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Research Article

Phenolic Content and Antioxidant Capacity of the Different Parts of *Atuna racemose* Raf. (Chrysobalanaceae)

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ABSTRACT

Growing concerns over synthetic antioxidants have increased interest towards plant-derived alternatives, particularly phenolic compounds. This study determines the Total Phenolic Content (TPC) and Total Antioxidative Capacity (TAC) of various parts of *A. racemosa* employing the Folin-Ciocalteu and phosphomolybdate methods, respectively, with samples extracted using 5% aqueous acetic acid.

Significant variations in total phenolic content (TPC) and total antioxidant capacity (TAC) were observed among different plant parts, expressed as milligrams of gallic acid equivalent (GAE) and ascorbic acid equivalent (AAE) per gram of dry weight sample, respectively. The TPC values were as follows: 102.12 mg GAE/g for fruits, 51.13 mg GAE/g for buds, 22.77 mg GAE/g for leaves, and 7.59 mg GAE/g for twigs. Similarly, the TAC values were 177.99 mg AAE/g for fruits, 93.46 mg AAE/g for buds, 33.33 mg AAE/g for leaves, and 12.14 mg AAE/g for twigs. A strong positive correlation between TPC and TAC (r = 0.997) indicates that phenolic compounds significantly contribute to the antioxidant activity of *A. racemosa*.

The study highlights the organ-specific distribution of phenolic compounds and antioxidants in *A. racemosa*, identifying its fruits as particularly rich reservoirs of these health-promoting compounds.

Keywords: antioxidant, Atuna racemosa, fruits, organ-specific distribution, phenolics

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1. INTRODUCTION

The rich medicinal history of plants is rooted in their diverse secondary metabolites, offering a wide array of pharmacological and biological benefits across traditional and modern healthcare practices. In recent years, there has been a notable shift towards exploring plant-based alternatives, particularly those known for strong antioxidant properties, due to concerns over the adverse side effects of synthetic products (Lourenço, 2019). Plant-derived phenolic compounds have emerged as potent antioxidants, providing a safer substitute to synthetic counterparts (Shahidi & Ambigaipalan, 2015) while exhibiting a broad range of biological activities (Li et al., 2014; Nina et al., 2020).

Antioxidants are crucial in regulating autoxidation by neutralizing harmful free radicals, which helps reduce oxidative stress, enhance immune function, and promote longevity (Tan et al., 2018). However, the balance between antioxidants and prooxidants can be disrupted by factors such as aging, environmental toxins, fatigue, excessive alcohol intake, and high-fat diets (Sindhi et al., 2013). Although the body has several endogenous antioxidant defense mechanisms, these may not always be sufficient, increasing the risk of diseases such as diabetes, atherosclerosis, cancer, and neurodegenerative disorders (Kruk et al., 2017; Pizzino et al., 2017; Yang et al., 2017; Ramalingam & Kim, 2012; Uttara et al., 2009; Evans et al., 2002). Consequently, supplementing with exogenous antioxidants may be essential for supporting optimal bodily function (Kurutas, 2016).

Atuna excelsa subsp. racemosa (Raf.) Prance, locally known as tabon-tabon, is a promising plant for exploring natural sources of antioxidants. Filipinos use the fruit in a local dish of raw fish in vinegar 'kinilaw' to remove fish odors (Tila et al., 2022). Ethnomedicinal records indicate that the plant has traditionally been used to treat body and abdominal pain, swelling, inflammation, and infections (Prance, 2004). Several studies have documented its antimicrobial activity (Buenz et al., 2007; Pacaña & Galarpe, 2017; Gentallan et al., 2019; Nadayag et al., 2019; Rizki, 2020; Tila et al., 2022). Additionally, previous research has highlighted the DPPH-radical-scavenging antioxidant potential of its fruits (Abug et al., 2012; Ang & Deocampo, 2019), inner bark (Nadayag et al., 2019), and leaves (Gicole, 2019). However, no comparative study has been conducted on the antioxidant properties of different parts of *A. racemosa*. Therefore, this study aimed to determine the phenolic content and evaluate the antioxidative capacity of aqueous acetic acid extracts from the buds, twigs, leaves, and fruits of *A. racemosa*.

2. METHODOLOGY

Plant Collection

Sampling was conducted in Bangcud, Malaybalay, Bukidnon, Philippines, at geographical coordinates 8° 0' 12" N and 125° 7' 58" E. Samples, including buds, twigs, leaves, and fruits (Figure 1), were randomly hand-collected from the tree early in the morning before sunrise. All samples were free from infection and insect damage, with mature leaves characterized by a dark green color and fully expanded shape and structure, while mature fruits displayed a dark brown color without any cracks.

Sample Preparation and Extraction

The collected plant parts were thoroughly washed with tap water to eliminate dirt and foreign substances, then rinsed with distilled water and dried with paper towels. The fruit samples were halved, and the endosperm was scraped out and mixed to create a composite sample. The leaves, buds, and twigs were chopped into small pieces.

The prepared samples were soaked in a 5% aqueous acetic acid solution at room temperature for 48 hours with occasional stirring. After soaking, they were sonicated for 30 minutes using a UC-305 40 kHz Ultrasonicator and then filtered through Whatman No. 1 filter paper. Finally, the moisture content of the samples was measured by oven-drying them at 105°C for 4 hours.

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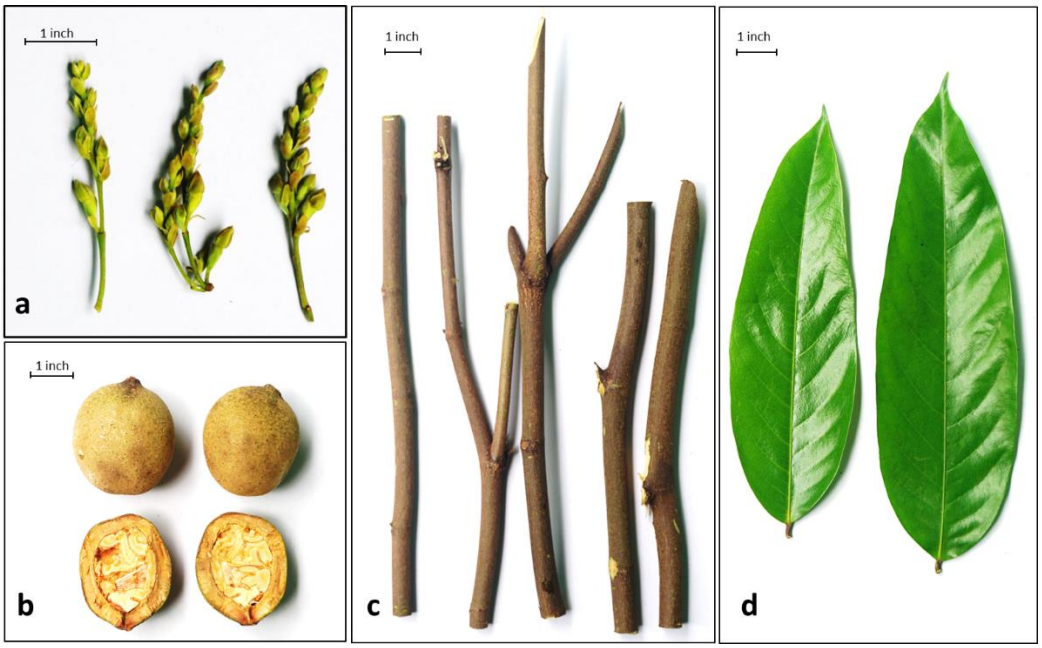

Figure 1. *Atuna racemosa* Raf. (a) buds, (b) fruits, (c) twigs, and (d) leaves.

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Determination of the Total Phenolic Content (TPC)

a. **Preparation of the standards and samples.** A 200 mg/L stock solution of gallic acid was prepared by dissolving 0.0200 g of standard gallic acid in 5% aqueous acetic acid and diluting it to 100 mL with the same solvent. From this stock solution, various concentrations (0, 8, 16, 32, 48, 64, and 72 mg/L) were prepared as working standards for the calibration curve. The sample extracts were diluted as needed.

b. **Assay**. TPC was determined using the Folin–Ciocalteu method with some modifications (Kaur & Kapoor, 2002). A 400 µL aliquot of the test sample extract, 400 µL of 10% Folin-Ciocalteu solution, and 1600 µL of 10% Na_2CO_3 solution were mixed and incubated for two hours at room temperature. After centrifugation for 2 minutes at 11,000 rpm, 200 µL of the reaction mixture was transferred to a 96-well plate, and absorbance was measured at 750 nm using a Thermoscientific Multiscan Skyhigh Microplate Reader. The same procedure was applied to the working standards (gallic acid) and the blank (5% aqueous acetic acid). The standard curve equation obtained was $y = 0.0087x + 0.0134$ with $R^2 = 0.9974$. TPC was expressed as mg gallic acid equivalent per gram of dry weight sample (mg GAE/g).

Determination of Total Antioxidant Capacity (TAC)

a. **Preparation of the standards and samples**. A 500 mg/L stock solution of L-ascorbic acid was prepared by dissolving 0.025 g of the standard gallic acid in 5% aqueous acetic acid, and then diluting it to 50 mL with the same solvent. From this stock solution, various concentrations (0, 20, 40, 60, 80, 100 mg/L) were prepared as working standards for the calibration curve. Sample extracts were diluted as needed.

b. **Assay.** TAC was determined using the phosphomolybdate method with some modification (Prieto et al., 1999). A 1 mL aliquot of the test sample extract and 3 mL of 1:1:1 solution of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM of ammonium molybdate were mixed and incubated for 90 minutes at 95°C. After cooling at room temperature and centrifugation for 3 minutes at 11,000 rpm, 200 µL of the reaction mixture was transferred to a 96-well plate. The absorbance was measured at 695 nm wavelength using a Microplate Reader. The same procedure was applied for the working standards (L-ascorbic

acid) and the blank (5% aqueous acetic acid). The standard curve equation was y = 0.0041x - 0.0099 with R^2 = 0.9972. TAC was expressed as mg L-ascorbic acid equivalent per gram dry weight sample (mg AAE/g).

Statistical Analysis

All analyses were performed in triplicate and expressed as mean ± standard deviation. Data were subjected to a One-way Analysis of Variance (ANOVA), followed by Tukey's Test to identify significant differences at a significance level of 0.05. The correlation between TPC and TAC was determined using Pearson's Correlation Test at a significance level of 0.01.

3. RESULTS AND DISCUSSION

Moisture Content

The moisture content results obtained from oven-drying at 105°C for 4 hours show significant differences among plant parts: buds at 70.03 ± 0.25%, fruits at 65.99 ± 1.89%, twigs at 51.8 ± 0.22%, and leaves at 46.42 ± 0.03%. Given this variability in water content, total phenolic content and total antioxidant capacity values were expressed on a dry weight basis for standardized comparisons among different plant parts.

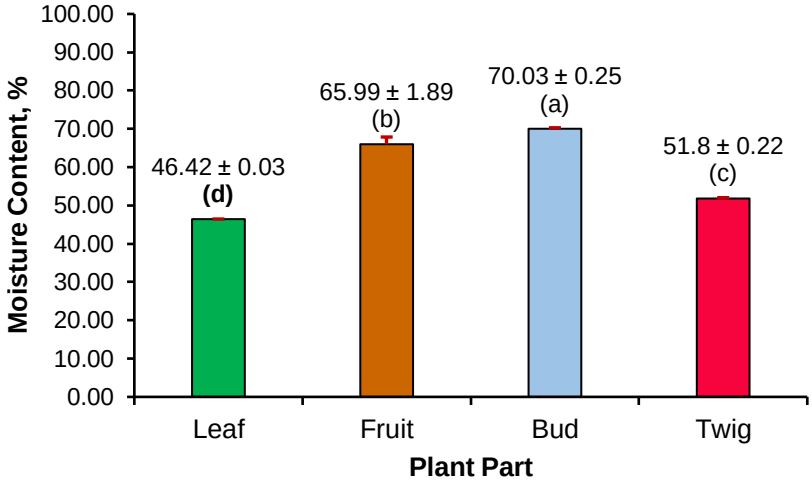

Figure 2. Moisture content (%) of the various parts of *A. racemosa*. Mean ± SD (n=3). Means with a different affixed letter are significantly different at 0.05 level of significance.

Total Phenolic Content (TPC)

Significant differences in the levels of phenolic compounds were observed among the various plant parts of *A. racemosa* (Figure 3). The fruits showed the highest TPC value at 102.12 ± 2.20 mg GAE/g, followed by buds at 51.13 ± 0.26 mg GAE/g, leaves at 22.77 ± 0.68 mg GAE/g, and twigs at 7.59 ± 0.20 mg GAE/g.

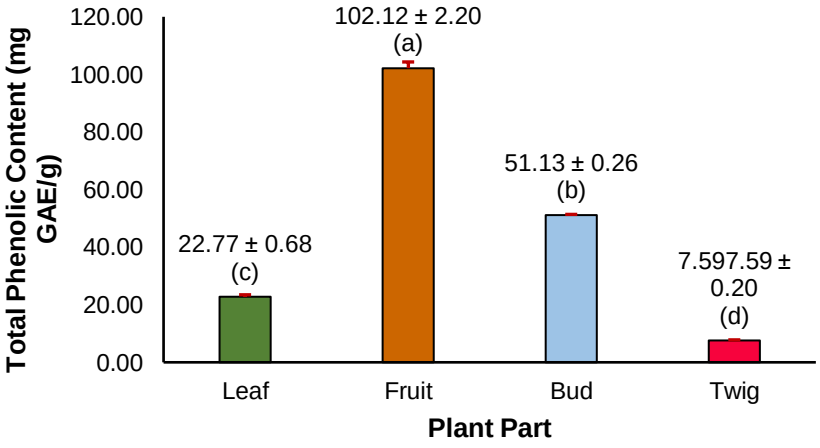

Figure 3. Total Phenolic Content (mg GAE/g) dry weight basis of the various parts of *A. racemosa*. Mean ± SD (n=3). Means with a different affixed letter are significantly different at 0.05 level of significance.

Phenolic compounds in plants are synthesized during normal tissue development and in response to environmental stresses, supporting optimal growth and survival (Ray et al., 2024; Kumar et al., 2023). This synthesis primarily occurs through pathways such as the pentose phosphate pathway, shikimate pathway, and phenylpropanoid pathway (Randhir et al., 2004). These compounds have diverse chemical structures and can be categorized into two main groups: flavonoids (e.g., anthocyanins, flavanols, flavanones, flavonols, and isoflavones) and non-flavonoids (e.g., phenolic acids, coumarins, stilbenes, lignans, lignins, and tannins) (Durazzo et al., 2019). Each type of phenolic compound has unique properties that determine its localization and function within the plant.

For example, phenolic acids like p-coumaric acid and ferulic acid are typically located in cell walls and vascular tissues, where they contribute to structural integrity (Pratyusha, 2022; Liu et al., 2018). In addition to structural roles, phenolic acids enhance color stability and influence aroma profiles, helping to attract pollinators.

Other phenolic compounds, including gallic acid, salicylic acid, flavonoids, and tannins, play a crucial role in protecting plants against various stressors. The increased synthesis of these compounds serves as a defense mechanism in response to conditions such as injury, infection, and UV radiation, enhancing the resilience of plants to environmental stress. Additionally, these phenolic compounds are involved in modulating stress response signaling pathways, which are vital for plant adaptability. They contribute to stabilizing cell membranes and scavenging reactive oxygen species, further supporting the ability of plants to withstand adverse conditions (Mazmanyan, 2024; Kumar et al., 2023; Chowdhary et al., 2022; Šamec et al., 2021; Lin et al., 2016). Moreover, flavonoids, articularly anthocyanins, are notably present in flowers and fruits, providing pigmentation that attracts pollinators and seed dispersers while also offering UV protection (Pratyusha, 2022; Zhang et al., 2022; Del Valle et al., 2020; Sulusoglu, 2014).

A noteworthy observation is the uneven distribution of phenolic compounds among different plant parts of *A. racemosa*. This organ-specific accumulation of phenolic compounds is consistent with findings reported in various studies (Savina et al., 2023; Chepel et al., 2020; Ghafoor et al., 2020; Grimalt et al., 2019; Feduraev et al., 2019; Kumar et al., 2018; Otles & Yalcin, 2012). The diverse qualitative and quantitative composition of phenolic compounds within plants contributes to this complexity in distribution which is closely related to the functional roles of these compounds throughout the life cycle and growth phase of plants (Cheynier et al., 2013; Feduraey et al., 2019).

The remarkably high phenolic content in the fruit of *A. racemosa* is of particular interest. Phenolic compounds enhance the fruit's organoleptic properties, pigmentation, firmness, and resistance to pathogens and adverse environmental conditions (Singh et al., 2010). The astringent taste of the fruit pulp extract, attributed to tannins and other polyphenolic compounds, helps neutralize any fishy odor (Sulusoglu, 2014; He et al., 2015; Li et al., 2011; Tila et al., 2022).

Previous studies have reported elevated levels of phenolic compounds in fully mature fruits (Abug et al., 2012; Gentallan et al., 2019). Additionally, different plant species exhibit varying phenolic content during the development and maturation of their fruits, as these processes involve significant biochemical, biophysical, and physiological changes that influence phytochemistry (Mahmood et al., 2012). For example, the maturation of mulberry, strawberry, and cherry fruits typically shows an initial decrease in total phenolic content, followed by an exponential increase toward the end of maturation. This increase correlates with the accumulation of anthocyanins and the darkening of the fruit (Mahmood et al., 2012; Pineli et al., 2011; Serrano et al., 2005). In blueberries, the maturation process involves an upregulation of transcript levels for genes encoding key enzymes involved in phenolic biosynthesis. This upregulation results in increased levels of phenolic compounds, such as quercetin, myricetin, and anthocyanins (Li et al., 2019). Furthermore, the activation of the phenolic biosynthetic pathway serves as an integrated defense mechanism, contributing to the accumulation of phenolics in plant tissues through enhanced activity of critical enzymes (Marchica et al., 2020; Wang et al., 2015; Arena et al., 2012).

Flower bud differentiation is a critical stage in floral development, representing a transition from nutritional to reproductive growth. Although not fully mature, buds contain substantial amounts of phenolic compounds. For instance, Tian et al. (2022) reported that buds of *S. japonica* and *R. pseudoacacia* have relatively higher levels of phenolic compounds compared to their corresponding flowers. Additionally, Schmitzer et al. (2009) observed a decrease in levels of phenolic acids, anthocyanins, quercetin, and catechins from rose buds to fully opened flowers. This decline can be attributed to several factors: the active degradation of anthocyanins, pigment dilution due to petal expansion (Vaknin et al., 2005), and a decrease in enzymatic activity and gene transcription (Dong et al., 1998).

Phenolic compounds are primarily synthesized in leaves, where the mesophyll, the central tissue responsible for their biosynthesis, is predominantly concentrated (Savina et al., 2023). While some phenolic compounds remain localized at their site of synthesis, others are translocated to different parts of the plant. Previous studies have shown that phenolic compounds produced in leaves can be translocated to fruits, which have higher metabolic

demands (Gutierrez et al., 2017; Petrussa et al., 2013). The translocation of phytochemicals among plant organs may also play a significant role in the accumulation of phenolics in fruits.

In the study, twigs, considered secondary stems, exhibited minimal phenolic content, which aligns with findings from previous comparative studies among different plant organs. For instance, Savina et al. (2023) noted minimal levels of hydroxycinnamic acids, flavonoids, catechins, proanthocyanidins, and tannins in the stems of meadowsweet. Similarly, Feduraev et al. (2019) reported the lowest accumulation of catechins, proanthocyanins, and phenolics in the stems of *R. crispus* and *R. obtusifolius*. As structural and supportive organs, stems typically have lower metabolic activity and fewer specialized cells involved in phenolic compound biosynthesis compared to other plant organs (Feduraev et al., 2019). The phenolic profile is primarily influenced by the photosynthetic mesophyll tissue and the metabolic characteristics of the phloem exudate (Savina et al., 2023) The limited presence of mesophyll in stem tissue suggests a reduction in phenolic biosynthesis leading to lower phenolic compound concentrations in stem tissues compared to leaves or fruits, potentially affecting the overall antioxidant capacity of the plant. Furthermore, the minimal phenolic composition in the phloem exudate is attributed to the alkaline reaction of the central cavity solution, which leads to the oxidation of plant phenolics into quinones, thereby hindering their free flow (Feduraev et al., 2019).

Total Antioxidant Capacity (TAC)

TAC reflects the collective (additive and potentially synergistic or antagonistic) actions of all antioxidants present in a complex sample (Apak et al., 2016). This measure is considered a more informative parameter for assessing antioxidant defenses than simply determining the concentrations of individual antioxidant constituents.

Significant variation in TAC was observed among the different plant parts of *A. racemosa* (Figure 4). Fruits exhibited the highest TAC, with 177.99 ± 6.97 mg AAE/g, followed by buds at 93.46 ± 2.77 mg AAE/g, leaves at 33.33 ± 0.95 mg AAE/g, and twigs at 12.14 ± 0.38 mg AAE/g. Similar variations in antioxidant activity among different plant organs have been reported in other plant species (Ghafoor et al., 2020; Feduraev et al., 2019; Otles & Yalcin, 2012).

The antioxidant capacity of a plant is influenced by the intrinsic metabolic activity of its cells and tissues, as well as the molecular composition of its chemical components (Feduraev et al., 2019). This includes the types, quantities, and distribution of antioxidant secondary metabolites throughout the plant. Among these, phenolic compounds are the most abundant secondary metabolites and are well-known for their potent antioxidant properties. They play a significant role in preventing various diseases associated with oxidative stress (Dai & Mumper, 2010). As antioxidants, phenolic compounds function as radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen quenchers (Liang et al., 2010; Santos-Sanchez et al., 2019).

Phenolic acids function as antioxidants primarily due to the activity of their phenol group, which contains a hydroxyl group attached to an aromatic ring. While various mechanisms contribute to their antioxidant effect, the main mechanism involves radical scavenging through hydrogen atom donation (Shahidi & Ambigaipalan, 2015). Additionally, these compounds exhibit antioxidant activity through mechanisms like electron donation and singlet oxygen quenching. The nature of substituents on the aromatic ring influences the stabilization of these compounds, thereby affecting their radical-quenching capacity. Consequently, different phenolic acids exhibit varying levels of antioxidant activity (Rice-Evans, Miller, & Paganga, 1996).

Pearson's correlation analysis of the relationship between phenolic compounds and antioxidant activity in *A. racemosa* revealed a significant positive correlation ($r = 0.997$) between total antioxidant capacity and total phenolic content. This strong linear relationship indicates that phenolic compounds play a substantial role in the antioxidant activity of *A. racemosa*. Similar findings have been reported in other plant species (Chandra et al., 2014; Feduraev et al., 2019; Kumar et al., 2014; Lim et al., 2019; Nurhasnawati et al., 2019; Yao, 2010), providing further scientific evidence that phenolic compounds significantly enhance the overall antioxidative potential of plants.

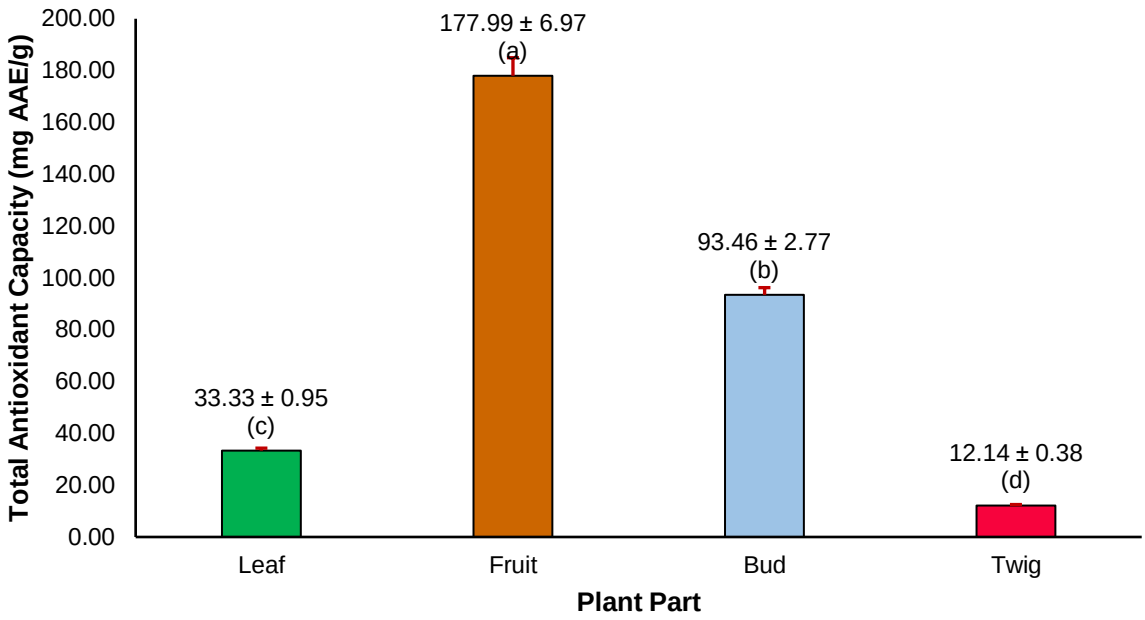

Figure 4. Total Antioxidant Capacity (mg AAE/g) dry weight basis of the various parts of *A. racemosa*. Mean ± SD (n=3). Means with a different affixed letter are significantly different at 0.05 level of significance.

4. CONCLUSION

The present study highlights significant variations in total phenolic content and total antioxidant capacity among different plant parts of *A. racemosa*, with fruits exhibiting the highest levels, followed by buds, leaves, and twigs. The notably high TPC and strong TAC of the fruits indicate their potential as valuable natural reservoirs of phenolic compounds and antioxidants. Therefore, further exploration into the development of innovative health-promoting and disease-preventing herbal preparations or medicinal products utilizing the fruits of *A. racemosa* is highly recommended.

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