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Total phenolic content and antioxidant activity of kesum (*Polygonum minus*), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) extract

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Abstract: Herb and spices namely kesum, ginger and turmeric were extracted by using juice extractor without the additional of solvent. These herb and spices were determined for moisture content and the extracts were analyzed for total phenolic content (TPC) and antioxidant activity (DPPH radical scavenging assay and FRAP ferric-reducing antioxidant power assay). The yield of kesum, ginger and turmeric extraction was 23.6%, 58.6% and 66.4%, respectively. The results showed that, there was significant difference ($P < 0.05$) in total phenolic content and antioxidant activity for kesum, ginger and turmeric extracts. Kesum extract had the highest total phenolic content followed by ginger and turmeric extract. A significant and positive high Pearson's correlations between TPC and DPPH assay ($r = 0.86$) and between TPC and FRAP assay ($r = 0.91$) respectively was observed for all plants extracts. This indicated that phenolic compounds were the main contributor of antioxidant activity in plants. However, there was no synergistic effect observed for all plants extract mixture.

Keywords: Kesum, ginger, turmeric, phenolic content, DPPH radical scavenging assay, ferric –reducing antioxidant power assay

Introduction

Antioxidants are recognized for their potential in promoting health and lowering the risk for cancer, hypertension and heart disease (Wolfe and Liu, 2003; Valko *et al*., 2007). The uses of natural antioxidants from plant extracts have experience growing interest due to some human health professionals and consumer's concern about the safety of synthetic antioxidants in foods (Sun and Ho, 2005; Suhaj, 2006). Antioxidant activities in plants have been identified by many researchers (Hinneburg *et al*., 2006; Kumar *et al*., 2006; Cousins *et al*., 2007) and their effects in chicken sausage have been studied by Noriham *et al*., (2005). The natural occurring antioxidant is focused more on edible plants, especially spices and herbs (Huda-Faujan *et al*., 2009; Nanasombat and Teckchuen, 2009). Spices and herbs are an excellent source of phenolic compounds (flavonoids, phenolic acid and alcohols, stilbenes, tocopherols, tocotrienols), ascorbic acid and carotenoids which have been reported to show good antioxidant activity (Zheng and Wang 2001). Generally, Malaysian foods are rich in spices and herbs including kesum, ginger and turmeric. The whole of kesum is normally use when making gravy for laksa (wet rice noodle), while ginger and turmeric can be added into various foods as whole spices, powder or extracts. However, direct use of these spices and herbs as antioxidant is limited due to their aromatic and pungent properties.

Numerous studies had showed good antioxidant activity of kesum (*polygonum minus*) (Vimala *et al*., 2003; Huda-Faujan *et al*., 2009), ginger (*zingiber officinale*) (Hinneburg *et al*., 2006; Kim *et al*., 2007; Kota *et al*., 2008) and turmeric (*curcuma longa*) (Kaur and Kapoor, 2002; Tangkanakul *et al*., 2009). In Thailand and Vietnam, kesum is known as Pak pai or Vietnamese mint (Rafi and Vastano, 2007). According to Nanasombat and Teckchuen (2009), kesum contained flavonoids such as rutin (3.77%), catechin (0.34%), quercetin (0.08%), isorhamnetin (0.01%) and kaempferol (0.01%) which had showed to have antioxidant activity.

Ginger is commonly use in food as spice. Kim *et al*., (2007) and Schwertner and Rios (2007) reported that the main components of ginger are 6-gingerol, 6-shogaol, 8-gingerol and 10- gingerol and these constituents had exhibited strong antioxidative activity.

Generally turmeric has been used as dye and spice. Turmeric is an important tropical spice mainly for its colour, aroma and antioxidant property. The yellow colour in turmeric are mainly due to the presence of 3 major pigments; curcumin 1,7-bis(4-hydroxy-3-methoxyfenil)-1,6-heptadiene-3,5-dione), demethoxy-curcumin and bis demethoxy-

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curcumin. These curcuminoids are known to have high antioxidant activities (Ishita *et al*., 2004; Sharma *et al*., 2005; Cousins *et al*., 2007).

The objectives of this research was to determine the total phenolic content (TPC) and antioxidant activity of kesum, ginger and turmeric by using Folin-Ciocalteau method, DPPH-free radical scavenging method and ferric-reducing antioxidant power assay (FRAP) method. The correlation of TPC with DPPH and FRAP assay of plants extracts were investigated.

Materials and Methods

Materials

Fresh plant materials namely kesum (*Polygonum minus*), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*) were purchased from local market in Selangor. Folin-Ciocalteu's (FC) phenol reagent was obtained from Merck (Darmstadt, Germany). Sodium carbonate, gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,4,6,-Tris (1-pyridyl)-5-triazine (TPTZ) were purchased from Sigma (Steinheim, Germany), and ferrous sulphate was obtained from R&M Chemicals (Essex, UK).

Determination of moisture content

Moisture content was determined by drying 5 g of samples (kesum, ginger and turmeric) at 105°C in a drying oven to a constant weight (AOAC, 1990).

Preparation of plant extracts and determination of yield

Two hundred gram of fresh kesum (Leaves and stem), peeled ginger and turmeric were washed with clean water followed by surface drying using oven at 37°C for 30 mins. The extracts were obtained by using juice extractor (Breville Juice Fountain® Plus Juice extractor, Australia) without any addition of water. Then, the pure plant extracts were filtered using filter paper (Whatman No 1) followed by centrifugation (Hermle GmbH, Germany) at 4750 g (4°C) for 15 mins. The extracts were collected and stored in air tight glass vials covered with aluminium foil and kept at - 4°C. Sample extract mixtures (kesum: ginger: turmeric) with different ratios (1:0:0, 1:1:0, 0:1:0, 0:1:1, 0:0:1, 1:0:1 and 1:1:1) were prepared before analyzed. The percentage of yield extracts was calculated as % yield = [Weight of sample extract / Initial weight of sample] x 100.

Determination of total phenolics

Total phenolic contents of all plants extracts were determined using Folin-Ciocalteu reagent as described by Singlaton and Rossi (1965). Samples were inserted into different test tube and mixed thoroughly with 5 ml Folin-Ciocalteu reagent (previously pre-dilute 10 times with distilled water). After 5 mins, 4 ml of 7.5% sodium carbonate (Na_2CO_3) was added and allowed to react for 2 hrs at room temperature. The absorbance was measure at 765 nm using microplate reader spectrophotometers (Molecular devices, VERSAmax tunable, California, USA). Samples were measured in three replicates. Standard curve of gallic acid solution (10, 20, 40, 60, 80 and 100 ppm) was prepared using the similar procedure. The results were expressed as mg GAE/100 g extract sample.

Determination of free radical scavenging using DPPH method

The antioxidant activities of all extracts were evaluated through free radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The determination was based on the method proposed by Akowuah *et al*. (2005). Two ml of 0.1mM DPPH methanolic solution was added into 200 µl of sample extracts and 0.8 ml methanol. The mixture was thoroughly mixed and kept in the dark for 1 hr. The control was prepared by mixing 2 ml of DPPH and 1 ml methanol. The absorbance was measure at 517 nm using microplate reader spectrophotometers (Molecular devices, VERSAmax tunable, California, USA). Samples were measured in three replicates. Percentage of DPPH scavenging activity was calculated as % inhibition of DPPH = [Abs control –Abs sample / Abs control] x 100.

Determination of ferric reducing/antioxidant power assay (FRAP)

FRAP assay was carried out according to the method of Benzie and Strain (1996). FRAP reagent was prepared from acetate buffer (1.6 g sodium acetate and 8 ml acetic acid make up to 500 ml) (pH 3.6), 10 mM TPTZ solution in 40 mM HCL and 20 mM iron (III) chloride solution in proportion of 10:1:1 (v/v) respectively. The FRAP reagent was prepared fresh daily and was warmed to 37°C in oven prior to use. A total of 50 µl samples extract were added to 1.5 ml of the FRAP reagent and mixed well. The absorbance was measured at 593 nm using using microplate reader spectrophotometers (Molecular devices, VERSAmax tunable, California, USA) after 4 mins. Samples were measured in three replicates. Standard curve of iron (II) sulfate solution (200, 400, 600, 800 and 1000 ppm) was prepared using the similar procedure. The results were expressed as µmol Fe (II) /100 g extract sample.

Statistical analysis

Experiment data were analyzed using Excel (Microsoft Inc.) and SPSS version 17.0 software. Significant differences between samples were analyzed using analysis of variance (ANOVA) and Duncan's multiple-range test ($P< 0.05$). Pearson's correlation was used to determine the correlation of data between DPPH free radical-scavenging activity (%) and ferric reducing/antioxidant power assay (μmol Fe (II)/g extract) on total phenolic content (mg GAE/100 g extracts). Data obtained were reported as mean ± standard deviation.

Results and Discussions

Moisture content and extraction yield

Results showed that, mean moisture content of kesum, ginger and turmeric in this study was 84.5%, 91.5% and 91.0 % respectively. Analysis of extraction showed that turmeric had the highest yield which is 66.2%. It seemed that more than 50% of moisture content of turmeric had been extracted. The yield of ginger extracted was 57.8%, while kesum had the lowest yield of extraction (23.6%).

Total phenolic content

Total phenolic contents of plants extract were tested using the diluted Folin-Ciocalteu reagent. Table 1 showed total phenolic content of plants extracts. Result clearly showed that kesum had the highest total phenolic content followed by ginger and turmeric which mean value of 165.34 mg GAE/100 g extract, 101.56 mgGAE/100 g extract and 67.89 mg GAE/100 g extract, respectively. In the present study, the mixture of kesum and ginger extract (132.0 mg GAE/100 g extract) showed significantly increased ($p<0.05$) in total phenolic content compared to single ginger extracts. There was no significant different ($P>0.05$) observed in total phenolic content for the mixture of kesum and turmeric (103.3 mg GAE/100 g extracts) with the total phenolic content for the mixture of kesum, ginger and turmeric (104.7 mg GAE/100 g extracts).

Antioxidant capacity

DPPH radical was used as a stable free radical to determined antioxidant activity of natural compounds (Ozturk *et al*., 2007). The antioxidant activity of plant extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals (Stoilova *et al*., 2007). Thus, the purple colour of 2,2-diphenyl-1-picryl hydrazyl (DPPH) will reduce to α, α-diphenyl-β-picrylhydrazine (yellow coloured) (Akowuah *et al*., 2005). According to Suhaj (2006) scavenging of the stable radical (DPPH) is considered a valid and easy assay to evaluate scavenging activity of antioxidants.

Results of the activity of free radical scavenging of plants extracts are presented in Table 2. Results showed that, kesum extract contained the highest DPPH radical scavenging activity (82.6 ± 0.7%), followed by ginger extract (79.0 ± 0.6%) and turmeric extract (64.6 ± 2.4%). Mixture of kesum and ginger (79.4 ± 1.2%) extract did not show significant ($P > 0.05$) increased in the antioxidant activity when compared to single ginger extract. In contrast, mixture of kesum and turmeric (73.4 ± 2.7%) extract and the mixture of ginger and turmeric (68.6 ± 1.8%) extract had showed significantly ($P<0.05$) higher antioxidant activity when compared to single turmeric extract.

The natural presence of antioxidants in plants and a combination with other antioxidants may have an additive effect (that is expected from a simple addition) and synergistic effect (an effect which is greater than individual or sum of the combination) (Fuhrman *et al.*, 2000). Studied by Graversen *et al*. (2008) and Roberts and Gordon (2003) found that plant polyphenols have a synergistic effect with other antioxidants present in plant material. In addition, other studies have also been carried out to analyze the synergistic effect of antioxidants (Liu *et al*., 2008; Altunkaya *et al*., 2009; Romano *et al*., 2009). However, antioxidant activity in this study did not show synergistic effect in plant extract mixture.

In this study, the antioxidant activity is also determined on the basis of the ability of antioxidant in this plants extracts to reduce ferric (III) iron to ferrous (II) iron in FRAP reagent (Alothman *et al*., 2009; Wong *et al*., 2006). Generally, FRAP assay was used due to its simplicity and reproducibility. Table 2 shows the antioxidant activity of plants extracts. The results indicated that kesum exhibited significantly ($P < 0.05$) higher antioxidant activity (46.3 ± 1.2 μmol Fe (II)/g) compared to ginger (26.2 ± 0.0 μmol Fe (II)/g) or turmeric (23.3 ± 0.9 μmol Fe (II)/g). Antioxidant activity of plants mixtures of kesum and ginger; kesum and turmeric; and ginger and turmeric were 34.4 μmol Fe (II)/g, 27.5 μmol Fe (II)/g and 25.3 μmol Fe (II)/g, respectively. There was no synergistic effect observed for antioxidant activity in any plants mixture. In addition, the result demonstrated that antioxidant activity of plants extracts by FRAP assay had similar trend with DPPH assay except for mixture of kesum, ginger and turmeric extracts.

Table 1. Mean ± SD of total phenolic content of fresh spices and herb extracts

Spices and herb extracts	Total phenolic (mg GAE/100g extracts)
Polygonum minus	165.3 ± 1.0[a]
Zingiber officinale	101.6 ± 0.6[d]
Curcuma longa	67.9 ± 1.0[f]
Polygonum minus: *Zingiber officinale* (1:1)	132.0 ± 1.9[b]
Polygonum minus: *Curcuma longa* (1:1)	103.3 ± 1.1[cd]
Zingiber officinale : *Curcuma longa* (1:1)	73.6 ± 1.2[e]
Polygonum minus: *Zingiber officinale* : *Curcuma longa* (1:1:1)	104.7 ± 1.2[c]

Values are mean (n=3) ± standard deviation. Values with the same superscript letter within each column are not significant different (p>0.05).

Table 2. DPPH inhibition and ferric reducing/antioxidant power assay of spices and herb extracts

Spices and herb extracts	DPPH inhibition (%)	FRAP (µmol Fe (II)/ g extracts
Polygonum minus	82.6 ± 0.7[a]	46.3 ± 1.2[a]
Zingiber officinale	79.0 ± 0.6[b]	26.2 ± 0.0[cd]
Curcuma longa	64.6 ± 2.4[e]	23.3 ± 0.9[e]
Polygonum minus: *Zingiber officinale* (1:1)	79.4 ± 1.2[b]	34.4 ± 1.1[b]
Polygonum minus: *Curcuma longa* (1:1)	73.4 ± 2.7[c]	27.5 ± 0.7[c]
Zingiber officinale : *Curcuma longa* (1:1)	68.6 ± 1.8[d]	25.3 ± 0.7[d]
Polygonum minus: *Zingiber officinale* : *Curcuma longa* (1:1:1)	78.1 ± 0.8[b]	23.1 ± 0.2[e]

Values are mean (n=3) ± standard deviation. Values with the same superscript letter within each column are not significant different (p>0.05).

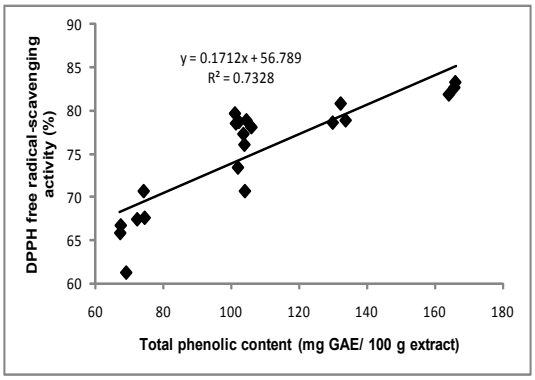

Figure 1. The correlation between total phenolic content and antioxidant activity (DPPH free radical-scavenging activity) of plants extracts

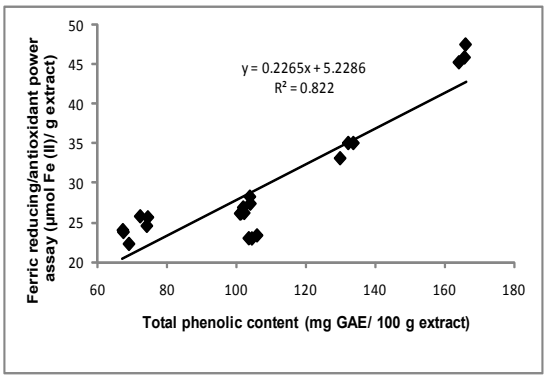

Figure 2. The correlation between total phenolic content and ferric reducing/ antioxidant power assay (FRAP) of plants extracts

The correlation between total phenolic content and antioxidant activity

Several studies (Shan *et al*., 2005; Wu *et al*., 2006; Wong *et al*., 2006) reported that phenolic compounds in spices and herbs significantly contributed to their antioxidant properties. Figure 1 shows the correlation between total phenolic content and DPPH assay of plants extracts. Results shows a positive correlation coefficient between the total phenolic content and DPPH assay of plants extracts ($r=0.86$) which is highly significant ($p<0.01$). Figure 2 shows the correlation between total phenolic content and FRAP assay of plants extracts and the results also demonstrated highly positive correlation coefficient between the total phenolic content and the FRAP assay of the plants extract ($r=0.91$), that was highly significant ($p<0.01$). Meanwhile, coefficient of determination (R^2) was measured on how well the regression line represents the data which shows the association between total phenolic content and DPPH assay ($R^2=0.73$) in Figure 1 and between total phenolic content and FRAP assay ($R^2=0.82$) in Figure 2. In this study, it seemed that, the higher total phenolic content of plants extracts resulted in higher antioxidant activity as similarly reported by Cai *et al*., (2004), Shan *et al*., (2005) and Wong *et al*., (2006). A significant and linear relationship existed between the antioxidant activity and phenolic content of kesum, ginger and turmeric, thus indicating that phenolic compounds are major contributors to antioxidant activity.

Conclusion

The results obtained demonstrated that kesum had the highest total phenolic content and antioxidant activity compared to ginger and turmeric. The mixture of plants extracts had showed no synergism effect. There was a good correlation between total phenol content and antioxidant activity (DPPH and FRAP assay) that support the idea of phenols as contributor of the antioxidant power of plants extracts.

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