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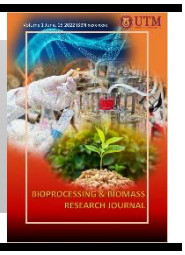

Research Article

Total Phenolic, Total Flavonoid and Antioxidant Activity of *Capsicum annuum* Seed and Pericarp Extracts

Muhammad Hafiz Ab Rahman[a], Yanti Maslina Mohd Jusoh[a], Zanariah Hashim[a,*]

[a] *Faculty of Chemical and Energy Engineering, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia*

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ABSTRACT

Capsicum annuum, a type of chilli, is a species that is extensively grown, a good source of bioactive chemicals, and an important component of diets that promotes health. Waste seeds from chilli processing have the potential to be used as an alternative source for antioxidants due to their chemical compositions, however, *C. annuum* seeds are still poorly studied. Thus, this study was carried out to evaluate the differences between *C. annuum* pericarp and seed extracts in terms of total phenolic and flavonoid contents and antioxidant activity. Antioxidant activity was measured using DPPH free radical scavenging assay. As a result, the pericarp extract exhibited a similar level of total phenolics (31.26 ± 6.71 µg GAE/mg dry weight) compared to the seeds (26.76 ± 2.08 µg GAE/mg dry weight, $p>0.05$), while a higher total flavonoid content was observed in the pericarp compared with the seed extract (21.65 ± 1.13 vs. 16.43 ± 0.37 µg QE/mg dry weight, $p<0.05$). Altogether, the seed extract demonstrated the same amount of DPPH radical scavenging activity as the pericarp extract, indicating its potential use as a source of phenolics or other chemicals rather than being discarded as waste. This study promotes sustainability and the conversion of waste product into valuable resources of that may be used in the nutraceutical, pharmaceutical and food industry.

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INTRODUCTION

Chilli is a plant that has been used extensively in medicine, spices, and flavoring. According to Berke et al. (2001), chilies from the *Capsicum* genus are among the world's oldest cultivated plants. This genus, which belongs to the nightshade family Solanaceae, has been endemic to Central and South America since pre-Columbian times. According to Ibiza et al. (2012), this genus currently includes 27 species, five of which are domesticated and utilized as fresh vegetables and spices, as well as approximately 3000 variations.

The five most frequently cited genera in the literature are *Capsicum annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens*. *C. annum* and *C. frutescens* were widely distributed from the New World to other continents by Spanish and Portuguese traders in the sixteenth century, and they quickly formed a fundamental component of the culinary habits of various countries, including Malaysia.

The dried, ripened red pod of *C. annuum* is known to contain pepper, which is used as a spice to flavour foods all around the world. Aside from that, chili is used as a flavoring and coloring ingredient, and it has ethnomedicinal properties. Numerous human diseases are treated using it. In Indian, Native American, and Chinese traditional medicine, *Capsicum* species have been used to cure rheumatism, arthritis, stomach aches, skin rashes, dog or snake bites, and lesions on the flesh.

Chillies such as *C. annuum*, *C. frutescens* and *C. chinense* are known for their antioxidant qualities (Hervert-Hernandez et al., 2010), and many compounds have been associated to this activity, including capsaicinoids and carotenoids (Deli et al., 2001), as well as phenolics and

*Corresponding Author
E-mail address: zanariah.hashim@utm.my
DOI address
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flavonoids, such as luteolin and quercetin (Howard et al., 2000; Marín et al., 2004; Materska and Perucka, 2005). Although mainly the flesh part or the pericarp of chillies is used for food and medicinal uses, the seeds are also rich in nutrition and beneficial compounds. Chili seeds contain a high concentration of bioactive chemicals, mainly phenolics and flavonoids that may act as health promoting agents with anti-inflammatory, antioxidant, anticancer, and antibacterial characteristics (Echave et al., 2020). In addition, flavonoids and phenolic acids have been described to possess the capacity to neutralize free radicals and reduce oxidative stress, which is a key root cause of numerous chronic diseases, including cardiovascular disease, diabetes, and cancer. According to recent studies, chili seeds which are usually discarded when making chili peppers, contain high concentrations of these healthy substances, including phenolic compounds like ferulic acid, chlorogenic acid, gallic acid, and caffeic acid which support the plant's strong antioxidant properties (Echave et al., 2020). Furthermore, flavonoids with anti-inflammatory, antibacterial, and anticancer properties, such as kaempferol, quercetin, and rutin, are found in chili seeds (Echave et al., 2020).

Despite several studies in the literature supporting the medicinal significance of chilli, it is worth noting that the chemical makeup and amount of distinct bioactivities are affected by the type of species, cultivar, and solvent studied, as well as the extraction method utilized in the experiments. So far, studies on the locally grown cultivar of *C. annuum* in Malaysia are limited, and further investigation is needed to appeal the use of local chillies. The Department of Agriculture Malaysia (2021) reports that 28,740 tons of *C. annuum* were produced in Malaysia in 2021 from 3,257 hectares of cultivation. Furthermore, up to 60% of the dry weight of the chilli comes from the seeds, which are frequently wasted as an agricultural byproduct during the production of chili peppers and chilli pastes (Siti Nadzirah et al., 2024). Finding useful applications for this waste stream not only helps to prevent environmental pollution, but it also contributes to a circular economy in which waste is transformed into valuable products. By extracting bioactive components from chili seed waste, the agricultural industry may turn a low-value byproduct into a valuable resource. Valorizing agricultural waste, such as chili seed waste, for the extraction of bioactive chemicals is a sustainable strategy for reducing the food industry's environmental impact while providing a steady supply of functional ingredients (Yasin et al., 2023).

Thus, in the present study, locally grown chilli of *C. annuum* was extracted according to the different parts, i.e. pericarp and seeds, to determine their total phenolic, total flavonoid and antioxidant activity. This study can provide proof for the long-term viability of waste-to-wealth practices in the chili processing industry.

MATERIALS AND METHOD

Sample Preparation

Fresh red chilli of *C. annuum* was obtained from a local supermarket (AEON Taman Universiti, Skudai, Johor, Malaysia). Meanwhile, methanol (99.9%) (molecular weight, MW=32.04 g/mol), 2,2-diphenyl-1-picrylhydrazil (DPPH) (MW=394.32 g/mol), Folin-Ciocalteu reagent (MW=239.27 g/mol), sodium carbonate (MW=105.98 g/mol), sodium nitrite (MW=68.99 g/mol), aluminium chloride (MW=133.34 g/mol), sodium hydroxide (MW=39.99 g/mol), quercetin (MW=302.23 g/mol), gallic acid (MW=170.12 g/mol) and ascorbic acid (MW=176.12 g/mol) were all purchased from Sigma-Aldrich (St. Louis, MO). The chilli was grown and packed by Monoluxury Sdn Bhd based in Genting Highland, Malaysia. It was immediately cleaned, seeds and pericarp separated, and dried at 60 °C using an oven the weight became constant. The dried seeds and pericarp were chopped and cut into pieces prior to grinding. The samples were ground into small and homogenous size using an immersion blender (Khind BH 600M, Selangor, Malaysia). **Figure 1** shows the chilli used in this study.

Figure 1 (a) *C. annuum* whole fruit, (b) pericarp and (c) seeds used in this study

Extraction Procedure

The extraction was conducted by using conventional Soxhlet extraction technique. Five grams (±0.05 g) of the plant sample (seed or pericarp powder) were weighed and inserted into a 500 ml round-bottom flask with 150 ml of methanol at 65 °C and extracted for 12 h. Next, the solution was placed in a rotary evaporator (BUCHI rotavapor, R-114, Switzerland) at 65 °C for 5 min to remove the solvent. Higher temperature was avoided to minimize the degradation of bioactive compounds. Samples were then stored at 4 °C in the refrigerator before further analysis.

Determination of Total Phenolic Content (TPC)

With some adjustments, the method outlined by Amin et al. (2006) was used to determine the total phenolic content (TPC). A final concentration of 1 mg/mL was obtained by dissolving 50 mg of dry extracts in 50 mL of methanol. 20 µL were then taken and combined with 100 µL of Folin-Ciocalteu reagent that had been diluted ten times. 80 µL of a 7.5% sodium carbonate solution were added to the mixture after 5 min. Methanol was present in the blank in place of the extract. The reaction mixtures were then let to sit at ambient temperature for 45 min. Finally, using a Bio-Tek ELX808 microplate reader, the absorbance was measured at 750 nm following a slow shaking. Gallic acid standard solutions in a range of values from 62.5 to 200 µg/mL were prepared. The results were averaged and expressed as gallic acid equivalents (µg-GAE/mg-dry mass of extract).

Determination of Total Flavonoid Content (TFC)

The measurement of total flavonoid content (TFC) was performed as described by Stankovic (2011). The solution of sodium nitrite 5% ($NaNO_2$) and aluminium chloride 10% ($AlCl_3$) were prepared by dissolving in a solvent mixed of methanol/water (50:50 v/v). Sodium hydroxide 4.3% (NaOH) was prepared using water as solvent. 50 mg of each extract dissolved in methanol/water (6/4). Working solution of

extract was at concentration of 200 ppm. To measure TFC, 1000 µL extract sample and 300 µL of $NaNO_2$ solution were placed in a test tube. After 5 min of standing, 50 µL of $AlCl_3$ was added, and it was incubated for 6 min before 500 µL of NaOH was added. Lastly, 10 µL of water were added to the test tube. The mixture was let to stand at room temperature for 30 min. Then, 200 µL of solution was pipetted into a 96-well plate, and the absorbance was read at 515 nm. Quercetin was used as the standard and the results were expressed as quercetin equivalents (µg-QE/mg-dry mass of extract).

Determination of Antioxidant Activity

With a few minor adjustments, the methodology described by Gurnani et al. (2016) was used to determine the antioxidant activity. Dry extract of pericarp and seeds (50 mg) was dissolved in 50 mL of methanol to a final concentration of 1 mg/mL. Next, 2 mL of a 0.004% methanolic solution of DPPH was combined with 2 mL of the extract solution at different concentrations (62.5-1000 µL). A spectrophotometer (Model UV-160, Shimadzu, Japan) was used to measure the absorbance at 517 nm after the mixture was vortexed for 1 min and left in the dark for 30 min at room temperature. Using the following formula, the crude extracts' capacity to scavenge free radicals was calculated.

$$\text{DPPH quenched (\%)} = \frac{\text{A0} - \text{A1}}{\text{A0}} \text{x100}$$

A0 represents the absorbance of the control, which is methanol substituted for the extracts, and A1 represents the absorbance of the sample. Ascorbic acid was used as positive control.

Statistical Analysis

Each measurement was carried out three times. To determine statistical significance, *p*-values were computed using the paired T-test.

RESULTS AND DISCUSSION

Extraction Yield

Figure 2 shows the extraction yields of seed and pericarp extracted using methanol as the solvent. It can be seen that the pericarp exhibited higher extraction yield, indicating that it has a higher content of compounds extractable using methanol and possibly higher amount of bioactive compounds.

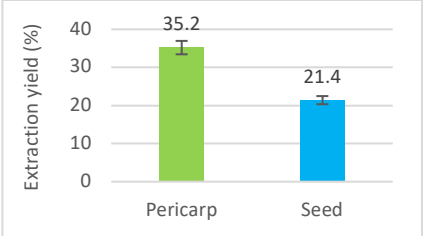

Figure 2 Percentage yield of crude extracts of the seed and pericarp of *C. annuum* extracted using methanol (*n*=3)

Total Phenolic and Total Flavonoid Contents of *C. annuum* Seed and Pericarp Extracts

Table 1 summarizes the total phenolic and flavonoid contents of *C. annuum* seed and pericarp extracts.

Table 1 Total phenolic and total flavonoid contents of extracts from chilli seed and chilli pericarp

	Total Phenolic (µg-GAE/mg-dry weight)	Total Flavonoid (µg-QE/mg-dry weight)
Seed	26.76 ± 2.08[a]	16.43 ± 0.37[a]
Pericarp	31.26 ± 6.71[a]	21.65 ± 1.13[b]
p-value	0.367	0.0016

Values are expressed as a mean of 3 replicates, different superscript letters indicate significant differences (p<0.05).

The total phenolic content (TPC) of chilli seed extract was found to be 26.76 ± 2.08 µg-GAE/mg-dry weight, whereas the total phenolic content of chilli pericarp extract was found to be 31.26 ± 6.71 µg-GAE/mg-dry weight, indicating that the pericarp extract has a slightly higher total phenolic content with no statistical difference. Such finding confirmed previous data reported by Chen et al. (2012) which indicated that the total phenols in the stalk and pericarp of chilli of *C. annuum* were higher than that of the seeds. Sim and Sil (2008) also stated that total phenolics in pericarp was higher than seed extracts. Because they can donate hydrogen, quench singlet oxygen, and function as metal chelators, phenolic compounds are secondary metabolites that have the potential to be antioxidants. Chillies' moderate to high phenolic acid content shows promise as a means of lowering the risk of degenerative illnesses.

The greater levels of phenolic compounds could have been explained by variations in the plant portion used and by variations in the varieties. It has been demonstrated that seeds from a variety of plant sources greatly contribute to the high total phenolic content. Marín et al. (2004), however, recently demonstrated a slight drop in the chilli's overall phenolic concentration as it matured from the green to the red stage. The primary factors influencing variations in phenolic content are plant maturity and age (Vallejo et al., 2003). The DPPH radical scavenging assay results show a close correlation between antioxidant activity and phenolic component levels, indicating that phenolic compounds are most likely what give chilli seeds and pericarp their antioxidant properties.

Similar to TPC, the total flavonoid contents (TFC) is accounted as an important parameter of antioxidant capacity of a plant. Here too, chilli pericarp demonstrated a higher flavonoid content of 21.65 ± 1.13 µg-QE/mg-dry weight, compared with chilli seeds with 16.43 ± 0.37 µg-QE/mg-dry weight (p<0.05). This result is consistent with previous data reported by Vera-Guzmán et al. (2017) which indicated that depending on the morphotype, landrace, and variation group, the flavonoid concentration of the chilli differed. Moreover, 23 flavonoids have been found and measured in the pericarp of chillies (*C. annuum* L. cv. Vergasa) using high-performance liquid chromatography. These consist of several C-glycosyl flavones as well as the O-glycosides of quercetin, luteolin, and chrysoeriol. Two most prevalent compounds accounting for 41% of the total flavonoids were identified, which were quercetin-3-O-rhamnoside and luteolin 7-O-(2-apiosyl-6-malonyl).

Antioxidant Activity of *C. annuum* Seed and Pericarp Extracts

The antioxidant activity of the chilli *C. annuum* extracts were measured by their scavenging activities against the DPPH radical. The results are shown in **Figure 3**.

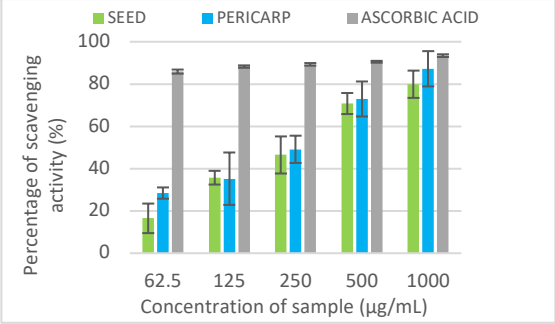

Figure 3 Percentage of DPPH scavenging activity of chilli *C. annuum* extracts

The chilli seed and chilli pericarp responded with the quenched DPPH radicals to varying degrees at all tested concentrations, with higher concentrations exhibiting greater activity. While not statistically significant, the scavenging activity of the chilli pericarp extracts was often higher than that of the chilli seed extracts. The scavenging activity of the chilli pericarp peaked at 87.22% at 1000 µg/mL, while for the chilli seed extract, it had a 79.96% scavenging effect at the same concentration. Consequently, based on these values, the IC_{50} values were determined, and were found to be 274.47 and 281.88 µg/mL for the pericarp and seed extracts, respectively (p>0.05). According to Deepa et al. (2006), the antioxidant activity of chillies fluctuated between 20% to 72% (% inhibition versus control) when measured by the DPPH radical. This variance could have resulted from variations in the amounts of reducing compounds (mostly phenolics) or in their efficacy.

Many studies reported that chilli was an effective free radical scavenger (Liu et al., 2020, Huei et al., 2020), and the same observation can also be seen in the chilli seed. In this study, although the DPPH radical scavenging activity of chilli seed extract of *C. annuum* was slightly lower compared to the chilli pericarp extract, but its percentage of scavenging activity was not relatively different with the pericarp extract. At 1000 µg/mL, there was only 9.07% difference of scavenging activity between the chilli seed and chilli pericarp extract.

The antioxidant properties of phenolic compounds in chili seeds are well-documented. Varieties of chillies are rich in phenolics and capsaicinoids and exhibited strong antioxidant activity (Liu et al., 2020, Azlan et al., 2022). Antioxidants counteract free radicals and lessen oxidative cell damage, which is connected to the emergence of a number of illnesses, including cardiovascular diseases and cancer, as well as neurological disorders. The findings of this study further affirm the nutritional and medicinal claims of chillies, particularly with regard to locally grown chilli variety and its waste seeds.

CONCLUSIONS

The use of chili seed waste as a source of bioactive chemicals like phenolics and flavonoids presents an opportunity to solve both sustainability and public health concerns. Chili seeds include bioactive compounds like flavonoids and phenolic acids, which give a variety of health advantages, including antioxidant, antibacterial, and anticancer activity. The extraction of these compounds from chili seed waste not only minimizes agricultural waste, but also helps to generate unique, sustainable bio-based products with substantial commercial and therapeutic applications. In this study, it was shown that waste chilli seeds from locally grown *C. annuum* contain a significant amount of phenolics and flavonoids, with the same level of DPPH scavenging activity compared with the pericarp extract. These results suggest that chilli seeds from the local market pose a great potential as a source of useful antioxidants. Using chili seed waste as a source of bioactive compounds, such as phenolics and flavonoids, has great potential for both the valorization of agricultural byproducts and the production of natural bioactive compounds with health benefits.

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