

Original Article

ANTIOXIDANT, FREE RADICAL SCAVENGING ACTIVITY AND GC-MS STUDIES ON  
PEDILANTHUS TITHYMALOIDES (L.) POIT

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ABSTRACT

**Objective:** To evaluate the methanolic extract of the leaves of *Pedilanthus tithymaloides* for total phenol, total flavonoid, total antioxidant and free radical scavenging ability and detect the phytoconstituents using GC-MS.

**Methods:** The total phenols were quantified using Folin-Ciocalteu method. Aluminium chloride method and Phosphomolybdenum method were used to quantify total flavonoid and total antioxidant content respectively. In addition to the above, Ferric thiocyanate assay, the thiobarbituric acid assay, Ferric Reducing Antioxidant Power assay and ABTS assay were performed to know the antioxidant potency of the methanolic extract of leaves of *Pedilanthus tithymaloides*. The phytoconstituents was detected using GC-MS.

**Results:** The leaves of *Pedilanthus tithymaloides* recorded a phenolic content of 10.98±0.08 mg TAE/g DW, flavonoid content of 11.49±0.15 µg QE/g DW and total antioxidant content of 6.64±0.05 mg TAE/g DW. The study also revealed significant free radical scavenging ability of the plant leaves as assessed by FTC, TBA, FRAP and ABTS assays. The structural elucidation by GC-MS analysis revealed five different compounds, including three esters, an amine and an alkaloid.

**Conclusion:** The study proves the anticipative potential ability of *Pedilanthus tithymaloides*, suggesting its exploitation in pharmaceutical applications.

**Keywords:** *Pedilanthus tithymaloides*, Antioxidant, FTC, TBA, FRAP, ABTS assays, GC-MS.

INTRODUCTION

The presence or generation of free radicals plays a vital role in several diseases like cancer, diabetes, sclerosis, Alzheimer's and Parkinson's [1]. An optimal dose of antioxidants is required so as to maintain a healthy system. Plants are a major source of antioxidants and they are exploited widely in the field of pharmaceutical and food industries. Hence, there is an increasing urge to detect natural resources for potent antioxidants. Antioxidant activity of several plants across different Nations such as Croatia [2], Egypt [3], Finland [4], Greece [5], India [6-10], Iran [11], Thailand [12], Turkey [13] etc., has been reported. However, in plants, complex system and diverse mechanisms owe to their antioxidant properties. Different reactive oxygen species may target different biomolecules, upon generation. Hence, characterization of plants through a variety of antioxidant assays is of importance [14] for their potential exploitation in industries. In this study, the plant, *Pedilanthus tithymaloides* was evaluated for its total antioxidant content, total phenolics and total flavonoids. Further, its free radical scavenging activity was determined through FTC, TBA, FRAP and ABTS assays. The phytoconstituents of the methanolic extract of the leaf are detected using GC-MS.

*Pedilanthus tithymaloides* (L.) Poit., belonging to Euphorbiaceae, is an erect shrub originating from Tropical America. Indian folklore uses *Pedilanthus tithymaloides* as antiviral, antibacterial, antihemorrhagic, antitumor, abortive, anticancer and anti-inflammatory agent [15]. Though the antibacterial [16], larvicidal [17], anti-inflammatory and antioxidant activities [18] of *Pedilanthus tithymaloides* have been reported, an extensive study dealing with different assays against free radicals and detection of constituents has not been analysed. Hence, the present study was carried.

MATERIALS AND METHODS

Plant source

Healthy, uninfected and undamaged leaves of *Pedilanthus tithymaloides* were collected from Thiruvallur district of Tamil Nadu, India. The leaves were cleaned, washed and allowed to dry in shade.

The dried leaves were pulverized using an electric blender and stored for further use.

Preparation of plant extract

The methanolic extract of the plant was prepared by cold percolation method. The pulverized plant material (20 g) was mixed with methanol (200 mL) and maintained for 48 h at 30±2°C, in a temperature controlled shaker. The crude extract obtained upon filtering and concentrating was reconstituted with the solvent for further analysis. The antioxidant potency of the plant was assessed by determining its phenolic, flavonoid and antioxidant content. The free radical scavenging ability was assessed by FTC, TBA, FRAP and ABTS assays. The experiments were performed in triplicates and the results are expressed as Mean±Standard Deviation.

Determination of total phenolic content

The total phenolic content (TPC) was determined by Folin-Ciocalteu method [19]. To the plant extract (100 µL), 500 µL of distilled water and 100 µL of Folin-Ciocalteu reagent were added and incubated for 6 min at room temperature. Addition of 1.25 mL of 7% sodium carbonate was followed by making up the final volume of the solution to 3 mL. The mixture was incubated for 90 min and the absorbance was measured at 760 nm using UV-Visible spectrophotometer (Cyberlab, USA). The total phenolic content was expressed as mg TAE (Tannic acid equivalents) per g of the dry weight of the plant, using a standard plot of Tannic acid.

Determination of total flavonoid content

The total flavonoid content (TFC) of the plant was determined by the method described by Moussa *et al.*, [3]. Two hundred microlitre of the plant extract was taken in a test tube and the solvent was allowed to evaporate. To the residue, 5 mL of 0.1M aluminium chloride was added and shaken well. The absorbance value was measured at 415 nm after incubation of the solution for 40 min, at

room temperature. Using a standard plot of Quercetin, the total flavonoid content was assessed and expressed as  $\mu\text{g}$  QE (Quercetin Equivalent) per gram dry weight of the plant material.

#### Determination of total antioxidants

Phosphomolybdenum method was employed for the estimation of total antioxidant activity [20]. Sulphuric acid (0.6 M), sodium phosphate (28 mM) and ammonium molybdate (4 mM) were used to prepare the reagent solution. To 0.5 mL of the plant extracts, 4.5 mL of the reagent solution was added and maintained at 95°C, in a boiling water bath for 90 min. Upon cooling the solution at room temperature, the absorbance value was measured at 695 nm. The total content of antioxidants in the plant was expressed as mg TAE (Tannic acid equivalent) per g of the dry weight of the plant material.

#### Ferric thiocyanate assay

The inhibitory effect of the plant against oxidation by peroxides was evaluated by Ferric Thiocyanate assay. 120  $\mu\text{L}$  of 98% ethanol, 100  $\mu\text{L}$  of 2.51% linoleic acid in ethanol and 9 mL of 40 mM phosphate buffer (pH 7) were successively added to 100  $\mu\text{L}$  of the plant extract. The mixed solution was maintained in dark, at 40°C. To 100  $\mu\text{L}$  of the mixture, 9.7 mL of 75% ethanol, 100  $\mu\text{L}$  of 30% ammonium thiocyanate and 100  $\mu\text{L}$  of 20 mM  $\text{FeCl}_3$  in 3.5% HCl were added. The absorbance of the solution was measured at 500 nm, after 3 min. The percentage of inhibition was calculated with Tannic acid as the standard [19].

% Inhibition

$$= \left( \frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \right) \times 100$$

#### Thiobarbituric acid assay

Two millilitres each of 20% trichloroacetic acid and 0.67% Thiobarbituric acid were mixed with 1 mL of 2.51% linoleic acid and 1 mL of plant extract. The solution was maintained in boiling water bath for 10 min. Upon cooling, the solution was centrifuged at 3000 rpm. The absorbance of the supernatant was measured at 532 nm. The percentage inhibition of the plant against the products in later stages of lipid peroxidation was evaluated with reference to the standard solution of Butylated hydroxyl toluene (BHT) [19].

% Inhibition

$$= \left( \frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \right) \times 100$$

#### Ferric Reducing Antioxidant Power (FRAP) Assay

A mixture of plant extract (1 mL), phosphate buffer - 2.5 mL (of 0.2 M, pH 7) and 1% potassium ferricyanide (2.5 mL) was incubated at 50°C for 30 min. To the solution, 2.5 mL of 10% Trichloroacetic acid was added, mixed and centrifuged for 10 min at 6500 rpm. To 2.5 mL of the supernatant, distilled water (2.5 mL) and 0.5 mL of 0.1%  $\text{FeCl}_3$  were added. The absorbance of the solution was measured at 700 nm. The reducing ability of the plant was expressed in terms of percentage by relating the absorbance value of the plant and the standard,  $\text{FeSO}_4$  [21].

% Reduction ability

$$= \left( \frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \right) \times 100$$

#### ABTS assay

A solution of 7 mM ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] mixed with 2.45 mM potassium persulphate was incubated in dark for 12-16 h. The solution was diluted with ethanol till the absorbance reached  $0.7 \pm 0.02$  at 734 nm. One mL of the diluted solution was mixed with 100  $\mu\text{L}$  of plant extract and the absorbance was read at 734 nm after 6 min. The percentage inhibition of ABTS was calculated with reference to the standard, Tannic acid [19].

% Inhibition

$$= \left( \frac{\text{Absorbance of the control} - \text{Absorbance of the sample}}{\text{Absorbance of the control}} \right) \times 100$$

#### Gas Chromatography (GC) and Mass Spectroscopy (MS) analysis:

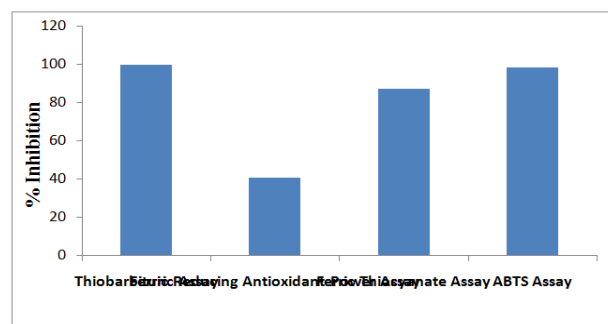
One  $\mu\text{L}$  of crude methanol extract of the plant was subjected to analysis of its constituents using Agilent Technologies 6890N GC system coupled with JEOL Mass spectroscopy. The sample was injected to the Agilent J&W HP-5 capillary column (30 m  $\times$  0.2 mm  $\times$  0.25  $\mu\text{m}$ ) fused with silica. The injection temperature was maintained at 220°C. The oven temperature of GC was programmed with an initial temperature of 50°C and increased up to 250°C at the rate of 10°C per min. Helium (He) was used as the carrier gas system with the flow rate of 1 mL/min. GC-MS interface temperature was maintained at 250°C. Identification of compounds was based on the comparison of the spectral values with the National Institute of Standards and Technology (NIST) Chemical Web book database. In addition, the peak area percentage contributed by each of the compounds detected was calculated.

#### RESULTS

The total phenolic content of *Pedilanthus tithymaloides* accounted to  $10.98 \pm 0.08$  mg TAE per gram dry weight of the plant. The total flavonoid content, as estimated by Aluminium chloride method, showed lower content ( $11.49 \pm 0.15$   $\mu\text{g}$  QE/g DW) than the phenolics. The total content of antioxidants, apart from phenols and flavonoids, accounted to  $6.64 \pm 0.05$  mg TAE/g DW of the plant. The plant is found to possess significant activity against the radicals of lipid peroxidation. This is evident from the results of TBA and FTC assays wherein the percentage inhibition was 99.73 and 87.25 respectively. Similarly, *Pedilanthus tithymaloides* inhibited the stable ABTS radical to 98.56%. However, the plant showed an average ability in reducing the ferric ions in the FRAP assay. The content of phenols, flavonoids and antioxidants is tabulated in table 1 and the percentage of inhibition acquired by the plants in TBA, FRAP, FTC and ABTS assays is depicted in Fig. 1.

**Table 1: Total Phenols, Total Flavonoids and Total Antioxidant content in the methanolic extract of *Pedilanthus tithymaloides***

	<i>Pedilanthus tithymaloides</i>
Total Phenolic Content (mg TAE/g DW)	$10.98 \pm 0.0876$
Total Flavonoid Content ( $\mu\text{g}$ QE/g DW)	$11.49 \pm 0.15$
Total Antioxidant Assay (mg TAE/g DW)	$6.64 \pm 0.05$



**Fig. 1: Percent inhibition recorded by *Pedilanthus tithymaloides* through TBA, FRAP, FTC and ABTS assays.**

The constituents of methanolic extract of *Pedilanthus tithymaloides* analysed by GC-MS resulted in the detection of 5 different compounds, of which three esters, an amine and an alkaloid. The maximum peak area was exhibited by 10-Octadecenoic acid, methyl ester with 75.37%. The lowest peak area with 2.53% was from (4,4-Diphenyl-butyl)-(3-phenyl-piperidin-4-yl)-amine.

The alkaloid, Rescinnamine has also been detected at the retention time of 26.23. The GC-MS spectra obtained is depicted in Fig. 2 and the compounds detected by GC-MS are listed in table 2.

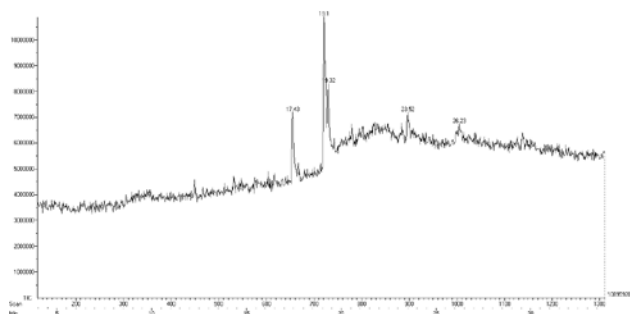


Fig. 2: GC-MS spectra of methanolic extract of *Pedilanthus tithymaloides*

## DISCUSSION

The principle metabolites that play a vital role in antioxidant defence mechanism include flavonoids, polyphenols, tannins and saponins. Plants scavenge free radicals by donating electrons or transferring hydrogen atoms, thereby neutralizing free radicals [22].

Hence, the quantification of phenols, flavonoids and other antioxidant constituents is necessary. Prevention of radicals formed by oxidative stress is of utmost importance so as to maintain a healthy system. Several plants are being screened for potent antioxidative properties. Secondary metabolites of plants, differing in their structure, mechanism of action and biological properties, possess several medicinal attributes [23]. The crude, methanolic and chloroform extracts of *Pedilanthus tithymaloides* possessed good wound healing properties, which might be attributed to the action of phytoconstituents like, phytosterols, flavonoids, alkaloids, triterpenoids and tannins, either individually or in combination [24-26]. The potential antioxidant ability of the plant as assessed in the present study is in accordance with significant antioxidant activity evaluated by the DPPH method [7]. Several compositions comprising *Pedilanthus tithymaloides* have been formulated by Raula [27] for the prevention of gastrointestinal disorders.

Abreu et al. [28] isolated flavonoids and coumarins from *Pedilanthus* sp. A major compound, cycloartanol triterpene, was isolated from the dichloromethane extract of *Pedilanthus tithymaloides* [29]. Similarly, Antimalarial and Antituberculous Poly-O-acetylated Jatrophone diterpenoids have been isolated from *Pedilanthus tithymaloides* [30]. In the present study, GC-MS analysis revealed five different compounds, including three esters, an amine and an alkaloid in the methanolic extract of the plant. The results suggest the possible utilization of the plant against different radicals involved in oxidation processes.

Table 2: GC-MS analysis of methanolic extract of *Pedilanthus tithymaloides*

Retention time	Name of the compound (IUPAC)	Molecular formula	Molecular weight (Da)	Peak Area (%)
17.43	Pentadecanoic acid, 14-methyl-, methyl ester	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270.45	5.14
19.1	10-Octadecenoic acid, methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296.49	75.37
19.32	Cyclopropanebutanoic acid, 2-[[2-[[2-[[2-pentylcyclopropyl)methyl]cyclopropyl]methyl]methyl]-, methyl ester	C <sub>25</sub> H <sub>42</sub> O <sub>2</sub>	374.59	14.33
23.52	(4,4-Diphenyl-butyl)-(3-phenyl-piperidin-4-yl)-amine	C <sub>27</sub> H <sub>32</sub> N <sub>2</sub>	384.56	2.53
26.23	Rescinnamine	C <sub>35</sub> H <sub>42</sub> N <sub>2</sub> O <sub>9</sub>	634.72	2.61

## CONCLUSION

The study on antioxidative properties of *Pedilanthus tithymaloides* has demonstrated significant ability as an antioxidant agent which can be used in pharmaceutical industries. Further, isolation of individual compounds responsible for such activity and their antioxidant potential as an individual compound is recommended.

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## CONFLICT OF INTERESTS

Declared None

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