Screening of Antioxidant Potential from Cereal Wastes and Fruit Peels

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Abstract

This research was designed to explore the potential of cereal wastes (CW) and fruit peels (FP) as a source of antioxidants. The optimized solvent extract and hot water extract was subjected to total phenolic content (TPC), total flavonoids content (TFC) and scavenging test of DPPH. Result showed that optimized solvent extraction, peanut skins revealed the highest value in TPC (13685.70mg GAE/g), TFC (635.69mg QEE/g) and DPPH (79.06%) among CW. Meanwhile, among the FP, guava peels showed the highest in TPC (219572mg GAE/g), TFC (3036.78mg QEE/g) and DPPH (79.16%). Similarly, in hot water extraction. peanut skins showed the highest TPC (20576.40mg GAE/g), TFC (250.49mg QEE/g) and DPPH (82.14%) among CW. However, mango peels showed the highest TPC (30444.20mg GAE/g), TFC (1670.79mg QEE/g) and DPPH (78.40%) among FP. In overall, peanut skins, guava peels and mango peels were the potent antioxidant resources among cereal wastes and fruit peels.

Keywords: Antioxidants, cereal wastes, fruit peels, total phenolic content, total flavonoid content

1. Introduction

Crop wastes are residues of high organic load and usually derived from raw material processing to foodstuff result in the form of liquid or solid [1]. Large quantities of crop wastes such as cereal wastes and fruit peels are generated by agriculture annually have become the main issue for worldwide [2]. Disposal of crop wastes leading to environment pollution such as land, water and air pollution due to burning of wastes. In recent years, public has increasingly aware about the environmental issue and shortage for land filling, therefore by-products or waste utilization has become an alternative to disposal [3].

Nowadays, crop wastes are concerned as valuable and useful resources due to their abundance in beneficial minerals. Clearing of wastes does not justification for the potentiality to reuse them inside the food chain. Benefits of crop wastes was due to their polyphenolic compounds which is a group of secondary plant metabolites including flavonoids, phenolic acids, dihydrochalcones and others [4]. High concentration of polyphenols contains in the crop wastes make them to be utilized as the source of natural antioxidants. Phenolic compounds which commonly found in the plants as well as flavonoids are well known with the potential of antioxidant activities [5][6][7]. Antioxidants are important for human health and play an important role in against disease such as cancer, oxidative stress coronary heart disease and others [8].

Nowadays, public are increasingly concern on the importance of natural and non-chemical antioxidant product due to a lot of emerging products in the market. Apart from this, increase production of large quantities crop wastes from industries leading to environment problem. Hence, investigation of antioxidant activity of cereal wastes and fruit peels is needed. Therefore, the aim of this paper was to investigate and compare the antioxidant properties of cereal wastes and fruit peels.

2. Materials and Methods

2.1. Material

Rice bran (1kg) obtain from local grocery Chop Siong Ho shop (Banting, Selangor), wheat bran (500g) from Tai Wan Natural Organic Health Food shop (Kuantan, Pahang), dry corn (1kg) from Aeon Big supermarket (Kuantan, Pahang), raw peanuts (2kg) and mango from Tunas Manja supermarket (Kuantan, Pahang) and papaya and banana from local fruit stall at Gambang,Pahang and guava obtained from Giant supermarket (Kuantan, Pahang)

2.2. Chemical

Methanol (Industrial grade), acetone, chloroform, ethyl acetate, Folin Ciocalteau's reagent, sodium carbonate, gallic acid, aluminum chloride, potassium acetate, DPPH free radicals, quercetin, ferulic acid standard, ascorbic acid and methanol (HPLC grade).

2.3. Sample preparation

All samples need to be prepared except wheat bran. The rice bran was heated in the oven at 60°C for 10 minutes to removed moisture, dry corn was grinded into powder form by using electrical blender and peanut skins were removed by roasting raw peanuts using oven at 160°C for one hour. The hot peanuts were left to cool down and obtained the skins by rubbing off the peanut skins from peanut. The dry peanut skins were blended into powdery form. Each of the cereal wastes powder samples was stored in different glass bottles at 4°C.

The fresh ripe mango, papaya, banana and guava were peeling off by using knife. Each type of fruit peels was washed separately with water to removed dust and cut into smaller pieces. Mango peels was dried at 40°C for 24 hours, papaya peels was dried at 50°C for 24 hours, banana peels was dried at 50°C for three days and guava peels was blended into juice and freeze dried until it become powdery. Each of the powdery fruit peels sample was stored in different glass bottle at 4°C.

2.4. Optimized solvent extraction of cereal wastes

Two gram of rice bran was extracted with 40 ml of 80% for 24 hours at room temperature and 150 rpm [9]. The extracted was centrifuged at 4000 g for 15 minutes and subjected to rotary evaporation. The crude extract was stored at -20°C.

Two gram of wheat bran was mixed with 40 ml of methanol and vortexed [10]. The mixture was extracted in hot water bath at 60°C for 20 minutes and vortex twice during incubation. Then, centrifuged at 7000 rpm for 10 minutes and the supernatant were collected into a clean glass sample bottle. The residue was re-extracted with same volume of methanol and vortexed. The supernatant was combined and rotary evaporate. The crude extract was stored in the glass sample bottle at -20°C.

Two gram of dry corn powder was extracted by using 40 ml of methanol and vortexed to mix thoroughly [11]. The mixture was extracted in 60°C hot water bath for 20 minutes and vortexed twice during incubation. The extracted was centrifuged at 2000 rpm for 15 minutes and the supernatant was collected. The residue was re-extracted with same volume of methanol and vortexed. The supernatant was combined and rotary evaporate. The crude extract was stored at -20°C.

Two gram of peanut skins powder extracted with 40 ml of distilled water in volumetric flask for 24 hours in room temperature at 150rpm and wrapped flask with aluminium foil [12]. The mixture was vacuum filtered through 0.22 µm Nylon membrane. The filter water layer to remove protein and lipid to obtain a good base line [13]. The water extraction layer was mixed with an equal volume of chloroform. The aqueous and organic phases are separated using separation funnel. Through this process the lipid and lipid soluble compounds can be removed with chloroform and the water layer will contain the entire hydrophilic compound. The chloroform layer was discarded and the water layer was kept for ethyl acetate phase separation. The water layer was added with equal amount of ethyl acetate and repeated phase separation using separation funnel. The ethyl acetate layer and water layer was collected. After partition and phase separation the water layer was stored in a glass sample bottle at -20°C. The ethyl acetate was rotary evaporated and was stored in bottle at -20°C.

2.5 Optimized solvent extraction of fruit peels

Two gram of mango peel powder was extracted with 40 ml of 60:40 v/v methanols: water by centrifuged at 10000 g for 15 minutes [14]. The supernatant was collected and adjusted to 25 ml using distilled water and subjected to rotary evaporation. The crude extract was stored in the glass sample bottle at -20°C.

Two gram of papaya peels powder was extracted with 40 ml of 90 % acetone for 60 minutes, 150 rpm at room temperature [15]. The extract was filtered through Whatman No.1 filter paper and filter was collected. The residue was re-extracted by using 10 ml of 90% acetone and extract under the same condition. The extract was filtered and mixed with previous extract then subjected to rotary evaporation. The crude extract was stored in the glass sample bottle at -20°C.

Two gram of banana peels powder was extracted with 40 ml of 70 % acetone in hot water bath at 55°C for 120 minutes [16]. The tube was sealed with aluminum foil. The mixture was centrifuged at 6000 rpm for 15 minutes and subjected to rotary evaporation. The crude extract was stored at glass sample bottle at -20°C.

Two gram of guava peels powder was extracted with 40 ml of 60: 40 v/v methanols in hot water bath at 50°C for 120 minutes [17]. The mixture was filtered through muslim cloth to separate the residue and extract. The filter liquid was centrifuged at 4750 rpm for 15 minutes and subjected to rotary evaporation. The crude extract was stored in the glass sample bottle at -20°C.

2.6. Hot water extraction of cereal wastes

Two gram of each rice bran, wheat bran, dry corn and peanut skins was extracted with 40 ml of 80°C boiling deionized water in hot water bath for 20 minutes at 80°C. The mixture was allowed to cool

down and centrifuged at 5000 rpm for 10 minutes. The mixture was vacuum filter via 0.45 µm Nylon membranes and rotary evaporate. The crude extract was stored in the glass sample bottle at -20°C [18].

2.7. Hot water extraction of fruit peels

Two gram of each mango peels, papaya peels, banana peels and guava peels was extracted with 40 ml of 90 oC deionized water in hot water bath at 90°C, 100 rpm for 1 hour. The mixture was filter through Whatmann No.1 filter paper and the filtrate was rotary evaporated. The crude extract was stored in the glass sample bottle at -20°C [12].

2.8. Determination of total phenolic content (TPC)

TPC was determined using Folin Ciocalteu's (FC) colorimetric assay with minor modification [19]. In this assay, 10 µl of extract and 100 µl of FC reagent were mixed well and allowed it to stand for 5 minutes. The 80 µl of 7.5 % sodium carbonate (Na_2CO_3) solution was added into the mixture and mix thoroughly. The mixture was incubated in dark for 30 minutes at room temperature. The blank of FC assay was the reagent without sample extract. The absorbance was measured using microtiter reader at 750 nm. TPC were expressed in gallic acid equivalents of mg GAE/g.

2.9. Determination of total flavonoid content (TFC)

TFC was determined using aluminum chloride colorimetric assay with minor modification [20]. In this assay, 10 µl of extract, 60 µl of methanol, 10 µl aluminium chlorides (10% w/v), 10 µl of potassium acetate (1 M) and 120 µl of distilled water was mixed well and incubated at room temperature for 30 min in dark. The absorbance was measured using microtiter reader at 415 nm. TFC were expressed in quercetin equivalents of mg QEE/g.

2.10.Determination of DPPH scavenging activity

In this, 5 µl of extract was mixed with 195 µl of DPPH solution (0.1 mM DPPH in methanol) and incubated at 25°C for 10 minutes in dark [21]. The DPPH dissolved in methanol was used as control and methanol was used as blank. The absorbance was measured using microplate reader at 520 nm. The percentage of DPPH radical scavenging activity of the sample was calculated by following formula:

Scavenging Activity (%) = (OD of control - OD of test)/(OD of control ×100)

The results were expressed in ascorbic acid equivalents of mg AAE/g.

2.11. Statistical analysis

All the experiments were carried out in triplicate and the data was analyzed by using SPSS software with Tukey HSD method. The mean and standard deviations of TPC, TFC and DPPH scavenging activity were calculated and analyzed by ANOVA. The statistical probability level of $p<0.05$ was considered as significant.

3. Result and Discussion

3.1 Comparison of TPC by using optimized solvent extraction and hot water extraction

Figure 1 indicates that peanut skins contain significant amounts of total phenolic compounds (10861.00 ± 0.22 mg GAE/g to13685.70 ± 0.05 mg GAE/g) among the cereal wastes by using optimized solvent extraction. High phenolic content of peanuts skins maybe due to the degradation or polymerization of polyphenol of peanut skins which are more soluble in distil water and cause it more easily and effective in reaction with FC reagent [22]. Moreover, during the sample preparation of peanut skin, the roasted brown peels cause by the Maillard reaction may also contribute to the increase of total phenolic content in the peanut skins [12]. Therefore, due to all this reason peanut skins show potent in phenolic content.

Peanut skins also showed the highest phenolic content of 20576.40 ± 0.17 mg GAE/ g as compared to the other three cereal wastes by using hot water extraction. All the result show positive correlation.

In overall, extraction of total phenolic content by using hot water extraction was better than optimized solvent extraction. Optimum temperature for polyphenol extraction should be range from 80°C to 100°C [23]. In this research 80°C of boiling deionized water was used in hot water extraction. Therefore, hot water extraction produce higher phenolic content may due to the high temperature used.

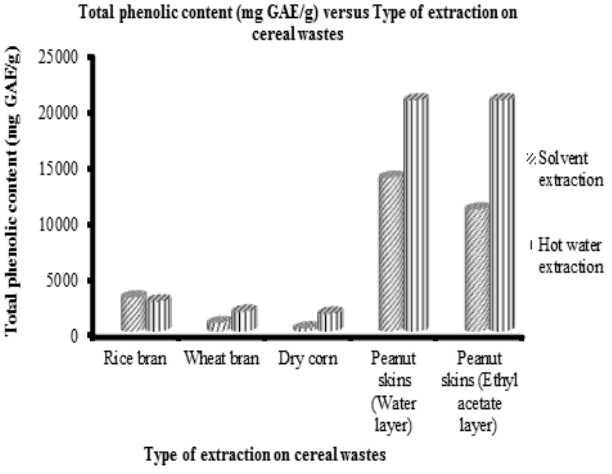

Figure 1: Total phenolic content against type of extraction methods on cereal wastes.

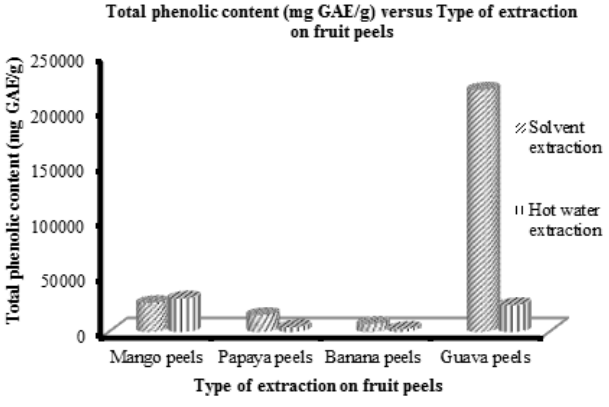

Figure 2: Total phenolic content against type of extraction methods on fruit peels.

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Based on Figure 2, guava peels showed the highest phenolic content of 219572 ± 0.06 mg GAE/ g in optimized solvent extraction. It also indicated a wide range of phenolic content between guava peels with other three fruit peels which contain lower amount of phenolic compounds. Phenolic compounds are the major contributor and found in abundance in guava peels [24]. Therefore, this compound make guava peels high potential in phenolic content among plant food [25].

However, mango peels showed the highest phenolic content with 30444.20 ± 0.21 mg GAE/g and followed by guava peels, papaya peels and banana peels in hot water extraction. The higher phenolic content in mango peels maybe due to the ripening stage [26] and sample preparation of peels [27]. Different ripeness stage of mango show different amount of phenolic content [26] and dried immediately is one ways to prevent degradation and microbial spoilage that affect the polyphenol content in the peels [27].

If for cereal waste, hot water extraction is better than solvent extraction, for fruit peels, it showed differently. Excellent result of solvent extraction cause by the properties of fruit peels more easily to be extracting by using polar solvent. Hot water extraction may cause some of the degradation of polyphenol in fruit residue and leading to low level of phenolic compounds produce [23].

3.2 Comparison of TFC by using optimized solvent extraction and hot water extraction

Figure 3 shows that rice bran and peanut skins (ethyl acetate layer) contain almost same high amount of flavonoids which is 604.51 ± 0.04 mg QEE/g and 635.69 ± 0.04 mg QEE/g in . It showed that flavonoids were present in all the cereal wastes. The better result of rice bran is due to size of bran used [28][29]. Rice bran with extremely small size or weight generally gave higher total phenolic content than those with large size [9]. Peanut skins indicated higher flavonoids content is due to some factors such as method of skin removal [30] and roasting time [31]. Roasting showed the better result in polyphenol including flavonoids content than direct peeled skin and exposure to heating improve the degradation of potent compounds into the extract solvent [32].

Peanut skins also showed the highest amount of flavonoid which was 250.49 ± 0.02 mg QEE/g among the cereal wastes in hot water extraction. From the result, it showed that flavonoids were present in all cereal wastes. Peanut skins with high flavonoids content are due to the solubility of phenolic compounds [33] and sample preparation [34]. Peanut skins water soluble extract containing flavonoids compounds and easily soluble in water. Besides that, the ways of peanut skin removal also have significant effect on the polyphenol content.

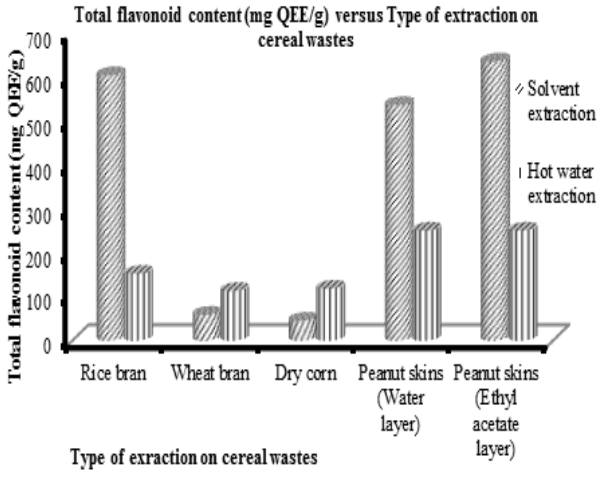

Figure 3: Total flavonoids content in cereal waste extracts using different extraction methods.

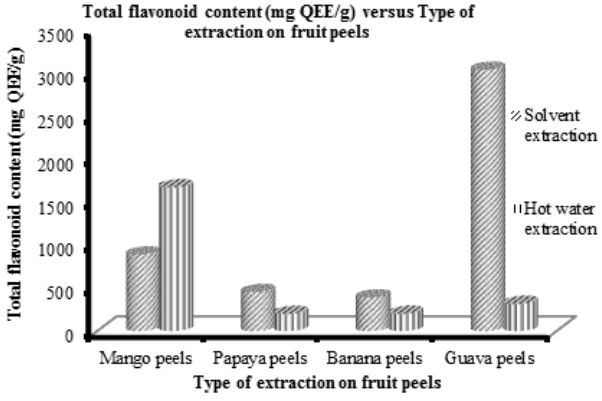

Figure 4: Total flavonoids content in the fruit peels extract using different extraction methods.

In Figure 4, guava peels showed the highest amount of flavonoids content of 3036.78 ± 0.05 mg QEE/ g. In this research guava peels was selected as the tested sample due to less research made on guava peels. Therefore, it proved that other than guava fruit, leaves, seed and bark, peels of guava was also potent in flavonoids content.

Among the fruit peels, mango peels indicated highest flavonoids content by using hot water extraction. Flavonoids compounds were present in all sample peels by using hot water extraction. Mango peel indicate higher flavonoids content maybe due the naturally abundance of polyphenol such as phenolic and flavonoid compounds [35]. The result obtained in this study was similar with previous study, claimed that mango peels shows highest flavonoids content [36].

In overall, total flavonoids content in cereal wastes and fruit peels were extracted best by using solvent extraction. Most plant extract was higher by using solvent extraction and lower by using water [37] and solvent polarity also plays an important role in phenolic compounds solubility and content [38]. Therefore, solvent extraction was better for cereal wastes in extract flavonoid compounds.

3.3. Comparison of DPPH by using optimized solvent extraction and hot water extraction

The scavenging activity percentage of cereal wastes is shown in Figure 5. All the cereal wastes showed ability of antioxidant inhibition. Peanut skins (water layer) indicated 79.06% (1708.97 ± 0.01 mg AAE/ g) and value for peanut skins (ethyl acetate layer) was 75.11% (1643.95 ± 0.02 mg AAE/ g) of scavenging activities. The high antioxidant activity of peanut skins was affect by several factors such as roasting of skins [12] and their nature abundance in polyphenol content [39].

Only rice bran and peanut skins displayed antioxidant activity in hot water extraction. Peanut skins indicated excellent result of 82.14% or 1551.29 ± 0.00 mg AAE/ g. Greater yield of phenolic compounds lead to high antioxidant activities and vice versa [40]. Wheat bran and dry corn indicated no antioxidant scavenging activity maybe due to the low concentration of phenolic acid including flavonoids that not strong enough to scavenging the free radical [41].

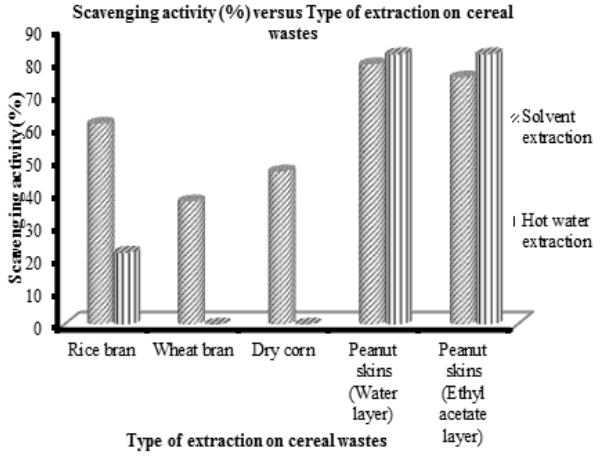

Figure 5. The comparisons of scavenging activity of cereal wastes extracted using solvent extraction and hot water extraction.

Among fruit peels (Figure 6), mango peels indicated highest scavenging activity of 79.16% or 1710.70 ± 0.00 mg AAE/g in solvent extraction. This result was similar with previous research on mango, result at the range of 61.74% to 91.57% [14].

Meanwhile, all the fruit peels indicated antioxidant activity and mango peels showed excellent result among the peels (78.40% or 1446.06 ± 0.02 mg AAE/ g) in hot water extraction. The drying effect of mango peels has relationship to the phenolic content of mango peels [42]. Large percentage of phenolic compounds including flavonoids bound to cellular structure capable to be release by heating treatment and easier to release bound phytochemical during extraction [36]. Hence, the drying effect has leading to the excellent antioxidant activity of mango peels.

In overall, scavenging activity of cereal wastes as well as fruit peels by using solvent extraction is higher compared to hot water extraction. This is maybe due to polarity of solvent and fruit characteristic [43]. Solvent polarity plays a key role in phenolic solubility and antioxidant activity [17][38]. When the polarity of solvent is higher, it will lead to increase phenolic solubility hence indirectly causing the antioxidant activities increase. Therefore, solvent extraction was performed better in cereal wastes and fruit peels scavenging activities.

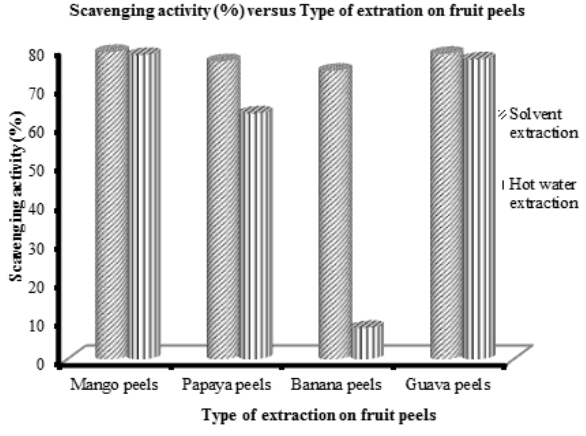

Figure 6: The scavenging activity of fruit peels by solvent extraction and hot water extraction.

4. Conclusion

Peanut skins were proven to become the most potential antioxidant resource among the cereal wastes follow by guava and mango peels. It was also found that antioxidant or scavenging activity was correlated with phenolic and flavonoids content in the crop wastes. It was also found that hot water extraction method was proven to show better result to extract phenolic compounds in cereal wastes and solvent extraction show better result in fruit peels. Flavonoids were excellent to be extract via solvent extraction. Whereas, solvent extraction was also shows excellent result than hot water extraction in scavenging activity.

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