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Correlation of Antioxidant Properties Between Immature and Mature Okra (*Abelmoschus esculentus*) Fruits

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Abstract. This study aimed to assess and compare the antioxidant activity and content (total flavonoid levels and total phenolic) of mature and immature okra. The antioxidant activity of okra fruits was assayed using four methods, namely: Aluminium Chloride Colorimetric assay, Folin-Ciocalteu assay, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), and Reducing Antioxidant Power assay (FRAP) assays. The immature, mature, and very mature okra samples (less than 8 days, 10-15 days, and more than 20 days, respectively) were extracted using two different solvents (65% ethanol and water). The sample that was extracted with mature ethanol had the highest Total Phenolic Content (TPC) at 21.564 ± 1.635 mg GAE/g, while the sample that was extracted with extremely mature ethanol had the highest TFC at 54.391 ± 8.224 mg QE/g. The mature 65% ethanolic extracted sample showed the lowest IC_{50} value of DPPH scavenging activity (0.920± 0.096 mg/ml), and the mature ethanol extracted sample had the highest FRAP value (232.018± 5.337 µmol Fe2+/g). These studies showed that ethanolic extracts of mature Abelmoschus esculentus had higher antioxidant content and activity than okra water extracts. Based on the DPPH Radical Scavenging Assay revealed favourable associations between TPC (r = 0.860), TFC (r = 0.742), and antioxidant activity as evaluated by FRAP, demonstrating that both phenolics and flavonoids contributed to the extract's antioxidant properties. Both TPC and TFC showed negative correlations with IC_{50} values (r = -0.766, r = -0.650, respectively). In conclusion, the mature okra fruits extracted with 65% ethanol give higher antioxidant content than the water extracts of okra fruits and potentially be used as a source of antioxidants rather than be discarded.

Keywords: antioxidant activity, ethanol-water solvent, okra fruits, total flavonoid content, total phenol content

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1. Introduction

A molecule having one or more unpaired electrons in its valency shell that can exist on its own is known as a free radical [1]. Briefly, a substance known as free radicals is short-lived, unstable, and particularly reactive because of its odd number of electrons [1]. Elevated levels of free radicals and oxidants can result in oxidative stress, which can produce adverse reactions that can damage different parts of the cell for instance lipids, membranes, lipoproteins, proteins, and DNA [1]. As a result, oxidative stress occurs when there exists an inequality between cells' ability to eliminate those and free radical formation [2]. Antioxidants are molecules that can neutralize free radicals, which are found in plants, and can either stop or slow down the oxidation of lipids and other materials [3]. Since consumers are generally advised to increase their intake of food rich in antioxidants, there is greater demand for natural and plant-based products than for synthetic ones [3] – [4].

Abelmoschus esculentus L. (Moench) is commonly found in tropical and subtropical locations and is known for its high level of antioxidants. It is commonly consumed in fresh form for its edible juvenile okra, but not the mature okra, which is often thrown [5]. To reduce postharvest losses, okra must be picked at a suitable stage of maturity. Since the ideal time to harvest crops greatly affects the texture and taste of the fruit, people usually eat the soft, immature okra as a vegetable while discarding or drying the tough, mature okra to produce seeds to grow further seedlings [5] – [7]. *Abelmoschus esculentus L. (Moench)* is a flowering plant widely known as lady finger or okra [5], Significant antioxidants found in okra fruit, include high levels of vitamin C, carotenoids, and flavonoids, have medicinal effects on conditions like diabetes, hyperlipidemia, infections, ulcers, and neurological disorders. [5]. As claimed in some traditional human treatments, okra is a great source of antioxidants and may even be a chemo-preventive agent, according to [8]. Nevertheless, Extraction is the first step in isolating wanted goods from raw materials. The resulting extract outputs and subsequent antioxidant value of plant materials are substantially influenced by the solvent employed for extraction since there are multiple antioxidant molecules with varied chemical properties and polarity that may or may not be soluble in a given solvent. According to the law of similarity and impermissibility, it is expected that solvents having polarity values near those of the solutes are predicted to perform better, or vice versa. Selectivity, solubility, cost, and danger are just a few of the variables that need to be considered when choosing a solvent for antioxidant extraction. Universal solvents having polarity values like the polarity of the solute, such as ethanol and methanol, are typically used for the extraction of antioxidant studies since they are likely to work effectively [9]. Therefore, it is essential to identify the characteristics of antioxidant properties (such as antioxidant activities, TPC, and TFC) based on the use of ethanol and water as solvent extraction to gain the maximum yields.

2. Materials and Methods

2.1. Materials

The mature (10–15 days) and immature (more than 8 days) Okra were bought from a local market in Serdang, Selangor, Malaysia while the extremely mature (more than 20 days) okras were hand-picked from a garden in Selangor, Malaysia. A convenient sample approach was used for this study.

2.2. Experimental Design

For sample preparation, the mature, immature, and very mature fresh okra was brought to the Department of Nutrition Laboratory, Faculty of Medicine and Health Sciences, University Putra Malaysia. After being freeze-dried at -80°C for 48 hours, the okra was ground into a powder and sieved to produce uniformly sized particles. The mature, immature, and very mature okra powders were obtained by grounding them in a blender. The sample was then sieved and stored in different airtight containers. Water extraction with minor modifications of the ratio of sample and water was used for this study [10]. In short, 200 mL of distilled water was used to soften 10g of okra powder, and then 2 mL of chloroform was added to inhibit the growth of microbial cells. Following a 72-hour incubation period in the combination, the extract was filtered through a fine-mesh sieve, and a vacuum system was used to remove the solvent before being freeze-dried to get a solid consistency. A mixture containing approximately 10g of powdered okra and 100 mL of 65% ethanol was stored at the ambient temperature for 72 hours, then the extract was filtered using a Whatman no.1 filter paper. The solvent was then removed under a vacuum and the yield was freeze-dried using a rotary evaporator.

2.3. Determination of Total Phenolic Content

With slight modifications, the total phenolic content (TPC) was determined using a method by Kumar *et al.* [11]. To sum up, 2.5 milliliters of the Folin-Ciocalteu reagent (0.2 N) were mixed with 500 microliters with various extract concentrations. After 5 min, 2 mL of Napolysaccharide CO_3 solution (75 g/L) was added. After the mixture was placed in darkness for 120 minutes, the optical density was measured using an emi The TPC was calculated and presented as gallic acid equivalents (GAE) in mg GAE/g of material using the gallic acid curve of calibration.

2.4. Determination of Total Flavonoid Content

The total flavonoid content (TFC) was determined using an Aluminium Chloride Colourimetric Test as previously described by Kamtekar *et al.* [12] with slight modifications. In general, test tubes were first filled with 4 ml of distilled water, 0.3 mL of 5% sodium nitrite solution, 1 mL of aliquots or 1 ml of standard quercetin solution (100, 200, 400, 600, 800, and 1000 μg/mL), and allowed to stand for 5 minutes. Then, 0.3 mL of 10% aluminum chloride and 2 mL of 1 M sodium hydroxide were added. Then, 2.4 mL of distilled water was added to raise the total amount to 10 mL, and the entire mixture was properly mixed. After 15 minutes of incubation at room

temperature, the absorbance of each sample was measured at 510 nm. To calculate the total flavonoids, quercetin equivalents (mg QE/g dry mass) were utilized. The standard deviation of three replications was used to present the findings.

2.5. The Assessment of Antioxidant Activity

2.5.1. Diphenyl picryl hydrazyl (DPPH) free radical scavenging assay

The Blois technique described by Hu *et al.* [13] was modified a bit to assess DPPH radical scavenging activity. About 0.1 mL of the experimental sample was mixed using 2.9 mL of the DPPH solution (previously prepared), giving it a good shake, vigorously, and allowing it to sit at room temperature in the dark for half an hour, the mixture was then allowed to settle. The working reagent in this investigation was 0.1 mM DPPH while methanol acted as the negative control and ascorbic acid was synthesized as the standard reference antioxidant. The following formula was used to calculate the extracts' antioxidant activity for DPPH free radical scavenging; the results are reported as a percentage of inhibition.

$$\text{Scavenging activity (\%)} = [(Abs^{control} - Abs^{sample}) / Abs^{control}] \times 100$$

Here, Abs^{sample} = the absorbance in the presence of an okra extract, and $Abs^{control}$ = the absorbance of the control reaction (without okra extract).

2.5.2. Ferric ion reducing antioxidant power (FRAP) assay

To test antioxidant activity, the FRAP assay was performed using the iron reduction technique proposed by Wahyuningsih *et al.* [14]. Freshly prepared FRAP reagent consisted of 20 mL of 300 mM pH 3.6 acetate buffer, 2 mL TPTZ solution (10 mM TPTZ in 40 mM HCl), and 2 mL FeCl3 (20 mM) in aqueous solution. An aliquot of about 50 µL okra extract was combined with 1 mL of FRAP reagent and 450 µL H20, and it was incubated in a water bath at 37°C for 30 minutes. Next, the intensity of absorption was determined with a UV/Vis microplate spectrophotometer. (Thermo Scientific TM Multiskan TMGO). Ferrous sulfate (100–2000 µM) was used to construct the calibration curve, and the results were expressed in µmol Fe2+/g.

2.6. Statistical Analysis

Every statistical analysis was carried out by IBM SPSS Statistics 26. The experiment's mean ± standard deviation was used to express the results. At a significant threshold of 0.05, significant differences between the means were determined. Pearson's correlation analysis was used to examine the association between antioxidant activity, TPC, and TFC.

3. Results and Discussion

3.1. Total Phenolic Content (TPC)

Different *Abelmoschus esculentus L.* mature extracts showed different TPC values. According to Table 1, the results were arranged in descending order: mature ethanol extract (21.56 ± 1.64 mg GAE/g) > immature ethanol extract (8.73 ± 0.08 mg GAE/g) > very mature ethanol extract (4.120 ± 0.420 mg GAE/g) > mature water extract (2.57 ± 0.16 mg GAE/g) > immature water extract (0.80 ± 0.08 mg GAE/g) > very mature water extract (0.73 ± 0.05mg GAE/g) (Table 1). The TPC values of the immature and mature okra that were determined using 65% ethanol and water showed statistically significant differences ($p>0.05$). While TPC in very mature water extract (>20 days) and immature water extract (<8 days) did not differ significantly ($p>0.05$).

In comparison to the water extracts of okra, the ethanolic extract demonstrates a greater TPC. The roles that ethanol and water play in the extraction of phenolic compounds are distinct, such as ethanol breaks down the solute's polyphenolic linkages, water plays a role in the solute's dispersion. Since ethanol increases solvent penetration into the solute, mass transfer is promoted [15], [16]. The extract of ethanol of the fully mature (10–15 days) showed the highest TPC, while the immature (<8 days) extract exhibited the lowest. Accordingly, the TPC increased with ripening but dropped after 15 days. Mphahlele *et al.* [17] reported in another study that different fruits may not develop at the same time, resulting in differences in the phenolic compounds examined. In this research, we discovered that, except for mature water extracts (10–15 days), compared to all other mature and immature okra, the TPC in highly mature okra ethanol extract (>20 days) was significantly different.

Table 1. Phenolic Content of Okra Extracts Expressed as Gallic Acid Equivalence (GAE)

Sample	Phenolic content (mg GAE/g)			Mean contents (Mean ± SD)
	1	2	3	
Very mature ethanol extract (>20 days)	3.72	4.56	4.09	4.12 ± 0.42^{a}
Very mature water extract (>20 days)	0.73	0.79	0.68	0.73 ± 0.05^{b}
Mature ethanol extract (10-15 days)	20.17	23.36	21.16	21.56 ± 1.64^{c}
Mature water extract (10-15 days)	2.57	2.72	2.41	2.57 ± 0.16^{a}
Immature ethanol extract (<8 days)	8.64	8.80	8.75	8.73 ± 0.08^{d}
Immature water extract (<8 days)	0.89	0.73	0.79	0.80 ± 0.08^{b}

3.2. Total Flavonoid Content (TFC)

The TFC was demonstrated in descending order for various maturities of the Okra extract, where the highest value was obtained for very mature ethanol extract (54.39± 8.22 mg QE/g) and the lowest value was found for immature water extract (4.36 ± 1.19 mg QE/g) (Table 2). The TFC in the ethanol extract of very mature okra (>20 days) differs significantly from all other mature and immature okra in both ethanol and water extracts, determined by a one-way ANOVA ($F(5,12) =$

71.336, $p<0.01$). In contrast, there was no statistically significant difference ($p>0.05$) in TFC between the immature ethanol extract and the mature water and ethanol extracts. Furthermore, there was no apparent difference in TFC among the immature and highly mature water extract. In the case of ethanol extract, TFC increased as okra gained maturity, from immature to very mature. In this research, we observed that ethanolic extraction was more successful than aqueous extraction as, in 65% of cases, ethanolic extracts had higher TFC than water extracts. It indicates that okra flavonoid mix is higher in organic solvents such as ethanol than in solutions of water [18]. Vuorinen *et al.* [19] found that the content of flavanol glycosides in black currants rose dramatically as the fruit matured. According to their variable oxidation susceptibilities after ripening, flavonol modifications may be related to the total flavonoid concentration in grapes and berries. Like TPC, the water extract rises with maturity and decreases after day 15. Our findings are consistent with [20], who found that flavonoid levels in *A. beccariana* leaves increased considerably from immature to mature leaves until decreasing from mature to older leaves.

Table 2. Flavonoid Content of Extract Okra Expressed as Quercetin Equivalence (QE)

Sample	Flavonoid content (mg QE/g)			Mean contents (Mean ± SD)
	1	2	3	
Very mature ethanol extract (>20 days)	46.17	62.62	54.39	54.39± 8.22a
Very mature water extract (>20 days)	11.21	7.10	5.30	7.79 ± 3.14b
Mature ethanol extract (10-15 days)	29.72	31.77	29.72	30.40 ± 1.19c
Mature water extract (10-15 days)	17.38	21.49	19.44	18.07 ± 1.19d
Immature ethanol extract (<8 days)	21.49	21.49	19.44	20.81 ± 1.19cd
Immature water extract (<8 days)	2.99	5.05	5.05	4.36 ± 1.19b

3.3. Antioxidant Activities Using DPPH Radical Scavenging Activity Assay

The IC_{50} values for okra extracts are presented in descending order, with the mature ethanol extract (0.92±0.10 mg/mL) having the lowest value and the extremely mature water extract (5.30 ± 0.20 mg/ml) containing the highest value (Table 3). One-way ANOVA ($F(5,12) = 795.717$, $p<0.01$) demonstrated significant differences in IC_{50} values between mature and immature okra extracted using 65% ethanol and water. Compared to the water-extracted samples, the mature okra extracted with 65% ethanol has the lowest IC_{50} value (0.920± 0.096 mg/ml), suggesting the best activity of antioxidant since a lower concentration is required to diminish the DPPH free radical by 50%. Yet between immature okra to mature okra to very mature okra, the antioxidant properties of water-extracted samples decreased. The oxidation of the bioactive molecules may be the reason for the decrease in antioxidant activity during withering [21]. Our findings are in align with [22], who published comparable findings from their research and suggested that binary

solvent systems were more effective than mono solvent systems for the extraction of antioxidant compounds. One notable constraint for evaluating the influence of hydrophilic antioxidants is that the antioxidant molecule used in the DPPH experiment was more easily soluble in organic solvents, especially ethanol than in aqueous solutions. Alcoholic solvents increase the interaction of DPPH radicals with antioxidants contained in the sample. Extracts derived from alcohol are expected to demonstrate greater antioxidant activity than water extracts [23].

Table 3. The IC_{50} Values of Okra Extracts

Sample	IC_{50} values (mg/mL)			Mean contents
	1	2	3	(Mean ± SD)
Very mature ethanol extract (>20 days)	2.00	1.80	1.90	1.90 ± 0.10^{a}
Very mature water extract (>20 days)	5.50	5.10	5.30	5.30 ± 0.20^{b}
Mature ethanol extract (10-15 days)	0.96	0.99	0.81	0.92 ± 0.10^{c}
Mature water extract (10-15 days)	4.70	4.54	4.56	4.60 ± 0.09^{d}
Immature ethanol extract (<8 days)	1.05	1.04	1.05	1.05 ± 0.01^{c}
Immature water extract (<8 days)	4.21	4.13	3.96	4.10 ± 0.13^{e}

3.4. Antioxidant Activities Using FRAP Assay

The FRAP values of various extracts of the okra sample are listed in Table 4. The highest value was obtained for very mature ethanol extract (232.02± 5.34 µmol Fe^{2+}/g), and the lowest value was obtained for very mature water extract (71.92± 2.67 µmol Fe^{2+}/g). The findings of a one-way ANOVA test showed that there were significant variations in the FRAP values between mature and immature okra that were extracted with water and 65% ethanol ($F(5,12) = 975.807$, $p<0.01$). The FRAP value of both the ethanolic and water extracts increases as the okra matures, but gradually falls after the 20th day. This result was similar to the prior investigation published by Ding and Syazwani [24], who found that the pineapple's antioxidant activity, as determined by the FRAP test, increased from stage 1 to stage 3, whereas water extract dropped from stage 4 to stage 5. Increasing then decreasing patterns in the FRAP value with maturity were also reported by Assanga *et al.* [25]. This research's findings are validated by another investigation, which found that employing 60% ethanol as the extraction solvent yields the highest antioxidant activity [26]. Yuon *et al.* [26] also showed that the level of antioxidant activity increases with the FRAP value. The mature okra has a higher level of antioxidant properties, demonstrated by the high FRAP values for mature ethanol and water extracts.

Table 4. The FRAP Values of Okra Extracts

Sample	FRAP values (µmol Fe^{2+}/g)			Mean contents (Mean ± SD)
	1	2	3	
Very mature ethanol extract (>20 days)	165.31	186.66	173.32	176.87 ± 4.08[a]
Very mature water extract (>20 days)	74.59	71.92	69.25	71.92± 2.67[b]
Mature ethanol extract (10-15 days)	237.35	232.02	226.68	232.02± 5.34[c]
Mature water extract (10-15 days)	106.61	101.27	103.94	103.94 ± 2.67[d]
Immature ethanol extract (<8 days)	125.29	125.29	122.62	124.40 ± 1.54[e]
Immature water extract (<8 days)	87.93	85.26	82.59	85.26 ± 2.67[f]

3.5. Correlation Between Antioxidant Capacity and Antioxidant Content

The Pearson correlation test was used to examine the relationship between antioxidant concentrations and antioxidant capacity (Table 5). There were strong and negative correlations between DPPH radical scavenging with TPC ($r = -0.766$, $p<0.01$) and TFC ($r = -0.650$, $p<0.01$); both were statistically significant. Instead, strong and positive correlations were found between FRAP assay with TPC ($r = 0.860$, $p<0.01$) and TFC ($r= 0.742$, $p<0.01$). In this study, the TFC and antioxidant activity by FRAP assay showed a significant positive relationship, which is in contrast with [27] – [28]. While in DPPH radical scavenging capability, TPC and antioxidant capability exhibited a greater negative correlation. These findings are consistent with earlier research [29] – [30]. Our findings demonstrated that the TFC and antioxidant activity by DPPH assay of plant extracts presented a significant correlation as previously documented by Nurcholis *et al.* [31].

Table 5. Pearson Correlation Between Antioxidant Content and Activities of *Abelmoschus esculentus L. (Moench*)

Antioxidant activities	Total phenolic content		Total flavonoid content	
	r-value	p-value	r-value	p-value
DPPH Radical Scavenging Assay (IC_{50} values)	-0.766**	0.000	-0.650**	0.004
Ferric Ion Reducing Antioxidant Power Assay (FRAP value)	0.860**	0.000	0.742**	0.000

4. Conclusion and Recommendation

The purpose of this research was to determine *Abelmoschus's Esculentus L.* antioxidant capacity and content in two solvent types and maturity stages. In this study, among the two solvents, 65% ethanolic extracted higher TFC and TPC than water extract. In this research, we observed that, except for mature water extracts (10–15 days), The amount of TPC in extremely mature okra extracted with ethanol (>20 days) was significantly different from the rest of mature and immature okra. The Pearson correlation analysis displayed a strong but negative relationship between the TPC and the values of IC_{50} ($r = -0.766$). Similarly, there is a somewhat unfavourable relationship

with the total flavonoid concentration and the IC_{50} values (r = -0.650). Moreover, FRAP evaluations of antioxidant capacity showed a favorable connection with TPC (r = 0.860), TFC concentration (r = 0.742), and antioxidant capacity. This strong correlation additionally indicates that increasing phenolic and flavonoid concentration may result in greater antioxidant activity. When the water content increased in organic solvents, the TPC, TFC, and antioxidant activity frequently decreased. Compared to other kinds of solvents, the water extract may include an increased amount of non-phenolic compounds or phenolic compounds with less active groups. Mature and extremely mature okra has the highest amounts of phenolic, flavonoid, and antioxidant activity. Accordingly, our study indicates that mature and very mature okra can be fully processed and used as one of the easily accessible sources of natural antioxidants rather than thrown away.

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