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Research Article

Total Phenolic Content, Total Flavonoid Content and Antioxidant Activity of Various Solvent Extracts from the Peels and Seeds of 'Marang' (*Artocarpus odoratissimus* Blanco)

Beatriz Danica Carating[1] and Nesteve John Agosto[1,2*]

[1]Department of Chemistry, University of Science and Technology of Southern Philippines, 9000, Cagayan de Oro City, Philippines
[2]Center for Natural Products Research, University of Science and Technology of Southern Philippines, 9000, Cagayan de Oro City, Philippines
*Corresponding author: nestevejohn.agosto@ustp.edu.ph

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ABSTRACT

Artocarpus odoratissimus, locally known as "marang," is a tropical fruit native to Mindanao, Philippines. As an underutilized fruit, it remains understudied and holds significant unexplored potential for drug discovery and pharmaceutical applications. This study evaluates the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity of *A. odoratissimus* peel and seed crude extracts obtained from using nonpolar and polar solvents. Results showed that the ethanol peel extract had the highest TPC of 129.26 mg GAE/g, while the aqueous peel extract had the highest TFC of 68.95 mg QE/g. Moreover, the aqueous peel extract exhibited very strong antioxidant activity (EC_{50} = 0.05 µg/mL). Overall, a general trend was observed showing that polar extracts exhibit higher TPC, higher TFC, and stronger antioxidant activities compared to the nonpolar extracts. These findings highlight the importance of selecting appropriate solvents to efficiently extract bioactive compounds, especially for maximizing antioxidant properties. Future research should investigate the aqueous peel extract, given its very strong antioxidant activity, which is more potent than that of ascorbic acid (EC_{50} = 6.84 µg/mL), and identify and characterize the specific bioactive compounds responsible for this potent activity.

Keywords: antioxidant activity; *Artocarpus odoratissimus*; peels and seeds; total flavonoid content; total phenolic content

1. INTRODUCTION

The increase in chronic illnesses continues to pose a substantial global healthcare issue. In the Philippines, conditions such as diabetes, cardiovascular diseases, cancer, and chronic respiratory diseases are among the leading causes of death. The World Health Organization (2019) reported that these diseases account for 68% of all deaths and carry a 29% risk of premature mortality before the age of 70. Given this evidence, the impact of these diseases is undeniably alarming. Oxidative stress has been extensively studied in relation to the development of various diseases. Research indicates that it plays a critical role in the pathogenesis of chronic diseases. Reuter et al. (2010) provided evidence linking oxidative stress

to the onset of metabolic disorders, chronic conditions, and cancer. Oxidative stress arises when excessive free radicals are produced in the body's cells, leading to an imbalance between antioxidants and oxidants (Pizzino et al., 2017). Free radicals are naturally generated through essential metabolic processes or from external sources such as radiation exposure, pollutants, and cigarette smoking. The body's antioxidant defenses help neutralize these harmful molecules by preventing their formation and interrupting chain reactions involving them (Sharifi-Rad et al., 2020). However, when natural antioxidant defenses are insufficient, supplemental antioxidants are often taken to counteract the harmful effects of free radicals.

These supplemental antioxidants are widely used due to their stability, performance, cost, and availability. However, concerns regarding the safety of synthetic antioxidants have emerged over time, as several studies have shown a direct correlation between their intake and various health issues. A study investigating the impact of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PG) on the endocrine system of female rats suggests that these substances have adverse effects, with PG exhibiting the most pronounced impact (Pop et al., 2013). Another study provided evidence that BHA stimulates reactive oxygen species (ROS) production, contradicting its intended antioxidant function (de Oliveira Pateis et al., 2018). The adverse consequences of synthetic antioxidants have prompted researchers to increasingly explore natural antioxidants as safer alternatives with minimal to no harmful effects.

The tradition of using plants to treat various illnesses has been practiced for a long time. It is estimated that over 80% of the global population residing in developing nations relies primarily on plants as remedies for basic healthcare needs (Ekor, 2014). Plants are a rich source of both food and medicinal compounds. Consuming antioxidant-rich foods has been shown to prevent oxidative stress and reduce the risk of developing diseases associated with oxidative damage. The antioxidant properties of plants are attributed to their abundance of phytochemicals, particularly polyphenols. Polyphenolic compounds exhibit strong electron-donating capabilities due to the presence of hydroxyl groups, which directly contribute to their antioxidant activity (Bendary et al., 2013). Among plant species, the genus *Artocarpus* (Moraceae) is recognized for its exceptional medicinal value and has been utilized as traditional folk medicine in several parts of the world. The substantial body of literature discussing the use of *Artocarpus* species in folkloric medicine has drawn considerable scientific interest to this genus (Jagtap & Bapat, 2010). However, despite extensive research, some *Artocarpus* species remain underexplored.

Artocarpus odoratissimus Blanco, locally known as "marang," is native to Mindanao, Philippines, yet it has not received the recognition and utilization it truly deserves, remaining both underrated and underused. Despite being an underutilized fruit, *A. odoratissimus* has the potential to offer significant medicinal benefits. Studies have shown that *A. odoratissimus* extracts exhibit potent antioxidant, anti-inflammatory, and antimicrobial activities due to their abundance of polyphenolic compounds. In a previous study, methanol seed extracts of *A. odoratissimus* demonstrated higher phenolic and flavonoid content, as well as greater antioxidant activity, compared to its methanol pulp extract (Bakar et al., 2009). Another study investigating the methanol peel, pulp, and seed extracts of different *Artocarpus* species, such as *A. integer*, *A. kemando*, and *A. odoratissimus*, revealed that the *A. odoratissimus* peel extract contained higher amounts of phenolic and flavonoid compounds, as well as greater antioxidant activity, compared to the other methanol extracts (Bakar et al., 2015). Additionally, the ethanol peel, pulp, and seed extracts of *A. odoratissimus* demonstrated antidiabetic activity via alpha-glucosidase inhibition and tested positive for the presence of phenolics and flavonoids (Jonatas et al., 2020).

These recent studies suggest that *A. odoratissimus* could serve as a potential source of lead compounds for drug discovery and pharmaceutical development. However, there is still

limited research on the variability of phytochemical composition, including phenolic and flavonoid content, among different parts (peels and seeds) of *A. odoratissimus* fruit, particularly when considering the effect of extraction solvents with varying polarity. Therefore, in this study, we specifically evaluated the total phenolic content, total flavonoid content, and antioxidant activity of the hexane, ethyl acetate, ethanol, and aqueous extracts of both peels and seeds of *A. odoratissimus*.

2. MATERIALS AND METHODS

2.1. Sample collection, preparation and extraction

The young fruits of *Artocarpus odoratissimus* were collected from Barangay Laturan, Libona, Bukidnon, Philippines, in February 2024 and were then authenticated at the Center for Biodiversity Research and Extension in Mindanao, Central Mindanao University, Musuan, Bukidnon, Philippines. The fruits were thoroughly rinsed with water, after which the peels and seeds were separated. The peels and seeds were cut into smaller pieces and dried in an oven at a maintained temperature of 40°C for three days until completely dry. The dried samples were then powdered using a mechanical grinder. The method of sample extract preparation followed Jonatas et al. (2020) with some modifications. Sixty grams of powdered samples were extracted via maceration at room temperature using four different solvents of varying polarity (Table 1): *n*-hexane, ethyl acetate, 95% ethanol, and distilled water, at a 1:10 w/v ratio for three days with occasional stirring. After maceration, the mixtures were filtered using Whatman filter paper No. 1. The hexane, ethyl acetate, and ethanol extracts were concentrated *in vacuo* using a rotary evaporator at 40°C, while the aqueous extract was subjected to freeze-drying. The crude concentrated peel and seed extracts were then stored in a refrigerator for further analysis.

Table 1. Polarity index of the extraction solvents used

Solvent	Polarity Index	Category
Hexane	0.1	Nonpolar
Ethyl acetate	4.4	Moderately polar
Ethanol	5.2	Polar
Water	10.2	Polar

2.2. Determination of total phenolic content (TPC)

TPC was determined using the Folin-Ciocalteu method (Castro and Lomonsod, 2021) with modifications. An aliquot of 1.5 mL of the crude extracts (1000 µg/mL prepared in 1% DMSO) or gallic acid standard (3.91–250 µg/mL prepared in 1% DMSO) was dispensed into 20 mL test tubes. Subsequently, 7.5 mL of 10% Folin-Ciocalteu reagent and 6 mL of 7.5% sodium carbonate solution were added to each tube. The reaction mixtures were then incubated at room temperature for 30 minutes. Following incubation, the absorbance was measured at 755 nm against the blank using a UV-Vis spectrophotometer. All analyses were carried out in triplicate. The TPC of the extracts was determined from the gallic acid standard calibration curve equation, $y = 0.0133x + 0.0561$, with $R^2 = 0.9986$, and was expressed in milligrams of gallic acid equivalents per gram of extract (mg GAE/g).

2.3. Determination of total flavonoid content (TFC)

TFC was determined using the aluminum chloride colorimetric method (Agosto, 2020) with modifications. An aliquot of 2 mL of the crude extracts (1000 µg/mL prepared in 1%

DMSO) or quercetin standard (3.91–250 µg/mL prepared in 1% DMSO) was initially dispensed into 10 mL test tubes. Subsequently, 6 mL of 1% DMSO solution was added, followed by the addition of 0.4 ml of 10% aluminum chloride solution and 0.4 mL of a 1M potassium acetate solution. The resulting reaction mixtures were incubated at room temperature for 1 h. Following incubation, absorbance was measured at 430 nm against the blank using a colorimeter. All analyses were conducted in triplicate. The TFC of the extracts was determined using the quercetin standard calibration curve equation, $y = 0.0013x + 0.0087$, with $R^2 = 0.9987$, and was expressed in milligrams of quercetin equivalents per gram of extract (mg QE/g).

2.4. Determination of antioxidant activity

The antioxidant activity was determined using the DPPH radical scavenging assay (Agosto, 2020) with modifications. A 0.2 mM DPPH solution was prepared in methanol and stored in the dark for 2 h. Then, 3 mL of this DPPH solution was mixed with serially diluted concentrations (7.81–1000 µg/mL) of each extract in 10 mL test tubes. DPPH in methanol without the extracts was used as the control. The reaction mixtures were incubated at room temperature for 30 minutes in the dark. After incubation, the absorbance was measured at 520 nm using a colorimeter. The percentage of DPPH radical scavenging activity (%RSA) was calculated using Eq. 1:

$$\%RSA = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \qquad \text{Eq. (1)}$$

where $A_{control}$ represents the absorbance of the control reaction and A_{sample} represents the absorbance of the extract. The effective concentration (EC_{50}) of sample required to scavenge DPPH radical by 50% was calculated by plotting %RSA versus concentration. All analyses were conducted in triplicate.

2.5. Statistical analysis

Results were expressed as mean ± standard deviation. Data were analyzed using two-way analysis of variance (ANOVA), followed by Tukey's test to determine significant differences among the means. Pearson's correlation analysis was performed to assess relationships between variables. Statistical significance was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Total phenolic content (TPC)

The present study quantifies the TPC of various solvent extracts from the peels and seeds of *A. odoratissimus*, as presented in Table 2. Based on the results, the total phenolic content varied among the different extracts, which, in decreasing order, were as follows: ethanol peel > aqueous peel > ethanol seed > aqueous seed > ethyl acetate peel > hexane seed > ethyl acetate seed > hexane peel. The classification of TPC values as high, medium, or low depends on various factors, such as the plant part, extraction method, and solvent type. Hence, this study followed the categorization established by Rufino et al. (2010), defining low TPC as <10 mg GAE/g crude extract, medium TPC as 10–50 mg GAE/g, and high TPC as >50 mg GAE/g. The type and polarity of the solvent influence the extract's quality, yield, and biological activity. This study employed four solvents with varying polarities to extract phenolic compounds from *A. odoratissimus* peels and seeds. The results demonstrated that solvent polarity significantly affected the extracted phenolic content, ranging from 0.52 ± 0.15 mg GAE/g to 129.27 ± 0.56

mg GAE/g. Extracts obtained using polar solvents exhibited higher TPC, with ethanol and aqueous peel extracts containing 129.27 ± 0.56 mg GAE/g and 107.01 ± 0.71 mg GAE/g, respectively. Additionally, peel extracts generally had higher TPC than seed extracts.

Table 2. Total phenolic content of the extracts

Extract	Part	TPC (mg GAE/g)	Classification
Hexane	Peel	0.52 ± 0.15^{f}	Low
	Seed	0.90 ± 0.45^{f}	Low
Ethyl acetate	Peel	8.60 ± 0.75^{e}	Low
	Seed	0.62 ± 0.23^{f}	Low
Ethanol	Peel	129.26 ± 0.56^{a}	High
	Seed	56.68 ± 0.30^{c}	High
Aqueous	Peel	107.01 ± 0.72^{b}	High
	Seed	16.48 ± 0.83^{d}	Medium

Different superscript letters indicate significant differences at $p < 0.05$

In most cases, TPC of the extracts increased with solvent polarity, with some exceptions. The four extracts with high to medium TPC (ethanol peel, aqueous peel, ethanol seed, aqueous seed) were all derived from polar solvents, whereas the extracts with low TPC came from moderately polar and nonpolar solvents like ethyl acetate and hexane. These findings align with those of Herrera-Pool et al. (2021), who also observed an increase in phenolic content with increasing solvent polarity. Since phenolic compounds are typically polar, they are more efficiently extracted using highly polar solvents. Although the general trend suggests a positive correlation between solvent polarity and TPC, exceptions may arise due to the selectivity and solubility of different phenolic compounds. Notably, despite water being more polar than ethanol, the ethanol extract exhibited a higher TPC. This observation aligns with Yusof et al. (2020), who reported that an 80% ethanol-water mixture yielded a higher TPC than a more polar 20% ethanol-water mixture. This may be attributed to ethanol's ability to dissolve a broader range of phenolic compounds due to its amphiphilic nature. Ethanol's molecular structure allows it to interact with both polar and less polar compounds, leading to greater phenolic extraction. Additionally, ethanol-water mixtures may provide a more favorable extraction environment than pure ethanol.

Several studies have shown that phytochemical content is generally higher in fruit peels and seeds than in the edible portions. In this study, peel extracts exhibited higher TPC than seed extracts, likely due to increased exposure to sunlight, which enhances phytochemical synthesis (Osorio-Esquivel et al., 2011). UV radiation in sunlight can damage fruit peels, prompting the synthesis of phenolic compounds as a defense mechanism. Furthermore, the results of this study exceeded those of Bakar et al. (2015), who quantified the TPC of methanol extracts from *A. odoratissimus* peels and seeds. They reported TPC values of 42.38 ± 0.20 mg GAE/g for the peel extract and 13.72 ± 0.87 mg GAE/g for the seed extract, both of which are lower than the ethanol extract values obtained in the present study. These findings suggest that ethanol is a more effective solvent for extracting phenolic compounds from *A. odoratissimus* compared to the other solvents tested. However, this conclusion applies specifically to *A. odoratissimus* peels and seeds and may not be generalizable to other plant materials.

3.2. Total flavonoid content (TFC)

Flavonoids are the most abundant class of phenolic compounds found in nearly all plants. This study quantifies the TFC of various solvent extracts from the peels and seeds of *A. odoratissimus*, as presented in Table 3. Based on the results, the extracts exhibited varying TFC values, arranged in decreasing order as follows: aqueous peel > ethanol peel > ethanol seed >

ethyl acetate peel > aqueous seed > ethyl acetate seed > hexane peel > hexane seed. Similar to total phenolics, TFC can be classified into three categories according to Recuenco et al. (2020): low TFC (<5 mg QE/g extract), medium TFC (5–25 mg QE/g extract), and high TFC (>25 mg QE/g extract). As with TPC, the polarity of the extracting solvents also influenced TFC, with values ranging from 0.49 ± 0.44 mg QE/g extract to 68.95 ± 1.60 mg QE/g extract. Extracts from polar solvents exhibited significantly higher TFC, with aqueous and ethanol peel extracts containing 68.95 ± 1.60 mg QE/g extract and 57.41 ± 7.27 mg QE/g extract, respectively. This indicates that polar solvents (water and ethanol) are more effective in extracting flavonoids compared to less polar and nonpolar solvents (ethyl acetate and hexane). Furthermore, the trend observed in TFC aligns with that of TPC, suggesting that both are similarly influenced by solvent polarity. This parallel trend may indicate a correlation between the extraction efficiency of flavonoids and phenolics in polar solvents, highlighting their potential role in maximizing the extraction of bioactive compounds from plant materials.

Table 3. Total flavonoid content of the extracts

Extract	Part	TFC (mg QE/g)	Classification
Hexane	Peel	3.05 ± 1.18^{d}	Low
	Seed	0.49 ± 0.44^{d}	Low
Ethyl acetate	Peel	51.00 ± 8.10^{a}	High
	Seed	18.95 ± 4.37^{c}	Medium
Ethanol	Peel	57.41 ± 7.27^{a}	High
	Seed	54.49 ± 7.31^{a}	High
Aqueous	Peel	68.95 ± 1.60^{a}	High
	Seed	34.85 ± 5.81^{b}	High

Different superscript letters indicate significant differences at $p < 0.05$.

The TFC of the extracts significantly increased with increasing solvent polarity, with few exceptions. According to Nugraha et al. (2023), the similarity in polarity between the solvent and flavonoids enhances extraction efficiency. This is evident in the results, which show that the top four extracts with the highest TFC levels were all obtained using polar solvents. In contrast, extracts with lower TFC levels were primarily obtained using nonpolar solvents, particularly hexane extracts. The findings of this study align with those of El Gamouz et al. (2022), which also reported an increase in flavonoid content with increasing solvent polarity. Flavonoids, a subclass of phenolic compounds, contain hydroxyl groups that contribute to their polar nature, making them more soluble in polar solvents. As a result, higher solvent polarity facilitates greater flavonoid extraction.

Considering both the TPC and TFC results, peel extracts exhibited significantly higher phenolic and flavonoid content than seed extracts. This trend is consistent with the findings of Nogata et al. (2006), which reported that the outer layers of fruits generally contain higher polyphenolic concentrations than the inner layers and pulp. A related study by Bakar et al. (2015) similarly demonstrated this pattern, revealing that the methanol peel extract of *A. odoratissimus* contained higher TPC and TFC than its methanol seed extract counterpart. The greater phenolic and flavonoid content in the outer layers is attributed to the plant's innate defense mechanisms against pathogens, microorganisms, and predators. As the outermost part of the fruit, the peel is more exposed to solar radiation, which stimulates the synthesis of a diverse and abundant range of phenolic compounds and flavonoids.

3.3. Antioxidant activity

In the DPPH radical scavenging assay, EC_{50} is defined as the effective concentration of a sample required to scavenge 50% of DPPH radicals. Lower EC_{50} values indicate higher

antioxidant activity. This study quantifies the antioxidant activity of various solvent extracts from the peels and seeds of *A. odoratissimus*, expressed as EC_{50}, as summarized in Table 4. According to Kannaian et al. (2020), EC_{50} values can be categorized into four antioxidant activity levels: very strong (<50 μg/mL), strong (50–100 μg/mL), medium (101–150 μg/mL), and weak (>150 μg/mL). Based on the results, the extracts demonstrated strong to very strong antioxidant activity. Several studies have shown a direct association between the amount of polyphenols present in plants and their antioxidant properties. The present study revealed that extracts with high levels of TPC and TFC exhibited very strong antioxidant activity. This finding aligns with the study of Aryal et al. (2019), which demonstrated a linear correlation between phenolic and flavonoid content and antioxidant activity. Their results further support this claim, showing that plants with higher polyphenolic content exhibit stronger antioxidant activity.

Table 4. EC_{50} value and antioxidant activity level of the extracts

Extract	Part	EC_{50} (μg/mL)	Classification
Hexane	Peel	66.72 ± 1.16^{b}	Strong
	Seed	65.32 ± 0.28^{c}	Strong
Ethyl acetate	Peel	35.02 ± 0.24^{d}	Very Strong
	Seed	80.46 ± 0.66^{a}	Strong
Ethanol	Peel	13.39 ± 0.14^{g}	Very Strong
	Seed	25.91 ± 0.17^{e}	Very Strong
Aqueous	Peel	0.05 ± 0.01^{h}	Very Strong
	Seed	23.32 ± 0.15^{f}	Very Strong
Ascorbic acid*	-	6.84 ± 0.08^{g}	Very Strong

*Positive control (reference antioxidant). Different superscript letters indicate significant differences at $p < 0.05$

Similarly, the polarity of the extraction solvent influences antioxidant activity. It was observed that polar extracts exhibited stronger antioxidant activity than nonpolar extracts. The antioxidant activity levels of the extracts, arranged in decreasing order, are as follows: aqueous peel > ethanol peel > aqueous seed > ethanol seed > ethyl acetate peel > hexane peel > ethyl acetate seed > hexane seed. The higher antioxidant activity of *A. odoratissimus* extracts obtained using polar solvents can be attributed to the stronger affinity of antioxidant compounds for polar solvents compared to nonpolar ones. The presence of polar functional groups in antioxidants facilitates the formation of hydrogen bonds and other polar interactions, enhancing their solubility in highly polar solvents, which, in turn, leads to stronger antioxidant activity (Palaiogiannis et al., 2023). Additionally, the antioxidant activities of the extracts were compared to the positive control, ascorbic acid. As shown in the results, the aqueous peel extract ($EC_{50} = 0.05 \pm 0.01$ μg/mL) exhibited antioxidant activity more potent than that of ascorbic acid ($EC_{50} = 6.84 \pm 0.08$ μg/mL), a well-established antioxidant standard. This finding suggests that the aqueous peel extract may serve as a potent natural antioxidant source for drug discovery and pharmaceutical applications.

Pearson correlation analysis was conducted to assess the relationship between total phenolic content (TPC), total flavonoid content (TFC), and the antioxidant activity (EC_{50}) of *A. odoratissimus* peel and seed extracts. As seen in the correlation graphs, the results showed strong negative correlations between TPC and EC_{50} (r = -0.8008) (Figure 1) and between TFC and EC_{50} (r = -0.8909) (Figure 2), suggesting that higher TPC and TFC values were associated with lower EC_{50} values, indicating stronger antioxidant activity. However, these correlations were not statistically significant ($p > 0.05$), suggesting that while a trend was observed, additional factors may influence the relationship, warranting further investigation.

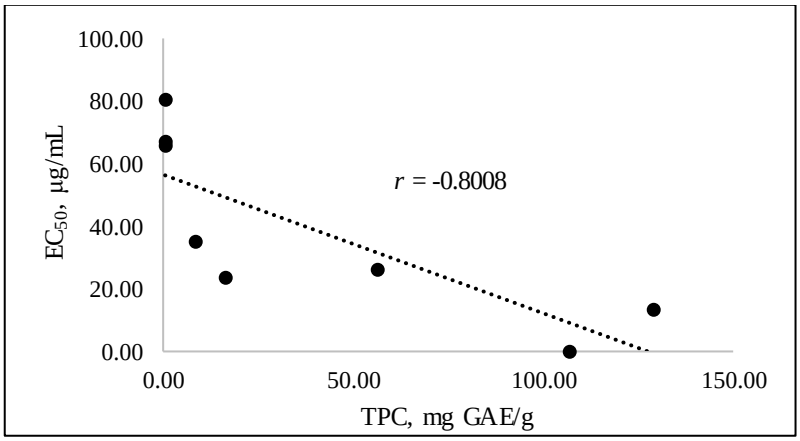

Figure 1. Correlation graph between TPC and EC_{50} values of the extracts

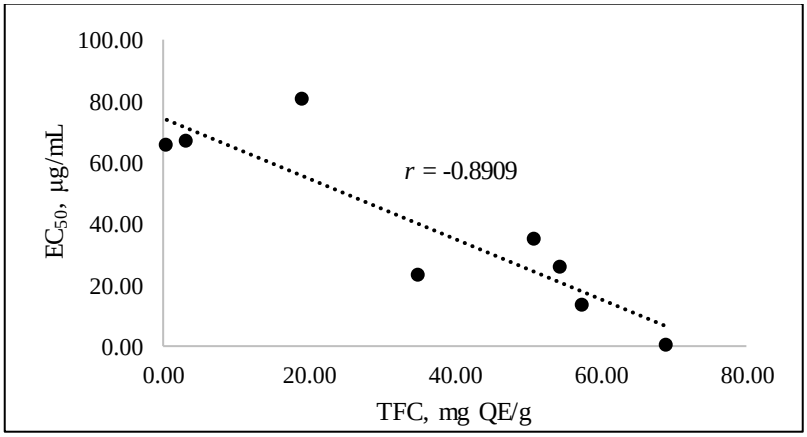

Figure 2. Correlation graph between TFC and EC_{50} values of the extracts

4. CONCLUSION

This study evaluated the TPC, TFC, and antioxidant activity of *Artocarpus odoratissimus* peel and seed crude extracts obtained using solvents of varying polarities, including hexane, ethyl acetate, ethanol, and water. The findings revealed that polar extracts exhibited higher TPC, TFC, and antioxidant activity than nonpolar extracts, with peel extracts demonstrating superior antioxidant potential compared to seed extracts. The strong antioxidant activity observed in *A. odoratissimus* extracts may be attributed to their phenolic and flavonoid content, reinforcing their potential as natural antioxidant sources. These results suggest that *A. odoratissimus* peels, in particular, could be further explored for applications in functional foods, nutraceuticals, or pharmaceutical formulations. Future studies should focus on the isolation of specific bioactive compounds responsible for the antioxidant activity and validate their efficacy using *in vitro* or *in vivo* antioxidant models, such as cell-based antioxidant assays and animal models, to confirm biological relevance beyond chemical assays.

Conflict of Interest
The authors declare no conflicts of interest.

Author Contribution Statement
Beatriz Danica Carating: Conceptualization, investigation, writing-original draft preparation, methodology, and formal analysis. Nesteve John Agosto: Conceptualization, writing-original draft preparation, methodology, formal analysis, writing-review and editing, visualization, and supervision.

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article.

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