Phytochemical profiling and *in vitro* antioxidant evaluation of methanol leaf extracts of the wild vegetable *Alternanthera sessilis*

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Wild leafy vegetables play an important role in food security and traditional health systems, yet their phytochemical composition and biofunctional potential are strongly influenced by ecological and geographical factors. *Alternanthera sessilis* is widely consumed and ethno-medicinally valued, but locality-specific quantitative data for wild populations from Nigeria remain limited. This study characterised the phytochemical composition and in vitro antioxidant potential of methanol leaf extracts of wild *Alternanthera sessilis* from southwest Nigeria. Leaves were air-dried, milled and extracted in methanol. Qualitative screening employed standard spot tests. Gravimetric assays quantified total alkaloids, saponins and terpenoids. Spectrophotometric methods determined tannin (tannic-acid equivalents), total flavonoids (quercetin equivalents) and total phenolics (gallic-acid equivalents). Antioxidant activity was assessed by DPPH radical scavenging, FRAP, and phosphomolybdenum (TAC) reducing-power assays. Qualitative screening detected alkaloids, flavonoids, terpenoids, tannins, saponins, steroids, and anthraquinones. Quantitative data indicated alkaloids > terpenoids > saponins (≈1.6%, 1.1% and 0.8% w/w, respectively). Spectrophotometric assays yielded mean (±SD) values of TPC 23.73 ± 0.50 mg GAE/g, TFC 84.73 ± 1.99 mg QE/g, and tannin 58.74 ± 1.73 mg TAE/g. DPPH IC_{50} values were 229.56 μg·mL^{-1} (extract) and 23.21 μg·mL^{-1} (ascorbic acid); FRAP assay produced ascorbic-acid equivalents (AAE) (≈12.04-25.42 μg AAE per mg extract) at higher assay concentrations (60-100 μg·mL^{-1}), while the TAC assay produced AAE values of 116.57-580.33 mg AAE/g across 20-100 μg·mL^{-1}. Overall, these results indicate that *A. sessilis* leaves constitute a significant reservoir of antioxidant compounds. These findings justify further chromatographic fractionation and compound-level analyses to isolate and characterise the major contributors to its antioxidant effects and assess their potential nutraceutical applications.

Keywords: *Alternanthera sessilis*; wild leafy vegetable; antioxidant activity; TPC; TFC; DPPH

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1. INTRODUCTION

Wild edible plants and underutilised leafy vegetables contribute materially to household food security, micronutrient supply and dietary diversity in many regions of the world. These species, often gathered from the wild or grown opportunistically in disturbed habitats, are typically rich in vitamins, minerals, fibre and antioxidant phytochemicals, and therefore represent important, low-cost sources of nutrients and bioactive compounds for rural and peri-urban populations. Several recent reviews and studies highlight the value of wild leafy vegetables for nutrition and resilience, and call for systematic integration of these species into food-security and public-health strategies (Bvenura and Afolayan, 2015; Kissanga et al., 2021; Tadesse et al., 2024). Plant-derived remedies and functional foods continue to play a central role in global healthcare and drug discovery. Traditional herbal medicines are widely used for their perceived safety and environmental advantages (Sreenivasa Nayak et al., 2020; Yuan et al., 2016), and isolated phytochemicals have furnished numerous lead compounds for modern therapeutics (Babalola et al., 2023). The pharmacological activities of medicinal plants are widely accepted to be principally determined by the diversity and abundance of secondary metabolites, alkaloids, terpenoids, glycosides and polyphenols, which together underpin their

antibacterial, anti-inflammatory, anticancer and antioxidant effects (Babalola et al., 2023; Malongane et al., 2017; Semwal et al., 2019). Despite their promise, only a fraction of plant secondary metabolites has been comprehensively characterised, making targeted screening and quantitative evaluation necessary steps in the discovery pipeline (Keskes et al., 2017; Yadav et al., 2017).

In light of this, wild leafy vegetables serve a dual role: they are both food and a potential source of nutraceuticals. Nutrient-dense vegetables can help reduce micronutrient deficiencies and provide consumers with dietary antioxidants that may contribute to long-term health. Recent surveys emphasise that integrating wild edible plants into local diets can improve nutrient intake and offer culturally acceptable, climate-resilient food options, particularly in low-resource settings where conventional horticulture is constrained (Casas et al., 2024; Tadesse et al., 2024).

Alternanthera sessilis L. R. Br.ex DC (Amaranthaceae) is an herb that can be either annual or perennial, grows to a height of 0.2-1 meters and is supported by robust taproots. Leaf growth occurs on opposing sides. It is sessile or short-leafstalk, 0.3 to 1 cm wide, 0.6 to 5 cm long, and widely subulate to spatulate to nearly linear (Ahmed et al., 2016). *A. sessilis* is a widely distributed wild leafy vegetable with a long history of ethnomedicinal use. Morphologically variable and frequently consumed as a green, the species is used in traditional medicine for wound care, gastrointestinal complaints and respiratory conditions, among a host of folkloric uses (Saqib and Janbaz, 2016). Phytochemical studies of *A. sessilis* report a recurring presence of alkaloids, flavonoids, terpenoids, steroids and other phenolic compounds, classes commonly associated with antioxidant and other bioactivities (Das et al., 2024; S. Nayak and Bhatta, 2020; Nikam and Namdas, 2022; Yap et al., 2019). Fractionation and activity-guided studies on red and green cultivars have identified antioxidant-rich fractions and implicated polyphenolic constituents as principal contributors to reducing power and radical-scavenging activity (Hazli et al., 2019; Hwong et al., 2023; Yap et al., 2019).

Nevertheless, constituent profiles and measured bioactivities of *A. sessilis* vary across studies, reflecting differences in cultivar, plant part, geographic provenance, harvest time and extraction protocol. Comparative studies have shown that solvent polarity and extraction method substantially influence the yield and class distribution of phytochemicals (Awotedu et al., 2020), factors that, in turn, affect spectrophotometric and chromatographic measures of antioxidant capacity. These sources of variation highlight the need for locality-specific, methodologically consistent analyses to characterise the nutraceutical value of wild greens destined for dietary or therapeutic use. Although comparable phytochemical and antioxidant surveys exist, this study provides locality-specific, quantitative data for wild *A. sessilis* from southwest Nigeria, information that captures ecological and cultivar-linked variation relevant to local dietary/nutraceutical use and that serves as a necessary baseline for prioritising regional germplasm, guiding compound-level isolation, and designing future comparative or translational studies. Given the species' dietary importance, ethnomedicinal pedigree and emerging evidence of antioxidant richness, the current study aims to assess the phytochemical profile and antioxidant potential of methanol extracts of the green cultivar of *A. sessilis* leaves from Nigeria.

2. MATERIALS AND METHODS

2.1. Materials

All chemicals and reagents used in this study were of analytical grade. The chemicals included methanol, ethanol, Ammonium hydroxide, and ferric chloride all obtained from BDH (England); butanol, sulphuric acid, and acetic acid obtained from Lobachem (China); Folin-Ciocalteu reagent and ascorbic acid were purchased from Merck KGaA (Darmstadt, Germany); anhydrous sodium carbonate, gallic acid, tannic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), aluminium chloride, sodium nitrite, and sodium hydroxide were from Sigma-Aldrich (St. Louis, MO, USA), whereas quercetin was bought from Biosynth (Staad, Switzerland), and UV Spectrophotometer Spectrumlab 752s (Spectrumlab, England).

2.2. Plant material collection and authentication

Fresh leaves of the wild vegetable *A. sessilis* L. R. Br.ex DC (Amaranthaceae) were harvested from natural populations at the Forestry Research Institute of Nigeria (Ibadan) during the early rainy season in May 2025. Voucher specimens were prepared and authenticated by taxonomists at the Forest Herbarium; Ibadan and were deposited in the institutional herbarium. The collected material was transported to the laboratory in paper bags, rinsed under running tap water to remove adhering debris, and air-dried in the shade at ambient laboratory temperature (25-30 °C). Dried material was milled to a fine powder using a mechanical grinder and stored in airtight, light-resistant containers until analysis.

2.3. Preparation of crude extract

One kilogram (1 kg) of the powdered leaf material was macerated in 5 L of methanol (1:5 w/v), and the mixture was agitated intermittently for 72 hours at room temperature. The crude extract was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at ≤ 40 °C to remove the solvent. The concentrated residue was transferred to amber vials and stored at 4 °C until use. Working solutions for phytochemical and antioxidant assays were prepared by dissolving a known mass of dried extract in methanol to obtain a 1 mg·ml^{-1} stock; serial dilutions were prepared freshly before assays (Harborne, 1998).

2.4. Qualitative phytochemical screening

Qualitative tests for major phytochemical classes, saponins, tannins, flavonoids, cardiac glycosides, anthraquinones, terpenoids, steroids, alkaloids were performed on the crude methanol extract of *A. sessilis* using standard chemical spot tests and precipitation reactions as described in classical phytochemical manuals (Harborne, 1998; Sofowora, 2008; Trease and Evans, 2002). Briefly, Wagner's and Dragendorff's reagents were used for alkaloids; frothing tests for saponins; ferric chloride tests for tannins; Shinoda/alkaline reagent tests for flavonoids; Keller-Kiliani and Kedde tests for cardiac glycosides; Borntträger's test for anthraquinones; Salkowski and Liebermann-Burchard reactions for terpenoids and steroids. All tests were conducted in triplicate and interpreted as present/absent or weak/moderate/strong based on colour intensity or precipitate formation.

2.5. Quantitative phytochemical determination

2.5.1. Total alkaloids

The alkaloid content was determined by the alkaline precipitation (gravimetric) procedure adapted from (Harborne, 1998). In brief, 2 g of crude methanol extract was acidified in 10% acetic acid in ethanol (200 mL), left to extract for 4 h, filtered, and concentrated on a water bath to about one-quarter of the original volume. The concentrated extract was made alkaline with concentrated ammonium hydroxide to precipitate alkaloids, which were collected on a pre-weighed filter paper, washed, oven-dried to constant weight (oven 40 – 60 °C/cooled in a desiccator). Total alkaloid was expressed as % (w/w) of the dry plant material.

2.5.2. Total saponins

Total saponin content was measured by a conventional gravimetric extraction. The 2 g of the crude extract was refluxed with 20% (v/v) aqueous ethanol, filtered, and the combined extracts partitioned with n-butanol. The butanolic fraction was washed with water, evaporated to dryness, and the residue was weighed. Saponin content was reported as % (w/w) of the dry plant material (Obadoni and Ochuko, 2002).

2.5.3. Total terpenoids

Total terpenoids were quantified by solvent extraction and gravimetric determination. Briefly, 2 g of crude methanol extract were extracted with a non-polar solvent (petroleum ether) under reflux in a Soxhlet apparatus; the solvent was evaporated under reduced pressure, and the residue (terpenoid-enriched fraction) was dried and weighed. Results were expressed as % (w/w) of the dried plant sample (Harborne, 1998).

2.5.4. Determination of total phenolic content (gallic-acid equivalents)

Total phenolics were measured using the Folin-Ciocalteu assay following standard practice (Kulkarni et al., 2015). Appropriate aliquots of extract (so that 200–1000 μg fell within the linear range) were mixed with 1.5 mL of 10% Folin-Ciocalteu reagent and left for 5 min. Next, 1.2 mL of 7.5% (w/v) Na_2CO_3 was added, and the volume was adjusted to 10 mL with distilled water. After incubation in the dark at room temperature for 30 min, absorbance was read at 765 nm against a blank. Gallic acid standards (0-100 μg·mL^{-1}) were processed in parallel to generate the calibration relationship ($y = 0.0124x - 1.0827$; $R^2 = 0.9593$). Phenolic concentrations were interpolated from the calibration curve and expressed as mg gallic-acid equivalents (GAE) per g of extract.

2.5.5. Determination of total flavonoid content (quercetin equivalents)

Total flavonoids were quantified by the aluminium-chloride colorimetric assay adapted from (Zhishen et al., 1999). An aliquot (4.0 mL) of each extract dilution (200-1000 μg/mL) was mixed with 0.3 mL 5% (w/v) $NaNO_2$ and left for 5 min. Then 0.3 mL 10% (w/v) $AlCl_3$ was added, and the mixture was incubated for a further 6 min, after which 2.0 mL 1 M NaOH was added and the volume brought to 10 mL with distilled water. After brief vortexing, absorbance was measured at 510 nm against a reagent blank. A quercetin standard series (5-100 μg·mL^{-1}) was run in the same conditions to construct the calibration curve ($y = 0.0012x + 0.0894$; $R = 0.9836$). Results were calculated from the standard curve and reported as mg quercetin equivalents (QE) per g of dry extract.

2.5.6. Determination of total tannin content (tannic-acid equivalents)

Tannin levels were estimated using a Folin-type colorimetric procedure adapted from (Amorim et al., 2008). In each assay, 0.1 mL of extract was combined with 7.5 mL of distilled water, 0.5 mL of Folin–Ciocalteu reagent and 1.0 mL of 35% (w/v) Na_2CO_3; the final volume was adjusted to 10 mL with distilled water. Samples were vortexed and allowed to develop for 30 min at ambient temperature before measurement. Absorbance was recorded at 725 nm against a reagent blank. Tannic acid standards were analysed concurrently to produce a calibration curve ($y = 0.0022x - 0.0565$; $R = 0.9926$). Sample absorbances were converted to tannic-acid equivalents using the calibration parameters and expressed as mg tannic acid equivalent per g of extract.

2.6 Results processing

All assays were performed in triplicates, in order to assess their linearity and mitigate the potential matrix interferences or non-specific absorbances. The respective TPC, TFC, and tannin content was quantified from the slope of the regression line in this validated linear range, expressed as milligrams of the standard equivalent per gram of dry extract. This approach provides a robust estimate by accounting for any fixed background absorbance (non-zero intercept) while ensuring the reported value is independent of the specific assay concentration tested (Matic et al., 2017; Ramos et al., 2017). The results are expressed as the mean ± standard deviation of the standard equivalents.

2.7. Determination of the antioxidant activity

2.7.1. DPPH radical-scavenging assay

The free-radical scavenging capacity of the *A. sessilis* crude methanol extract was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method with ascorbic acid as a positive control. A 0.01 mM DPPH working solution was prepared in methanol and kept protected from light. Aliquots (100 μL) of each test sample (prepared at the desired concentration) or the ascorbic acid standard were added to 2.0 mL of the DPPH solution in amber vials. After incubating the mixtures at room temperature in the dark for 30 minutes, the reduction in DPPH absorbance was measured at 517 nm against a DPPH reagent blank (containing solvent but no sample). Radical scavenging activity was expressed as percentage inhibition and calculated as:

$$\%\ \text{Inhibiion} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

where $A_{control}$ is the absorbance of the DPPH solution without sample, and A_{sample} is the absorbance in the presence of extract or standard (Gulcin and Alwasel, 2023)

2.7.2. Ferric reducing antioxidant power (FRAP) assay

FRAP activity was measured using ascorbic acid as the reference reductant in a modification of the standard protocol (Benzie and Strain, 1996). The FRAP working reagent was prepared immediately before use by mixing TPTZ (20 mM in 40 mM HCl), $FeCl_3 \cdot 6H_2O$ (20 mM) and 0.3 M acetate buffer (pH 3.6) in the ratio 1:1:10 (v/v/v) and equilibrating to 37 °C. For each determination, 30 μL of sample was combined with 90 μL distilled water and 900 μL pre-warmed FRAP reagent (final assay volume = 1.0 mL), incubated at 37 °C for 30 min, then cooled briefly and read at 593 nm against a reagent blank prepared in the same way but substituting the sample with water. Ascorbic acid standards (0.5-100 μg·mL^{-1}) were processed in parallel to construct a calibration curve. The blank-corrected absorbances were plotted against ascorbic acid concentration to obtain the linear regression ($y=0.0148y + 0.317$, $R^2 = 0.9388$). The results are expressed as mg AAE (ascorbic acid equivalents)/ g.

2.7.3. Total antioxidant capacity (phosphomolybdenum; TAC)

TAC was determined by the phosphomolybdenum method adapted from (Prieto et al., 1999). Fresh reagent was prepared containing sulfuric acid, sodium phosphate, and ammonium molybdate according to the original procedure. In each assay, 0.5 mL of sample was mixed with 3.0 mL of the reagent, incubated at 95 °C for 90 min to allow reduction of Mo(VI) to Mo(V), cooled to room temperature, and measured at 695 nm against a reagent blank prepared identically but without sample. Ascorbic acid standards (1-10 μg·mL^{-1}) were run in the same manner to generate a calibration relationship ($y = 0.1725x - 0.1067$; $R^2 = 0.9984$). The results are presented as ascorbic-acid equivalents (AAE) per unit mass of extract (mg AAE·g^{-1}) by accounting for assay volume and the mass of extract used in the assay.

Table 1. Phytochemical screening of *Alternanthera sessilis*.

Test	Results
Saponins	+ve
Tannins	++ve
Flavonoids	++ve
Cardiac glycosides	+ve
Anthraquinones	+ve
Terpenoids	++ve
Steroids	+ve
Alkaloids	++ve

Note: +ve: present; ++ve: abundant; -ve: absent

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1. Qualitative Analysis

Qualitative phytochemical screening of the crude methanol leaf extract of *A. sessilis* (Table 1.) revealed the presence of saponins, cardiac glycosides, anthraquinones and steroids, while phenolic compounds (tannins and flavonoids), terpenoids, and alkaloids were found in comparatively higher abundance. The rich occurrence of flavonoids, tannins, and alkaloids is consistent with the extract's demonstrated reducing capacity in the antioxidant assays (FRAP, TAC), and supports further quantitative and chromatographic characterisation of these classes.

3.1.2. Quantitative Analysis

Quantitative determinations showed that alkaloids were the most abundant of the three classes quantified by gravimetry, followed by terpenoids and saponins (Figure 1.). These values indicate a substantive presence of nitrogen-containing and terpenoid constituents in the leafy part of *A. sessilis*. Such constituents are plausible contributors to the extract's reducing capacity observed in antioxidant assays. The spectroscopic estimation of phytochemical constituents in the methanol leaf extract of *A. sessilis* yielded the following values: total phenolic content of 23.73 ± 0.50 mg gallic acid equivalent per gram of dry extract, total flavonoid content of 84.73 ± 1.99 mg quercetin equivalent per gram of dry extract, and tannin content of 58.74 ± 1.73 mg tannic acid equivalent per gram of dry extract (Table 2.).

3.1.3. Antioxidant Activity

The methanol extract of *A. sessilis* and ascorbic acid (standard antioxidant), as assayed by DPPH to determine free-radical scavenging potency, showed a concentration-dependent inhibition (Figure 2.). The linear regression of the percentage inhibition and concentration for the methanol extract of *A. sessilis* ($y = 0.0137x + 46.855$, $R = 0.9092$) yielded an IC_{50} of 229.59 μg·mL^{-1} against that of the ascorbic acid standard ($y = 0.1049x + 47.565$, $R = 0.9371$), which returned an IC_{50} of 23.21 μg·mL^{-1}. These values indicate that the crude methanol extract exhibits measurable DPPH scavenging activity but is substantially less potent than ascorbic acid under the assay conditions employed.

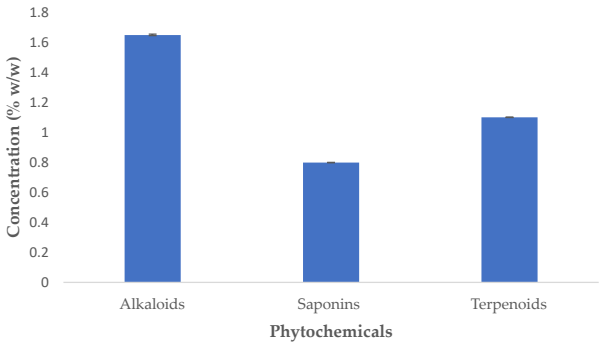

Figure 1. Quantitative estimation of alkaloids, saponins, and terpenoids in the methanol leaf extract of *Alternanthera sessilis*.
*Bars show mean concentration expressed as % w/w, error bars indicate ± SD (n = 3).

Table 2. Concentration of tannin, total flavonoid and total phenolic contents in *Alternanthera sessilis* extracts.

Phytochemical	Standard Equivalent	Content (mg/g)
Total Phenolic Content (TPC)	Gallic Acid	23.73 ± 0.50
Total Flavonoid Content (TFC)	Quercetin	84.73 ± 1.99
Tannin	Tannic Acid	58.7 ± 1.73

The FRAP assay calibration using ascorbic acid produced a linear regression model ($Abs = 0.0148C + 0.317$, $R^2 = 0.9388$). Using this regression, the antioxidant capacity of the *A. sessilis* methanol extract was expressed as ascorbic acid equivalents (AAE). Extract concentrations ≤ 40 μg/mL produced absorbances below the assay quantification limit (BQL). For the measurable concentrations, the extract exhibited FRAP activities of 12.03, 15.88, and 25.41 mg AAE/g extract at 60, 80, and 100 μg/mL extract, respectively (Table 3.). These results demonstrate a concentration-dependent increase in ferric-reducing antioxidant power. The total antioxidant capacity (TAC) of the *A. sessilis* methanol extract, as evaluated using the phosphomolybdenum method and quantified against an ascorbic acid calibration curve (1-10 μg/mL) yielded a linear relationship ($Abs = 0.1725C - 0.1067$; $R^2 = 0.9984$). Sample absorbances were converted to ascorbic acid equivalents (AAE) using the regression parameters and expressed as milligrams of AAE per gram of extract. The extract showed a concentration-dependent increase in TAC, with values ranging from 116.57-580.33 mg AAE/g at an extract concentration range of 20-100 μg/mL (Table 4.). These results indicate that the methanol extract of *A. sessilis* possesses a strong, concentration-dependant capacity to reduce the phosphomolybdenum complex, reflecting a substantial pool of redox-active constituents within the extract.

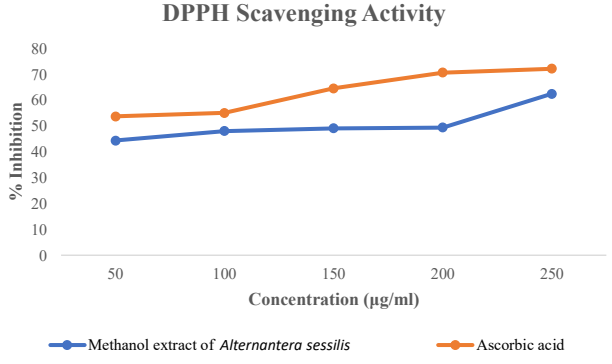

Figure 2. DPPH radical scavenging activity of *Alternanthera sessilis* extract compared to ascorbic acid standard.

4. DISCUSSION

The crude methanol extract of *A. sessilis* was found to contain a broad spectrum of secondary metabolites, including alkaloids, flavonoids, terpenoids, tannins, saponins, steroids, and even

Table 3. FRAP antioxidant capacity of *Alternanthera sessilis* methanol extract (Ascorbic Acid Equivalents (AAE))

Concentration (μg/mL)	AAE (mg/g)
20	BQL
40	BQL
60	12.03
80	15.88
100	25.41

BQL – below quantification limit

Table 4. Total Antioxidant Capacity (TAC) of *Alternanthera sessilis* methanol extract (Ascorbic Acid Equivalents (AAE))

Concentration (μg/mL)	AAE (mg/g)
20	116.57
40	232.50
60	348.44
80	464.39
100	580.33

anthraquinones. This profile largely concurs with prior reports. For example, *A. sessilis* leaf extracts are well-known to be rich in alkaloids, flavonoids, terpenoids, and steroids (Das et al., 2024; Monroy and Limsiaco, 2016; Nikam and Namdas, 2022; Singla et al., 2022). Nayak and Bhatta (2020) similarly detected alkaloids, terpenoids, phenolic compounds and saponins in a methanol extract, while Jeeva and Rani (2024) observed alkaloids and glycosides in an extract of *A. sessilis*, with flavonoids, steroids, and terpenoids absent.

Notable differences arise in the occurrence of tannin and anthraquinone. Positive tannin test in the methanol extract was observed, which aligns with the study of Nikam and Namdas (2022) and Das et al (2024), whereas some authors report tannins absent in the methanol extract (Monroy and Limsiaco, 2016; S. Nayak and Bhatta, 2020; Pathak et al., 2020). This may reflect methodological variation where certain organic solvents often fail to precipitate high-molecular polyphenolic tannins, whereas aqueous or hydroalcoholic solvents do. Conversely, anthraquinones were faintly positive in our methanol extract. Monroy and Limsiaco (2016),reported anthraquinones in all extracts, while they are absent in other studies (Babu et al., 2018; Umate and Marathe, 2017). Such discrepancies likely stem from differences in *A. sessilis* chemotypes and solvent polarity. Regional factors may also alter secondary metabolism. Moreover, extraction conditions (cold maceration vs. Soxhlet, solvent concentration, plant age) will influence which compounds are solubilised.

Quantitative analysis yielded measurable yields for each class. Alkaloids were the most abundant, followed by moderate terpenoid content and lower saponin levels. Quantitative data for *A. sessilis* are scarce, but these value are broadly in line with one literature report, where (Nigam et al., 2023) found~2% saponins (w/w) in *A. sessilis* extract. The alkaloid yields are less frequently reported; overall, the gravimetric quantification confirms that *A. sessilis* is moderately rich in alkaloids and terpenoids, with saponins at lower levels, a pattern qualitatively matching prior observations of its phytochemical profile.

The spectrophotometric assays quantified the total phenolic, flavonoid, and tannin contents (expressed as standard equivalents) in the methanol leaf extract of *A. sessilis*. Linearity was confirmed through regression analysis of equivalent concentrations against extract concentrations within the validated range, yielding high coefficients of determination ($R^2 \geq 0.9$). The slope-derived values demonstrated consistent intrinsic content independent of assay concentration, adhering to established dose-response principles. Importantly, the total values obtained for TPC, TFC, and tannins falls within the moderate to lower range reported in the literature for this species. In earlier work, (Kota et al., 2017) found a range of 0.013-67.75 mg GAE/g TPC in various extracts of *A. sessilis*, the mid-range consistent with this study. Similarly, (Hazli et al., 2019) also reported 77.29 ± 1.02 mg GAE/g extract in the ethanol extract of *A. sessilis*, however, higher levels within the range (264.19-292.65 mg GAE/g) were reported in the study by (Amrutanand et al., 2024) and (Aryal et al., 2019), while a study on cooked *A. sessilis* leaves also reported a high TPC of ~113.75 mg GAE (Babu et al., 2018).

For TFC, (Aryal et al., 2019) reported a lower TFC (21.51±0.46 mg QE/g) in the methanol extract, while (Amrutanand et al., 2024) observed a substantially higher TFC level (605.91±6.42 mg QE/g). Other studies, including Das et al. (2024) and Shreshtha et al. (2017), have similarly indicated lower levels of both TPC and TFC, consistent with the moderate range observed in this study. The total tannin values in this study's methanol extract were modest, consistent with the literature notes that tannins may be under-reported in polar extracts

The observed variability among studies can be attributed to several factors. Cultivar and environment differences, and also methodology variations, also play a role. Solvent strength, extraction time, and even calibration standards can shift the reported values. Despite such differences, the concentration-dependent increase and assay linearity in this study are well within ranges reported for *A. sessilis* and related leafy greens.

The result of the antioxidant activity of the plant was evaluated by three complementary assays. The DPPH data show that the crude methanol extract of wild *A. sessilis* possesses moderate radical-scavenging activity ($IC_{50} \approx 230$ μg·mL^{-1}), substantially weaker than the ascorbic acid reference, which is typical for complex crude extracts where active principles are present at low concentrations relative to a pure antioxidant standard. This DPPH outcome aligns with other studies that reported a range of 82.6-587.09 μg·mL^{-1}, showing the plant's good antioxidant profile (Borah et al., 2011; Hwong et al., 2023; Yap et al., 2019). In the phosphomolybdenum (total antioxidant capacity, TAC) assay, the extract exhibited a strong, concentration-dependent AAE (ascorbic acid equivalent) value, reflecting its overall antioxidant load. Borah et al. (2011) reported 12.044 mM AAE per gram for the *A. sessilis* methanol extract, which is higher when converted to equivalent units and compared with the values observed at the highest test concentration in this study, indicating robust reducing capacity. Likewise, in the FRAP assay (expressed as AAE), substantial activity was observed only at high concentrations, which correlates positively with the sample concentration. Although direct FRAP values for *A. sessilis* are less commonly reported, studies consistently show a close correlation between phenolic content and reducing power in this species (Hwong et al., 2022). Indeed, the high TPC and TFC measured provided a logical basis for the observed antioxidant effects: phenolic soumpaunds (such as flavonoids) and alkaloids are known reductants and free-radical scavengers, so their abundance translates into strong FRAP/TAC signals. Hwong et al. (2022) emphasised that *A. sessilis*'s potent antioxidant activity is a result of its diverse phytochemical constituents, such as polyphenols, terpenes, and alkaloids. Findings from this study are fully consistent with this: the significant AAE values measured stem directly from the abundant phenolics and related compounds in the extract. Small differences can be ascribed to solvent effects, seasonal/geographic variation, and inherent assay variability, where different laboratories use different standards and conditions in the various antioxidant assays. The stronger reducing-power signals observed in FRAP and TAC compared with the DPPH IC_{50} pattern reflect assay-specific chemistry: FRAP and TAC measure overall reducing capacity under acidic or strongly reducing conditions, and therefore,

often correlate closely with total phenolics, whereas DPPH specifically reports the ability to quench a stable nitrogen radical and is more sensitive to certain flavonoid structures and small-molecule antioxidants.

Finally, methodological limitations are observed. Both FRAP and TAC are broad reducing power assays and may over- or under-estimate specific antioxidant species. FRAP measures only those compounds able to reduce Fe^{3+} to Fe^{2+} under the assay conditions, while the phosphomolybdenum method depends on Mo reduction at high acidity; compounds that act by other mechanisms (e.g. direct radical quenching) may not be fully captured. Matrix interferences (e.g. coloured extract) can also affect absorbance. Nonetheless, in the scope of the *A. sessilis* study, these assays are standard, and results from this study fit the expected pattern: the measured antioxidant capacity scales with phenolic and flavonoid content, as documented by prior studies. In a nutshell, the strong FRAP and TAC values of our methanol extract are consistent with its phytochemical richness, and compare favourably with those reported for *A. sessilis* by others, strengthening the fact that solvent polarity and ecological factors modulate the plant's antioxidant potential.

5. CONCLUSION

The study has established that the methanol extract of the wild vegetable *A. sessilis* is chemically diverse, containing multiple classes of secondary metabolites including phenolics, flavonoids, alkaloids, terpenoids, saponins, steroids and anthraquinones. The quantitative estimation indicated that nitrogenous and isoprenoid constituents constitute appreciable fractions of the extract, while spectrophotometric assays confirmed a moderate phenolic content with consistent intrinsic levels in flavonoids, and tannins, independent of assay concentration through validated linear regression. Complementary antioxidant assays demonstrated a clear, concentration-responsive antioxidant capacity that is consistent with the observed phytochemical profile. When taken together, these findings identify *A. sessilis* leaves as a rich source of redox-active phytochemicals and justify further work to complete kinetic-based radical-scavenging assays, perform compound-level characterisation, and evaluate the biological relevance through bioavailability, safety and *in vivo* studies aimed at validating nutritional or therapeutic potential.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests in relation to this study.

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