Assessment of Total Phenolic Contents and in Vitro Evaluation of Free Radical Scavenging Potentials of Cucumber

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Abstract : Cucumber (*Cucumis sativus* Linn.) which belongs to cucurbitaceae is one of many vegetables in Myanmar. In the present study, the expressed juices of fresh cucumber with/without internal organ distributed in Mandalay Region were subjected to the total phenol content and the potential antioxidant activities. The fresh cucumbers were purchased from Mingalar Market, Chan Aye Thar Zan Township, Mandalay Region, in Myanmar. Firstly, the fresh juices of selected samples with/without internal organ which are the liquid products by crushing were prepared. Furthermore, these fresh juices were checked for qualitative test of phenols. The natural product phenolic compounds that are secondary metabolites are antioxidants. So, the total phenolic contents of expressed juices were determined by spectrophotometric method with UV spectrophotometer (PD-303 UV Visible spectrophotometer) using Folin-Ciocalteau reagent at 765 nm. Moreover, the antioxidant capacities of these fresh juices were also evaluated by DPPH Assay method using the same UV spectrophotometer.

***Index Terms - Cucumis sativus* Linn., antioxidant activities, secondary metabolites, spectrophotometric method, UV spectrophotometer, Folin Ciocalteau reagent, DPPH Assay.**

I. INTRODUCTION

Fruits and vegetables have been considered as functional foods due to their health benefits besides nutritional content. Polyphenols are the most popular antioxidants mainly present in fruits and vegetables. Regular eating of fruits and vegetables confers benefits to human health [1]. Epidemiological studies reported that foods containing phytochemicals with antioxidant capacity have strong protective effects against several diseases including cardiovascular diseases and certain cancers [2, 3]. The protective action of fruits and vegetables has been attributed to the presence of antioxidants, most especially antioxidant vitamins [4,5]. However, several types of research reported that most of the antioxidant capacity may be from phenolic compounds such as flavonoids, rather than from Vitamins [6].

Antioxidants help organisms deal with oxidative stress caused by free radical damage. Free radicals are chemical species, which contain one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immune suppression, neurodegenerative diseases and others [7]. Antioxidants are the agents that can interfere with the oxidation process by various mechanisms, such as, reacting with free radicals, chelating free catalytic metals, and acting as oxygen scavengers [8, 9].

There is an increasing interest in natural antioxidants, namely phenols, present in medicinal and dietary plants, that might help prevent oxidative damage [10, 11]. The administration of an antioxidant source comprising of multiple components could offer protection against cancer and combat oxidative stress –induced physiological malfunctions [12]. In situations of increased free radical generation, the reinforcement of endogenous antioxidants via intake of dietary antioxidants may be of particular importance in attenuating the cumulative effects of oxidatively damaged molecules. Therefore, the studies of possible new sources of antioxidants have become important in the last few years. The new sources of the antioxidants could be used for direct consumption or for the production of food supplements which could be used for enriching foods with the aim of increasing their nutritional value. Medicinal plants used in the traditional medicine and healing are one of these sources of antioxidants. In many countries, screening studies were carried out for the comparison of antioxidant activities of medicinal plants typical for the respective country [13-16].

Presently, the use of synthetic antioxidants has been criticized. It is usually implied that regular consumption of natural antioxidants from vegetables, fruit, tea, and herbs may contribute to a shift in balance toward an ample antioxidant status. The interest in natural antioxidants, especially phytochemicals has greatly increased in recent years [17]. Many phytochemicals including phenolics, flavonoids, tannins, proanthocyanidins, and various herbal extracts have been reported as antioxidants [18,19].

Cucumis sativus commonly known as Cucumber is well known plant belonging to family Cucurbitaceae. The plant is widely cultivated in Myanmar and throughout the world. The fruit obtained from the plant is widely consumed throughout the world. The plant is attributed to various uses in Avurveda. Seeds are highly nourishing. Leaves boiled in water and mixed with cumin seeds are used for throat infection. Seed oil is used for burning, insomnia and frontal headache. Plant is also used for jaundice, bleeding disorders and anuria. The seeds are used as diuretic, tonic, anthelmintic and also as taeniacide. The leaf juice is emetic and is used to treat dyspepsia in children [20, 21].

Cucumber is the fourth most important vegetable crop after tomato, cabbage, and onion [22]. Although its calorie and nutritional value is very low, it is a primary source of vitamins and minerals in the human diet [23]. Due to high content of potassium (50-80 mg/100g), cucumber can highly be useful for both high and low blood pressures [24]. Cucumber is a widely

cultivated plant of gourd family which is eaten in the unripe, green form. Its fruit extract has shown free radical scavenging and analgesic activities in mice [25], carminative and antacid property [26].

Traditionally, this plant is used for headaches; the seeds are cooling and diuretic, the fruit juice of this plant is used as a nutritive and as a demulcent in anti-acne lotions. The fruits contain an enzyme, erepsin, Vitamin B1 and C, ascorbic acid, proteolytic enzyme, rutin, oxidase, succinic and maleic dehydrogenases, and so on. The seeds contain α- and β-amyrin, sitosterols and cucurbitasides, whereas, the leaves contain free cucurbitasides B and C and ferredoxin [27, 28]. According to the traditional use and phytoconstituents, cucumber was selected and screened for free radical scavenging activities using *in-vitro* model.

In the present work, the fresh cucumber with/without internal organ was selected for the total phenolic content and evaluation of their antioxidant activities because it is one of the most commonly consumed vegetables by the Myanmar population, and the rich sources of phenolic compounds which possess antioxidant properties. Based on the content of phenols in the cucumber the conducted research trails on the antioxidant activity of the fresh cucumber with/without internal organ by using DPPH.

II. MATERIALS AND METHODS

2.1 Instrumentation

Absorbance and λ_{max} were recorded on PD-303 UV Visible Spectrophotometer.

2.2 Plant Material

The fresh cucumbers (*Cucumis sativus* Linn.) were collected from Mingalar Market, Chan Aye Thar Zan Township, Mandalay Region, in Myanmar.

2.3 Preparation of Fresh Cucumber Juices

Extraction Procedure

100 g of fresh cucumbers with/without internal organ were crushed by blender. These Juices were squeezed, filtered and then centrifuged with 5000 rpm for 30 minutes. (51) mL of expressed juice of fresh cucumbers with internal organ and (56) mL of expressed juice of fresh cucumbers without internal organ which are the liquid product were obtained.

2.4 Qualitative Test for Phenols

Group Test:

The fresh juice of pomegranate was tested by blue litmus paper. This blue litmus paper turns red.

Colour with $FeCl_3$:

1mL of fresh juice of pomegranate was taken and a few drops of very dilute solution of ferric chloride were added. The colour changes to brown which indicates the presence of phenol. The reaction takes place as follows [29].

$$6C_6H_5OH + FeCl_3 \longrightarrow 3H^+ + [Fe(OC_6H_5)_6]^{3-} + 3HCl$$

(phenol) (brown)

2.5 Quantitative Determination of Total Phenolic Content

2.5.1 Principle

Phenols in alkaline medium react with phosphomolybdic acid of Folin- Ciocalteau reagent producing a blue coloured complex.

2.5.2 Preparation and Determination of Standard Gallic Acid

10 mg of the standard gallic acid was taken in a test tube. 10 mL of distilled water was added to the standard compound. 1 mL of this standard solution was taken in another test tube. The volume of this solution was made up to 10 mL with distilled water. The standard solution was taken by micro-pipette into a series of test tubes 20 µL, 40 µL, 60 µL, 80 µL and 100 µL respectively.

The volume was made up to 1.6 mL with distilled water in each test tube. And then, 100 µL of Folin-Ciocalteau reagent and 300 µL of saturated Na_2CO_3 (20%) solution were added. After each standard solution was heated in the water bath at 40ºC for 30 minutes, they were cooled at room temperature. [30-32].

2.5.3 Estimation of λ_{max} for Gallic Acid

To determine the absorption maximum, standard solution of gallic acid in concentration 7.5 µg/mL was prepared. Spectrum of Folin-Ciocalteau reagent solution was measured according to the above procedure in the wavelength interval 700 to 800 nm. The results were described in Figure 1.

2.5.4 Determination of Standard Gallic Acid

The absorbance values of prepared standard gallic acid solutions were measured by PD-303 UV visible spectrophotometer at 765 nm with respect to the blank solution. The calibration curve of standard gallic acid was shown in Figure 2. [30-32].

2.5.5 Determination of Total Phenolic Content of *Cucumis sativus* Linn.

The total phenolic content of expressed fresh cucumber juice was measured with the Folin-Ciocalteau reagent. Firstly, 40 µL of expressed juice of fresh cucumber with internal organ was taken in a test tube. It was made up to 1.6 mL with distilled water. 100 µL of Folin-Ciocalteau reagent was mixed, then 300 µL of saturated Na_2CO_3 (20%) solution was added.

The mixture was heated in a water bath at 40°C for 30 minutes and then cooled at room temperature. The absorbance of this prepared sample solution was measured at 765 nm, using a UV spectrophotometer. The assay was carried out in triplicate.

The total phenolic content of fresh cucumber without internal organ was measured as the same procedure. The total phenolic content of fresh cucumbers was expressed as mg gallic acid equivalent per 100 g fresh weight. The results were shown in Table 2.

2.5.6 Calculation

The total phenolic content of fresh cucumbers was calculated from the standard curve prepared from different concentrations of gallic acid in which the same spectrophotometric procedure was followed for the working standard.

2.6 Preliminary Screening of Radical Scavenging Activity by Spectrophotometric Method

2.6.1 Chemicals

DPPH, 95% Ethanol, Ascorbic acid

2.6.2 Preparation of 60 µM DPPH Solution

0.0024 g (2.4 mg) of DPPH powder was weighed and it was thoroughly and gently dissolved in 100 mL of 95% ethanol and stored in brown coloured reagent bottle. It must be kept in the fridge for no longer than 24 hours before use.

2.6.3 Preparation of Standard Ascorbic Acid Solution

0.01 g (10 mg) of ascorbic acid was weighed and was dissolved in 100 mL of 95 % ethanol. It was diluted with 50% ethanol in various ratios to obtain five ranges of concentration, such as 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL and 10µg/mL respectively and the same volume 5.0 mL of standard ascorbic acid solution was prepared for each concentration.

2.6.4 Preparation of Test Sample Solution

(i) Preparation of Fresh Cucumber with Internal Organ Solution

The fresh juice of cucumber with internal organ was diluted with 50% ethanol in various ratios to obtain five ranges of concentration, such as 50 µg/mL, 75 µg/mL, 100 µg/mL, 125 µg/mL, and 150 µg/mL expressed as gallic acid equivalent respectively. Then, 5.0 mL of ethanol solution was prepared for each concentration.

(ii) Preparation of Fresh Cucumber without Internal Organ Solution

The fresh juice of cucumber without internal organ was diluted with 50% ethanol in various ratios to obtain four ranges of concentration, such as 20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, and 100 µg/mL expressed as gallic acid equivalent respectively. Then, 5.0 mL of ethanol solution was prepared for each concentration.

2.6.5 Measurement of DPPH Radical Scavenging Activity by Spectrophotometric Method

The control solution was prepared by mixing 2 mL of 60 µM DPPH solution and 2.0 mL of 95% ethanol using vortex mixer. Moreover, the blank solution could be prepared by mixing 2.0 mL of test sample solution and 2.0 mL of 95% ethanol thoroughly in the vortex mixer. Furthermore, the prepared standard ascorbic acid solutions and the test sample solutions (cucumber with/without internal organ) were also prepared by mixing gently 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. 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 Spectrophotometer.

The absorbance values obtained were applied to calculate percent inhibition by the following formula. [33, 34]

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

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

III. RESULTS AND DISCUSSION

The total phenolic content of *Cucumis sativus* Linn. was qualitatively and quantitatively investigated. Firstly, the fresh juices obtained by crushing from the fresh cucumber with/without internal organ were examined by using the special qualitative tests of phenol. The resulted data were tabulated in Table 1.

Table 1 Special Qualitative Test for Phenol

No	Experiment	Observation	Inference
1.	Group Test	Blue litmus paper turns red	Phenol may be present.
2.	Color with $FeCl_3$	Brown color was observed	Phenol is present.

From these results, it was observed that the fresh juices of selected samples consist of phenolic compounds.

The candidate phenolic antiodxidants in foods and vegetables included flavonoids, anthocyanins, catechins, chalcones and hydroxybenzoic and hydroxycinnamic acids. Total phenol contents (TPC) in fresh juices of cucumber with/without internal organ were determined spectrophotometrically according to the Folin-Ciocalteau spectrophotometric method using gallic acid as the standard. The Folin-Ciocalteau method is a rapid and widely-used assay to investigate the total phenolic content. Phenols react with an oxidizing agent phosphomolybdate in Folin-Ciocalteau reagent under alkaline conditions and result in the formation of blue coloured complex, the molybdenum blue which is measured at 765 nm spectrophotometrically. Scanning of the complex in a wavelength range from 700 nm to 800 nm showed a maximum absorbance (λ_{max}) at 765 nm as shown in Figure 1. The standard gallic acid solutions at concentration 2 to 10 µg/mL in distilled water were measured to know their absorbance by PD-303 UV visible spectrophotometer. The calibration curve was plotted against by using the resulting data of standard gallic acid solution as shown in Figure 2.

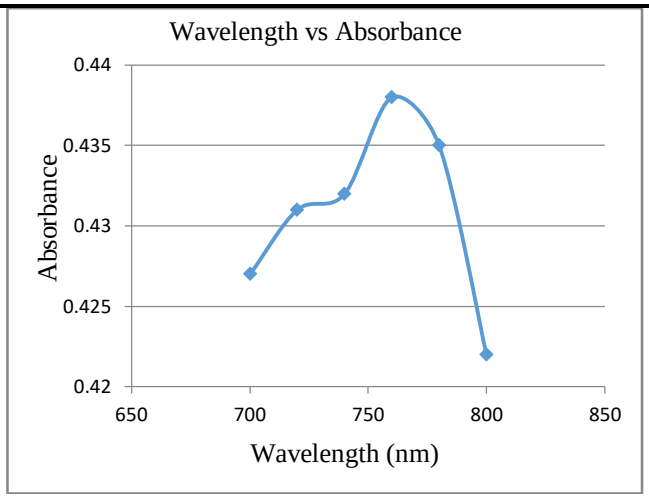

Figure 1 Maximum Wavelength of Standard Gallic Acid

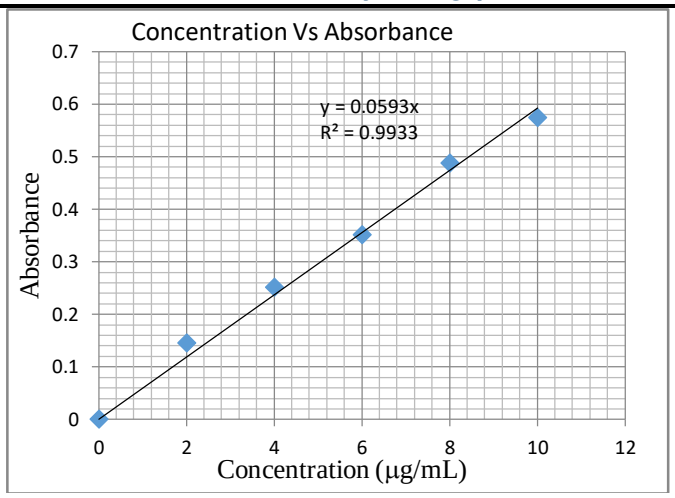

Figure 2 Concentration and Absorbance Calibration Curve for Standard Gallic Acid

The absorbance values of prepared sample solutions (40 µL of each fresh juice) were measured with UV- visible spectrophotometer at 765 nm with respect to the blank solution. From these results, the amount of total phenolic content of analyzed samples was obtained by using the standard curve (Figure 2). The results were described in Table 2.

Table 2 The Results of Absorbance Values, Concentrations and Phenolic Content of Fresh Juice Solutions of Cucumber with/without Internal Organ

No.	Test Sample	Absorbance	Concentration (µg/mL)	Total Phenol Content[a]
1.	Fresh cucumber with internal organ (40 µL)	0.379	6.42	7.72 ± 0.164
		0.348	5.90	
		0.345	5.85	
2.	Fresh cucumber without internal organ(40 µL)	0.271	4.59	6.35 ± 0.116
		0.270	4.58	
		0.262	4.44	

Each total phenolic content is expressed as mean ± standard deviation (n = 3),
[a] mg of gallic acid equivalent per 100 g fresh weight

The content of total soluble phenol in fresh cucumber with/without internal organ was expressed as gallic acid equivalent. The total phenolic content of fresh cucumber with/without internal organ was found to be 7.72 ± 0.164 mg of gallic acid equivalent per 100 g fresh weight and 6.35 ± 0.116 mg of gallic acid equivalent per 100 g fresh weight, respectively.

Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals. The antioxidative effect is mainly due to phenolic components, such as penolic acids, and phenolic diterpenes. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

With the increasing interest in function and diversity of antioxidants, some methods have been developed in order to determine this activity in food, beverages and biological samples. Among chemical methods applied to determine the antioxidant activity of a compound, DPPH, (2,2-diphenyl-1-picrylhydrazyl) is one of the most used methods because it is practical, fast and stable.

The present study was done for the investigation of antioxidant effects of fresh cucumber with/without internal organ. 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 juices were assessed on the basis of the scavenging activity of the stable DPPH free radicals.

Antioxidant activities of fresh cucumber with/without internal organ were expressed as percentage of DPPH radical inhibition and IC_{50} values (µg/mL). The results of antioxidant activity using DPPH method in fresh cucumber with/without internal organ using ascorbic acid as a positive control were shown in Figure 3, 4 and 5.

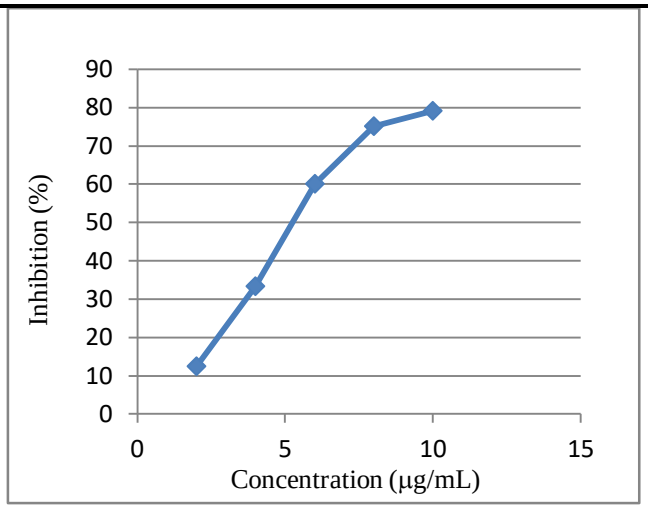

Figure 3 Plot of % Inhibition vs concentration of standard ascorbic acid

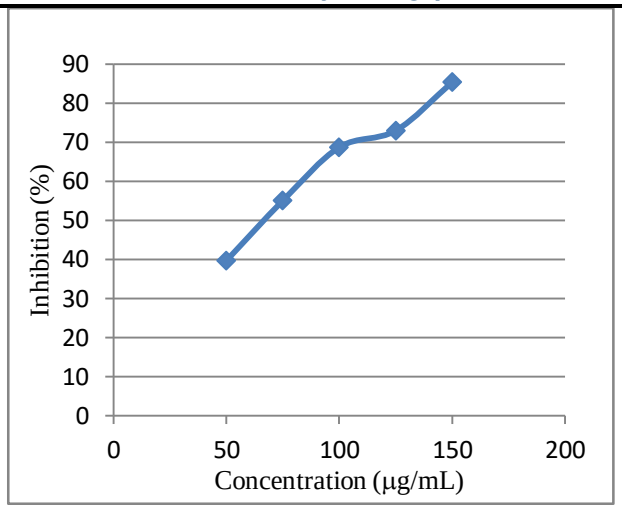

Figure 4 Plot of % inhibition vs concentration of fresh juices of cucumber with internal organ

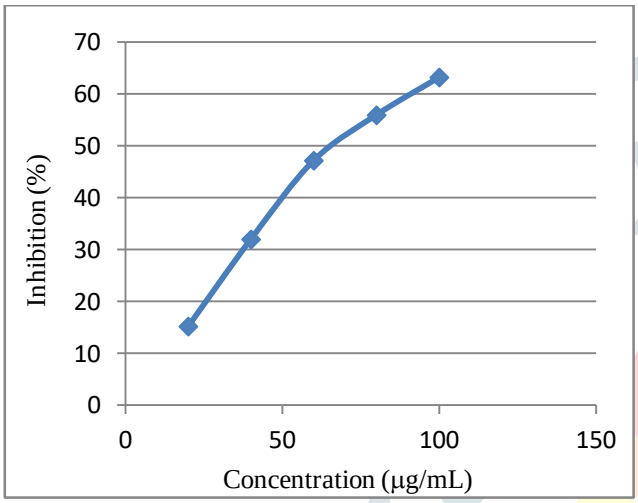

Figure 5 Plot of %inhibition vs concentration of fresh juices of cucumber without internal organ

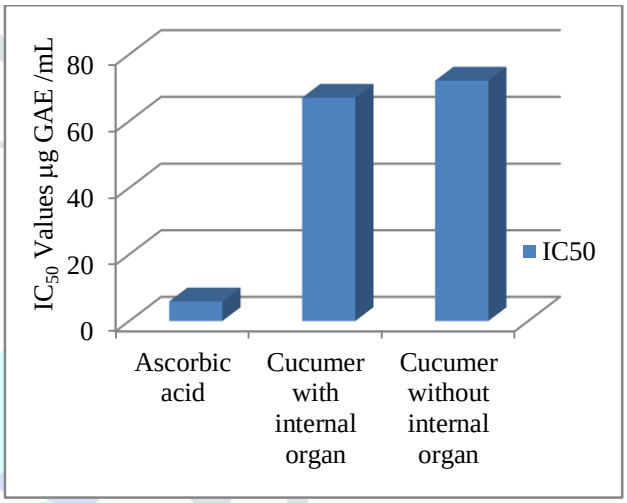

Figure 6 IC_{50} Values of Standard Ascorbic Acid and Fresh Cucumber with/without Internal Organ

Table 3 IC_{50} Values of Standard Ascorbic Acid and Fresh Cucumber with/without Internal Organ

Test Samples	**IC_{50} Values**
Ascorbic acid	5.89 µg /mL
Fresh cucumber with internal organ	67.01µg GAE /mL
Fresh cucumber without internal organ	72.12µg GAE /mL

The IC_{50} value is a parameter used to measure antioxidative activity and it is defined as the juice 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 fresh juices was shown to be influenced by the total phenolic content. Fresh juices 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 fresh juices and phenolic contents.

In this present research, the fresh juices of cucumber with/without internal organ showed scavenging against DPPH radical. The lowest IC_{50} value was acquired by cucumber with internal organ, indicating it has the highest antioxidant capacity to scavenge DPPH radicals. A higher antioxidant capacity in cucumber with internal organ was expected because it contained the highest total phenolic content. Higher phenolic contents generally indicated stronger antioxidant capacities. These results revealed that antioxidant activity of cucumber with internal organ is higher than that of cucumber without internal organ but lower than ascorbic acid as positive control (IC_{50} = 5.89 µg /mL). The fresh juice of cucumber with internal organ contains significant amount of antioxidant agents. Therefore, the study suggests that the selected cucumber with internal organ might be a potential source of natural antioxidants.

IV. CONCLUSION

The results of the fresh cucumber with/without internal organ showed that the highest antioxidant activity presented in fresh cucumber with internal organ in accordance with IC_{50} value of 67.01 µg GAE /mL, but still lower than ascorbic acid (5.89 µg /mL) which is a positive control. Based on the obtained results, it can be concluded that phenol-enriched fresh cucumber

with internal organ was more effective on DPPH radicals than the fresh cucumber without internal organ. It has been suggested that the antioxidant capacity of fresh cucumber with/without internal organ is strongly correlated to the type of phenolic compounds which are present in fresh cucumber with/without internal organ. The fresh cucumber with internal organ can be regarded as promising candidates for natural plant sources of antioxidants with high value. Further studies are required and are in progress here.

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