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TOTAL PHENOLIC CONTENT, FLAVONOID CONTENT, AND ANTIOXIDANT ACTIVITY OF *Alternanthera ficoidea (L.) P. Beauv*

T.V. MAHALAKSHMI, E. SUJATHA* AND B. RAMADEVI

Department of Botany, Osmania University, Telangana, India, 500007

***Corresponding Author: Sujatha E: E Mail: sujatha@osmania.ac.in**

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ABSTRACT

Stress is a physiological and psychological response that alters the homeostasis in the body. Cellular stress can trigger several chronic illnesses and need to be addressed in the initial stages. Polyphenolics such as tannins and flavonoids are proven powerful antioxidants to resolve cellular stress, sustain homeostasis in the body and prevent stress-associated disorders. Plant-based medicine gained importance due to its safety margin and multiple benefits. Regular intake of antioxidants is an alternative to avoid serious illness. The current investigation is aimed to measure the phenolic, flavonoid content, the antioxidant activity of *Alternanthera ficoidea (L.) P. Beauv*.

Total phenolic content was estimated by Folin–Ciocalteu colorimetric method taking gallic acid as standard. Whereas, total flavonoid content was determined by Aluminum chloride colorimetric assay using Rutin as standard. The absorbance was measured at 760 nm and 510 nm respectively. Antioxidant activity was measured by two *In vitro* methods (DPPH and NO free radical scavenging assay) using standard protocols, where Ascorbic acid served as a reference standard.

Results disclosed that total phenolic content and flavonoid content for the ethanolic extract of *A. ficoidea* are 45.61±1.08 GAE and 29±1.32 GER respectively. In DPPH assay, ethanolic extract is effective to inhibit (72.17%) the free radicals with IC_{50} values 45.67µg/ml at higher doses that are comparable to standard ascorbic acid (80.15%, IC_{50} =36.59 µg/ml). Similarly, in NO free radical scavenging assay, *A. ficoidea* (70.36%) exhibited free radical scavenging

activity with IC_{50} values 45.61µg/ml. Whereas, ascorbic acid is found to inhibit 80.15% with IC_{50} values 36.59µg/ml.

The presence of various secondary metabolites such as alkaloids, carbohydrates, phenols, steroids, terpenoids, glycosides, saponins, especially tannins and flavonoids, either alone or in combination may be responsible for the observed scavenging property. The quantity of the phenolic compounds and flavonoids can be directly correlated to the exhibited activity.

Keywords: ***Alternanthera ficoidea (L.) P. Beauv*****, antioxidant, total phenolic content, total flavonoid content, free radical scavenging, amaranthaceae**

INTRODUCTION

The precious herbal knowledge has been practiced for centuries for the management of social and community health. Man has adopted various systems of medicine and cultivated different herbs for regular medicinal necessities **[1]**. Regarding its safety, the beneficiaries of herbal medicine are directing the whole world towards plant-based medicine for chronic and lifestyle diseases. Currently, drug discovery research is taking cues from traditional and folklore knowledge to generate molecules with better therapeutic outcome **[2, 3]**.

Alternanthera ficoidea (L.) P. Beauv of the Amaranthaceae family is an invasive alien weed that can grow up to 45cm in diversified habitats. Leaves are elliptical with acute to acuminate apex **[4]**. Traditionally, the leaves are edible and are used either in raw form or boiled. The plant has ameliorative effects on cardiovascular diseases, viral infections, and cancer **[5, 6]**.

Reactive oxygen species are one of the free radicals that are useful in the defensive mechanisms of the cell. The imbalance between the production and neutralization of the generated free radicals can trigger chronic disorders. When free radical scavenging enzymes are incapable of limiting cellular stress, external antioxidants are needed. Flavonoids and tannins are plant-derived polyphenolic compounds that ameliorate cellular stress and prevent various neurological and cardiovascular disorders **[7-9]**.

In this connection, the present work aims to screen total phenolic content, total flavonoid content and estimate the antioxidant potential of aerial parts of *A. ficoidea* using *In vitro* models such as DPPH assay and nitric oxide free radical scavenging assay.

MATERIALS AND METHODS

Plant material

Alternanthera ficoidea (L.) P. Beauv was collected from Osmania University campus, Hyderabad, and authenticated by Dr. L. Rasingam, Scientist-E, Botanical

survey of India, Deccan section, Hyderabad, India. A voucher specimen (BSI/DRC/2021-22/Tech/369) was deposited in the Herbarium for future reference.

Reagents and chemicals

All the chemicals and reagents were procured from Sigma Aldrich (laboratory grade).

Preparation of extracts

The shade dried plant was powdered and subjected to Soxhlet extraction with ethanol. The solvent was evaporated to dryness to get solid extract and percentage yield was calculated.

Phytochemical screening

The preliminary phytochemical investigation of ethanolic extract of *A. ficoidea* was carried out by employing standard protocols **[10]**.

Estimation of total phenolic content

Folin-Ciocalteu (FC) assay was used to estimate the total phenolic content of *A. ficoidea*. 200µl of the extract solution was mixed with 2.5ml of FC reagent (diluted to 10 times) and 2 ml of Na_2CO_3 solution (7.5% w/v) followed by proper mixing and incubation at 30°C for 90 minutes. Absorbance for all the samples was recorded at 765 nm and expressed in terms of mg equivalents of gallic acid. The calibration curve was constructed by plotting the absorbance against concentration and the values are calculated for triplicates **[11]**.

Estimation of total flavonoid content

The flavonoid content was determined by a colorimetric method using Aluminium trichloride method (Zhishen method). A volume of 125µL of the extract is added to 75 µL of a 5% Sodium nitrite ($NaNO_2$) solution. After 6 min, 150 µL of $AlCl_3$ solution (10%) was added followed by the addition of 750 µL of NaOH (1M). The final volume of the solution was made to 2500 µL with distilled water. After 15 min of incubation, the mixture turned pink and the absorbance was measured at 510 nm. The total flavonoids content was expressed as gram equivalence of Rutin per gram dry weight **[12]**.

***In vitro* antioxidant assay**

DPPH radical scavenging assay:

Plant extracts of different concentrations were prepared using DMSO, whereas a solution of 25mg/L DPPH was prepared by using ethanol. In 96-well plate, 5µl of the extract solution followed by 195 µl of DPPH solution was added and incubated for 20 minutes at room temperature. The absorbance was measured at 515 nm for individual extracts and the free radical scavenging activity was recorded by comparing the absorbance values with the blank. The above procedures were repeated using ascorbic acid as positive controls in

triplicates. The antioxidant activity was calculated using the formula given below **[13]**.

% Free radical scavenging activity = $[(A_0-A_s)/A_0] \times 100$

Where,

A_0 is the absorbance of blank (DPPH solution alone)

A_0 is the absorbance of extracts (DPPH + sample)

Nitric oxide radical scavenging assay:

In nitric oxide radical scavenging activity assay, 2 mL of sodium nitroprusside (10 mM) and 0.5 mL of phosphate buffer (pH-7.4) was mixed with 0.5 mL of the test solution and incubated for 150 min at 25 °C. Ascorbic acid solution and DMSO served as standard and control respectively. Sulfanilic acid reagent (1mL 0.33% of sulfanilic acid in 2% glacial acetic acid) was added to 0.5 mL of nitrite and kept for 5 min. Naphthyl ethylene diamine dihydrochloride (NEDD, 1 mL of 1%) was added and incubated for 30 min at 25 °C **[14]**. The absorbance was recorded at 540 nm and the percentage of nitric oxide inhibition was calculated as:

Percentage of nitric oxide radical scavenging assay = $[(A_0-A_s)/A_0] \times 100$

Where,

A_0 was the absorbance of control

A_s was the absorbance of the treated sample

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical study of ethanolic extracts of *A. ficoidea* exposed that the extracts are instituted with various secondary metabolites such as alkaloids, carbohydrates, flavonoids, phenols, steroids, terpenoids, glycosides, tannins, saponins **(Table 1)**.

Total phenolic content

Phenolic compounds can be directly correlated to their protective effect against the cellular stress in the body. Total phenolic contents of ethanolic extract of *A. ficoidea* was evaluated by Folin–Ciocalteu method taking gallic acid as the reference standard. A calibration curve was plotted against the absorbance values versus different concentrations of gallic acid **(Figure 1)**. Total phenolic content of the extracts was calculated from the regression equation of calibration curve ($Y = 0.005x + 0.0223$; $R^2 = 0.999$) and expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). Total phenolic content in the ethanol extract was found to be 45.61±1.08 GAE **(Table 2)**.

Total flavonoid content

Total flavonoid content of *A. ficoidea* was determined by Zhishen technique and is found to be 29±1.32 of gram equivalence of Rutin at 510 nm **(Table 2)**. The calibration curve was made by linear regression and the results represented the average of three

determinations to each concentration **(Figure 2)**. Total phenolic content of the extracts was calculated from the regression equation of calibration curve (Y = 3.51x + 0.054; R^2 = 0.9964) and expressed as mg Rutin equivalents (RE) per gram of sample in dry weight (mg/g).

DPPH radical scavenging assay

In the present study, when compared to the standard Ascorbic acid, *A. ficoidea* has exhibited significant free radical scavenging activity in a dose-dependent manner. A standard curve was plotted using various concentrations of ascorbic acid **(Figure 3)**. At a higher concentration (100 µg/mL), the *A. ficoidea* ethanol extract exhibited 70.36% of inhibition next to ascorbic acid (84.11%) **(Table 3)**. IC_{50} value for ascorbic acid and *A. ficoidea* were found to be 41.37 µg/mL 50.23 µg/mL.

Nitric oxide radical scavenging activity

A. ficoidea ethanol extract exhibited NO free radical scavenging activity in a dose-dependent manner compared to standard ascorbic acid **(Table 4)**. At a higher concentration 100 µg/mL, *A. ficoidea* is showing 70.36% of inhibition with IC_{50} values 53.44 µg/ml. Whereas for ascorbic acid it is found to be 84.11% with IC_{50} values 41.37µg/ml.

Table 1: Phytochemical screening of *A. ficoidea*

Phytochemicals	Ethanol extract of *A. ficoidea*
Alkaloids	+
Glycosides	-
Flavonoids	++
Terpenoids	+
Steroids	+
Tannins	++
Proteins	-
Carbohydrates	+
Amino acids	+
Saponins	++

+ indicates the presence and – indicates the absence of phytochemicals

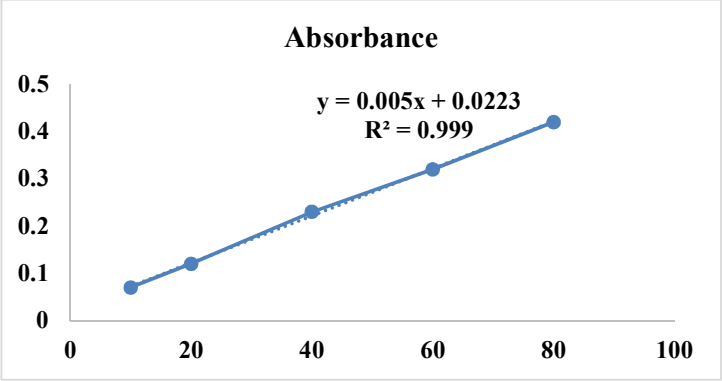

Figure 1: Calibration curve for Gallic acid

Table 2: The total phenolic and flavonoid content of *A. ficoidea*

Extract	Ethanol extract of *A. ficoidea*
Total phenolic content mg/ml	2.26±0.83 mg/ml
Total flavonoid content mg/ml	28.57±0.91 mg/ml

*All values are expressed as mean±SD for three determinations

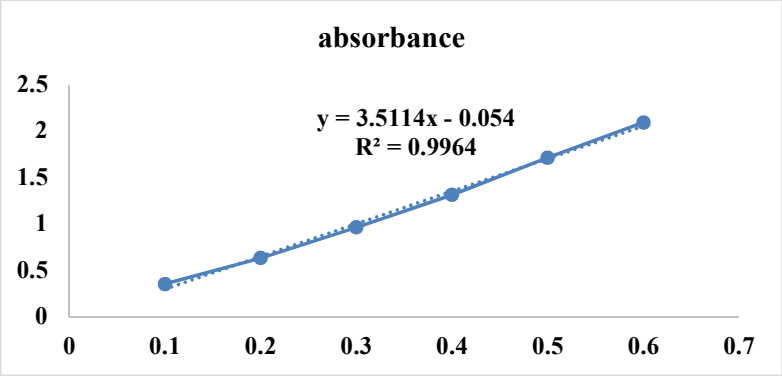

Figure 2: Calibration curve for Rutin

Table 3: Percentage inhibition and IC_{50} values of *A. ficoidea* and ascorbic acid at different concentrations in DPPH assay

Concentration µg/mL	Ascorbic acid	*A. ficoidea*
100	80.15	72.17
75	78.36	68.07
50	75.41	65.19
25	63.15	58.11
IC_{50} µg/ml	36.59	45.61

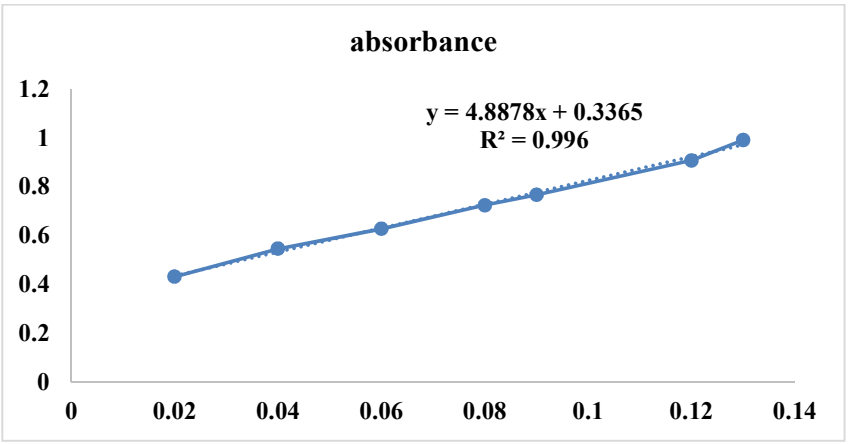

Figure 3: Calibration curve for Ascorbic acid

Table 4: Percentage inhibition and IC_{50} values of *A. ficoidea* and ascorbic acid at different concentrations in NO free radical scavenging assay

Concentration µg/mL	Ascorbic acid	*A. ficoidea*
100	84.11	70.36
75	70.23	63.44
50	65.16	57.13
25	61.09	48.32
IC_{50} µg/ml	41.37	53.44

DISCUSSIONS

The plant detoxification mechanism produces various biologically active molecules. Polyphenols such as flavonoids are important plant derived antioxidants that help to scavenge free radicals in humans to mitigate stress associated disorders. [22]

In the current work, the ethanol extract of *A. ficoidea* was studied for qualitative and quantitative phytochemicals. From the results it is apparent that *A. ficoidea* is opulent with diversified plant metabolites. Especially, ethanol extract is rich in phenolic compounds, including flavonoids. The ethanol extract also exhibited significant antioxidant activity in the DPPH assay and NO free radical scavenging assay.

Further investigation is in progress to evaluate the complete phytochemical and pharmacological profile of the plant to justify its traditional applications and the reported antioxidant activity.

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