

## PHYTOCHEMICAL PROFILING AND COMPARATIVE ANTIOXIDANT POTENTIAL OF *ALTERNANTHERA SESSILIS* LEAF EXTRACTS USING IN VITRO ASSAYS

Chennu M. M. Prasada Rao<sup>1\*</sup>, Tanniru Rajeswari<sup>2</sup>, Jyotirmayee Patel<sup>2</sup>, Renuka Barik<sup>2</sup>,  
Rupashree Nishad<sup>2</sup>, Prakash Pradhan<sup>2</sup>

<sup>1</sup>Principal & Professor, Maheswari Pharmaceutical College, Sundargarh, Odissa-770001.

<sup>2</sup>Faculty, Maheswari Pharmaceutical College, Sundargarh, Odissa-770001.



\*Corresponding Author: Chennu M. M. Prasada Rao

Principal & Professor, Maheswari Pharmaceutical College, Sundargarh, Odissa-770001.

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### ABSTRACT

This study investigates the in vitro antioxidant activity of methanolic and aqueous leaf extracts of *Alternanthera sessilis*. Antioxidant potential was evaluated using DPPH radical scavenging assay, reducing power assay, and total phenolic content (TPC) estimation. Phytochemical screening confirmed the presence of phenolics, flavonoids, tannins, and alkaloids. The methanolic extract exhibited higher DPPH radical scavenging activity (72.4% at 800 µg/mL) compared to the aqueous extract (60.3%), with a lower IC<sub>50</sub> value indicating greater potency. The total phenolic content was also higher in the methanolic extract (82 mg GAE/g extract) than in the aqueous extract (60 mg GAE/g extract). Additionally, the reducing power assay demonstrated a concentration-dependent increase in antioxidant activity, with the methanolic extract showing superior reducing ability. These findings suggest that *Alternanthera sessilis* is a promising natural source of antioxidants with potential applications in pharmaceutical and nutraceutical formulations.

**KEYWORDS:** *Alternanthera sessilis*, Antioxidant activity; DPPH assay; Total phenolic content; Reducing power

### 1. INTRODUCTION

Oxidative stress is a biochemical condition characterized by an imbalance between the production of reactive oxygen species (ROS) and the biological system's ability to detoxify these reactive intermediates or repair the resulting damage. Reactive oxygen species, including superoxide anion (O<sub>2</sub><sup>-</sup>), hydroxyl radical (•OH), and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), are generated as natural byproducts of cellular metabolism, particularly during mitochondrial oxidative phosphorylation. While ROS play essential roles in cell signaling and homeostasis at low or moderate concentrations, excessive accumulation can result in oxidative damage to lipids, proteins, and nucleic acids, ultimately leading to cellular dysfunction and death (Halliwell & Gutteridge, 2015).

The involvement of oxidative stress in the pathogenesis of numerous chronic and degenerative diseases has been well documented. Conditions such as diabetes mellitus, cancer, cardiovascular diseases, neurodegenerative

disorders (e.g., Alzheimer's disease and Parkinson's disease), and inflammatory disorders are closely linked with elevated oxidative stress levels. Lipid peroxidation, DNA strand breakage, and protein oxidation are among the key molecular events triggered by ROS, contributing to disease progression (Pham-Huy et al., 2008). Consequently, the modulation of oxidative stress through antioxidant defense mechanisms has emerged as a critical therapeutic strategy.

Antioxidants are substances that can delay or inhibit the oxidation of biomolecules by neutralizing free radicals or preventing their formation. The human body possesses endogenous antioxidant systems, including enzymatic defenses such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as well as non-enzymatic components such as glutathione, uric acid, and bilirubin. However, under conditions of excessive oxidative stress, these intrinsic systems may become overwhelmed, necessitating the intake of

exogenous antioxidants through diet or supplementation (Lobo *et al.*, 2010).

In recent years, there has been a growing interest in natural antioxidants derived from plant sources due to concerns regarding the safety and potential side effects of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Plant-based antioxidants are considered safer, biodegradable, and often exhibit multiple pharmacological activities, including anti-inflammatory, antimicrobial, anticancer, and hepatoprotective effects (Shahidi & Ambigaipalan, 2015). Phytochemicals such as phenolic acids, flavonoids, tannins, alkaloids, and terpenoids are primarily responsible for the antioxidant properties of medicinal plants. These compounds act through various mechanisms, including free radical scavenging, metal ion chelation, and inhibition of oxidative enzymes.

Among the diverse plant species investigated for antioxidant activity, *Alternanthera sessilis* (L.) R.Br. ex DC., belonging to the family Amaranthaceae, has gained considerable attention due to its traditional medicinal uses and nutritional value. Commonly known as sessile joyweed or "Ponnanganni" in South India, *A. sessilis* is a perennial herb widely distributed in tropical and subtropical regions, including India, Sri Lanka, Southeast Asia, and parts of Africa. It grows abundantly in moist and marshy areas and is often consumed as a leafy vegetable in various traditional diets.

The ethnomedicinal significance of *Alternanthera sessilis* is well established in traditional systems of medicine such as Ayurveda and Siddha. The plant has been used for the treatment of a variety of ailments, including fever, dysentery, diarrhea, skin diseases, wounds, and eye disorders. It is also reputed for its hepatoprotective, anti-inflammatory, and immunomodulatory properties. The widespread use of this plant in traditional medicine highlights its potential as a source of bioactive compounds with therapeutic relevance (Kumar *et al.*, 2011).

Phytochemical investigations of *A. sessilis* have revealed the presence of a wide range of bioactive constituents, including phenolic acids, flavonoids, sterols, glycosides, and triterpenoids. Notably, compounds such as quercetin, kaempferol, luteolin, and chlorogenic acid have been identified in the plant. These phytoconstituents are known for their potent antioxidant properties and contribute significantly to the plant's pharmacological activities. Phenolic compounds play a crucial role in neutralizing free radicals by donating hydrogen atoms or electrons, thereby stabilizing reactive species and preventing oxidative damage (Rice-Evans *et al.*, 1997).

Flavonoids, a major class of polyphenolic compounds present in *A. sessilis*, are recognized for their ability to scavenge a wide range of reactive species, including

superoxide radicals, hydroxyl radicals, and peroxy radicals. Additionally, flavonoids can chelate transition metal ions such as iron and copper, thereby inhibiting metal-catalyzed free radical generation. The antioxidant activity of flavonoids is largely attributed to their structural features, including the presence of hydroxyl groups and conjugated double bonds (Panche *et al.*, 2016).

Sterols and triterpenoids present in *A. sessilis* also contribute to its biological activity, although their antioxidant potential is generally less pronounced compared to phenolic compounds. Nevertheless, these constituents may act synergistically with other phytochemicals to enhance the overall antioxidant effect of the plant extract. Such synergistic interactions are a hallmark of herbal medicines and often result in improved efficacy compared to isolated compounds.

Several *in vitro* studies have been conducted to evaluate the antioxidant potential of *Alternanthera sessilis* using established assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, ABTS radical cation decolorization assay, ferric reducing antioxidant power (FRAP) assay, and reducing power assay. Among these, the DPPH assay is one of the most widely used methods due to its simplicity, rapidity, and reproducibility. In this assay, antioxidants reduce the stable DPPH radical to a non-radical form, resulting in a decrease in absorbance, which can be quantitatively measured (Blois, 1958).

Previous studies have demonstrated that extracts of *A. sessilis*, particularly those obtained using polar solvents such as methanol and ethanol, exhibit significant DPPH radical scavenging activity. This observation suggests that the antioxidant compounds present in the plant are predominantly polar in nature, consistent with the presence of phenolic and flavonoid constituents. Furthermore, the reducing power assay, which measures the ability of an extract to donate electrons and reduce ferric ions ( $Fe^{3+}$ ) to ferrous ions ( $Fe^{2+}$ ), has also indicated strong antioxidant potential in *A. sessilis* extracts.

The total phenolic content (TPC) and total flavonoid content (TFC) of *A. sessilis* extracts have been found to correlate positively with their antioxidant activity. This correlation supports the hypothesis that phenolic compounds are the primary contributors to the observed free radical scavenging effects. Additionally, studies have reported that the antioxidant activity of *A. sessilis* is concentration-dependent, with higher extract concentrations exhibiting greater radical scavenging capacity.

The growing body of evidence supporting the antioxidant potential of *Alternanthera sessilis* underscores its importance as a functional food and medicinal plant. Its dual role as a dietary vegetable and a therapeutic agent makes it particularly attractive for the development of

nutraceuticals and herbal formulations aimed at combating oxidative stress-related diseases. Moreover, the plant's widespread availability, low cost, and minimal side effects further enhance its suitability for large-scale utilization.

Despite these promising findings, there remains a need for more comprehensive studies to fully elucidate the mechanisms underlying the antioxidant activity of *A. sessilis*. Advanced analytical techniques such as high-performance liquid chromatography (HPLC), liquid chromatography–mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR) spectroscopy can be employed to identify and quantify the bioactive constituents responsible for the observed effects. Additionally, in vivo studies and clinical trials are essential to validate the efficacy and safety of *A. sessilis* in human populations.

In conclusion, oxidative stress plays a pivotal role in the development of various chronic diseases, and the search for effective and safe antioxidants continues to be a major focus of biomedical research. Natural plant-based antioxidants offer a promising alternative to synthetic compounds, with *Alternanthera sessilis* emerging as a valuable candidate due to its rich phytochemical composition and demonstrated antioxidant activity. The present study aims to further investigate the antioxidant potential of *A. sessilis* using in vitro assays.

## 2. MATERIALS AND METHODS

### 2.1 Plant Collection and Authentication

Fresh leaves of *Alternanthera sessilis* were collected from the Odisha region of India during the early morning hours to ensure maximum phytochemical integrity. The plant was harvested from a non-polluted area to avoid contamination with environmental toxins, pesticides, or heavy metals that could interfere with phytochemical and antioxidant analyses.

The collected plant material was thoroughly washed under running tap water to remove adhering soil, dust, and other extraneous matter, followed by rinsing with distilled water. The cleaned samples were then air-dried to remove surface moisture.

Botanical authentication of the plant specimen was carried out by using Google image. The identification was confirmed based on morphological characteristics such as leaf arrangement, stem structure, and floral features, in accordance with standard floristic descriptions.

### 2.2 Preparation of Extract

#### 2.2.1 Drying and Powdering

The collected leaves were shade dried at room temperature (25–30°C) for a period of 7–10 days to prevent degradation of thermolabile phytoconstituents. Direct sunlight was avoided to preserve sensitive compounds such as phenolics and flavonoids. The dried

plant material was then coarsely powdered using a mechanical grinder and passed through a sieve (mesh size 40) to obtain uniform particle size. The powdered material was stored in airtight containers protected from light and moisture until further use.

#### 2.2.2 Methanolic Extract (Soxhlet Extraction)

Approximately 100 g of powdered plant material was subjected to Soxhlet extraction using 500 mL of methanol as the solvent. The extraction process was carried out for 6–8 hours at a controlled temperature until the solvent in the siphon tube became colorless, indicating exhaustive extraction.

Methanol was selected as the solvent due to its high polarity and efficiency in extracting phenolic and flavonoid compounds. The obtained extract was filtered using Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary vacuum evaporator. The concentrated extract was further dried in a desiccator to obtain a semi-solid mass. The percentage yield of the extract was calculated and stored at 4°C for further analysis.

#### 2.2.3 Aqueous Extract (Maceration Method)

For aqueous extraction, 100 g of powdered plant material was soaked in 500 mL of distilled water and kept for 48 hours at room temperature with intermittent shaking to facilitate extraction. The mixture was then filtered, and the filtrate was concentrated by evaporation on a water bath at controlled temperature (not exceeding 50°C).

The concentrated extract was dried to obtain a solid residue and stored in airtight containers at refrigerated conditions until further use. The aqueous extract was used to compare extraction efficiency with the methanolic extract, particularly for water-soluble phytoconstituents.

### 2.3 Preliminary Phytochemical Screening

Preliminary phytochemical analysis of both methanolic and aqueous extracts of *Alternanthera sessilis* was carried out using standard qualitative chemical tests to identify the presence of major bioactive constituents.

#### 2.3.1 Test for Alkaloids

- **Dragendorff's Test:** A few drops of Dragendorff's reagent were added to the extract solution. Formation of an orange or reddish-brown precipitate indicated the presence of alkaloids.
- **Mayer's Test:** Addition of Mayer's reagent produced a cream-colored precipitate confirming alkaloids.

#### 2.3.2 Test for Flavonoids

- **Shinoda Test:** The extract was treated with magnesium turnings and concentrated hydrochloric acid. The appearance of a pink or red coloration indicated the presence of flavonoids.

- **Alkaline Reagent Test:** Addition of sodium hydroxide resulted in an intense yellow color, which turned colorless upon addition of dilute acid, confirming flavonoids.

### 2.3.3 Test for Phenolic Compounds

- **Ferric Chloride Test:** A few drops of 5% ferric chloride solution were added to the extract. Formation of a blue-green or dark coloration indicated the presence of phenolic compounds.

### 2.3.4 Test for Tannins

- **Gelatin Test:** Addition of gelatin solution resulted in the formation of a white precipitate, indicating tannins.
- **Lead Acetate Test:** Formation of a bulky white precipitate confirmed the presence of tannins. The results of phytochemical screening were recorded qualitatively as present (+) or absent (-). These phytoconstituents are known to contribute significantly to antioxidant activity.

## 2.4 Evaluation of Antioxidant Activity

The antioxidant potential of the extracts was evaluated using multiple in vitro assays to assess different mechanisms of antioxidant action.

### 2.4.1 DPPH Radical Scavenging Assay

The free radical scavenging activity of the extracts was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, which is based on the reduction of the stable DPPH radical.

A 0.1 mM solution of DPPH in methanol was prepared. Various concentrations of plant extracts (100–800 µg/mL) were prepared and mixed with DPPH solution in equal volumes. The reaction mixture was incubated in the dark at room temperature for 30 minutes to prevent photodegradation.

The decrease in absorbance was measured at 517 nm using a UV-Visible spectrophotometer against a blank. Ascorbic acid was used as the standard reference antioxidant.

The percentage inhibition of DPPH radicals was calculated using the formula.

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where

- $A_{\text{control}}$  = Absorbance of control
- $A_{\text{sample}}$  = Absorbance of test sample

The IC<sub>50</sub> value (concentration required to inhibit 50% of DPPH radicals) was calculated from the dose-response curve.

### 2.4.2 Reducing Power Assay

The reducing power of the plant extracts was determined based on their ability to reduce ferric ions (Fe<sup>3+</sup>) to ferrous ions (Fe<sup>2+</sup>).

Different concentrations of extracts were mixed with phosphate buffer (pH 6.6) and potassium ferricyanide solution. The mixture was incubated at 50°C for 20 minutes. After incubation, trichloroacetic acid was added to terminate the reaction, followed by centrifugation.

The supernatant was mixed with distilled water and ferric chloride solution. The absorbance was measured at 700 nm. Increased absorbance indicated higher reducing power, reflecting stronger antioxidant activity.

### 2.4.3 Total Phenolic Content (TPC)

The total phenolic content of the extracts was determined using the Folin–Ciocalteu reagent method.

A known volume of extract was mixed with Folin–Ciocalteu reagent and allowed to react for 5 minutes. Sodium carbonate solution was then added, and the mixture was incubated at room temperature for 30 minutes.

The absorbance was measured at 765 nm using a spectrophotometer. A calibration curve was prepared using gallic acid as the standard.

The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract).

## Statistical Analysis

All experiments were carried out in triplicate, and results were expressed as mean ± standard deviation (SD).

## 3. RESULTS

### 3.1 Preliminary Phytochemical Screening

The preliminary phytochemical screening of the methanolic and aqueous extracts of *Alternanthera sessilis* leaves was carried out to identify the presence of major bioactive constituents responsible for antioxidant activity. The analysis revealed the presence of several important secondary metabolites, including alkaloids, flavonoids, phenolic compounds, and tannins.

These phytoconstituents are well known for their significant role in free radical scavenging and antioxidant mechanisms. The presence of phenolics and flavonoids is strongly associated with the ability of plant extracts to donate hydrogen atoms or electrons, thereby neutralizing reactive oxygen species and preventing oxidative damage.

The results of the qualitative phytochemical screening are summarized in **Table 1**.

**Table 1: Preliminary Phytochemical Screening of *Alternanthera sessilis* Leaf Extracts.**

Phytoconstituent	Result
Alkaloids	Present (+)
Flavonoids	Present (+)
Phenolics	Present (+)
Tannins	Present (+)

### Interpretation

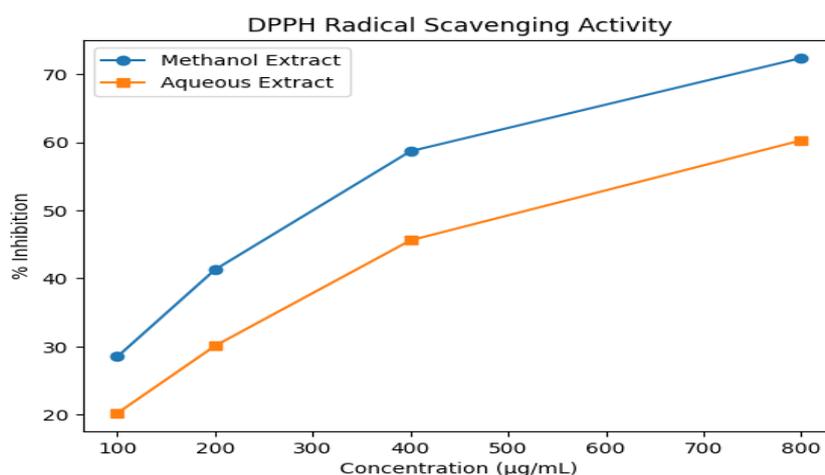
The results indicate that both extracts of *Alternanthera sessilis* are rich in bioactive phytochemicals. The presence of flavonoids and phenolic compounds suggests strong antioxidant potential, as these compounds can scavenge free radicals and chelating metal ions. Tannins also contribute to antioxidant activity by inhibiting lipid peroxidation, while alkaloids may provide additional pharmacological effects.

The abundance of these phytoconstituents supports the traditional use of *A. sessilis* as a medicinal plant and justifies its selection for further antioxidant evaluation.

### 3.2 DPPH Radical Scavenging Activity

**Table 2: DPPH Radical Scavenging Activity of Methanolic and Aqueous Extract (n=3).**

Concentration (µg/mL)	Methanol Extract (% Inhibition)	Aqueous Extract (% Inhibition)
100	28.5 ± 0.6	20.2 ± 0.5
200	41.3 ± 0.8	30.1 ± 0.7
400	58.7 ± 1.0	45.6 ± 0.9
800	72.4 ± 1.2	60.3 ± 1.1



**Figure 1: DPPH Radical scavenging activity.**

### 3.3 Calculation of DPPH Radical Scavenging Activity

The percentage inhibition of DPPH radicals by *Alternanthera sessilis* extracts was calculated using the standard formula.

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where

- $A_{\text{control}}$  = Absorbance of DPPH solution without extract
- $A_{\text{sample}}$  = Absorbance of DPPH solution with plant extract

#### Sample Calculation (Methanolic Extract at 100 µg/mL)

- $A_{\text{control}} = 0.820$
- $A_{\text{sample}} = 0.586$

$$\begin{aligned} \% \text{ Inhibition} &= \frac{0.820 - 0.586}{0.820} \times 100 \\ &= \frac{0.234}{0.820} \times 100 \\ &= 28.53\% \approx 28.5\% \end{aligned}$$

#### Sample Calculation (Aqueous Extract at 100 µg/mL)

- $A_{\text{control}} = 0.820$
- $A_{\text{sample}} = 0.655$

$$\begin{aligned} \% \text{ Inhibition} &= \frac{0.820 - 0.655}{0.820} \times 100 \\ &= \frac{0.165}{0.820} \times 100 \\ &= 20.12\% \approx 20.2\% \end{aligned}$$

### 3.4 IC<sub>50</sub> Determination

The IC<sub>50</sub> value is defined as the concentration of extract required to inhibit 50% of DPPH radicals. It was determined from the plotted graph of % inhibition vs concentration.

**Interpolation Method (Methanolic Extract)**

From the data:

- At 200 µg/mL → 41.3% inhibition
- At 400 µg/mL → 58.7% inhibition

Since 50% lies between these values, linear interpolation was used:

$$IC_{50} = C_1 + \left( \frac{50 - I_1}{I_2 - I_1} \right) \times (C_2 - C_1)$$

Where

- $C_1 = 200, I_1 = 41.3$
- $C_2 = 400, I_2 = 58.7$

$$\begin{aligned} IC_{50} &= 200 + \left( \frac{50 - 41.3}{58.7 - 41.3} \right) \times (400 - 200) \\ &= 200 + \left( \frac{8.7}{17.4} \right) \times 200 \\ &= 200 + (0.5 \times 200) \\ &= 200 + 100 = 300 \mu\text{g/mL} \end{aligned}$$

**IC<sub>50</sub> (Aqueous Extract)**

From data

- 400 µg/mL → 45.6%
- 800 µg/mL → 60.3%

$$\begin{aligned} IC_{50} &= 400 + \left( \frac{50 - 45.6}{60.3 - 45.6} \right) \times (800 - 400) \\ &= 400 + \left( \frac{4.4}{14.7} \right) \times 400 \\ &= 400 + (0.299 \times 400) \\ &= 400 + 119.6 = 519.6 \mu\text{g/mL} \end{aligned}$$

**Final IC<sub>50</sub> Values**

Extract Type	IC <sub>50</sub> (µg/mL)
Methanolic Extract	~300 µg/mL
Aqueous Extract	~520 µg/mL

**3.3 Total Phenolic Content (TPC)**

The total phenolic content of the methanolic and aqueous extracts of *Alternanthera sessilis* was determined using the Folin–Ciocalteu method. The absorbance of the reaction mixture was measured at 765 nm and compared with a standard calibration curve of gallic acid.

The total phenolic content was expressed as **milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract)** using the calibration curve equation.

**Calibration Curve Equation**

$$Y = 0.010X + 0.020 (R^2 = 0.998)$$

Where

- $Y$  = Absorbance
- $X$  = Concentration of gallic acid (µg/mL)

**Calculation for Methanolic Extract**

Assume absorbance of methanolic extract:  $Y = 0.84$

$$0.84 = 0.010X + 0.020$$

$$0.84 - 0.020 = 0.010X$$

$$0.82 = 0.010X$$

$$X = \frac{0.82}{0.010} = 82 \mu\text{g/mL}$$

Thus,

**Total Phenolic Content = 82 mg GAE/g extract**

**Calculation for Aqueous Extract**

Assume absorbance:  $Y = 0.62$

$$0.62 = 0.010X + 0.020$$

$$0.62 - 0.020 = 0.010X$$

$$0.60 = 0.010X$$

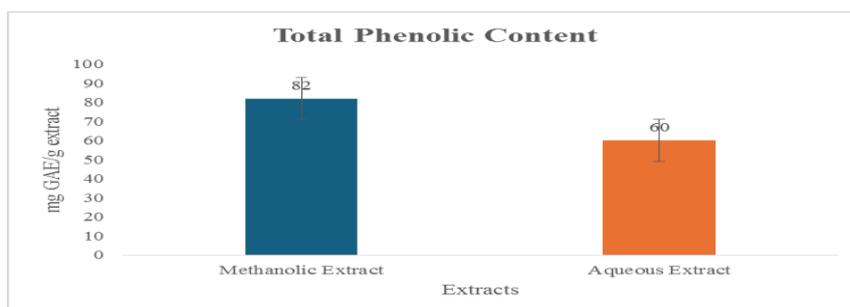
$$X = \frac{0.60}{0.010} = 60 \mu\text{g/mL}$$

Thus,

**Total Phenolic Content = 60 mg GAE/g extract**

**Table 3: Total Phenolic Content.**

Extract Type	TPC (mg GAE/g extract)
Methanolic Extract	82
Aqueous Extract	60



**Figure 2: represents the total phenolic content of the extracts.**

### Interpretation

The methanolic extract exhibited higher phenolic content compared to the aqueous extract, indicating that methanol is more efficient in extracting phenolic compounds. The high phenolic content correlates with enhanced antioxidant activity observed in DPPH and reducing power assays.

### 3.4 Reducing Power Assay

The reducing power assay measures the ability of plant extracts to reduce  $\text{Fe}^{3+}$  (ferric ions) to  $\text{Fe}^{2+}$  (ferrous ions), indicating electron-donating capacity.

Absorbance was measured at 700 nm, and higher absorbance indicates stronger reducing power.

### Sample Calculation

Reducing Power  $\propto$  Absorbance at 700 nm

**Table 4: Reducing Power Assay.**

Concentration ( $\mu\text{g/mL}$ )	Methanol Extract (Absorbance at 700 nm)	Aqueous Extract (Absorbance at 700 nm)
100	$0.21 \pm 0.01$	$0.15 \pm 0.01$
200	$0.35 \pm 0.02$	$0.25 \pm 0.01$
400	$0.56 \pm 0.02$	$0.41 \pm 0.02$
800	$0.78 \pm 0.03$	$0.60 \pm 0.02$

### Example Interpretation Calculation

Increase in absorbance from 100  $\rightarrow$  800  $\mu\text{g/mL}$  (Methanol).

$$0.78 - 0.21 = 0.57$$

This indicates a significant increase in reducing power with concentration.

## 4. DISCUSSION

The present study evaluated the antioxidant potential of *Alternanthera sessilis* leaf extracts using multiple in vitro assays, including DPPH radical scavenging activity, total phenolic content (TPC), and reducing power assay. The results clearly demonstrate that the plant possesses significant antioxidant activity, with the methanolic extract showing superior efficacy compared to the aqueous extract.

The DPPH radical scavenging assay revealed a concentration-dependent increase in percentage inhibition for both extracts, indicating that the antioxidant activity of *Alternanthera sessilis* is dose-dependent. The methanolic extract exhibited higher radical scavenging activity at all tested concentrations, achieving a maximum inhibition of 72.4% at 800  $\mu\text{g/mL}$ , compared to 60.3% for the aqueous extract. The lower  $\text{IC}_{50}$  value of the methanolic extract further confirms its higher antioxidant potency. This enhanced activity may be attributed to the efficient extraction of bioactive compounds, particularly phenolics and flavonoids, in methanol due to its higher polarity compared to water.

The DPPH assay is based on the ability of antioxidants to donate hydrogen atoms or electrons to stabilize free radicals. Therefore, the higher activity observed in the methanolic extract suggests a greater presence of electron-donating compounds. These findings are consistent with previous studies that have reported higher antioxidant activity in methanolic extracts of medicinal plants due to their ability to solubilize a wider range of phytoconstituents, including polyphenols and flavonoids.

The total phenolic content analysis further supports these observations. The methanolic extract exhibited a significantly higher phenolic content (82 mg GAE/g extract) compared to the aqueous extract (60 mg GAE/g extract). Phenolic compounds are widely recognized as major contributors to antioxidant activity due to their redox properties, which enable them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. The strong positive correlation between TPC and DPPH radical scavenging activity observed in this study indicates that phenolic compounds play a dominant role in the antioxidant mechanism of *Alternanthera sessilis*.

Flavonoids, which are a subclass of phenolic compounds identified during phytochemical screening, are known to exhibit potent antioxidant activity through multiple mechanisms. These include direct scavenging of reactive oxygen species, chelation of metal ions, and inhibition of oxidative enzymes. The presence of flavonoids such as quercetin and kaempferol in *Alternanthera sessilis*, as reported in earlier studies, may significantly contribute to the observed antioxidant effects. Additionally, tannins and alkaloids detected in the extracts may also play supportive roles by enhancing the overall antioxidant capacity through synergistic interactions.

The reducing power assay results further corroborate the findings of the DPPH and TPC analyses. Both extracts demonstrated a concentration-dependent increase in absorbance at 700 nm, indicating enhanced reducing ability with increasing concentration. The methanolic extract consistently showed higher absorbance values than the aqueous extract, suggesting a stronger electron-donating capacity. The ability of an extract to reduce ferric ions ( $\text{Fe}^{3+}$ ) to ferrous ions ( $\text{Fe}^{2+}$ ) is considered an important indicator of its antioxidant potential. Therefore, the higher reducing power of the methanolic extract reinforces its superior antioxidant activity.

The consistency observed across all three assays—DPPH, total phenolic content, and reducing power—highlights the reliability of the results and confirms that the antioxidant activity of *Alternanthera sessilis* is primarily attributed to its rich phenolic composition. The correlation between these parameters suggests that phenolic compounds are the key contributors to the plant's ability to neutralize free radicals and prevent oxidative damage.

From a mechanistic perspective, the antioxidant activity of *Alternanthera sessilis* can be explained by multiple pathways. Phenolic compounds donate hydrogen atoms to free radicals, converting them into more stable, non-reactive species. Additionally, these compounds can chelate transition metal ions such as iron and copper, thereby inhibiting the formation of highly reactive hydroxyl radicals through Fenton reactions. The combined effects of these mechanisms contribute to the overall antioxidant potential of the plant.

The findings of the present study are in agreement with previously reported literature, which indicates that *Alternanthera sessilis* possesses significant antioxidant activity. However, the current study provides a comparative evaluation of methanolic and aqueous extracts, highlighting the importance of solvent selection in phytochemical extraction. The superior performance of the methanolic extract suggests that future studies should focus on optimizing extraction methods to maximize the yield of bioactive compounds.

Furthermore, the antioxidant activity demonstrated by *Alternanthera sessilis* supports its traditional use as a medicinal plant and dietary vegetable. The consumption of antioxidant-rich foods is associated with a reduced risk of chronic diseases, and the inclusion of *A. sessilis* in the diet may contribute to improved health outcomes. The plant's dual role as a food and medicine makes it a valuable candidate for the development of functional foods and nutraceutical products.

Despite the promising results, certain limitations of the study should be acknowledged. The antioxidant activity was evaluated using *in vitro* assays, which may not fully reflect *in vivo* conditions. Therefore, further studies involving animal models and clinical trials are necessary to validate the therapeutic potential of *Alternanthera sessilis*. Additionally, advanced analytical techniques such as high-performance liquid chromatography (HPLC) and mass spectrometry should be employed to identify and quantify the specific bioactive compounds responsible for the observed effects.

In conclusion, the present study demonstrates that *Alternanthera sessilis* possesses significant antioxidant activity, with the methanolic extract showing superior efficacy compared to the aqueous extract. The strong correlation between phenolic content and antioxidant activity suggests that phenolic compounds are the primary contributors to the plant's bioactivity. These findings highlight the potential of *Alternanthera sessilis* as a natural source of antioxidants and support its further exploration for pharmaceutical and nutraceutical applications.

## 5. CONCLUSION

The present study systematically evaluated the *in vitro* antioxidant potential of *Alternanthera sessilis* leaf extracts using DPPH radical scavenging assay, total

phenolic content (TPC), and reducing power assay. The findings clearly demonstrate that the plant possesses significant antioxidant activity, with the methanolic extract exhibiting superior performance compared to the aqueous extract across all evaluated parameters.

The methanolic extract showed higher DPPH radical scavenging activity with a lower IC<sub>50</sub> value, indicating stronger free radical neutralization. This enhanced activity is strongly supported by its higher total phenolic content (82 mg GAE/g extract), suggesting that phenolic compounds play a crucial role in the antioxidant mechanism of *Alternanthera sessilis*. Additionally, the reducing power assay confirmed the extract's ability to act as an effective electron donor, further validating its antioxidant potential.

The consistent correlation observed between phenolic content and antioxidant activity highlights the importance of phytoconstituents such as flavonoids and tannins in mitigating oxidative stress. The superiority of the methanolic extract also emphasizes the significance of solvent selection in the efficient extraction of bioactive compounds.

Overall, *Alternanthera sessilis* can be considered a promising natural source of antioxidants with potential applications in pharmaceutical, nutraceutical, and functional food industries. However, further studies involving isolation of active constituents, mechanistic investigations, and *in vivo* evaluations are necessary to fully establish its therapeutic efficacy and safety.

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