Investigation of Some Physicochemical Properties, Antimicrobial Activities and Antioxidant Activity of the *Citrus paradisi* Macfad (Grapefruit)

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Abstract

In this research work, the Grapefruit (*Citrus paradisi* Macfad), one of the edible fruits was chosen for chemical analysis. According to the phytochemicals tests, alkaloid, flavonoid, glycoside, steroid, phenolic compound, polyphenol, protein and saponin were found to be present in these grapefruits. The mineral composition of sample was measured by EDXRF spectroscopy. The determination of vitamin C contents in fruit juice by an iodometric titration. Antimicrobial activities of sample were examined by Agar-well diffusion method. Moreover, the antioxidant activity of the sample was investigated in vitro by DPPH assay.

Keywords – *Citrus paradisi* Macfad, Phytochemical test, Vitamin C, EDXRF, Antimicrobial, DPPH assay, Antioxidant

I. Introduction

Grapefruit (*Citrus paradisi* Macfad) has been known for its accumulation of flavonoids and ascorbic acid. These contents are important because of their nutritional and antioxidant properties. In recent years, the grapefruit (*Citrus paradisi* Macfad) has received much attention because of its nutritional and antioxidant properties. Besides ascorbic acid, it also contains abundant flavonoids, which are reported to be the important part of active ingredients. The potential health benefits of flavonoids in food and beverages have been discovered for antioxidative effect [1]. Grapefruit is cultivated principally to obtain the juice. Flavonoid components, particularly naringin, are contained abundantly in this juice. Now, more reporters are mainly focused on the beneficial effects of the grapefruit juice on human health, such as antioxidant, antiallergic and anticarcinogenic benefits as well as protection against high blood pressure or cholesterol increase [2].

Antioxidants can protect lipids and oils in food against oxidative degradation. When added to food, antioxidants control rancidity development, retard the formation of toxic oxidation products, maintain nutritional quality, and extend the shelf-life of products. Because of safety concerns, synthetic antioxidants are limited to be used as food preservatives [3]. Natural antioxidants obtained from edible materials such as spices and herbs, have been of increasing interest.

Natural antioxidants contained in spices help to reduce oxidative stress. Oxidative stress, which is caused by high concentration of free radicals in cells and tissues, can be induced by various negative factors, such as gamma, UV and X-ray radiation, psycho-emotional stress, polluted food, adverse environmental conditions, intensive physical, intensive physical exertion, smoking and alcoholism [4].

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Botanical Description of Citrus paradisi Macfad

Family	:	Rutaceae
Genus	:	Citrus
Scientific name	:	*Citrus paradisi*
Botanical name	:	*Citrus paradisi* Macfad
Local Name	:	Grapefruit
Common name	:	Grapefruit, pomelo, smaller shaddock
Part Used	:	Fruit

Figure 1 Fruit of Grapefruit

II. Aim

The main aim of this paper is to investigate of some physicochemical properties, antimicrobial activities and antioxidant activity of the *Citrus paradisi* Macfad (grapefruit).

III. Expermental

Sample Collection

The Grapefruit (*Citrus paradisi* Macfad) were collected from Putao Township, Kachin State.

Phytochemical Tests for the Grapefruit

Test for Alkaloid

About 2 mL of juice sample was boiled with 10 mL of 1 % hydrochloric acid for about 10 minutes and was allowed to cool and filtered. Then the filtrate was tested with Dragendorff's reagent. Orange precipitate was formed indicating that alkaloid was present in the sample.

Test for Flavonoid

About 1 to 2 mL of concentrated hydrochloric acid was added into a test tube containing 2 mL of the juice sample. Then small pieces of magnesium turnings were added to the test tube and heated in a water bath. The solution in the test tube turned pale red indicating the presence of flavonoid.

Test for Glycoside

About 2 mL of juice sample was boiled with 10 mL of distilled water for about 10 minutes and then allowed to cool and filtered. The filtrate was mixed with a few drops of 10 % lead acetate white precipitate was obtained due to the presence of glycoside in the sample.

Test for Steroid

About 2 mL of juice sample was boiled with 10 mL of ethanol. The solution was filtered and filtrate was mixed with 2 mL of acetic anhydride and concentrated sulphuric acid. Pale green colour appeared and steroid was present in the sample.

Test for Phenolic Compound

About 2 mL of juice sample was boiled with distilled water for about 10 minutes and allowed to cool and filtered. The filtrate was treated with 10 % $FeCl_3$ solution. A purplish colour showed the presence of phenolic compound in the sample.

Test for Protein

The juice sample 2 mL was dissolved in hot water and heated in a water-bath. The solution was filtered and 5 to 8 drops of NaOH solution and 1 to 2 drops of $CuSO_4$ solution were added. Pale green colour appeared indicating the presence of protein in the sample.

Test for Polyphenol

The juice sample 2 mL was dissolved in 10 mL ethanol and heated in water-bath for about 10 minutes. The solution was filtered. A mixture of 1 mL of 1 % $FeCl_3$ and 3 drops of 1 % $K_3Fe(CN)_6$ solution were added to each of the filtrate. A dark green colour was appeared. Thus the sample contained polyphenol.

Test for Saponin

The juice sample 2 mL was dissolved in 10 mL of distilled water and heated to 10 minutes in water-bath. The slolution was filtered and shaked 3 mins. Frothy appeared and therefore saponin was present in the sample

Test for Resin

The juice sample 2 mL was dissolved in distilled water and heated in a water-bath. The solution was filtered and 1 mL of acetone was added to the filtrate. No brownish turbidity appeared indicating the absence of resin in the sample.

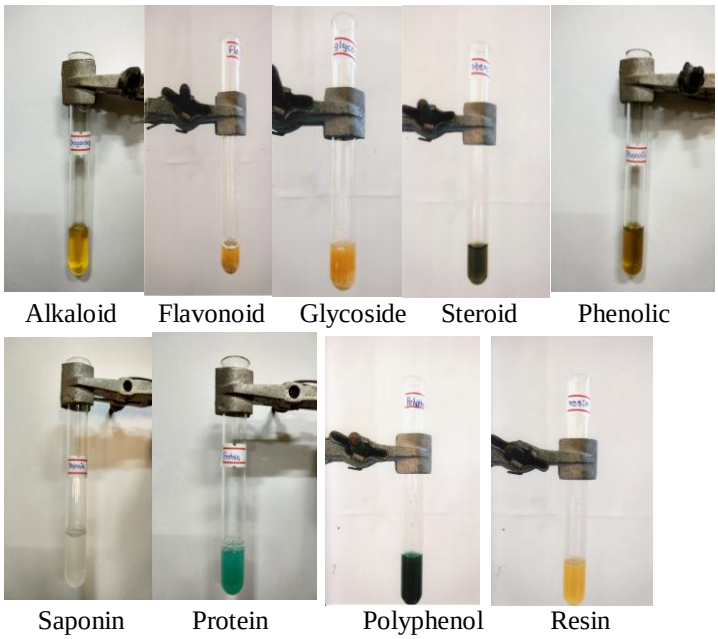


Figure 2. Phytochemical Tests for the Grapefruit

Determination of Mineral Contents from Grapefruit

The mineral contents of grapefruit (juice) were determined by EDXRF method at the Department of Chemistry, West Yangon University.

Determination of Vitamin C Content in Fruit Juice by Iodometric Titration

Iodometric Titration Method

Vitamin C can be determined in fruit juice by use of an oxidation-reduction reaction. The redox reaction is preferable to an acid-base titration because a number of other species in juices can act as acids, but relatively few interfere with the oxidation of ascorbic acid by iodine. The endpoint is indicated by the reaction of iodine with starch, which produces a blue product.

Preparation of Solutions

(a) 1% Starch Indicator Solution

Add 0.5 g soluble starch to 50 mL of near-boiling water. Mix well and allow to cool before use.

(b) Iodine Solution

Dissolve 1.6 g of KI, 0.2 g of KIO_3 and 1 mL of conc: H_2SO_4 dissolved in 500 mL of distilled water.

(c) Standard Vitamin C Solution

Standard ascorbic acid (0.25 g) was dissolved in distilled water and the volume makes up to 250 mL in a volumetric flask.

Standardization of Iodine Solution with Vitamin C Standard Solution

Add 20 mL of vitamin C standard solution to a 150 ml conical flask. Add 1 ml of starch solution. Rinse the burette with a small volume of the iodine solution and the fill it. Titrate the solution until the endpoint is reached. Record the initial volume and final volume. Repeat the titration at least three times.

Titration of Fruit Juice Sample

Add 20 ml of fruit juice sample into 150 mL conical flask. Titrated with iodine solution until the endpoint is reached. Record the initial volume and final volume. Repeat the titration at least three times.

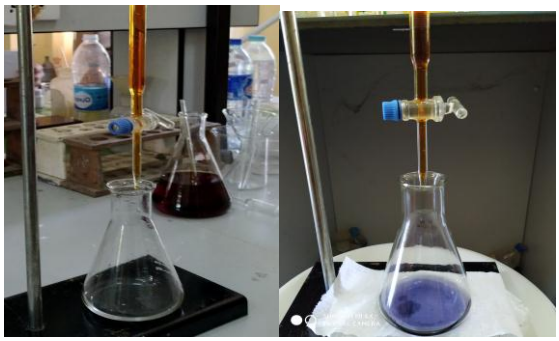


Figure 3. Experiment for Iodometric Titration

Investigation of Antimicrobial Activities of Grapefruit

The antimicrobial activities of the grapefruit (juice) were investigated by applying Agar-well diffusion method at MIT (Mandalay Institute of Technology), Mandalay.

Determination of Antioxidant Activity of Grapefruit

DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging assay was chosen to assess the antioxidant activity of grapefruit (juice).

Preparation of Test Sample Solution

Fresh juice (1 mL) was dissolved in 95 % ethanol (10 mL) under vigorous shaking. This solution was used as a stock solution. The stock solution was diluted with ethanol to obtain various concentrations of test sample solutions.

Preparation of 60 μM DPPH Solution

DPPH (0.0023 g) was thoroughly dissolved in 95 % ethanol (100 mL). This solution was freshly prepared in the brown coloured flask and kept in refrigerator for no longer than 24 hours.

Preparation of Standard Solution

Ascorbic acid (0.001 g) was dissolved in 100 mL of 95 % ethanol and it was used as a standard solution.

Procedure

(i) Control solution was prepared by mixing 60 μM DPPH solution (2.0 mL) and 95 % ethanol (2.0 mL).

(ii) Similarly, the blank solution was prepared by mixing the test solution (2.0 mL) and 95 % ethanol (2.0 mL).

(iii) The sample solution was also prepared by mixing the test solution (2.0 mL) with 60 μM DPPH solution (2.0 mL).

All these solutions were allowed to stand at room temperature for 30 minutes. Then, the absorbance was measured at 517 nm using UV-2550 spectrophotometer.

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

% inhibition = percent inhibition of test sample, DPPH alone = absorbance of control solution

Sample = absorbance of test sample solution, Blank = absorbance of blank solution

IV. Results and Disscussion

RESULTS OF PRELIMINARY PHYTOCHEMICAL SCREENING OF GRAPEFRUIT

Phytochemical investigation on the sample indicated that alkaloid, flavonoid, glycoside, steroid, phenolic compound, polyphenol, protein and saponin were present. Resin was absent.

Table 4.1 The Results of Phytochemical Test of Grapefruit

No.	Text	Extract	Test reagent	Observation	Results
1.	Alkaloid	HCl	Dragendorff's	Orange precipitate	+
2.	Flavonoid	Conc. HCl	Mg turning	Red colour solution	+
3.	Glycoside	H_2O	10 % $Pb(CH_3COO)_2$	White precipitate	+
4.	Steroid	EtOH	Acetic anhydride and conc: H_2SO_4	Pale green colour solution	+
5.	Phenolic compound	H_2O	10 % $FeCl_3$	Purplish colour solution	+
6	Polyphenol	EtOH	1 % $FeCl_3$, 1 % $K_3[Fe(CN)_6]$	Dark green colour solution	+
7.	Protein	hot water	NaOH $CuSO_4$	Pale green colour solution	+
8.	Saponin	H_2O	Shake 3 mins	Frothy	+
9	Resin	H_2O	Acetone	No brownish turbidity	–

Determination of Mineral Contents from Grapefruit

As described in experimental section, the mineral contents of grapefruit (juice) were determined by EDXRF method. The observed data were listed in Table (4.2).

Table 4.2 Results of Mineral Content of Grapefruit

No.	Element	Symbol	Content (%)
1.	Potassium	K	0.090
2.	Silicon	Si	0.022
3.	Carbon (C-H)	C	99.888

Determination of Vitamin C Content in Grapefruit Juice

In the present work, the vitamin C content in fruit juice was measured by iodometric titration. In iodometric titration method, the mixture of potassium iodate and iodine were used as titrant.

From the experimental results, it was observed that the content of vitamin C was found that 51.7 mg in 100 g of grapefruit.

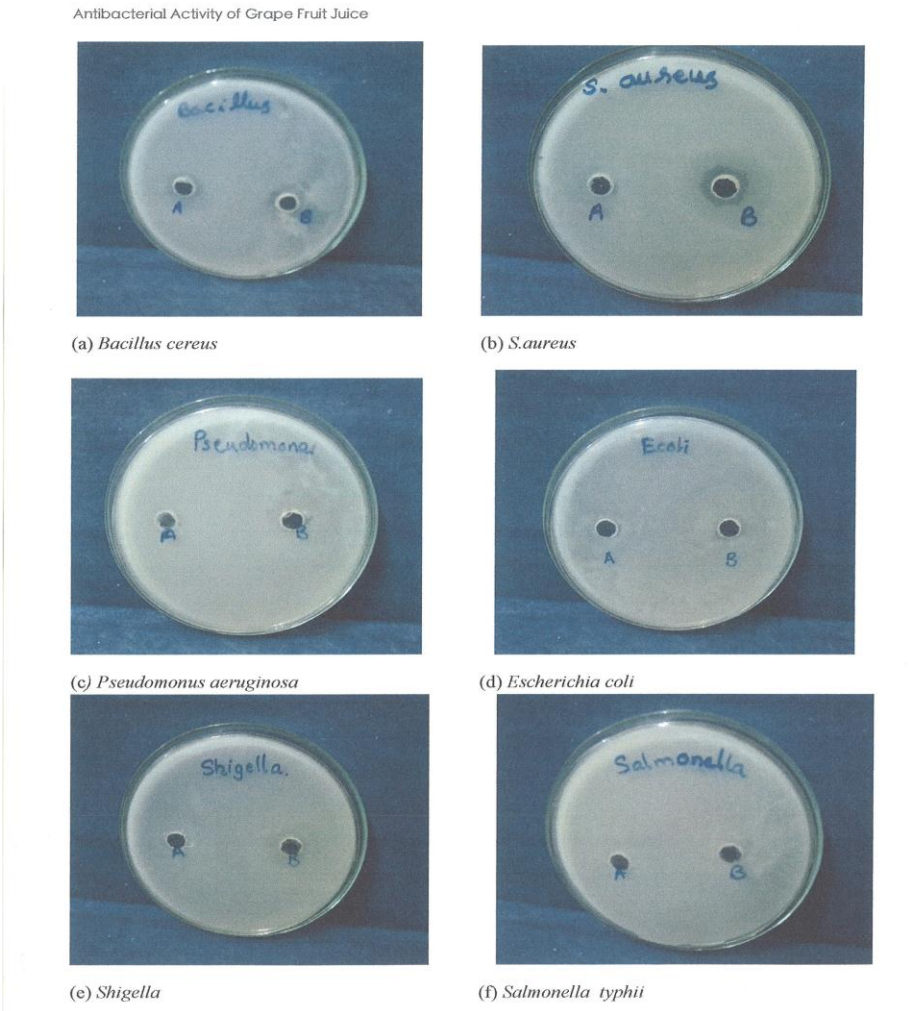
Antimicrobial Activities of Grapefruit

The study of antimicrobial activities of grapefruit (juice) was performed by Agar-well diffusion method on six microorganisms. The results were shown in Table (4.3).

Table 4.3 Results of Antimicrobial Activities of Grapefruit

Organism	Inhibition Zone (cm)	
	Control (A)	Grapefruit Juice (B)
Bacillus cereus	-	0.8
Staphylococcus aureus	-	0.9
Pseudomonas aeruginosa	-	-
Escherichia coli	-	0.4
Shigella	-	0.5
Salmonella typhii	-	-

(-) = No Inhibition



(a) *Bacillus cereus* (b) *S.aureus*

(c) *Pseudomonus aeruginosa* (d) *Escherichia coli*

(e) *Shigella* (f) *Salmonella typhii*

Figure 4.1 Antimircobial Activities of Grapefruit

Antioxidant Activity of Grapefruit

Screening of radical scavenging activity (RSA) was performed by using DPPH Assay. This assay was chosen to apply because of its simplicity and applicability either an antioxidant in its pure state or in a mixture. This method based on the capability of test sample to inhibit stable free radical (DPPH) that was followed by decrease in absorbance at 517 nm. Low absorbance value of test sample indicates high % inhibition against DPPH radical which in

turn reveals high radical scavenging activity of that sample at constant concentration. Ascorbic acid was used as a standard antioxidant for comparison purpose.

Table 4.4 % Inhibition of Various Concentrations and IC_{50} Value for Standard Ascorbic Acid

Sample Concentration (µg/mL)	Absorbance	% inhibition	IC_{50} (µg/mL)
50	0.168	82.80	8.23
25	0.279	71.44	
12.5	0.4	59.06	
6.25	0.506	48.21	
3.125	0.608	37.77	

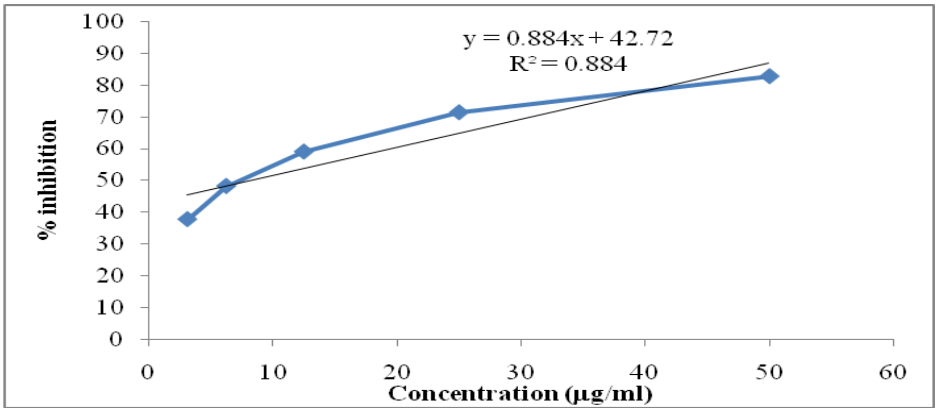


Figure 4.2 Plot of Concentration Vs % Inhibition of Standard Ascorbic Acid

Table 4.5 Radical Scavenging Activity of Various Concentrations of Grapefruit and IC_{50}

Sample Concentration (µg/mL) (10^{-3})	Absorbance	% inhibition	IC_{50} (µg/mL)
1	0.0680	82.7874	43.469
0.5	0.1830	53.6709	
0.25	0.2320	41.2658	
0.125	0.2740	30.6329	
0.0625	0.2850	27.8481	

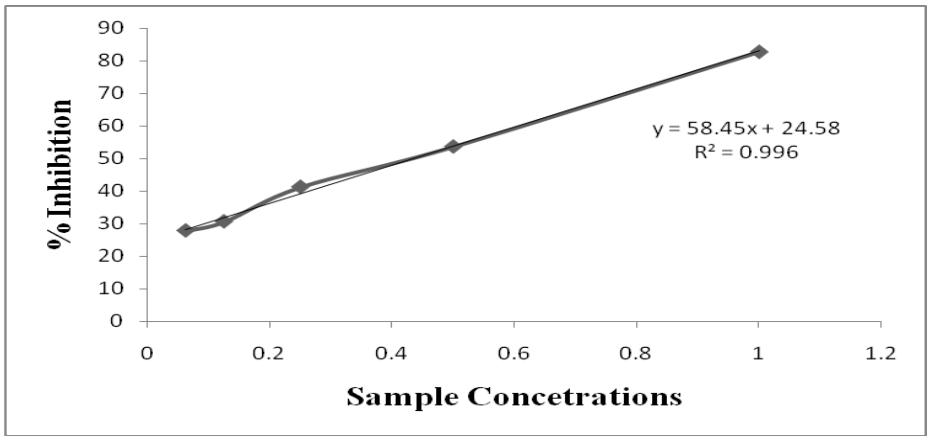


Figure 4.3 Plot of Concentration Vs % Inhibition of Grapefruit

V. Conclusion

According to preliminary phytochemical test, alkaloid, flavonoid, glycoside, steroid, phenolic compound, polyphenol, protein and saponin were found to be present in these grapefruit, while resin was absent. From EDXRF analysis the carbon content is the highest value in this sample. The content of vitamin C was found to be 51.7 mg in 100 g of grapefruit.

Antimicrobial activities of the sample were determined on six selected organisms. In the sample, *Pseudomonas aeruginosa and Salmonella typhii* exhibited no activity, *Escherichia coli* and *Shigella* exhibited lower activities and, *Staphylococcus aureus* and *Bacillus cereus* exhibited medium acitivities.

According to the study of antioxidant activity, IC_{50} value of grapefruit sample was found to be 43.469 μg/mL. These in vitro assays showed that the grapefruit exhibited the high antioxidant activity. From the results obtained in the present study, it is concluded that the grapefruit is good source of nutrient and natural antioxidant. Therefore, the consuming the grapefruit is effective and efficient for good health.

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