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TOTAL PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF DIFFERENT PARTS OF CUCUMBER (*Cucumis sativus* L.)

– Research paper –

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Abstract: The aims of this research were to estimate the polyphenol content and antioxidant capacity from different parts of cucumber. The antioxidant activity was investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP), total flavonoid and phenolic contents were estimated using aluminium chloride and Folin-Ciocalteau reagents assays, respectively. Our finding showed that the ethanolic peel extract contained the highest phenolic (23.08 mg GAE/g) and flavonoids (14.02 mg QE/g). Also, ethanolic peel extract demonstrated significantly ($p<0.05$) higher FRAP value. Pearson correlation revealed that there were positive correlations ($p<0.01$) between TPC and TFC with FRAP assay. These findings suggest that consumption of cucumber with peel may provide optimum health benefit than its peeled counterpart.

Keywords: antioxidant capacity, cucumber, total phenolic content, total flavonoid content

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 (Asghar et al., 2016). Regular eating of fruits and vegetables confers benefits to human health (Asghar et al., 2016). Epidemiological studies reported that foods containing phytochemicals with antioxidant capacity have strong protective effects against several diseases including cardiovascular diseases and certain cancers (Kaur and Kapoor, 2002; Vissotto et al., 2013). The protective action of fruits and vegetables has been attributed to the presence of antioxidants, most especially antioxidant vitamins (Kalt and Kushad, 2000; Prior and Cao, 2000). However, several types of research reported that most of the antioxidant capacity may be from phenolic compounds such as flavonoids, rather than from Vitamins (Kahkonen et al., 1999).

Cucumber (*Cucumis sativus* L.), belongs to the Cucurbitaceae family. The family includes several species of cultivated plants of great economic importance, such as cantaloupe (*Cucumis melo* L.), squash (*Cucurbita maxima* L.), and watermelon (*Citrullus lanatus* L.) (Ritschel et al., 2004). Cucumber is native to north western India (Kumaraswamy, 2016). Traditionally, it is used as a cooling agent in both rural and urban areas. Cucurbitacins is the active compound present in *C. sativus* and demonstrated cytotoxicity. Cucumber extract showed antioxidant capacities against various in vitro methods such as DPPH radical scavenging activity, total radical-trapping antioxidant parameter (TRAP), Trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP) (Stratil et al., 2006). The total phenolic contents, proanthocyanidins and flavonols in cucumber extract were found to be 9.05 ± 0.83, 2.06 ± 0.09 and 55.66 ± 1.52 mg/100g respectively (Melo et al., 2006).

Vegetables are consumed more often compared to fruits probably due to their availability and low price (Deng et al., 2013). The antioxidant capacity in cucumber has been reported in the literature. However, antioxidant activities and bioactive compounds in different parts of fruits

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and vegetables are varied. According to Pantelic´ et al., (2016) phenolic compounds are distributed in all parts of plant food with varying composition; for example, the authors reported flavonols were the dominant phenolics found in grape skins which may contribute the antioxidant activity. Researcher's interest in functional food is currently receiving a great momentum, consumption of cucumber is varied; peeled cucumber sometimes is preferred by consumers which may be due to the lack of information on the phytochemicals contents and health benefit of each part. Moreover, the peel discarded may be useful in the development of functional food or may be used to replace the synthetic antioxidant used in the preservation of food. Therefore, the following research was aimed to evaluate the phenolic contents and antioxidant activity from different parts of cucumber (peel, flesh, seed and whole).

MATERIALS AND METHODS

Sample

The cucumber fruit was purchased from the shop in Kampung Gong Badak Terengganu, Malaysia. The whole cucumber was washed under tap water to remove any foreign material and carefully peeled; the seeds were separated manually. All the cucumber parts were cut into pieces and dried. The dried parts were ground and kept at -20 °C before analysis.

Chemical Reagents

Folin–Ciocalteu's phenol reagent, 2,4,6-tri(2-pyridyl)-S-triazine (TPTZ) were purchased from Sigma–Aldrich (USA). Acetic acid, hydrochloric acid, iron (III) chloride hexahydrate, iron (II) sulfate heptahydrate, sodium carbonate, sodium acetate, ethanol and other solvents were of analytical grade.

Extraction

The extraction of phenolic compounds was based on the protocol described by Asghar et al., (2016) with slight modification. Dried and ground parts of cucumber were extracted each with ethanol and water at 1:10 (w/v) ratio of cucumber part to solvent, for three days with shaking at intervals. The contents were filtered through Whatman # 1 filter paper. All the filtrates were concentrated with a rotary evaporator under vacuum at 40 °C (Heidolph, Hei-VAP, Germany) and the extracts were kept at -20 °C before analysis.

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) was estimated with Folin–Ciocalteu reagent according to Singleton and Rossi, (1965) slightly modified by Deng et al., (2013). Briefly, 30 µL of extract (1 mg/mL) was mixed with 150 µL Folin-Ciocalteu reagent (10%) (v/v). After 4 min, 120 µL of 7.5% Na_2CO_3 was then added. The resulting mixture was kept in the dark for 45 min at ambient condition; the optical density was read at 760 nm. Calibration was done using Gallic acid (Figure 1A). The result was expressed as (mg GAE)/g of extract.

Determination Total Flavonoid Content (TFC)

The TFC was estimated using the protocol adopted by Jakovljević et al., (2013) with modification. Briefly, 150 µL (1 mg/mL) of the extract was mixed with an equal volume of 2% $AlCl_3$ solution dissolved in methanol. The mixture was kept for 30 min at ambient condition. The optical density was read using a microplate reader at 415 nm. The standard curve was generated using quercetin (Figure 1B). The flavonoid content (TFC) in the extract was expressed in terms of quercetin equivalent (mg of QE/g of extract).

DPPH Radical Scavenging Activity Assay

Free radical scavenging activity of the samples was estimated with a modified method (Hafsé et al., 2017). A volume of 0.1 mL was added to 0.2 mL of a methanol solution of DPPH (0.04%) at different concentrations; the mixture was vigorously shaken and incubated at room temperature for 30 min in the dark. The percentage of scavenging activity was evaluated by comparing with the control. The absorbance was read at 517 nm, and the antioxidant activity estimated using the equation:

% antioxidant activity = (Abs control - Abs sample / Abs control) x 100

Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric reducing antioxidant power was evaluated following the protocol of Benzie and Strain (1996) with slight modification. A volume of 15 µL of the extract was mixed with 285 µL of FRAP reagent, the mixture was kept at ambient condition for 30 min in the dark and

the absorbance was read at 593 nm. The result was expressed as mmol Fe^{2+}/g extract using Iron(II) sulfate heptahydrate calibration curve (Figure 1C).

Statistical Analysis

Statistical package for social science (SPSS, version 20.0 for Windows) was used for statistical analysis. Results were reported as mean ± SD of three measurements. The alpha level was at 0.05. The relationship between TPC, TFC and antioxidant activity (DPPH and FRAP) was assessed using Pearson correlation analysis ($p<0.01$).

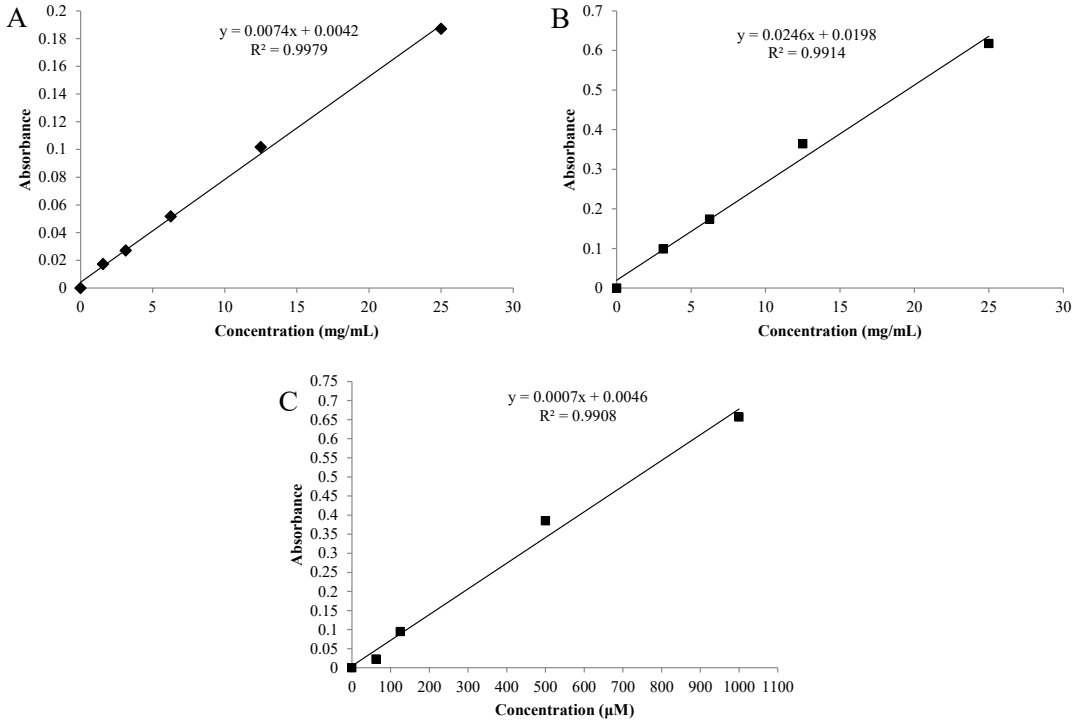

Figure 1. Calibration curve for (A) Gallic acid, (B) Quercetin and (C) Iron(II) sulfate heptahydrate

RESULTS AND DISCUSSION

RESULTS

Total Phenolic Flavonoid Contents

Different parts of cucumber recorded a variation in total phenolic contents as shown in Table 1. Ethanoic peel extract recorded the highest phenolic ($p<0.05$) followed by ethanolic whole extract, while the lowest phenolic content was found in ethanolic flesh extract. The TPC were in the decreasing order: ethanolic peel extract > ethanolic whole extract > aqueous seed extract > aqueous peel extract ≥ aqueous whole extract ≥ ethanolic seed extract > aqueous flesh extract ≥ ethanolic flesh extract.

Among the different parts of cucumber studied, ethanolic peel extract contained significantly ($p<0.05$) higher TFC (14.02 ± 0.87 mg QE/g), the lowest TFC was detected in aqueous whole, flesh and seed extracts which were respectively, 0.10, 0.09 and 0.09 mg QE/g ($p>0.05$) (Table 1).

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of different parts of cucumber ranged from 6.61 to 20.18%. Higher inhibition was observed in the ethanolic whole extract, while aqueous flesh extract possessed the lowest inhibition. The DPPH radical scavenging activity followed the order: ethanolic whole extract ≥ ethanolic flesh extract ≥ aqueous seed extract ≥ ethanolic seed extract ≥ ethanolic peel extract ≥ aqueous peel extract ≥ aqueous whole extract ≥ aqueous flesh extract (Table 2).

FRAP

The FRAP values of both ethanolic and aqueous extracts of different parts of cucumber are presented in Table 2. The FRAP values

varied from 0.03 to 0.12 mmol Fe^{2+}/g, the mean value of ethanolic peel extract was significantly ($p<0.05$) higher than the other extracts, and it followed the order: ethanolic peel extract > aqueous seed extract ≥ ethanolic whole extract ≥ ethanolic seed extract > aqueous whole extract ≥ ethanolic flesh extract ≥ aqueous peel extract ≥ aqueous flesh extract.

Correlation

The relationship between the polyphenolic and antioxidant activity (DPPH and FRAP) was analysed and presented in Figures 2 and 3.

Table 1. TPC and TFC of ethanolic and aqueous extracts of different parts of cucumber

Cucumber part	Solvent	TPC (mg GAE/g)	TFC (mg QE/g)
Peel	Ethanol	23.08 ± 1.02a	14.02 ± 0.87a
	Water	13.22 ± 0.75d	0.83 ± 0.13c
Flesh	Ethanol	9.65 ± 0.34e	0.27 ± 0.04cd
	Water	10.02 ± 0.28e	0.09 ± 0.03d
Seed	Ethanol	12.00 ± 0.84d	0.09 ± 0.01d
	Water	17.59 ± 0.51c	0.32 ± 0.06cd
Whole	Ethanol	19.16 ± 1.07b	3.00 ± 0.38b
	Water	12.41 ± 0.72d	0.10 ± 0.04d

Values are the means ± standard deviation based on three readings. Superscript letter refers to significant different ($p<0.05$) by comparing among solvents/cucumber parts. Means with different superscript letters were significantly different ($p<0.05$). QE: Quercetin equivalent. GAE: Gallic acid equivalent.

Table 2. FRAP values and DPPH radical scavenging capacity of ethanolic and aqueous extracts of cucumber parts

Cucumber part	Solvent	DPPH scavenging activity (%)	FRAP (mmol Fe^{2+}/g)
Peel	Ethanol	8.11 ± 3.40c	0.12 ± 0.03a
	Water	8.09 ± 1.57c	0.03 ± 0.00c
Flesh	Ethanol	13.51 ± 5.49bc	0.03 ± 0.00c
	Water	6.19 ± 5.34c	0.02 ± 0.00c
Seed	Ethanol	12.79 ± 2.78bc	0.06 ± 0.01b
	Water	13.05 ± 2.74bc	0.07 ± 0.02b
Whole	Ethanol	20.18 ± 6.07b	0.06 ± 0.01b
	Water	6.61 ± 3.39c	0.03 ± 0.01c
Quercetin		87.45 ± 0.68a	ND

Values are the means ± standard deviation based on three readings. Superscript letter refers to significant different ($p<0.05$) by comparing among solvents/cucumber parts. Means with different superscript letters were significantly different ($p<0.05$). ND; not determined

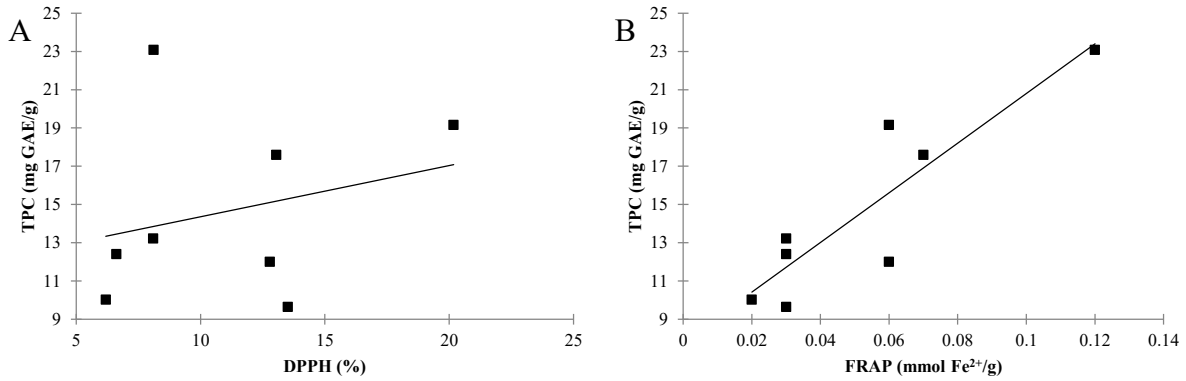

Figure 2. Correlation of TPC versus (A) DPPH radical scavenging assay, (B) FRAP of different parts of cucumber. Correlation coefficient r = 0.265 and r = 0.890 for DPPH and FRAP, respectively

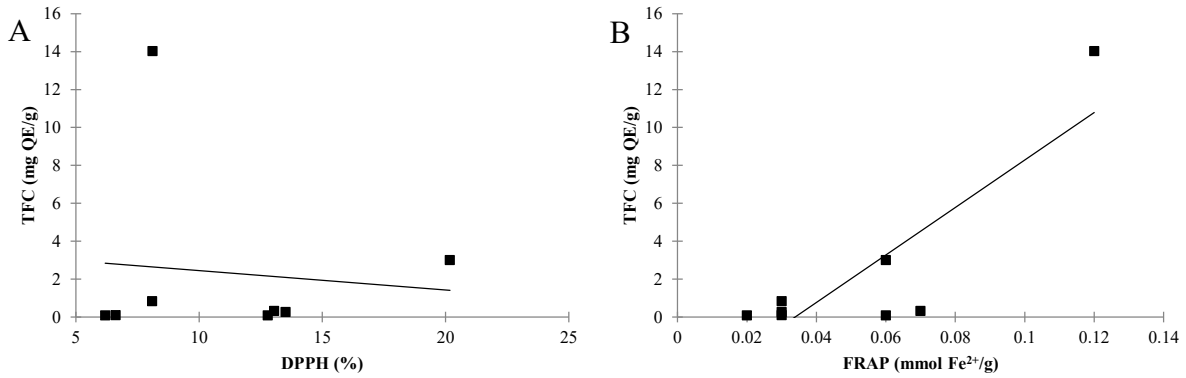

Figure 3. Correlation of TFC versus (A) DPPH radical scavenging assay, (B) FRAP of different parts of cucumber. Correlation coefficient r = -0.100 and r = 0.853, for DPPH and FRAP, respectively

There was a strong relationship between TPC and antioxidant activity assayed by FRAP (r = 0.890), similar trend was also found between TFC and FRAP (r = 0.853). On the other hand, TPC and DPPH showed weaker correlation (r = 0.265), while TFC and DPPH showed a negative correlation (r = -0.100).

DISCUSSION

Total Phenolic and Flavonoid Contents

In the present study, ethanolic peel extract was found to contain the highest TPC ($p<0.05$) compared to other parts. Peel of several fruits have reported to contained higher phenolic content than their flesh counterpart. These include rambutan (Yoswathana and Eshtiaghi, 2013) onion (Albishi et al., 2013). Cantaloupe (*Cucumis melo*) skin extract also recorded the highest TPC compared to the other parts (Ibrahim and El-masry, 2016). In contrast, Sotiroudis et al., (2010) observed higher phenolic contents in the pulp which was twofold than that of the peel. Kaur and Aggarwal, 2013 reported the TPC of methanolic extract of cucumber (41.47 mg GAE/g) which was higher than the values obtained in present study. However, the TPC recorded in the present study was higher than that reported by (Sreeramulu and Raghunath, 2010). Ikram et al., (2009) observed a variation in TPC among nine underutilized fruits and the authors attributed the difference due to the blue, purple and red color pigments present. Moreover, the variation may be due to the presence of lipophilic compounds which contribute to the highest phenolic content. Ethanol and water are the most commonly used solvents to extract phytochemicals due to the absence of toxicity (Yoswathana and Eshtiaghi, 2013). It can be seen that the highest TPC was found in the ethanolic peel extract, while lowest was found in the flesh part. Pantelic´ et al., (2016) observed similar trend in which they found higher TPC in the seeds and skins of grape, whereas very low contents were found in the pulp.

Previous findings identified more than 6000 flavonoids in plants, in which most of them are present in fruits and vegetables (Asghar et al., 2016). In the present study, the TFC showed similar trend as observed in TPC in which the ethanolic peel extract was a potential source of polyphenolic compounds and recorded significantly ($p<0.05$) higher TFC. Peel of *Pouteria campechiana* fruit gave remarkable TPC and TFC in both ethanolic and methanolic extracts (Kong et al., 2013). The ethanolic peel extract had the highest TFC compared to its aqueous counterpart. Asghar et al., (2016) also observed higher TFC in ethanolic extract of *Carica papaya* leaves followed by the methanolic extract.

DPPH Scavenging Activity

Different antioxidant assays are available with a different mechanism. Ikram et al., (2009) reported that it is recommended to use different methods instead of using a single assay for determination and comparison of the antioxidant capacity in food or plant extract. 1,1-diphenyl-2-picrylhydrazyl (DPPH) is a stable organic free radical which is commonly used to determine the free radical scavenging activity of food (Abozed et al., 2014). In the present study, despite higher TPC, the free radical scavenging activity was found to be very low in both ethanolic and aqueous extracts and hence revealed weaker correlation. Similar trend was also reported in sea cucumber (Zhong

et al., 2007). Also, Sotiroudis et al., (2010) observed lowest antioxidant activity in the methanolic extract of edible parts of cucumber. Cucumber showed poor antioxidant activity among the 18 vegetables studied by Yamaguchi et al., (2001), also, according to Qusti et al., (2010) cucumber showed lowest antioxidant activity compared to the vegetables cited in the Holy Quran. Meanwhile, Kaur and Aggarwal, (2013) observed higher DPPH scavenging activity. Also, The DPPH radical scavenging activity of non-chilled radicles cucumber seedling was 92% (Kang and Saltveit, 2002), which was higher than the value obtained in the present study. Some of the factors contributed to the variation in the antioxidant activity are environmental factors such as climate, soils and light exposure (Ikram et al., 2009). However, it was in line with most of the 30 aqueous plant extracts (Dudonné et al., 2009).

FRAP

In this study, the FRAP value of the ethanolic peel extract was higher than the remaining parts. It consistently agreed with those reported in apple (Henríquez et al., 2010) where the peel part was found to possess higher reducing power than pulp and whole fruit. FRAP value of *Abelmoschus moschatus* seed and *Lavandula augustifolia* flower were found to be 0.08 and 0.14 mmol/g, respectively (Dudonné et al., 2009) and consistently agreed with our present study.

Pearson Correlation

To establish a justification on the correlation between TPC and antioxidant activity, proper characterization of individual phenolic compounds is required (Ikram et al., 2009). Vissotto et al., (2013) observed a positive correlation between the scavenging capacity against ROO• and the contents of TP and TF with the r value similar to what we obtained in the present study. Our correlation coefficient was also in line with those reported in grape where they found a significant correlation between TPC with free radical scavenging activity with value r value of 0.76 and 0.98, for seeds and skins, respectively (Pantelic´ et al., 2016). Phenolic compounds found in plant are considered as the main active components with antioxidant capacity (Stagos et al., 2012). Positive correlation between TPC and antioxidant activities (FRAP, DPPH• and ORAC) was found in black mulberry, blackberry and strawberry (Boeing et al., 2014). Moreover, consistently agreed with the present work. In the same manner, Maisarah et al., (2013) reported a positive correlation between TPC, TFC and DPPH ($r = 0.846$ and $r = 0.873$, respectively) in different parts of papaya. On the other hand, TPC presented the lowest contribution to the DPPH scavenging activity. This indicates that the antioxidant activity of plant extracts depend not only on the phenolic constituents but also on the antioxidant assay (Dudonné et al., 2009). Non-phenolic compounds may also contribute to the antioxidant activity. According to Sotiroudis et al., (2010) uracil and 24- methylenecycloartenol were among the non-phenolic compounds contribute to the antioxidant activity in cucumber, as Uracil demonstrated high antioxidant activity evaluated using deoxyribose method. For the correlation between scavenging capacity assayed using DPPH and TFC, a negative correlation was found ($r = -0.100$). Stagos et al., (2012) observed a negative correlation between TPC and DPPH in Lamiaceae species. Simalarly, Sotiroudis et al., (2010) observed a negative relationship between TPC and radical scavenging activity ($r = –0.92$).

CONCLUSIONS

The antioxidant capacity and total phenolic content of ethanolic and water extracts of different parts of cucumber were evaluated. The study revealed that ethanolic peel and whole extracts exhibited the most potent antioxidant activity; as such the consumption of cucumber with peel may contribute to the prevention of several ailments caused by oxidative stress. The correlation results indicated that TPC and TFC contributed highly to the reducing activity, while TPC showed little contribution against DPPH scavenging activity. Further studies for the individual compounds in the ethanolic peel extract are recommended.

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