzerland), high vacuum pump (Edwards High Vacuum Ltd., U.K), vortex mixer (K 550-G, 50-60 Hz, scientific industries, Inc., U.S.A), Ultraviolet lamp (UV-254/365 nm, 50/60 Hz, Gibthai Co. Ltd., U.S.A) and UV-Vis Spectrophotometer (Shimadzu Co.Ltd., Japan).

Preparation of Various Crude Extracts

Dried sample of *C. cathayensis* fruit (*ca.* 20 g) was percolated with 95% ethanol for one week, filtered and centrifuged. The filtrate was concentrated under reduced pressure by using rotatory evaporator and partitioned with petroleum ether. Half portion of the residue after partitioning with petroleum ether was slowly evaporated to form dried defatted 95% ethanol extract. The other half of the above residue was partitioned with ethyl acetate. The ethyl acetate solution was concentrated under reduced pressure and evaporated to dryness to form dried defatted ethyl acetate extract. Dried powered sample (*ca.* 10 g) was heated with water for one hour, filtered and centrifuged. The filtrate was slowly evaporated to dryness to obtain dried watery extract.

Rapid Screening of Antioxidant Activity by Dot-Blot and DPPH Staining

40, 20, 10 and 5 mg dried matter/mL solutions of ethyl acetate, 95% ethanol and watery extracts of the fruit were prepared by using their respective solvent. 5 µL each of these prepared solutions containing 200, 100, 50 and 25 µg of each extract of fruit was carefully loaded within each dot area of 1 cm diameter on a TLC plate.

After air-drying the loaded spots, the TLC plate was placed upside and sprayed with 0.4 mM DPPH solution prepared in methanol solvent. Excess DPPH solution was removed with a tissue paper and the plate was dried by blowing cold air with a hair-dryer. The loaded spots appeared as yellow spots on the purple colour background TLC plate. 5 minutes later, the diameter of each yellow spot was measured.

Radical Scavenging Activity by DPPH Assay

Antioxidant activities of various crude extracts of the fruit were determined by DPPH assay.

Preparation of 60 μM DPPH Solution

100 mL of 60 μM DPPH solution was prepared in the brown coloured bottle by using 95% ethanol solvent. Thorough mixing was made by using vortex mixer. This prepared solution should be used within 24 hours.

Preparation of Standard Ascorbic Acid Solution

160 μg/mL stock solution of standard ascorbic acid was prepared in a 100 mL volumetric flask by dissolving 16 mg of ascorbic acid in distilled water and the volume was made up to 100 mL. 80, 40, 20, 10 and 5 μg/mL solutions were prepared by serial dilution of the stock solution.

Preparation of Test Sample Solutions

160 μg/mL stock solutions of defatted ethyl acetate, defatted 95% ethanol and watery extracts of the fruit were prepared as similar manner in ascorbic acid preparation. The clear stock solutions were made by using 50% ethanol solvent for ethyl acetate and ethanol extract. Distilled water was used for watery extract and standard ascorbic acid. Each stock solution obtained was serially diluted with respective solvents to obtain desired concentration of 80, 40, 20, 10 and 5 μg/mL test sample solutions.

Preparation of Control Solution

The control solution was prepared by thorough mixing of 1.5 mL of 60 μM DPPH solution and 1.5 mL of 95% ethanol solvent with the aid of vortex mixer. This solution was allowed to stand for 30 minutes at room temperature and measured its absorbance at λ_{max} 517 nm.

Preparation of Blank Solutions

Each blank solution was prepared by thorough mixing of 1.5 mL of each test sample solution and 1.5 mL of its respective solvent. After 30 minutes, the absorbances of these solutions were measured at 517nm by using UV-Vis spectrophotometer.

Measurement of DPPH Radical Scavenging Activity by UV-Vis Spectrophotometric Method

1.5 mL each of 60 μM DPPH solution and 1.5 mL each of test samples or standard ascorbic acid solution were thoroughy mixed by using vortex mixer. These solutions were allowed to stand at room temperature

for 30 minutes. Then the absorbances of these sample solutions were measured at 517nm by using UV-Vis spectrophotometer. All the sample solutions were tested in triplicate.

Absorbance values so obtained were used to calculate percent inhibitions and standard deviation (SD) by using the formula, % Inhibition = [(C-S) / C] x 100; where C = the net absorbance of the control; S = the net absorbance of the sample. Percent inhibition is plotted against concentration and the equation for the line obtained is used to get the 50 % inhibition concentration (IC_{50}) value. A lower IC_{50} value indicates greater radical scavenging activity. IC_{50} values in µg/mL were calculated by computer program called linear regressive excel program (Reynertson, 2007).

Results and Discussion

Rapid Screening of Antioxidant Activity by Dot-Blot and DPPH Staining

To make a semi-quantitative visualization possible, 95% ethanol, ethyl acetate and watery extracts of *C. cathayensis* fruit were detected on the TLC plates by DPPH staining method. For rapid screening, each diluted fruit extract was applied as a dot on a TLC plate followed by staining with DPPH solution. The appearance of yellow colored spots has a potential value of antioxidant activity for the indirect evaluation of the different extracts of the fruit.

After staining, yellow spots with strong intensity appeared fast up to the amount of 50 µg of dry matter for ethyl acetate extract, 200 µg for ethanol and watery extracts of *C. cathayensis* fruit. Thus the fruit extracts were found to possess the antioxidant activity (Reynertson, 2007).

Measurement of DPPH Radical Scavenging Activity by UV-Vis Spectrophotometric Method

It was observed that the decrease in absorbance indicated the increase in radical scavenging activity. In this DPPH assay, ascorbic acid was used as a standard. So antioxidant activity of the present research work was evaluated by vitamin C equivalent antioxidant capacity (VCEAC) assay.

From the absorbances measured, percent inhibition was calculated. Percent inhibitions were plotted against concentrations and the equation for

the line resulted was used to obtain the 50% inhibition concentration (IC_{50}) value. Percent inhibitions and IC_{50} values of various extracts of *C. cathayensis* fruit and standard ascorbic acid were tabulated in Table 1. Percent inhibitions were presented as a line graph shown in Fig. 2. and IC_{50} values were exhibited as a bar graph shown in Fig. 3.

From these results, it can generally be inferred that the higher the concentration of the extract, the greater is the percent inhibition *i.e.*, the greater is the free radical scavenging activity. The lower IC_{50} value indicates the greater antioxidant activity. $IC_{50} < 50$ μg /mL is very active; 50 μg/mL $< IC_{50} <$ 100 μg/mL is active; 100 μg/mL $< IC_{50} <$ 200 μg/mL is moderately active; $IC_{50} > 200$ μg/mL is not active (Reynertson, 2007).

IC_{50} values for ethyl acetate, 95% ethanol and watery extracts of *C. cathayensis* fruit were 32.51, 20.51 and 34.12 μg/mL respectively. According to these results, 95% ethanol extract showed the most potent antioxidant activity followed by ethyl acetate and watery extracts. It could be deduced that all these extracts were very active in radical scavenging activity because their IC_{50} values were less than 50 μg/mL.

Conclusion

In the determination antioxidant activity measured by DPPH assay, it was observed that IC_{50} values for ethyl acetate, ethanol and watery extracts of *C. cathayensis* fruit were 32.51, 20.51 and 34.12 μg/mL respectively. 95% Ethanol is found to be most potent antioxidant activity followed by ethyl acetate, and watery extracts.

According to these results that showed the IC_{50} values of these fruit extracts is less than 50 μg/ mL, *C. cathayensis* fruit is very active in antioxidant acitvity. The results from in vitro experiments of DPPH assay demonstrated that the phytochemicals in *C. cathayensis* fruit may contribute a significant effect on antioxidant activity. Hence, chinsaw-ga (*C. cathayensis*) fruit can be used as an easy accessible source of natural antioxidants or a food supplement.

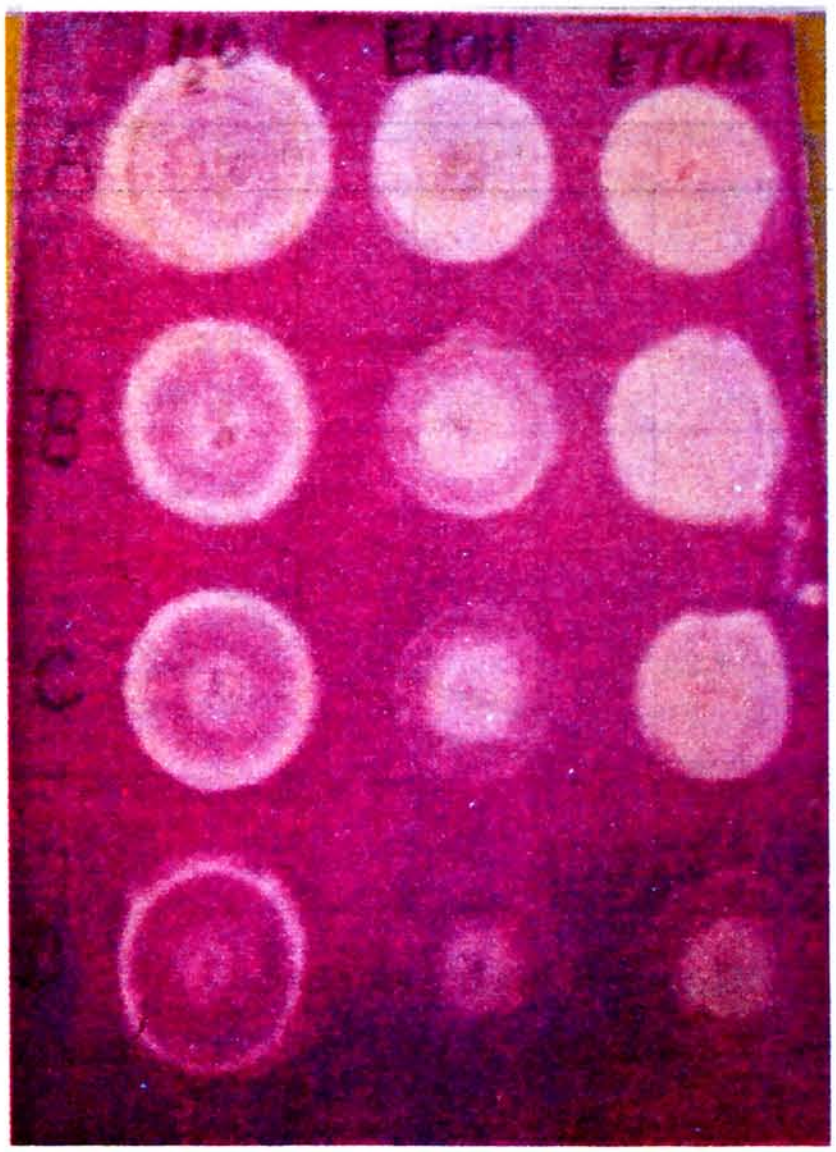

Fig. 1. Screening of antioxidant activity of *C. cathayensis* fruit extracts by dot-blot and DPPH staining (A= 200 µg; B= 100 µg; C= 50 µg; D= 25 µg)

Table 1. Percent Inhibition, IC_{50} values of various extracts of *Cydonia cathayensis* fruit compared with standard ascorbic acid

	Extracts/ Conc. (µg/mL)	% Inhibition (mean ± SD)					IC_{50}
		5	10	20	40	80	
Cydonia cathayensis Fruit	EtOAc	22.33 ±0.50	25.08 ±0.23	38.72 ±0.50	56.77 ±0.66	61.28 ±0.69	32.51 ±0.25
	EtOH	37.03 ±0.78	43.79 ±0.47	49.44 ±0.47	68.31 ±0.31	81.54 ±0.62	20.51 ±0.48
	H_2O	18.66 ±0.66	22.85 ±0.48	29.35 ±0.65	58.60 ±0.66	71.59 ±0.48	34.12 ±0.28
Ascorbic acid		88.58 ±1.46	93.24 ±0.40	94.87 ±0.40	96.50 ±0.70	102.33 ±1.07	2.62 ±0.09

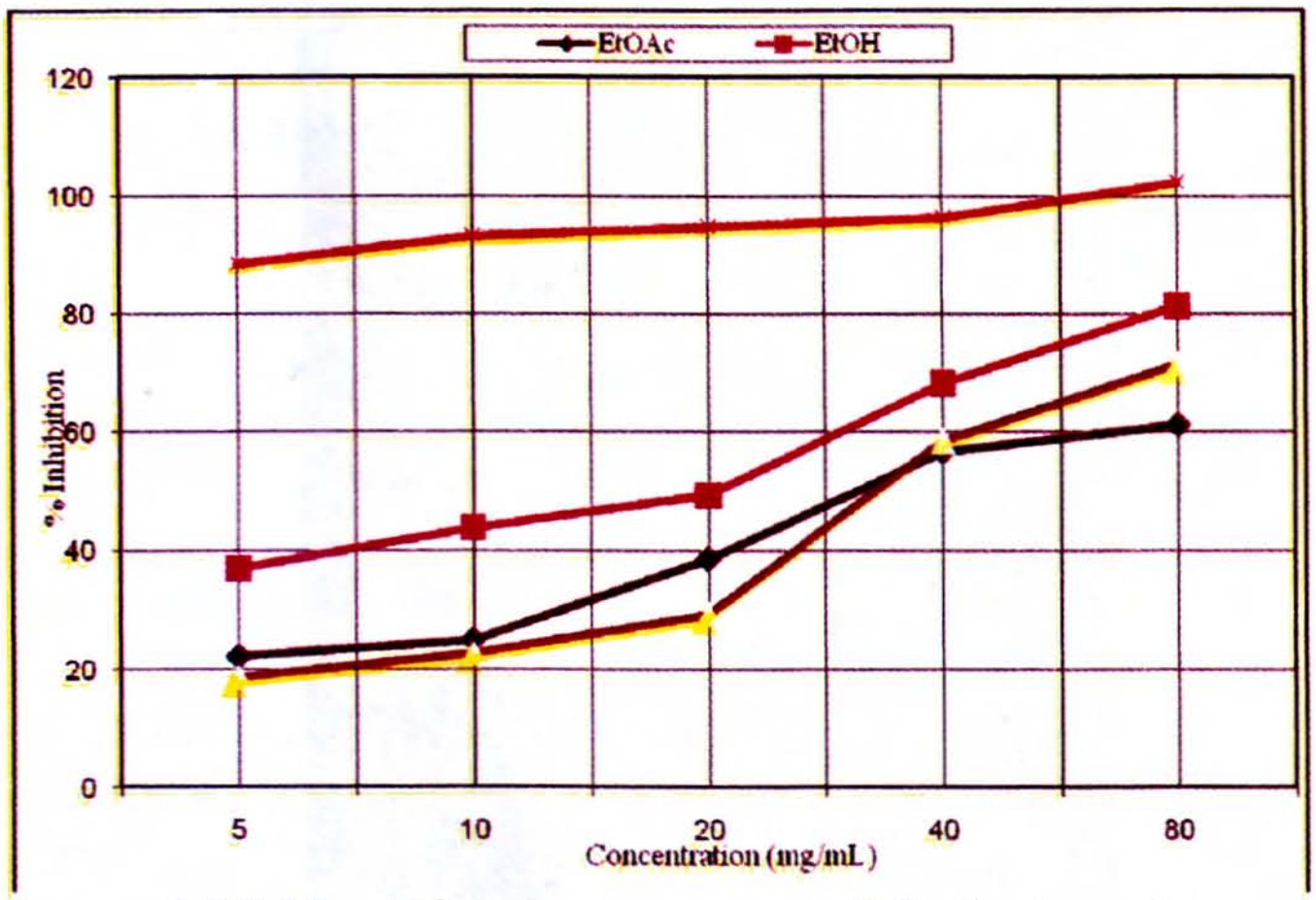

Fig. 2. Percent inhibition of various extracts of *Cydonia cathayensis* fruit compared with that of standard ascorbic acid

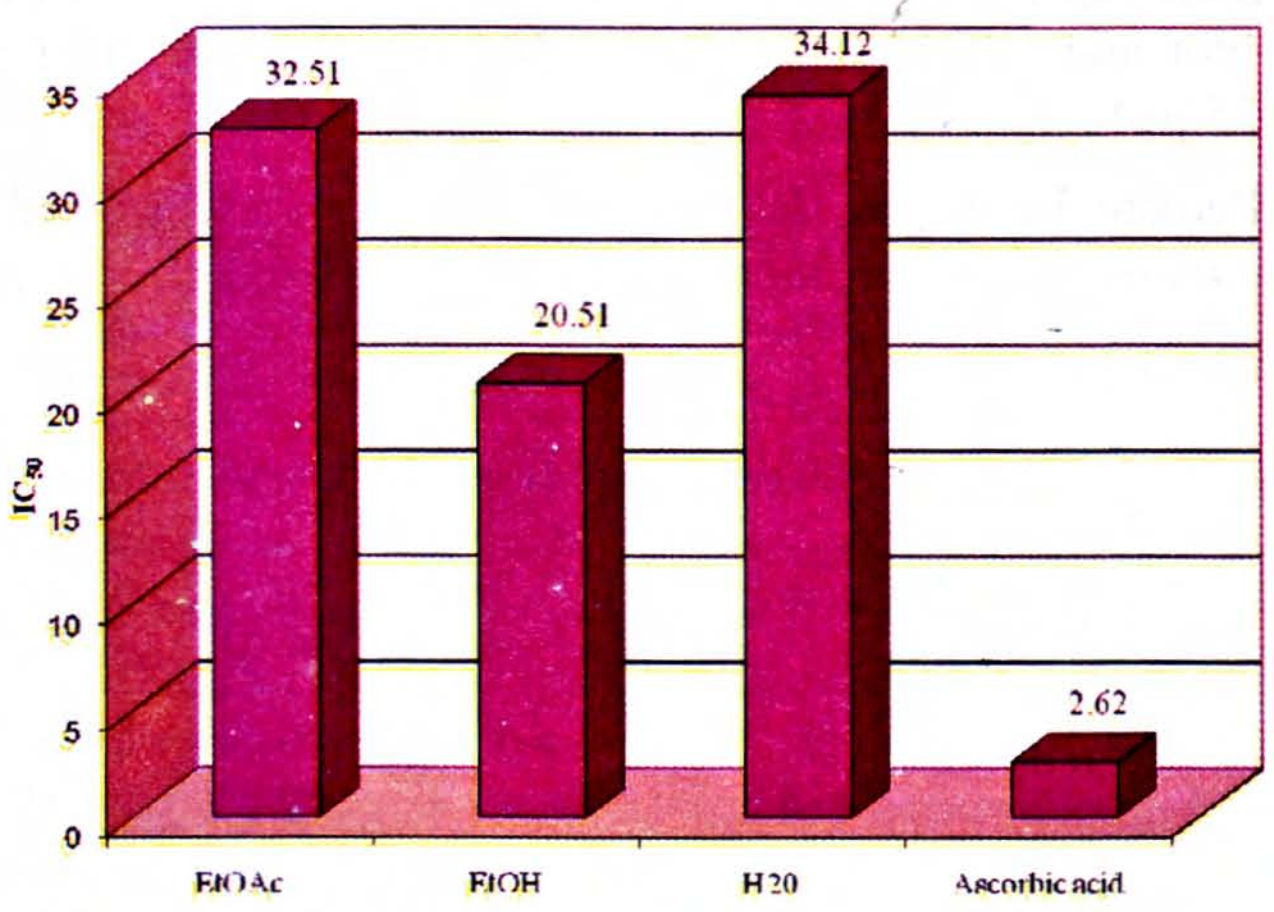

Fig. 3. 50 % inhibition (IC_{50}) of various extracts of *Cydonia cathayensis* fruit compared with that of standard ascorbic acid

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