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PHYTOCHEMICAL INVESTIGATION AND ANTIOXIDANT ACTIVITY OF *THUNBERGIA ALATA* AND *TABERNAEMONTANA CORONARIA*

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ABSTRACT

Thunbergia alata and *Tabernaemontana coronaria* are used in traditional and folklore medicine but not systematically studied for their traditional claims. Polyphenolics such as tannins and flavonoids are proven as powerful antioxidants and antiseptic agents to treat skin infections. The current investigation is aimed to determine the phenolic, flavonoid content, the antioxidant activity of *Thunbergia alata* and *Tabernaemontana coronaria* leaves.

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 for the ethanol extracts of *Tabernaemontana coronaria* (95.25±2.06) and *Thunbergia alata* (62.74±1.28) was found to be higher than petroleum ether extracts. Similarly, of *Tabernaemontana coronaria* (25.64±1.92) is richer in flavonoids than *Thunbergia alata* (13.73±1.14). In DPPH assay, ethanol extract of *Tabernaemontana coronaria*

(72.37%) is more effective to inhibit the free radicals than *Thunbergia alata* (61.72%) with IC_{50} values 50.06µg/ml & 74.77µg/ml respectively. Similarly, in NO free radical scavenging assay, ethanol extract of *Tabernaemontana coronaria* (75.37%) stood best among all to scavenge the free radicals with IC_{50} values 47.15µg/ml.

The presence of various secondary metabolites alone or in combination may be responsible for the observed scavenging property. The quantity of the phenolic compounds and flavonoids can directly correlate to the exhibited activity.

Keywords: *Thunbergia alata, Tabernaemontana coronaria*, antioxidant, total phenolic content, total flavonoid content, free radical scavenging, extraction

INTRODUCTION

Infections are the major global challenges that need to be addressed in synthetic and herbal approaches. Plants have been the primary source of medicine for centuries for human health care. Traditional healers and practitioners utilize various plant parts to get rid of acute and chronic diseases. The phytochemistry and pharmacological profile of the plants need to be established to make the proper utilization of the plants with the best therapeutic output.[1-2] *Thunbergia alata* of the family Acanthaceae is a perennial vine that grows 1-5 meters long propagated through seeds. Five nerved, simple leaves with an entire margin and opposite arrangement. The flowers are yellow to orange in color and bloom throughout the year. The fruits are depressed globous capsule bears four seeds and fruiting throughout the year. The plant is distributed in Eastern and Western ghats. *Thunbergia alata* is important in folklore medicine for inflammations, fevers, dysentery, cough, pains, skin infections, etc. phytochemical reports on *Thunbergia alata* revealed the presence of glycosides, polyphenoliccompounds.[3-7]

Tabernaemontana coronaria of the family Apocynaceae is an evergreen shrub that can spread to 3 meters. The shiny dark green leaves are oblong and with wavy margins. The plant is ornamental and also with a huge number of alkaloids. Plant parts are reported to possess various phenolic compounds, glycosides, steroids, terpenoids etc. In traditional medicine, it is used to treat hypertension, cancer, skin infections, inflammations, gastric problems, sore eyes, snake and scorpion poisoning.[8-12]

The current research was planned to explore the total phenolic and flavonoid content and estimate the antioxidant potential of various extracts of leaves of *Thunbergia alata* and *Tabernaemontana coronaria* using *In vitro* models such as DPPH assay and nitric oxide free radical scavenging assay. The extracts were also subjected to preliminary phytochemical

screening to determine the presence of secondary metabolites.

MATERIALS AND METHODS

Plant material

Thunbergia alata and *Tabernaemontana coronaria* leaves were collected from rural areas of Tirupati, Chittoor and authenticated by Dr. K. Venkata Ratnam, Assistant Professor, Department of Botany, Rayalaseema University, Kurnool; voucher specimens have been submitted (RU/BD/VSN-142&163) for future reference.

Reagents and chemicals

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

Preparation of extracts

The leaves of *Thunbergia alata* and *Tabernaemontana coronaria* were collected and dried under shade. The dried leaves were powdered and subjected to exhaustive extraction with petroleum ether and ethanol, respectively, using the Soxhlet apparatus. The solvent was evaporated to dryness to get the solid extract, and percentage yield was calculated.[13]

Phytochemical screening

The preliminary phytochemical investigation of petroleum ether and ethanol leaf extract of *Thunbergia alata* and *Tabernaemontana coronaria* was carried out by employing standard protocols.[14]

Estimation of total phenolic content

Folin-Ciocalteu (FC) assay was used to estimate the total phenolic content of extracts of *Thunbergia alata,* and *Tabernaemontana coronaria* leaves. 200µl of the extract solutions were 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 760 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.[15]

Estimation of total flavonoid content

The flavonoid content of the leaf extracts was determined by a colorimetric method using Aluminium trichloride method (Zhishen method). A volume of 125µL of the extracts 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 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.[16]

***In vitro* antioxidant assay**

DPPH radical scavenging assay:

The free radical scavenging activity of *Thunbergia alata* and *Tabernaemontana coronaria* leaf extracts was estimated. 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. [17]

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

Where,

A_0 is the absorbance of blank (DPPH solution alone)

A_s is the absorbance of extracts (DPPH + sample)

Nitric oxide radical scavenging assay:

Leaf extracts of *Thunbergia alata* and *Tabernaemontana coronaria* were also screened for nitric oxide radical scavenging activity using sodium nitroprusside. In this experiment, 2 mL of sodium nitroprusside (10 mM) and 0.5 mL of phosphate buffer (pH-7.4) will be 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.[18] 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 percentage yield of the plant was calculated **(Table 1)** and the preliminary phytochemical study of petroleum ether and ethanol extracts of *Thunbergia alata* and *Tabernaemontana coronaria* exposed that the extracts are instituted with various secondary metabolites such as alkaloids, carbohydrates, flavonoids, phenols, steroids, terpenoids, glycosides, tannins, saponins **(Table 2)**.

Total phenolic content

Total phenolic contents of petroleum ether and ethanol extracts of *Thunbergia alata* and *Tabernaemontana coronaria* leaves were 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)**. The total phenolic content of the extracts was calculated from the regression equation of calibration curve ($Y = 0.0049x + 0.0284$; $R^2 = 0.9989$) and expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g) **(Table 3)**. Total phenolic content values were observed high in ethanol extract of *Tabernaemontana coronaria* (95.25±2.06) than *Thunbergia alata* (62.74±1.28).

The flavonoid content

The flavonoid content of the leaf extracts of *Thunbergia alata* and *Tabernaemontana coronaria* was determined by a colorimetric method using Zhishen technique. The calibration curve was made by linear regression, and the results represented the average of three determinations to each concentration. The total flavonoid content of the extracts was calculated from the regression equation of the calibration curve ($Y = 3.5486x + 0.0653$; $R^2 = 0.9981$) and expressed as mg Rutin equivalents (RE) per gram of sample in dry weight (mg/g) at 510 nm **(Table 4 and Figure 2)**.

The results show that the ethanol extracts of both plants are rich in flavonoids compared to the petroleum ether extracts. The polarity of the ethanol is sufficient to solubilize the majority of the flavonoids, while petroleum ether failed to do may be a reason to get higher values for these extracts. Moreover, ethanol leaf extracts of *Tabernaemontana coronaria* (25.64±1.92) have more flavonoid content than *Thunbergia alata* (13.73±1.14). Petroleum ether extract of *Thunbergia alata* (3±1.24) has a very low concentration of flavonoids among all.

DPPH radical scavenging assay

In the present study, compared to the standard Ascorbic acid, *Tabernaemontana coronaria* has exhibited significant free radical scavenging activity in a dose-dependent manner than *Thunbergia alata* leaf. A standard curve was plotted **(Figure 3)** using various ascorbic acid concentrations. At a higher concentration (100 µg/mL), the *Tabernaemontana coronaria* extract with 72.37% of inhibition stood high among the extracts next to ascorbic acid (79.11%) **(Table 5 and Figure 4)**. Ethanol extracts of *Tabernaemontana coronaria* (IC_{50} = 50.06) and *Thunbergia alata* (IC_{50} = 74.77) were

inferred to be potent antioxidants than the petroleum ether extracts **(Figure 5)**.

Nitric oxide radical scavenging activity

When compared to the standard Ascorbic acid, *Tabernaemontana coronaria* leaf extract exhibited high NO free radical scavenging activity in a dose-dependent manner than *Thunbergia alata* leaf extract **(Table 6 and Figure 6)**. At a higher concentration of 100 µg/mL, the ethanol extract of *Tabernaemontana coronaria* is showing 75.37% of inhibition with IC_{50} values 47.15 µg/ml followed by petroleum ether extract 59.11% with IC_{50} values 77.23µg/ml, whereas for ascorbic acid, it is 81.11% with IC_{50} values 41.22µg/ml **(Figure 7)**.

Table 1: Percentage yield of *Thunbergia alata* and *Tabernaemontana coronaria*

Extract	*Thunbergia alata*		*Tabernaemontana coronaria*	
	Petroleum ether extract	Ethanol extract	Petroleum ether extract	Ethanol extract
Percentage yield	1.94	7.15	2.56	8.73

Table 2: Phytochemical screening of *Thunbergia alata* and *Tabernaemontana coronaria*

Phytochemicals	*Thunbergia alata*		*Tabernaemontana coronaria*	
	Petroleum ether Extract	Ethanol extract	Petroleum ether extract	Ethanol extract
Alkaloids	-	+	+	+
Glycosides	-	+	-	+
Flavonoids	-	+	+	+
Terpenoids	+	+	+	+
Steroids	+	+	+	-
Tannins	-	+	-	+
Proteins	-	-	-	+
Carbohydrates	-	+	-	+
Amino acids	-	-	-	+
Saponins	-	+	-	+

+ indicates the presence and – indicates the absence of phytochemicals

Table 3: The total phenolic content of leaf extracts of *Thunbergia alata* and *Tabernaemontana coronaria*

Extract	Total phenolic content mg/ml
TAPE (*T. alata* petroleum ether extract)	2.26±0.83
TAE (*T. alata* ethanol extract)	28.57±0.91
TCPE (*T. coronaria* petroleum ether extract)	5.18±0.14
TCE (*T. coronaria* ethanol extract)	36.14±1.72

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

Table 4: The total flavonoid content of leaf extracts of *Thunbergia alata* and *Tabernaemontana coronaria*

Extract	Total flavonoid content mg/ml
TAPE	2±0.64
TAE	29±1.32
TCPE	27.54±1.15
TCE	107±0.96

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

Table 5: Percentage inhibition and IC_{50} values of extracts and ascorbic acid at different concentrations in DPPH assay

Concentration µg/mL	Ascorbic acid	*Thunbergia alata*		*Tabernaemontana coronaria*	
		Petroleum ether extract	Ethanol extract	Petroleum ether extract	Ethanol extract
100	84.34	40.19	73.05	68.07	78.21
75	79.87	28.07	58.84	59.37	73.64
50	74.53	19.48	47.82	45.24	68.27
25	62.36	11.14	38.63	39.06	58.81
IC_{50} µg/ml	36.27	127.66	59.52	62.22	41.55

Table 6: Percentage inhibition and IC_{50} values of extracts and ascorbic acid at different concentrations in NO free radical scavenging assay

Concentration µg/mL	Ascorbic acid	*Thunbergia alata*		*Tabernaemontana coronaria*	
		Petroleum ether extract	Ethanol extract	Petroleum ether extract	Ethanol extract
100	83.26	39.37	69.39	71.34	72.54
75	72.64	24.19	54.51	58.11	69.82
50	68.21	14.21	46.17	45.27	58.25
25	62.14	10.24	29.54	38.35	49.17
IC_{50} µg/ml	39.75	132.31	65.38	61.36	50.06

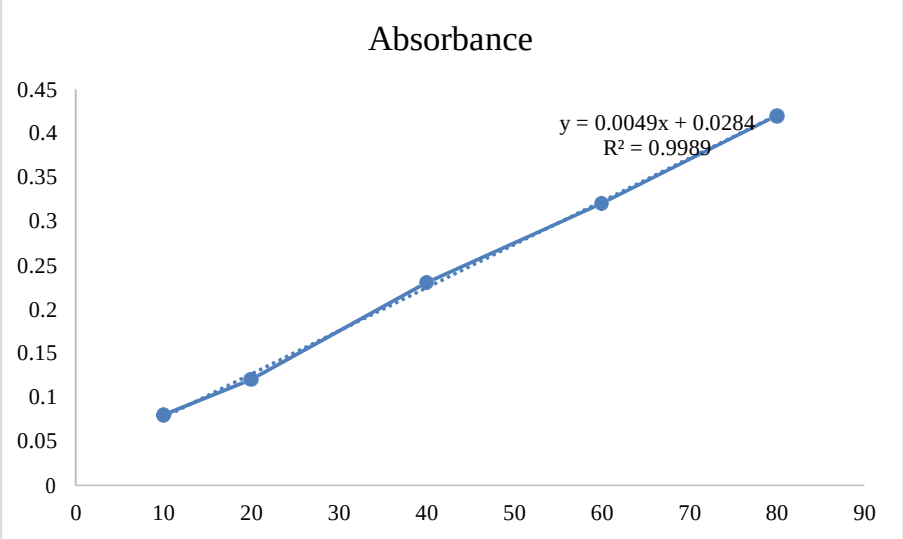

Figure 1: Calibration curve for Gallic acid

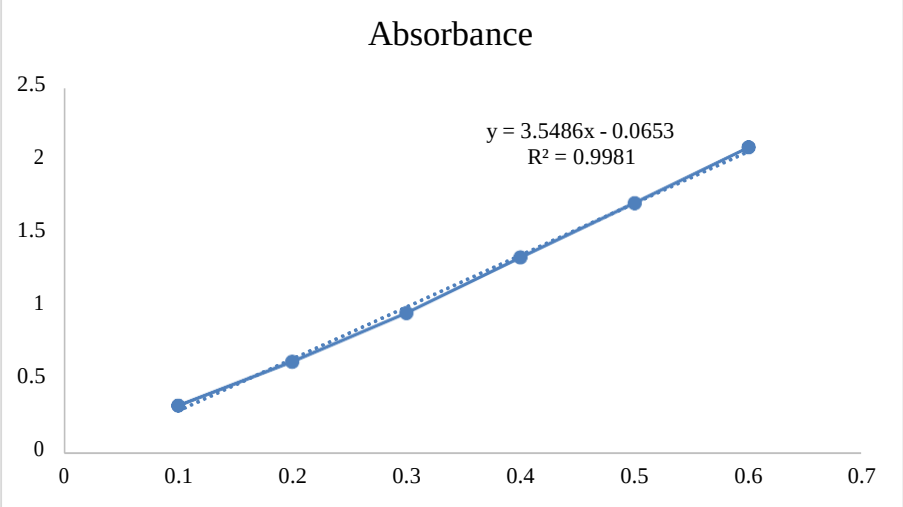

Figure 2: Calibration curve for Rutin

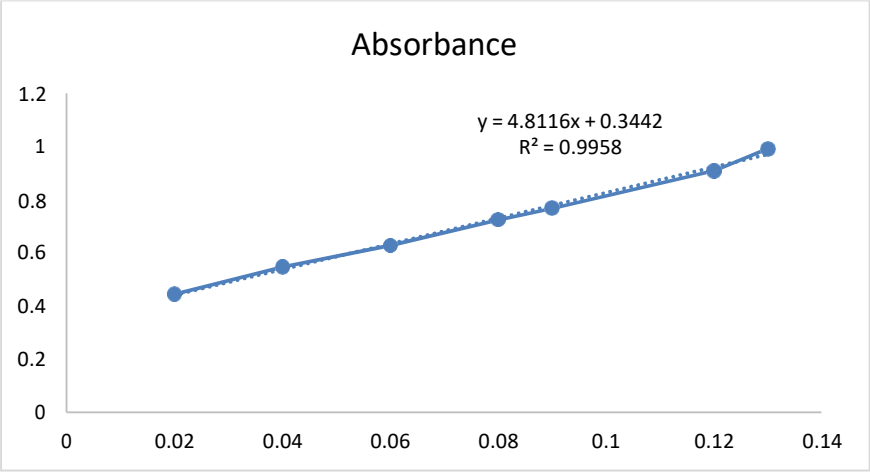

Figure 3: Calibration curve for Ascorbic acid

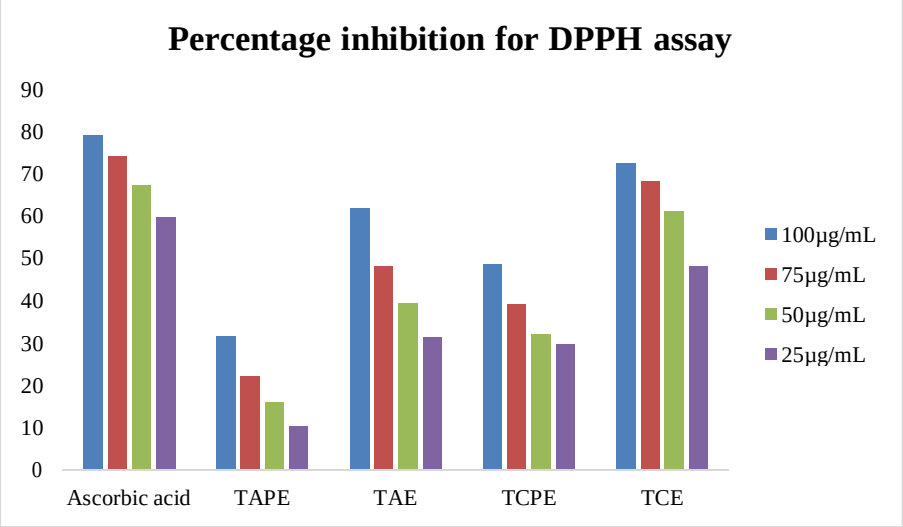

Figure 4: Percentage inhibition of extracts at different concentrations for DPPH assay

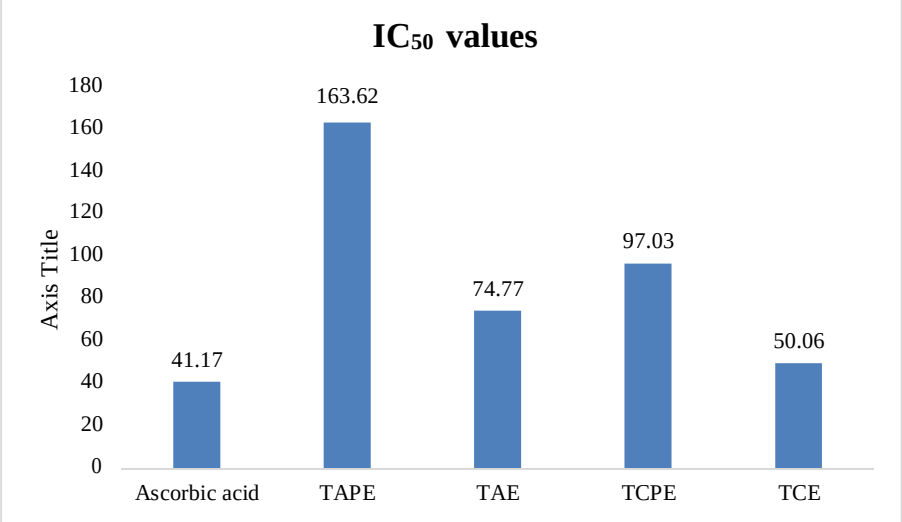

Figure 5: IC_{50} values of extracts for DPPH assay

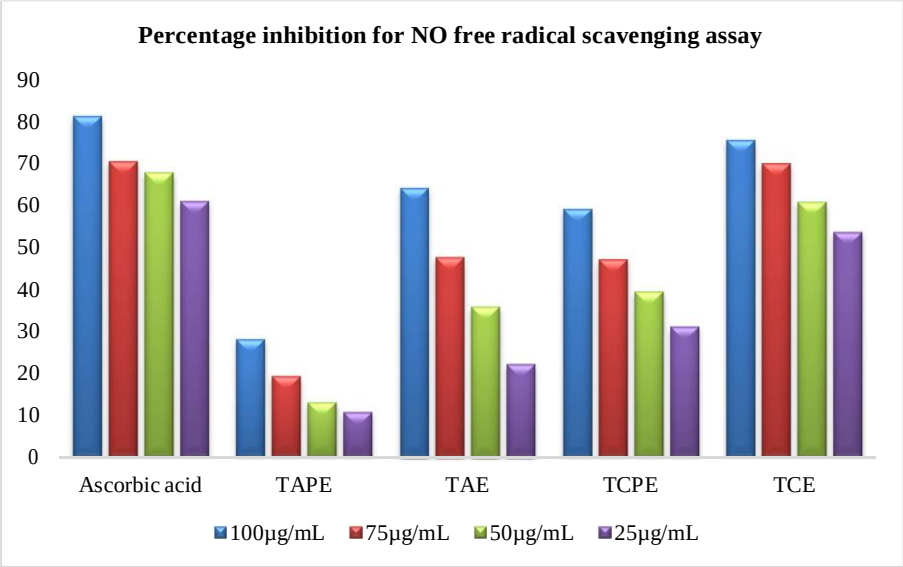

Figure 6: Percentage inhibition of extracts at different concentrations for NO free radical scavenging assay

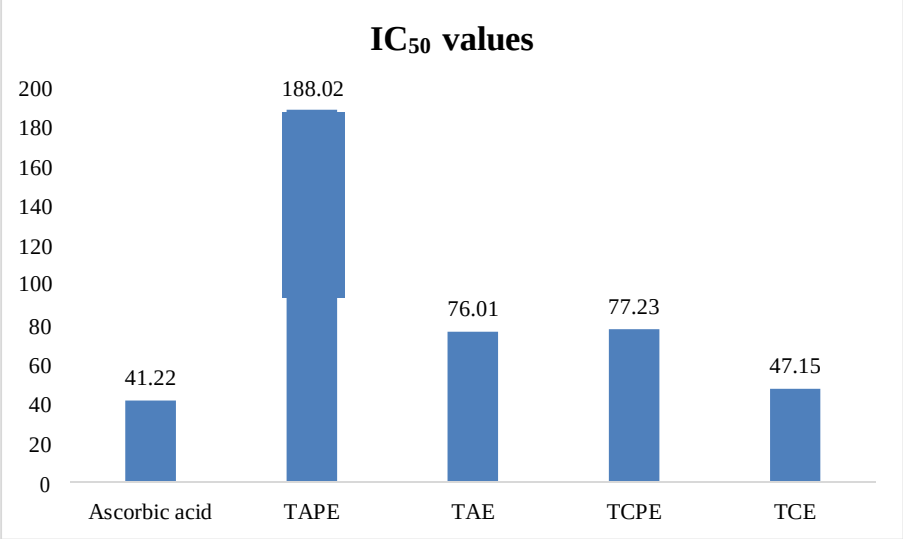

Figure 7: IC_{50} values of extracts for NO free radical scavenging assay

DISCUSSIONS

Polyphenolic compounds such as flavonoids, tannins, and lignans are important secondary metabolites that can have a protective effect on human diseases. Since the phenolic groups are proved to be powerful antibacterial, various formulations are using them as preservatives. Polyphenolics are also renowned as powerful antioxidants and useful to prevent the rancidity of oils. It is important to study the phenolic and flavonoid content in the plants to use them as protective agents. Since plant-based antioxidants are safer than synthetic agents, they are preferred in nutraceuticals and cosmeceuticals.[19-20] Total phenolic, total flavonoid content for petroleum ether and ethanol extracts of *Thunbergia alata* and *Tabernaemontana coronaria* were evaluated using standard

protocols. *In vitro* antioxidant screening in DPPH and NO free radical assay was performed for all extracts. To conclude, it is clear from the results that *Tabernaemontana coronaria* is rich in phenols and flavonoids and can be correlated to the high antioxidant activity observed in both methods. Further investigation of these extracts against a few pathogenic organisms is in progress to evaluate the antibacterial profile of the plant to make use of the selected plants to treat infectious diseases.

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