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Total Phenolic Content and antioxidant activity of pure and formulated extracts of kesum (*Polygonum Minus*), bawang putih (*Allivium Sativum*), pegaga (*Centella Asiatica*), and ulam raja (*Cosmos Caudatus*)

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GRAPHICAL ABSTRACT

ABSTRACT

The use of herbs, spices, vegetables and medicinal plants have been traditionally utilize as the alternate medicinal to treat many of diseases by virtue of their antioxidant actions. Kesum, bawang putih, pegaga and ulam raja were extracted by using juice extractor without additional of solvent. The pure extracts were determined for moisture content and the pure and formulation extracts were analyzed for total phenolic content (TPC) and antioxidant activity (DPPH radical scavenging assay). The yield showed that kesum, bawang putih, pegaga and ulam raja extraction yield at 8.5%, 12%, 22.5% and 24% respectively. The results showed that, there was significant difference ($P < 0.05$) in total phenolic content and antioxidant activity between pure and formulation extracts. Formulated kesum and bawang putih (1:0:0:1) extract had the highest total phenolic content (1703.59 ± 11 GAE/100mg) followed by kesum pure extract (1388.19 ± 11 GAE/100mg) and bawang putih pure extract (1177.87 ± 138.82 GAE/100mg). No significant was noted and positive Pearson's correlations between TPC and DPPH assay ($r = 0.293$) was observed for all plants extract. The statistical indicated that phenolic compounds were not the main contributor of antioxidant activity in plants. Further, there was no synergistic effect observed for pure and formulation extracts.

Keywords: Kesum; bawang putih; ulam raja; pegaga; total phenolic content and DPPH radical scavenging assay

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1. INTRODUCTION

Phenolic compounds as a second metabolites, ubiquitous in herbs, spices and medicinal plants which have potential in promoting health and medical benefits [1]. Besides that, phenolic compounds contribute as an antioxidant by scavenging the superoxide anion, hydroxy radical, peroxy radical by inhibiting lipid peroxidation in the biological system [2]. Antioxidants activities in plants have been recognized by many researchers; for their potential in promoting health such as anti-viral [3]; anti-cancer [4]; anti-inflammatory [5]; anti-diabetic [6]; anti-ulcer [7] and etc. Synthetic antioxidants, such as butylated hygroxyanisole (BHA), butylated hydroxytoluene (BHT) and ter-butylhydroquinone (TBHQ) are commercially available currently in use. Unfortunately, it has been shown that they promote toxicity [8] and development of cancerous cells in rats [9]. In consequence, due to concern safety of synthetic antioxidants in food [10-12] and consumer demand for natural products [8]; have leads many researchers towards safer and more effective natural antioxidants from the edible plants as resources [12-14].

Numerous studies were conducted and had showed good antioxidant activities of kesum (*Polygonum minus*) [12, 15]; bawang putih (*Allium sativum*) [16]; pegaga (*Centella asiatica*) [17]; and ulam raja (*Cosmos Caudatus*) [18-19]. Kesum or scientific name *Polygonum minus* Huds is a medicinal herb and originated from Southeast Asia countries like Malaysia, Thailand, Vietnam and Indonesia. Among bioactive compounds reported was isolated from kesum that showed to have antioxidant activities, i.e: rutin, catechin, quercetin, isohamnetin, kaempherol [20], gallic acid, coumaric acid, rutin and quercetin [21]. Most of these compounds have been found valuable to use in pharmaceuticals, agrochemical, cosmetic, perfumery and food flavouring. For instance, farnesol has been has been suggested as anti-tumor agent and anti-bacterial activity [22]. Also, the gallic acid, coumaric acid, rutin and quercetin have possesses the anti-ulcer healing activities [21]. Bawang putih is a strongly aromatic bulb that has long been used in cooking and medicine. Lanzotti, 2012a [23], reported two major metabolites of bawang putih are sapogenin and saponin compounds such as furostane [24], spirostane [25] and cholestane [26]. These constituents had exhibited antispasmodic [27], antifungal [25], anti-ischemia [28], cytotoxicity [29], haemolytic [30], and platelet anti-aggregating activity [24].

Pegaga and ulam raja are commonly taken as traditional vegetable or 'ulam'. Pegaga has been reported to have sedative and anyxiolytic properties [17] and healing gastric ulcers [31]. Besides that, ulam raja is a rich source of bioactive compounds, including phenolics, flavonoids, carbohydrates, proteins, minerals and vitamins, increasing its nutritional value [32]. Subjected by Ragasa *et al*. (1999) [33] have reported several anti-mutagen and anti-fungal compounds identified from ulam raja, e.g. cotunolide, stigmasterol, lutein and 4, 4′-bipyridine.

The objectives of this research was to determine the total phenolic content (TPC) and antioxidant activity (DPPH) of kesum, bawang putih, pegaga dan ulam raja extracts and formulated mixture extracts. The correlation of TPC with DPPH assay of pure and formulated extracts were investigated.

2. EXPERIMENTS

Materials. Fresh plant materials such as kesum (*Polygonum minus*), bawang putih (*Allium sativum*), pegaga (*Centella asiatica*) and ulam raja (*Cosmos caudatus*) were purchased from local market in Johor. Follin-Ciocalteu's (FC) phenol reagent was purchased from Merck, sodium carbonate anhydrous (QRëC), garlic acid (Sigma), methanol (Labscan), DPPH (2-2-Diphenyl-1-picrylhydrazyl) (Sigma Aldrich) and L-ascorbic acid (Sigma Aldrich).

Determination of moisture content. About 5g each single sample was dry under 105°C. The weight of each sample was taken every one hour till the constant weight achieved [34].

Preparation of plant extracts and determination of yield. About one hundreds grams of fresh leaves kesum (*Polygonum minus*), pegaga (*Centella asiatica*), ulam raja (*Cosmos caudatus*) and peeled bawang putih (*Allium sativum*) were washed with clean water and drying the surface at 37°C for 30 minutes. For each plant was blended using a juice extractor without any addition of water. The juice was then filter using a whatman filter paper followed by centrifugation at 4800 rcf at 4°C for 15 minutes. The filtrate were collated and used for moisture content, measuring percentage of the yield extracted. After that, the extracts were collected and store in tight glass covered with aluminum foil and kept under -20°C. The yield of extraction calculated as follow:

$$\%\ \text{yield} = \frac{W_{SE}}{W_{IE}} \times 100\% \qquad (1)$$

where W_{SE} is weight of sample extract and W_{IE} is weight of initial sample. Pure extracts (kesum: pegaga: ulam raja: bawang putih) were formulated with different ratios (1:0:0:0, 0:1:0:0, 0:0:1:0, 0:0:0:1, 1:1:0:0, 1:0:1:0, 1:0:0:1, 0:1:1:0, 0:1:0:1, 0:0:1:1, 1:1:1:0, 1:1:0:1, 1:0:1:1, 0:1:1:1, 1:1:1:1) were prepared before analyzed for total phenolic contents (TPC) and antioxidant radical scavenging (DPPH) activity.

Determination of total phenolic content (TPC). Total phenolic content for pure and formulated extracts were determined using Folin-ciocalteu following the method of Singleton (1999) [35] and Jamal *et al*., (2010) [36] with slightly modification. Approximately, 30µl (1mg/ml) of samples were inserted into different test tube and diluted by 2370µl of deionized distilled water and followed by 150µl of Folin-Ciocalteu reagent. Then, samples mixed well thoroughly by using vortex for 15s in the dark. After one minute incubation, 450µl of 20% (w/v) of sodium carbonate was added and allowed to react for 30 minutes at 40°C in the dark. The absorbance was taken at 750nm. All measurements were performed in three duplicate. The total phenolic acids concentration was calculated from the calibration curve, using gallic acid as the standard and the results were expressed as mg L^{-1} of gallic acid equivalents (GAE mg L^{-1}).

Determination of free radical scavenging activity by DPPH assay. 2-2-diphenyl-1-1picrylhydrazyl (DPPH) assay was carried out to measure the antioxidant activity of pure, formulated extracts and L-ascorbic acid in terms of hydrogen donating or free radical scavenging ability [37]. The method established was based on the method described by Mavundza *et al*., (2010) [38] with minor modification. Sample stock solution (1mg/mL) were freshly prepared and diluted to 2-fold dilution of methanol. An aliquot of each dilution (100 µL) was added with 100 µL of 0.04% DPPH (Sigma Aldrich, 90%) to each well to give final volume of 200 µL equally in each well in a 96-well plate. The plate was gently shaken and incubated in dark for 30 minutes at room temperature. The absorbance (A) was measured at 517nm using a microplate reader (Biotek Epoch, USA) against methanolic solution as blank. The experiment were done in triplicates. The percentage of DPPH inhibition was calculated using following formula:

$$\%\ \text{inhibition} = \frac{[A_C - A_S]}{A_C} \times 100\% \qquad (2)$$

where A_C is the absorbance of the control reaction (containing all reagents except the test compound) and A_S is the absorbance of the tested compound.

Statistical Analysis. All data obtained were analyzed using SPSS version 15.0 software and Execl (Microsoft Inc). Analysis of varience (ANOVA) and Duncan Dunnet's multiple- range test were use to analyzed significant differences between samples. Pearson's correlation was used to determine the correlation between

DPPH free radical scavenging activity (%) on total phenolic content. Data were reported as mean ± standard deviation.

3. RESULTS AND DISCUSSION

Measurement of moisture content showed that ulam raja had the highest moisture content (89.6%), followed by pagaga, kesum and bawang putih was 89.4%, 82.6% and 69% respectively. The yield of extraction showed that ulam raja had the highest yield which is 24% followed by pegaga 22.5% and bawah putih 12% while the kesum had the lowest yield of extraction (8.5%). Also, Maizura *et al.*, (2011) [12] shown that the kesum have lowest yield of extraction compared to ginger and turmeric.

Total phenolic content of pure and formulated extracts (kesum: pegaga: ulam raja: bawang putih) with several combination were tested using the Folin-Ciocalteu method. Table 1 shown the distribution of total phenolic content of pure and formulated extracts. For the pure extracts, the highest total phenolic content is kesum, followed by bawang putih, ulam raja and pegaga was at 1388.19 ± 111 mg GAE/100g extract, 1177.87 ± 138.82 mg GAE/100g extract, 323.59 ± 7.19 mg GAE/100g extract and 150.01 ± 37.93 mgGAE/100g extract, respectively. Based on the highest phenolics content in kesum extract obtained, the kesum extract was subjected as control for the statistical analysis one-way ANOVA of Duncan and Dunnet multiple-range test. Besides that, the formulated extracts shown the highest total phenolic content is formulated kesum and bawang putih (1:0:0:1) was at 1703.59 ± 152.2 mg GAE/100g extract compare to others formulation extracts. However, this formulation does not significantly increased ($p>0.05$) compare to a pure kesum and bawang putih extracts. Then, followed by formulated kesum and pegaga was at 999.14± 172.317 mg GAE/100g extract and pegaga and bawang putih which was at 993.91 ± 16.67mg GAE/100g extract. Other than that, the comparison between the pure extracts such as pegaga and ulam raja; and formulated combination extracts except for formulated kesum and bawang putih shown decrease significantly difference ($p<0.05$) of total phenolic content presence in extracts. According to Bolling *et al.,* (2010) [39] the way of cultivation and climate can be counted as factors affecting the structure of phenolics and bioactive compounds. This can partly explain the wide range of variation in total phenolic content values obtained from different studies, which used the same evaluation methods.

Table 1. Total phenolic content of fresh plants extracts

Plants extracts	Total phenolic content (mg GAE/100g extracts)
Kesum (*Polygonum minus*) (1:0:0:0)	1388.19 ± 111^{a}
Pegaga (*Centella asiatica*) (0:1:0:0)	150.01 ± 37.93^{f}
Ulam raja (*Cosmos caudatus*) (0:0:1:0)	323.59 ± 7.19^{e}
Bawang putih (*Allium sativum*) (0:0:0:1)	1177.87 ± 138.82^{a}
Kesum: Pegaga (1:1:0:0)	999.14± 172.317^{b}
Kesum: Ulam raja (1:0:1:0)	730.57 ± 73.46^{cd}
Kesum: Bawang putih (1:0:0:1)	1703.59 ± 152.2^{a}
Pegaga: Ulam raja (0:1:1:0)	218.35 ± 18.36^{ef}
Pegaga: Bawang putih (0:1:0:1)	607.56 ± 14.56^{d}
Ulam raja: Bawang putih (0:0:1:1)	710.10 ± 120.69^{cd}
Kesum: Pegaga : Ulam raja (1:1:1:0)	642.32± 93.48^{d}
Kesum: Pegaga : Bawang putih (1:1:0:1)	993.91 ± 16.67^{b}
Kesum: Ulam raja: Bawang putih (1:0:1:1)	852.63 ± 39.97^{bc}
Kesum:Ulam raja : Bawang putih (0:1:1:1)	574.06 ± 123.53^{d}
Kesum: Pegaga : Ulam raja : Bawang putih (1:1:1:1)	585.97 ± 11.96^{d}

Values are mean (n=3) ± standard deviation. Value with the different letter superscript are significantly different ($p<0.05$) and same superscript letter are not significant different ($p>0.05$) within each column evaluated in one-way ANOVA (Duncan and Dunnet's multiple-range test).

The antioxidant activities of pure and formulated extracts were measured by scavenging activities of the stable radical (DPPH) [40]. Table 2 presented results of the activity of free radical scavenging of plants extracts. Results showed that kesum extract had the highest DPPH radical scavenging activity (84.45 ± 7.33%), followed by garlic (74.76 ± 4.76%), ulam raja (60.71 ± 6.74%) and the lowest is pegaga (40.83 ± 26.95%). Overall results showed that kesum had highest antioxidants among the others. Previous studied by Maizura *et al.,* (2010) [12] also found that kesum had highest antioxidant activity compare to ginger and turmeric and mixture of their extracts. This present study showed that all extracts were decrease significant different ($p<0.05$) compared to kesum extract (control). Subjected by Fuhrman *et al.,* (2000) [41] reported that "the natural presence of antioxidant in plants and combination with other antioxidants may have an additive effect and synergistic effect". Thus, it is expected that from a simple addition will resulted greater synergistic effects in combination compared to individual extract. Also, the synergistic effect between plant polyphenols with other antioxidant present in plant material was found by Gravesan *et al.,* (2008) [42]. In addition, Romano *et al.,* (2009) [43] also were proved that synergistic effect occurred in combination of two chemicals. However, in this study, there are no synergistic effects of antioxidant activity for the mixture of plants extracts.

Table 2. Percentage of DPPH inhibition of plants extracts

Plant extracts	DPPH inhibition (%)
Kesum (*Polygonum minus*) (1:0:0:0)	84.45 ± 7.33^{a}
Pegaga (*Centella asiatica*) (0:1:0:0)	40.83 ± 26.95^{d}
Ulam raja (*Cosmos caudatus*) (0:0:1:0)	60.71 ± 6.74^{bcd}
Bawang putih (*Allium sativum*) (0:0:0:1)	74.76 ± 4.76^{abc}
Kesum: Pegaga (1:1:0:0)	60.99 ± 0.13^{bcd}
Kesum: Ulam raja (1:0:1:0)	-49.51 ± 22.67^{e}
Kesum: Bawang putih (1:0:0:1)	77.72 ± 5.59^{ab}
Pegaga: Ulam raja (0:1:1:0)	53.78 ± 7.07^{cd}
Pegaga: Bawang putih (0:1:0:1)	54.98 ± 11.48^{bcd}
Ulam raja: Bawang putih (0:0:1:1)	59.18 ± 2.72^{bcd}
Kesum: Pegaga : Ulam raja (1:1:1:0)	58.17 ± 10.05^{bcd}
Kesum: Pegaga : Bawang putih (1:1:0:1)	62.94 ± 9.39^{abcd}
Kesum: Ulam raja: Bawang putih (1:0:1:1)	60.72 ± 0.90^{bcd}
Kesum:Ulam raja : Bawang putih (0:1:1:1)	55.98 ± 18.79^{bcd}
Kesum: Pegaga : Ulam raja : Bawang putih (1:1:1:1)	52.98 ± 8.93^{cd}

Values are mean (n=3) ± standard deviation. Value with the different letter superscript are significantly different ($p<0.05$) and same superscript letter are not significant different ($p>0.05$) within each column evaluated in one-way ANOVA (Duncan and Dunnet's multiple-range test).

As proposed from many studies, stated that plants, herbs and spices extracts that contain a high amount of phenolic also exhibit high antioxidant activity (12, 41). Pearson's correlation between total phenolic content and antioxidant activity (DPPH) indicated weak relationship where the r=0.293. According to Shaida *et al.*, (2011) [44], the low correlations confirm that phenolic compounds are not the only contributor to the antioxidant activities but the type and quantity of phenolic compounds and the presence of non-phenolic antioxidants may also contribute to the antioxidant activity of the extracts. Meanwhile, coefficient of determination (r^2) was measured on how well regression line represent the data which shows the non-association between total phenolic content and DPPH assay (r^2=0.115). Based on studies of Huang *et al.*, (2005) [45], DPPH reaction presents the disadvantages which can underestimate the antioxidant capacity such as it may react slowly or be inert to many antioxidants. Reaction kinetic with antioxidants appears not linear to DPPH concentrations, and reaction of DPPH with some phenolic structures could not go to completion, reaching an equilibrium state, as found for eugenol.

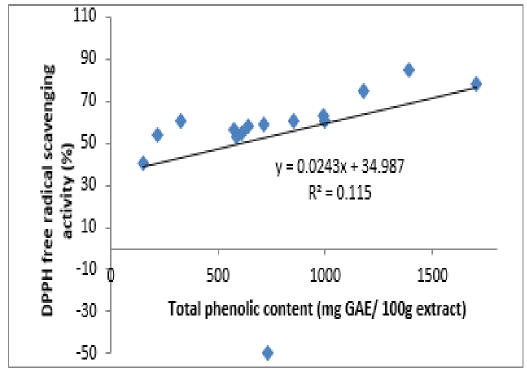

Figure 1. The correlation between total phenolic content and antioxidant activity (DPPH fre radical-scavenging activity) of plants extracts

4. CONCLUSION

The results obtained demonstrated that kesum extract had highest of total phenolic content and antioxidant activity, compare to pegaga, ulam raja, bawang putih and formulated extracts. Moreover, it is found that the formulated extracts does not show any synergism effect.

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