



Flavonoid-to-Phenol Ratios in Methanolic Extracts of Aspilia africana: Implications for Managing Oxidative Stress

Owo, Gogo James ^{a*}, Karibo, Amakiri Okari ^b and Kpomah Enyowho Dennis ^c

 ^a Department of Biology, Faculty of Natural and Applied Sciences, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Rivers State, Nigeria.
 ^b Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria.
 ^c Department of Biochemistry, Faculty of Sciences, Federal University, Otuoke, Bayelsa State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author OGJ designed the study, performed the statistical analysis, wrote the protocol, and drafted the first version of the manuscript. Author KAO managed the analyses of the study and conducted literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Oxidative stress results from the imbalance between the production and regulation of reactive oxygen species or free radicals and causes harmful effects such as cancer, Alzheimer's disease, cardiac diseases, kidney disorders, liver diseases, fibrosis, atherosclerosis, arthritis, neurodegenerative disorders, aging and so on. Plant phenolics and flavonoids play a significant role

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^{*}Corresponding author: Email: gogojames76@gmail.com;

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in scavenging free radicals in the body and act as antioxidants. This study aimed to quantify the total phenolic and flavonoid contents in different parts of Aspilia africana and evaluate their potential antioxidative properties through flavonoid-to-phenol (F/P) ratios. Total phenolic and flavonoid contents of the extracts of Aspilia africana were determined using spectrophotometric methods. The results obtained showed that the highest total phenolic content value was observed for the roots extracts (150.75 ± 0.11 mg GAE/g) followed by the leaves extracts (112.17 ± 1.62 mg GAE/g), and the lowest was for the stems extracts (98.34 \pm 0.19 mg GAE/g). The high phenolic content of the root (150.75 ± 0.11 mg GAE/g) indicates that the root of A. africana may have a high antioxidant capacity. The leaf of A. africana had the highest total flavonoid content while the stem had the lowest total flavonoid content. This is an indication that the methanolic root extract has antioxidative potential. The F/P ratio ranges from 1.17 to 2.51, with the leaf extract having the highest flavonoid/phenolic ratio of 2.51 and the root extract having the lowest flavonoid/phenolic ratio of 1.17. The high F/P ratio of the leaves extract shows that it contains more flavonoids than the stems and roots. Hence, the leaf extracts of A. africana, with their elevated flavonoid content, could be used in developing dietary supplements or therapeutic formulations aimed at managing oxidative stress and enhancing overall health. This practical application of the study's findings underscores the importance of harnessing natural sources of antioxidants. Most importantly, the study points to the need for further research to elucidate the specific mechanisms through which flavonoids and phenolics exert their antioxidant effects. Such research could help optimize the use of A. africana extracts in managing oxidative stress and improving health outcomes related to oxidative damage.

Keywords: Total phenolic; total flavonoid; Aspilia africana; oxidative stress; antioxidant.

1. INTRODUCTION

"Medicinal plants are the richest sources of phytochemicals and have been used in various traditional herbal practices since ancient times to treat numerous diseases associated with oxidative stress" (Ashraf et al.. 2024). "Phytochemicals bioactive compounds are obtained from plants" (Demir & Akpınar, n.d.). "These compounds are also known as secondary metabolites because the plants that produce them might have little need for them. They are naturally found in all parts of plants, including the bark, leaves, stems, roots, flowers, fruits, seeds, and other tissues" (Gebreslassie & Eyasu, 2019).

Among the known classes of phytochemicals are phenolics such as polyphenols and flavonoids, significantly present in fruits, which are vegetables, and various medicinal and aromatic plants (Garg et al., 2019; Prabhu et al., 2021) and are widely recognized for their antioxidant properties (Makhaik et al., 2021; Nwozo et al., 2023). "Antioxidants are substances that prevent oxidative damage or reduce to cellular components by neutralizing free radicals, including peroxides and hydroperoxides" (Yadav et al., 2016). "This process helps lower the risk of diseases associated with oxidative stress" (Engwa et al., 2022; Kıran et al., 2023).

Oxidative stress itself is a condition characterised by an imbalance between the production of reactive oxygen species (ROS) or free radicals and the body's ability to effectively neutralise or repair the resulting damage (Phull et al., 2018; Pizzino et al., 2017). "It may cause several severe human diseases such as cancer, Alzheimer's disease, cardiac diseases, kidney disorders, liver diseases, fibrosis, atherosclerosis, arthritis, neurodegenerative disorders, and aging" (Hajam et al., 2022).

Oxidative stress plays a pivotal role in the development of various human pathologies. including cardiovascular diseases. neurodegenerative disorders, and aging-related conditions. Hajam et al. (2022) highlight the mechanisms underlying oxidative molecular stress and its connection to disease progression, emphasizing the importance of antioxidants in mitigating its harmful effects. Here, plant-derived antioxidants, particularly phenolic compounds flavonoids, and have garnered significant attention for their potential to reduce oxidative damage. According to Pizzino et al. (2017), oxidative stress shows a complex dual role in human health. On one hand, excessive oxidative stress, caused by an imbalance between reactive oxygen species (ROS) and antioxidant defenses, can lead to cellular damage and contribute to the development of chronic diseases such as cardiovascular diseases, cancer, diabetes, and neurodegenerative conditions. On the other hand. ROS also have beneficial roles in cellular signaling, immune responses, and the regulation

of gene expression. When controlled, ROS are essential for immune defense and the activation of cellular responses to external stressors. However, prolonged or excessive oxidative stress can overwhelm the defense mechanisms of the body, leading to tissue damage and the progression of various diseases.

Polyphenols are considered among the most compounds bioactive with exceptional antioxidant properties. They primarily function as antioxidants by scavenging free radicals and chelating metals in both in vitro and in vivo systems (Bhuyan & Handique, 2022). According to Alotaibi et al. (2021), a high intake of phenolic compounds is associated with a reduced risk of cardiovascular diseases and cancers. Plant phenolics are known to exhibit a variety of biological activities, including anti-carcinogenic, antioxidant, antibacterial, anti-atherosclerotic, anti-inflammatory, and antiviral effects. Flavonoids are a prominent subgroup of phenolic compounds that have recently attracted significant interest due to their diverse pharmacological and biological activities. They are particularly well-known for their potent antioxidant properties (Makhaik et al., 2021; Tariq et al., 2021).

Aspilia africana is a semi-woody, low-toxicity herb belonging to the Asteraceae family and found predominantly in tropical regions. It is traditionally used to treat wounds and other ailments due to its richness in various bioactive compounds, such as flavonoids and phenolics (Komakech et al., 2019; Okello et al., 2020; Popoola et al., 2021). Ajeigbe et al. (2014) reported that Α. africana possesses antimicrobial. hemostatic. anti-fertility. antiulcerogenic, antioxidant, and anti-inflammatory properties.

The wound healing properties of *A. africana* are well-established, with research indicating its ability to promote tissue regeneration and

alleviate inflammation. This supports the notion that the plant's bioactive compounds, such as flavonoids and phenolics, may play a role not only in antioxidant activity but also in a range of therapeutic applications (Komakech et al., 2019).

The traditional use of the plant in treating various ailments, including inflammatory conditions, is supported by its ethnopharmacological profile, which includes compounds like flavonoids and phenolic acids. These compounds are believed to modulate inflammatory pathways, reduce oxidative stress, and provide therapeutic effects in conditions such as rheumatoid arthritis and other inflammatory disorders (Hajam et al., 2022).

The current study aims to quantitatively determine the flavonoid-to-phenolic ratios in methanolic extracts of the leaves, stems, and roots of *A. africana*.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The leaves, stems, and roots of *A. africana* were collected from abandoned building site in Oyigbo West, Oyigbo Local Government Area, Rivers State, Nigeria as shown in Fig. 1.

The plant was identified and authenticated by Dr. A.O Wokoma, Head of the Department of Biology, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt, Rivers State, Nigeria. Fig. 1 depicts A. africana thriving in an abandoned building site. This setting is particularly relevant as it highlights the plant's resilience and adaptability to harsh and disturbed environments. The ability of A. africana to grow in such a location suggests that the plant can endure stressful conditions, potentially leading to increased production of secondarv an metabolites like phenolics and flavonoids, which are critical for its defense mechanisms.



Fig. 1. Aspilia africana growing in an abandoned building site

2.2 Preparation of Plant Extracts

The plant material was shade dried for about 15 days. The dried sample was then coarsely powdered and stored in a sterile container. The successive extraction of the samples was done by using methanol using the standard technique of maceration. The extracts thus obtained were evaporated to dryness at room temperature and stored in a sterile airtight container. The concentrated mass obtained, i.e. the crude methanolic extracts were weighed and kept in a refrigerator for a further experimental procedure.

2.3 Spectrophotometric Estimation of Total Phenolic Content

The total phenolic content (TPC) in Aspilia africana extracts was quantified using a spectrophotometric technique with the Folin-Ciocalteu reagent, following the method of Siddiqui et al. (2017) with minor adjustments. Gallic acid served as the reference standard for constructing a calibration curve ranging from 0.1 to 1.0 mg/mL. A 0.5 mL aliquot of the plant extract (100 µg/mL) was mixed with 2 mL of the Folin-Ciocalteu reagent, diluted 1:10 with deionized water, and neutralized by adding 4 mL of 7.5% (w/v) sodium carbonate solution. The reaction mixture was incubated at room temperature for 30 minutes with periodic shaking to enhance color development. The absorbance of the developed blue color was measured at 765 using а UV-Vis spectrophotometer nm (Shimadzu UV-1800). The total phenolic content was calculated using the linear regression

equation obtained from the gallic acid calibration curve and expressed as mg of gallic acid equivalents (GAE) per gram of extract. Triplicate measurements ensured reproducibility. The results and spectra for the TPC estimation are presented in Fig. 3.

2.4 Spectrophotometric Estimation of Total Flavonoid Content

The total flavonoid content (TFC) in Aspilia africana extracts was determined using an aluminum chloride assay, as described by Ayele et al. (2022), with minor modifications. A 0.5 mL aliquot of each extract was mixed with 2 mL of distilled water in separate test tubes. Subsequently, 0.15 mL of 5% (w/v) sodium nitrite solution was added and left to react for 6 minutes. Then, 0.15 mL of 10% (w/v) aluminum chloride solution was introduced, followed by incubation for another 6 minutes. Finally, 2 mL of 4% (w/v) sodium hydroxide solution was added, and the volume was adjusted to 5 mL with distilled water. After 15 minutes of incubation, the developed a pink color. mixture whose absorbance was measured at 510 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). The total flavonoid content was expressed as mg of quercetin equivalents (QE) per gram of extract. Calibration curves with quercetin standards (0.1-1.0 mg/mL) ensured accuracy, and blank samples were used for baseline correction. The reproducibility of the results was confirmed through triplicate experiments. The results and spectra for the TFC estimation are presented in Fig. 4.



Fig. 2. Pictures of different extracts of *A. africana*. SE=Stem extract, RE=Root extract, LE=Leaf extract



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Fig. 3. Uv-vis Spectra for the estimation of total phenolic content



Fig. 4. Uv-vis spectra for the estimation of total flavonoid content

3. RESULTS AND DISCUSSION

The total phenolic and flavonoid contents in methanolic leaf stem, and root extracts of *Aspilia african* are presented in Table 1.

3.1 Total Phenolic and Flavonoid Contents

Total phenolic contents (TPC) in different extracts of leaves, stems, and roots of *A. africana* were determined by Folin–Ciocalteu (F–

C) method using gallic acid as the standard. The absorbance values gotten at different concentrations of gallic acid were used to determine the calibration curve. Total phenolic content of the extracts was calculated from the regression equation of the calibration curve (Y =0.0104x; R²=0.988) and expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g) where y is the absorbance at 765 nm and x is total phenolic content in the extracts of A. africana extract expressed in mg/g equivalent of Gallic acid. TPC values varied

Part of the plant	Total Phenolics (mg GAE/g)	Total flavonoids (mg QE/g)	Flavonoids/Phenolics (F/P ratio)
Leaves	112.17 ± 1.62	281.30 ± 0.20	2.51
Stems	98.34 ± 0.19	166.44 ± 1.43	1.69
Roots	150.75 ± 0.11	176.14 ± 0.35	1.17

 Table 1. Total phenolic and flavonoid contents in leaf, stem, and root methanolic extracts of

 Aspilia africana

between 98.34 ± 0.19 and 150.75 ± 0.11 mg gallic acid equivalent per gram of extract as shown in Table 1. TPC values were higher in the roots and leaves extracts than in the stem extracts. The highest TPC value was observed for the roots extracts (150.75 \pm 0.11 mg GAE/g) followed by the leaves extracts (112.17 \pm 1.62 mg GAE/g), and the lowest was for the stems extracts (98.34 \pm 0.19 mg GAE/g). The high phenolic content of the root $(150.75 \pm 0.11 \text{ mg})$ GAE/g) indicates that the root of A. africana may have a high antioxidant capacity. This agrees with Phuyal et al. (2020), who opined that the phenolic content of the part of any plant is directly related to its antioxidant properties. This implies that the roots of A. africana have high antioxidant properties than the other parts studied. Antioxidants are extremely important substances, which can defend the body from impairment caused by free radical-induced oxidative stress (Tarig et al., 2021).

Also, the content of total flavonoid of the methanolic extracts of the three different parts of the plant measured spectrophotometrically by using the aluminum chloride assay is shown in Table 1. The flavonoid content of the extracts was expressed as mg quercetin equivalent per gram of the extract. The total flavonoid content varied between 166.44 ± 1.43 and 281.30 ± 0.20 mg quercetin equivalent per gram of extract. The leaf of *A. africana* had the highest total flavonoid content while the stem had the lowest total flavonoid content.

A study carried out by Ajeigbe et al. (2014) has shown that leaf extract of *A. africana* significantly increased malondialdehyde (MDA) levels and decrease superoxide dismutase (SOD) and catalase (CAT) activities in experimental rats. This is an indication that the extract has antioxidative potential which is attributed to the presence of flavonoids in the extract, thus supporting my present findings.

3.2 Flavonoid/Phenol (F/P) Ratio

The calculated flavonoid/phenolic ratio to ascertain the extracts that are rich in flavonoids

is shown in Table 1. The F/P ratio ranges from 1.17 to 2.51, with the leaf extract having the highest flavonoid/phenolic ratio of 2.51 and the root extract having the lowest flavonoid/phenolic ratio of 1.17. This high F/P ratio of the leaf extract shows that it contains more rich flavonoids than the stem and roots. This agrees with the study carried out by Agbo et al. (2015), who investigated the antioxidant, total phenolic contents, and total flavonoid contents of selected Nigerian medicinal plants and observed that the leaves extracts of the plants studied had higher F/P ratio than the stem, although the roots of the selected plants were not included in the study. the best of our knowledge, Τo no flavonoid/phenolic ratio in A. africana extracts has been reported.

The F/P ratio serves as an indicator of the balance between flavonoids and phenolics in contributing to the antioxidative properties of plant extracts. While phenolics generally provide broad-spectrum antioxidant activity, flavonoids exhibit targeted free-radical scavenging due to their specific hydroxyl group arrangements. Thus, calculating the F/P ratio offers insight into the functional synergy of these compounds in managing oxidative stress-related diseases. Unlike previous studies on A. africana that primarily focused on its antimicrobial and antiinflammatory properties (Ajeigbe et al., 2014), this study uniquely highlights the antioxidative significance of flavonoid-to-phenolic ratios in the plant's extracts. Compared to related species like Helianthus annuus, which demonstrates a higher phenolic dominance, Aspilia africana exhibits a more balanced F/P ratio, underscoring its potential for holistic oxidative stress management.

4. CONCLUSION

Methanolic extracts of the leaves, stems, and roots of *A. africana* were investigated for their total flavonoid contents, total phenolic contents, and flavonoid/phenolic content ratio. The leaf extract was found to be rich in flavonoids since it had high flavonoids/phenolics ratio. Hence, the

leaf extract can be used in the management of oxidative stress.

5. IMPLICATION OF THE STUDY ON THE MANAGEMENT OF OXIDATIVE STRESS

Oxidative stress is associated with numerous health issues, including cardiovascular diseases, cancer, neurodegenerative disorders, and diabetes (Kıran et al., 2023). For instance, in cardiovascular diseases. oxidative stress can lead to the oxidation of low-density lipoprotein contributing to the formation (LDL). of atherosclerotic plaques. In cancer, oxidative stress can cause mutations in DNA, leading to uncontrolled cell growth and tumour formation. Similarly, in neurodegenerative disorders such as Alzheimer's disease, oxidative stress can result in neuronal damage and cognitive decline (Pizzino et al., 2017; Reddy, 2023).

Moreover, oxidative stress is a significant factor in aging, accelerating the aging process and contributing age-related diseases. The to continuous oxidative damage to cellular components over time can lead to a gradual decline in cellular function and integrity, manifesting as aging symptoms and ageassociated diseases (Liguori et al., 2018). The ethnopharmacological properties of A. africana have been explored in various studies, including its potential for treating inflammatory diseases (Komakech et al., 2019). This aligns with our findings, which suggest that the antioxidant activity of the plant's methanolic extracts may contribute to its therapeutic effects, particularly in managing oxidative stress-related disorders.

The findings of this study regarding the flavonoid/phenolic content ratios in methanolic extracts from the leaves, stems, and roots of *A. africana* (Wild Sunflower) have significant implications for managing oxidative stress. The high ratio observed in the leaf extracts indicates a potent antioxidant capacity, suggesting that these extracts may offer considerable benefits in reducing oxidative stress. This potential makes *A. africana* leaves a valuable contender for developing antioxidant therapies aimed at mitigating the harmful effects associated with oxidative stress.

The study also highlights the potential of *A. africana* leaf extracts in managing diseases linked to oxidative stress, such as cardiovascular

conditions, cancer, neurodegenerative disorders, and diabetes.

The leaf extracts of *A. africana*, with their elevated flavonoid content, could be used in developing dietary supplements or therapeutic formulations aimed at managing oxidative stress and enhancing overall health. This practical application of the study's findings underscores the importance of harnessing natural sources of antioxidants.

Above all, the study points to the need for further research to elucidate the specific mechanisms through which flavonoids and phenolics exert their antioxidant effects. Such research could help optimize the use of *A. africana* extracts in managing oxidative stress and improving health outcomes related to oxidative damage.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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