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Comparative Studies on the Total Phenolic and Antioxidant Activities of White and Red Pomelo Fruits

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Abstract: *In this study, the total phenolic content and antioxidant activities of two kinds of Citrus decumana Linn. (Pomelo) were comparatively determined by spectrophotometric methods. Total phenolic content of white and red fruit juice were determined by Folin-Ciocalteu reagent and UV spectrophotometer at 765 nm. The phenolic contents were calculated from the standard quarcetin curve which obtained the 1.58 mg/mL and 1.31 mg/mL. Moreover the antioxidant activities of these fruits were measured by DPPH radical scavenging assay method. IC_{50} values of white and red pomelo fruits are 1.42 µg/mL and 2.75 µg/mL. According to the comparison of IC_{50} values from experiment, white pomelo fruit has higher antioxidant activity than red pomelo fruit which indicates that the phenolic content of white pomelo fruit is slightly higher than that of red pomelo fruit.*

Keywords : *Citrus decumana* Linn.; Folin-Ciocalteu reagent; quarcetin; DPPH radical scavenging assay; antioxidant activities.

1. Introduction

The genus of citrus belonging to the family Rutaceae comprises about 40 species which are distributed in India, China, Malaysia, Srilanka and Australia. Citrus one of the most important world fruit crops and is consumed mostly as fresh or as juice because of its nutritional value and special flavor. Consumption of citrus juice is found to be beneficial in preventing coronary diseases and chronic asthma [1]. It is believed that most of the species under genus Citrus are native to tropical and subtropical regions of South East Asia, particularly India. North East India and South East Asia are considered to be the centre of origin and diversity of Citrus species [2]. In Myanmar pomelo fruits are seasonal fruits which have two main varieties. Citrus fruits are rich sources of useful phytochemicals, such as vitamins A, C and E, mineral elements, flavonoids, coumarins, limonoids, carotenoids, pectins, and other compounds [3].

These phytochemicals, consumed through fresh fruits or their derived products, have been suggested to have a wide variety of biological functions including antioxidant, antiinflammation, antimutagenicity, anticarcinogenicity and anti-aging to human health [4-6]. *C. decumana* Linn. is a common citrus species in the country, thought to be the local version of grapefruit.

In view of previous findings, ascertaining the high antimicrobial and anti-oxidant activities of a number of phytochemicals inherent in plant food like citrus and the fact that all citrus have similar complex structure regardless of cultivars, it can therefore be a potential replacement for synthetic preservatives therefore providing multiple benefits to consumers by way of furthering its possible usage in the fields of medicine, therapeutics and food technology [7-8].

Since the beginning of this century, ethnobotanical and traditional uses of natural compounds, mainly of plant origin established much interest as they are well tested for their efficacy and generally believed to be safe for human use. Thorough screening of literature available on *C. decumana* Linn. depicted the fact that it is used as a cure for variety of ailments. Following the traditional and folk claims, very little efforts have been made by the researchers to explore the therapeutic potential of this plant [9]. *C. decumana* Linn. are a perennial shrub commonly known as Papanus, distributed throughout India [10]. Like other citrus plant *C. decumana* Linn. are rich in Vitamin C. They are generally used eaten as fruit. It has been used in indigenous system of medicine as sedative in nervous affections, convulsive cough and in the treatment of hemorrhagic diseases and epilepsy. It is said to poses appetizing, cardiac stimulant and antitoxic property [11]. *C. decumana* Linn. fruits also contains high amount of polyphenolic compound like hesperidin, naringin, caffeic acid, P-Coumaric acid, Ferulic acid and vanillic acid [10].

Many studies had revealed that phenolic content in plants could be correlated to their antioxidant

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activities. Plants contained phenolic and polyphenol compounds can act as antioxidant [12-14]. Plants are potential sources of natural antioxidants such as ascorbic acid, tocopherol, carotenoids. flavonoids and phenolic acids [15].

Oxidative stress has related with many degenerative diseases. Antioxidant has potency to mobilize protective effects against oxidative stress. Phenolic compounds are commonly found in plants, and they have revealed to have multiple biological effects, including antioxidant activity [16-17].

This study aimed to investigate the differences of phenolic content and antioxidant activity of white and red pomelo fruits.

1.1 Botanical Description of *Citrus decumana* Linn.

Botanical name	:*Citrus decumana* Linn.
Family	:Rutaceae
Genus	:*Citrus*
Species	:*C. decumana*
Synonyms	:*Citrus grandis*(L.),*Citrus maxima* (Burm.) Merr.
Common name	:Pomelo, Pummelo, Chinese grapefruit, Pommelo, Shaddock, Jambola
Myanmar Name	:Kywe-gaw , Shauk-pan
Parts used	:Fresh juice

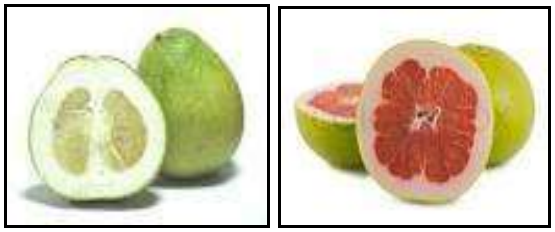

Figure 1. White and red fruits of *Citrus decumana* Linn. (Pomelo)

2. Materials and Methods

2.1 Plant Materials

The white and red fruits of *C. decumana* Linn. (pomelo) were collected from Mawlamyine Township, Mon State in Myanmar.

2.2 Materials and Instrumentation

Ethanol and DPPH were used to determine the antioxidant activities of pomelo fruit juices. Sodium Carbonate and Folin- Ciocalteu reagent were used for the measurement of total phenol content in pomelo fruit juice. Some chemical reagents and common laboratory apparatus were used for physico-chemical tests. pH meter (pH-700, Eutach), refractometer (Pal-I, Atago), UV-1601 Shimadzu spectrophotometer and Vortex mixer (MODEL K 550-G) were used for experiment.

2.3 Determination of Yield Percent in Pomelo Fruit Juice

Percent juice was calculated as (total juice volume / total fruit weight) × 100 and these are tabulated in Table 1.

2.4 Determination of Physico-chemical Characterization

2.4.1 Soluble Matter

The fruit juice (ca. 100 mL) was gently heated on a water bath for about 4 hours. And then, the liquid was dried off and the residue was obtained [18]. From these results, the soluble matter was obtained and tabulated in Table 2.

2.4.2 pH value

Determination of pH value in preparation of fruit juice was done by pH meter (pH-700, Eutach) and these results are shown in Table 2 [18].

2.4.3 Sugar Content

Determination of sugar content in preparation of fruit juice was tested by refractometer (Pal-I, Atago) and these results are tabulated in Table 2 [19-21].

2.5 Quantitative Determination of Total Phenol Content in Pomelo Fruit Juice

The total phenol contents of pomelo fruit juices (white and red) were measured with the Folin-Ciocalteu reagent. Firstly, 1.6 mL of diluted pomelo fruit juices and 100 µL of Folin-Ciocalteu reagent were mixed, then 300 µL of saturated Na_2CO_3 (20 %) was added. After the solution was incubated 40ºC for 30 minute, the absorbance of the solution was measured at 765 nm with the UV spectrophotometer (UV-1601) [22-24]. Total phenol contents of pomelo fruit juices (white and red) were calculated from quercetin standard curve was shown in Figure 2.

2.6 Determination of Antioxidant Activity of Pomelo Fruits by DPPH Assay Method

DPPH (1,1-Diphenyl-2-Picryl-Hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of pomelo fruit juice [25].

2.6.1 Preparation of 60 µM DPPH Solution

DPPH powder 0.00236 g (2.36 mg) was weighed and it was thoroughly and gently dissolved in 100 mL of 95 % ethanol and stored in brown colored volumetric flask. It must be kept in the fridge for no longer than 24 hours before use.

2.6.2 Preparation of Test Sample Solution

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Fruit juices (white-8.9 mg and red- 8.6 mg) and 10 mL of 95 % ethanol were gently mixed by vortex mixer (MODEL K-550-G). The stock solution was obtained. It was diluted with 50 % ethanol in various rations to obtain five ranges of concentration, such as 4 µg/mL, 2 µg/mL, 1 µg/mL, 0.5 µg/ mL and 0.025 µg/ mL respectively. Then, 5.0 mL of ethanol solution was prepared for each concentration.

2.6.3 Measurement of DPPH Radical Scavenging Activity by Spectrophotometric method

After mixing of 60 µM DPPH solution 2.0 mL and 2.0 mL of 95 % ethanol by vortex mixer (MODEL K-550-G) gave rise to the control solution. Moreover, the blank solution could be prepared by mixing 2.0 mL of test sample solution and 2.0 mL of 50 % ethanol thoroughly in the vortex mixer (MODEL K-550-G). Furthermore, the test sample solutions were also prepared by gently mixing each of 2.0 mL of 60 µM DPPH solution and 2.0 mL of test sample solution with various concentrations by applying vortex mixer (MODEL K 550-G). After that, the solutions were allowed to stand for 30 minutes at room temperature. Then, the absorbance value of each solution at 517 nm was measured by UV-1601 Shimadzu spectrophotometer.

These measurements were performed triplicate for each solution. The absorbance values obtained were applied to calculate percent inhibition by the following formula.

$$\% \text{ inhibition} = \frac{\text{DPPH}_{\text{alone}} - (\text{Sample} - \text{Blank})}{\text{DPPH}_{\text{alone}}} \times 100$$

% inhibition = percent inhibition of test sample
DPPH = absorbance of control solution
Sample = absorbance of test sample solution
Blank = absorbance of blank solution

Finally, IC_{50} (50 % inhibition concentration) was determined by using linear progressive excel program.

3. Results and Discussion

3.1 Yield Percent of Pomelo Fruit Juice

The obtained results of yield percent of pomelo fruit juices were shown in Table 1.

Table 1. Yield Percent of Pomelo fruit juice.

No	Sample	Yield Percent (%)
1	White pomelo	51.87
2	Red pomelo	53.94

3.2 Determination of Physico-chemical Characterization

Some physico-chemical characterization of pomelo fruit juice such as soluble matter, pH value and sugar content were determined and the observed data were listed in Table 2.

Table 2. Some physico-chemical characterization of *Citrus decumana* Linn.

No	Physico-chemical Test	Results	
		White pomelo	Red pomelo
1	Soluble matter (g/100 mL)	8.85	8.6
2	pH value	5.29 (27.4 ºC)	4.02 (27.2 ºC)
3	Sugar content (%)	7.9	7.3

3.3 Determination of Total Phenol Content in Pomelo Fruit Juice

The total phenol content of pomelo fruit extracts was determined by Folin-Ciocalteu reagent. A plot of absorbance Vs concentration of standard quercetin curve was shown in Figure 2.

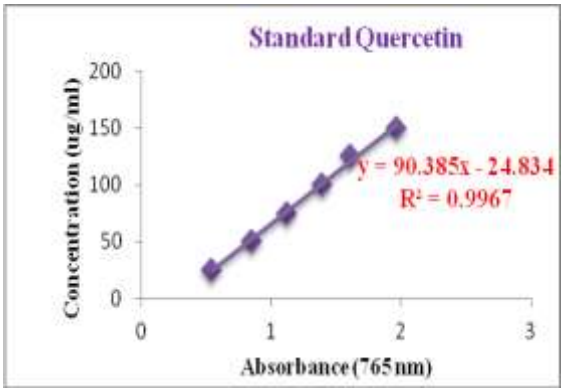

Figure 2. A plot of absorbance vs concentration of standard quercetin curve.

Total phenol contents of pomelo fruit juices (white and red) were calculated from quercetin standard curve. These results were shown in Table 3.

Table 3. Total phenol content of pomelo fruit juice.

No	Sample	Total phenol (mg/mL)
1	White pomelo	1.58
2	Red pomelo	1.31

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3.4 Determination of Antioxidant Activity of Pomelo Fruit by DPPH Assay Method

The present study was done for the investigation of antioxidant effects of white and red pomelo fruits. In this study, ascorbic acid was used as a standard antioxidant. Ascorbic acid is a water soluble antioxidant that maintains many cofactors in the reduced state. The potential antioxidant activities of selected samples were assessed on the basis of the scavenging activity of the stable DPPH free radicals. Antioxidant activities of white and red pomelo fruits were expressed as percentage of DPPH radical inhibition and IC_{50} values (µg/mL). The results of antioxidant activity using DPPH method in white and red pomelo fruits using ascorbic acid as a positive control are showed in Figure 3 and 4.

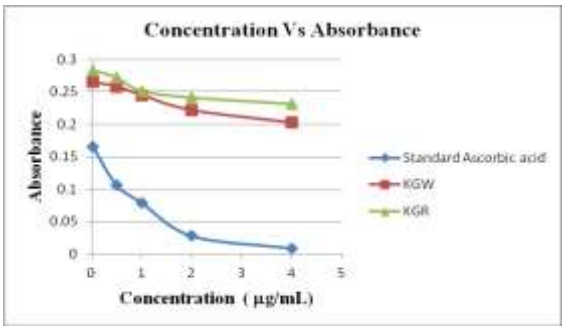

Figure 3. Plot of different concentrations vs absorbance of Pomelo fruits and standard ascorbic acid.

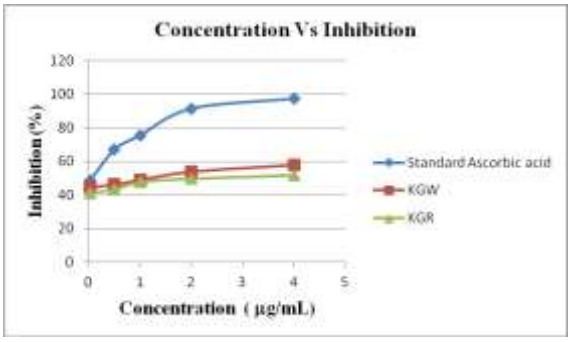

Figure 4. A plot of concentrations vs %inhibition of Pomelo fruits and standard ascorbic acid.

In accordance with Figure 3 and 4, decrease in absorbance implies increase in % inhibition of oxidation. From these results, it is also observed that increase in concentration shows to increase in % inhibition, it means that increase the free radical scavenging activity.

IC_{50} values of white and red pomelo fruits were determined by the linear progressive excel program. These results were described in Table 4.

Table 4. IC_{50} values of standard ascobic acid, white and red pomelo fruits.

Test Samples	IC_{50} Values (µg/mL)
Ascorbic Acid	0.19
Pomelo fruit (White)	1.42
Pomelo fruit (Red)	2.75

The IC_{50} value is a parameter used to measure antioxidative activity and it is defined as the concentration required for 50% scavenging of DPPH radicals under experimental condition employed. A smaller IC_{50} value corresponds to a higher antioxidant activity.

In this study, the antioxidant activity of selected fruits was shown to be influenced by the total phenolic content. Pomelo fruits containing high phenolic contents have been found to exert high antioxidant potential. The study of present research has shown a direct relation between antioxidant activity of selected fruits and phenolic contents.

In this present research, the white and red pomelo fruits showed scavenging against DPPH radical. From the results revealed that antioxidant activity of white pomelo fruit is higher than that of red pomelo fruit but lower than ascorbic acid as positive control (IC_{50} = 0.19 µg/mL). The white pomelo fruit contains significant amount of antioxidant agents. Therefore, the study suggests that the selected white pomelo fruit might be a potential source of natural antioxidants.

4. Conclusion

In this study, one of citrus fruits, *C. decumana* Linn., pomelo, Myanmar name Kywe-gaw, was selected for determination of some physicochemical properties, total phenolic content and antioxidant activities. The phenolic contents were calculated from the standard quarcetin curve which obtained the 1.58 mg/mL and 1.31 mg/mL. In addition, the antioxidant activities of white and red pomelo fruit juice were measured by DPPH assay method. IC_{50} values of white and red pomelo fruit juices are 1.42 µg/mL and 2.75 µg/mL. According to the comparison of IC_{50} values from experiment, white pomelo fruit has higher antioxidant activity than red pomelo fruit

which means that the phenolic content of white pomelo fruit is slightly higher than that of red pomelo fruit.

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