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Research Article

ANTIOXIDANT ACTIVITY OF TOTAL PHENOLIC COMPOUNDS OF *TECOMELLA UNDULATA*

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ABSTRACT

Objective: the study aims to investigate the methanolic extract of different plant parts (leaves, stems, bark and roots) of *Tecomella undulata* (family Bignoniaceae), for their antioxidant activity and were used for quantitative estimation of the total phenolics as Gallic Acid Equivalent (GAE) per gram dry weight and total flavonoid as Quericitin Equivalent (QE)) per gram dry weight

Methods: The methanol extract of the leaves, stem, root and bark of *Tecomella undulata* were estimated for total antioxidant capacity using 1,1-diphenyl picrylhydrazyl (DPPH) free radical scavenging assay, total phenolic and flavonoid contents using spectrophotometric methods.

Results: maximum total phenolic content was recorded in stems (13.75±0.125 mgGAE/gdw) While maximum total flavonoid content was found in leaves (71.875±18.393 mQE/gdw) and Highest radical scavenging activity is observed in stems with IC_{50} value 92.29±7.693.

Conclusion: It signifies that the Plant-derived phenolics and flavonoids represents good sources of natural antioxidants. From the above results it seen that this plant exhibits significant antioxidant activity.

Keywords: *Tecomella undulata.,* TPC, TFC, DPPH radical scavenging assay.

INTRODUCTION

*Tecomella undulata (*family: *Bignoniaceae*) is a tree species produces quality timber is also known as roheda, honey tree, desert teak, marwar teak (English), roheda, rohida (Hindi), rakhtroda, raktarohida (Marathi), chalachhada, dadimacchada, dadimapuspaka (Sanskrit). Distribution of *Tecomella undulata* is restricted to the drier parts of the Arabia, southern Pakistan and Northwest India up to an elevation of 1200 meters. In Pakistan it is found in Attock kala chita mountain it is found in Baluchistan and Sindh. In India, it occurs naturally in Maharashtra, Gujarat, Rajasthan, Punjab and Haryana. Pharmacologically it is seen Methanolic extracts of *Tecomella undulata* are known for their analgesic and anti-inflammatory potential **[1, 2]** . It was observed that the traditional medicinal practitioners use a total of seven Bignoniaceae family species for treatment of ailments like cancer, snake bite, skin disorders, gastrointestinal disorders, respiratory tract disorders, gynecological disorders, hepatic disorders, epilepsy, cholera, pain, urinary problems, malaria, heart problems, and sexually transmitted diseases **[3].**

Free radicals or reactive oxygen species, (ROS) or activated oxygen species (AOS) are produced as byproduct of normal metabolism also by xenobiotic compounds, drugs or ionizing radiations**[4].** Plants also generate ROS as signaling molecules to control various processes such as programmed cell death, pathogen defence, and stomatal behavior **[5].**ROS or free radicals are highly toxic these cause damage to genetic material and lipid peroxidation also inactivate membrane bound enzymes **[6].** They also cause chronic and degenerative disease like Alzheimer's, ageing, pulmonary disease, cardiovascular disease, cancer, rheumatoid arthritis **[7].**Many medicinal plants have great antioxidant potential, antioxidants reduce oxidative stress in cells and therefore are useful in treatment of disease like cancer, cardiovascular and inflammatory diseases**[8].**

MATERIALS AND METHODS

Plant material

The different plant parts (roots, stems, leaves and bark) of *Tecomella undulata* were collected in month of October - December from University of Rajasthan campus. It was washed with tap water, dried at room temperature and ground to fine powder. The species specimen was submitted to herbarium, Department of Botany, University of Rajasthan. Jaipur, Rajasthan, India and got the voucher specimen no. **RUBL211300**

Chemicals

All the chemicals used were of analytical grade and purchased from Hi Media from Hi-media Laboratory Pvt. Ltd. Mumbai.

Total phenolic and flavanoidal content

Plant extraction

2gm each of the dry material (leaves, stems, roots and bark) was extracted with 25ml of methanol at room temperature for 48 hours, filtered through Whatman paper no 1 filter paper, stored and used for quantification

Total phenolic content

Total phenolic compound contents were determined by the Folin-Ciocalteau method **[9,10,11,12].**The extract samples (0.5 ml; 1;10 diluted) were mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (4 ml, 1 M) were then added. The mixture was allowed to stand for 15 min and the phenols were determined by colorimetric method at 765 nm. The standard curve was prepared by 0, 50, 100, 150, 200, and 250 mg/ ml solutions of Gallic acid in methanol. Total phenol values are expressed in terms of Gallic acid equivalent (mg/ g of dry mass), which is a common reference compound. Total phenolic content can be calculated from the formula:

$$T = \frac{C.V}{M}$$

Where,

T=Total Phenolic concentration

C= Concentration of Gallic acid from caliberation curve (mg/ml)

V= Volume of extract (ml)

M= Wt of methanol plant extract

Total flavanoidal content

Total Flavonoid content was determined by using aluminium chloride colorimetric method ($AlCl_3$) according to the known method **[13, 14]** with slight modifications using Quercetin as standard. 1ml of test material was added to 10ml volumetric flask containing 4ml of water. To above mixture, 0.3ml of 5% $NaNO_2$ was added. After 5mins 0.3ml of 10% AlCl3 was added. After 6min, 2ml

of 1M NaOH was added and the total volume was made up to 10ml with distilled water. Then the solutions were mixed well and absorbance was measured against blank at 510nm. Total Flavanoidal content of the extracts was expressed in milligram of Quercetin equivalents/g dw. Total Flavanoidal content can be calculated from the formula:

$$T = \frac{C.V}{M}$$

Where,

T=Total Flavanoidal concentration

C= Concentration of Gallic acid from calibration curve (mg/ml)

V= Volume of extract (ml)

M= Wt of methanol plant extract

DPPH Radical Scavenging Activity

Plant extraction

10gm each of the plant material was soxhlet extracted with methanol for 24hours. The extract was filtered with Whatman filter paper no 1 and the crude extract was concentrated to dryness in a rotary flash evaporator under reduced pressure and controlled temperature (40–50°C). The extract was preserved in vacuum desiccators for subsequent use in antioxidant assay.

Assay

The antioxidant activities were determined using 1, 1,diphenyl-2-picryhydrazyl (DPPH) as a free radical. Experiments were initiated by preparing a 0.25mM solution of DPPH and 1mg/ml solution of different plant parts extracts (stock) in methanol was prepared. To the methanolic solutions of DPPH an equal volume of the extract dissolved in methanol was added at various concentrations. An equal amount of alcohol was added to the control. The setup was left at dark in room temperature and the absorption was monitored after 20 minutes. Ascorbic acid was used as a control. Experiment was performed in triplicate **[15,16]**. A control reaction was carried out without the test sample. Absorbance values were corrected for radicals decay using blank solution. The inhibitory effect of DPPH was calculated according to the following formula:

Linear graph of concentration Vs percentage inhibition was prepared IC_{50} values was calculated:

% Inhibition = $[1 - (Abs_{SAMPLE} / Abs_{CONTROL})] \times 100$

The antioxidant activity of each sample was expressed in terms of IC_{50} (micromolar concentration required to inhibit DPPH radical formation by 50%), calculated from the inhibition curve **[17, 18, 19]**

Statistical analysis

Experimental results are expressed as means ± standard deviation (SD). All measurements were replicated three times. IC_{50} values were also calculated by linear regression analysis. Experiments results were further analyzed for Pearson correlation coefficient(r) between Total Phenolic, Flavanoid and DPPH radical scavenging assay using the Microsoft Excel 2007 software and two way analysis of variance (ANOVA) was applied to investigate the differences among means by using Microsoft excel 2007 software. The values were considered to be significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

Screening of methanolic extracts of different parts (leaves, stems, bark and roots) of *T.undulata* were used for quantitative estimation of the Total Phenolics as Gallic Acid Equivalent (GAE) per gram dry weight and total Flavonoid as Querctin Equivalent (QE) per gram dry weight. Maximum Total Phenolic content was recorded in stems (13.75±0.125 mgGAE/gdw whereas lowest Total Phenolic content was recorded in roots (7 ±0.946) 125 mgGAE/gdw. Maximum total Flavonoid content was recorded in leaves(71.875 ±18.393) mgQE/gdw, whereas lowest in roots(11.25 ±0.721)) mgQE/gdw (Table 1).

Table 1: Total phenolic and flavonoidal content in different plant parts of *T.undulata*

Plant Part	Total Phenolic Content (mg GAE/gdw)	Total Flavonoidal Content (mg QE/gdw)
Leaves	12.7 ±0.288	71.875 ±18.393
Stem	13.75±0.125	38.75 ±1.895
Root	7 ±0.946	11.25 ±0.721
Bark	10.25±1.063	15.625 ±1.644

Antioxidant activity of methanolic extracts of different plant parts was measured by the using DPPH free radical scavenging assay. IC_{50} values of different plant parts are shown in TABLE 2. While graphs for %inhibition at different concentrations of different plant parts are shown in (Figure1-4). Antioxidant acitivity decreases in the order stem>bark>roots>leaves. Stems show highest antioxidant acitivity (92.29±7.693) while leaves show lowest antioxidant activity (277.82 ±8.135).

Table 2: The IC_{50} values of different plant parts of *T.undulata* of DPPH radical scavenging assay

Plant part	IC_{50} values (µg/ml)
Leaves	277.82 ±8.135
Stem	92.29 ±7.693
Root	230.16 ±9.083
Bark	196.1 ±2.744

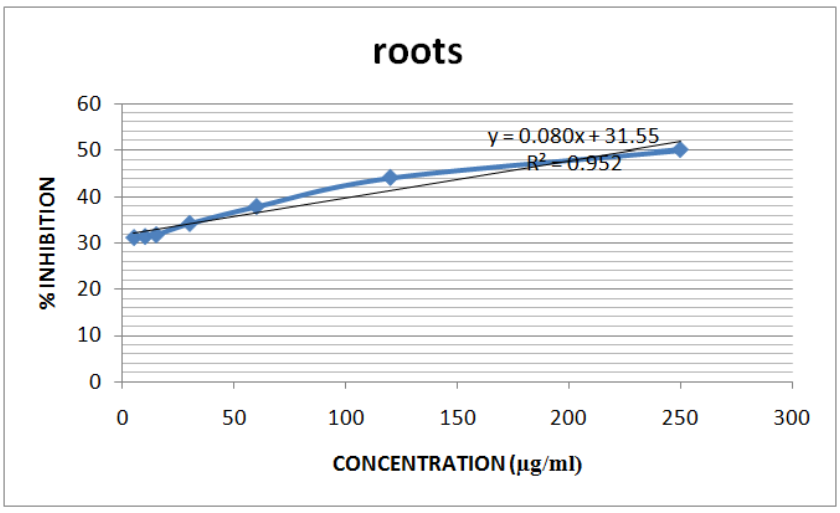

Fig. 1: DPPH Scavenger Assay of the methanol extract of roots of *T.undulata*

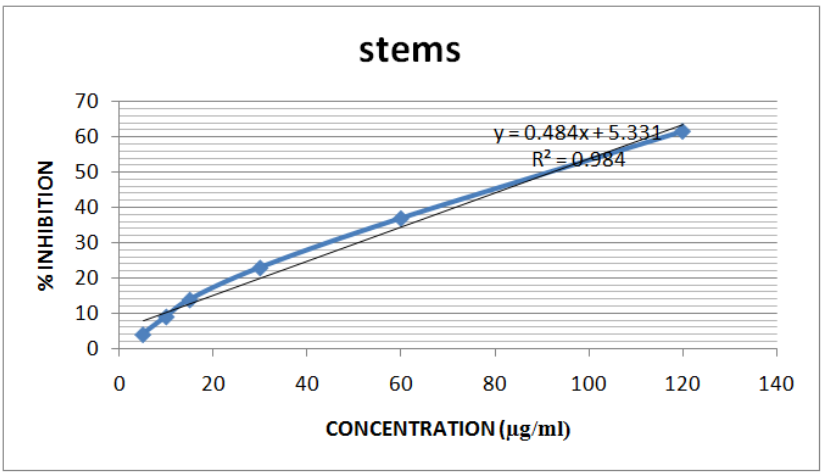

Fig. 2: DPPH Scavenger Assay of the methanol extract of stems of *T.undulata*

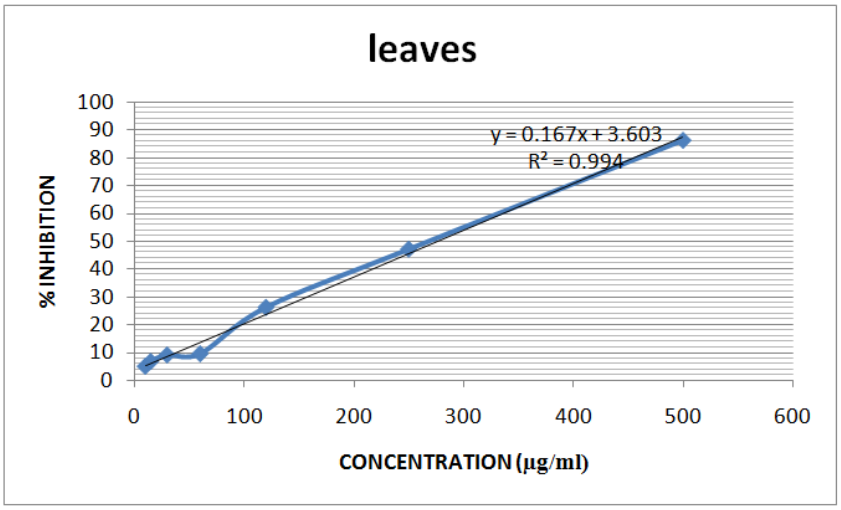

Fig 3: DPPH Scavenger Assay of the methanol extract of leaves of *T.undulata*

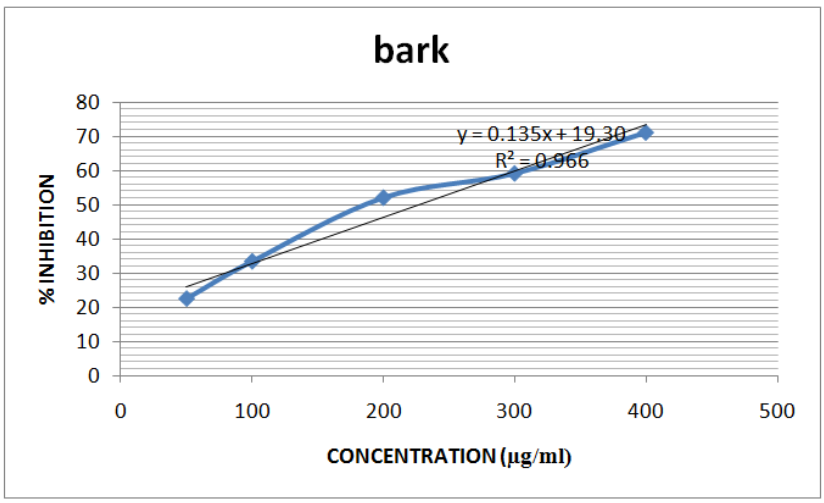

Fig 4: DPPH Scavenger Assay of the methanol extract of bark of *T.undulata*

Antioxidant activity is also correlated to the Total Phenolic and Flavonoidal content of the plant for this we performed linear regression and correlation analysis of the values of the DPPH with TPC and TFC (TABLE 3). These analysis reveal that there is a positive correlation between IC_{50} and TPC of leaves stem and root while this is negative for bark . There is positive correlation between

IC_{50} and TFC for leaves root and bark while negative correlation for stems. The high antioxidant activity in stems might be due to presence of high phenolic content. The medicinal actions of phenolics is mostly ascribed to their antioxidant capacity, free radical scavenging, chelation of redox active metal ions, modulation of gene expression and interaction with the cell signaling pathways. The free radical scavenging and antioxidant activities of phenolics are dependent upon the arrangement of functional groups about the nuclear structure. Both the number and configuration of H-donating hydroxyl groups are the main structural features influencing the antioxidant capacity of phenolics **[20, 21].**

ANNOVA analysis from Table 4 shows that statistically there is no significant difference between rows i.e. the content of Phenols and Flavonoids and DPPH free radical scavenging activity of plant parts is similar, as from table (F value < F crit), that is Fvalue of rows is acceptable. But when columns are taken into account there is a very significant difference between them i.e. content the content of phenols and flavonoids and DPPH free radical scavenging activity of different plant parts vary largely as F value > F crit for columns.

Table 3: Correlation between IC_{50} values of DPPH assay, phenolic and flavonoid content of *T.undulata*

Plant part	Phenols	Flavonoids
Leaves	0.9432	0.8830
Stem	0.90881	-0.029
Root	0.9352	0.9963
Bark	-0.2921	0.9756

Table 4: ANNOVA analysis for DPPH, Phenolic and Flavonoidal content of *T.undulata*

Source of Variation	SS	df	MS	F	P-value	F crit
Rows	8121.63	3	2707.21	1.269381	0.36632	4.757063
Columns	84117.41	2	42058.71	19.72086	0.002302	5.143253
Error	12796.21	6	2132.701			
Total	105035.2	11				

Rows and columns of ANNOVA analysis.			
Plant Part	**Phenols**	**Flavonoids**	**DPPH**
Leaves	12.7	71.88	277.82
Stem	13.75	38.75	92.29
Root	7	11.25	230.16
Bark	10.25	15.63	196.1

Also the positive correlation is seen between TFC and IC_{50} this reveals that antioxidant properties are also due to flavonoids as Flavonoids are an antioxidant group of compounds composed of flavonols, flavanols, anthocyanins, isoflavonoids, flavanones and flavones. All these sub-groups of compounds share the same diphenylpropane (C6C3C6) skeleton. The antioxidant properties are conferred on flavonoids by the phenolic hydroxyl groups attached to ring structures and they can act as reducing agents **[22].**

Due to presence of phenols and flavonoids and their correlation with TPC and TFC these studies suggest that phenols and flavonoids can be used to develop the novel phenolic synthetic antioxidants aimed at retarding the effects of free radicals and oxidants **[23, 24, 25].**

Also ethanolic extracts of leaves of T.undulata has shown to have significant effect on invivo antihyperglycemic and antioxidant potential **[26]**. Polyphenols are possess antioxidative properties and occur ubiquitously in plants **[27]**. hepatoprotective activity of *Tecomella undulata* bark may be due to its free radical-scavenging and antioxidant activity **[28].**

CONCLUSION

The Total Phenolic and Flavanoidal content and antioxidant activity of different plant parts was measured in the methanol extract of *T.undulata.* All these vary significantly in different plant parts. Significant correlations was found between the antioxidant activities and Total Phenolic and Flavanoid contents indicating that these phytocompounds are the major contributors of antioxidant capacities of this plant. The results of the present study suggests that *T.undulata* contained potential antioxidant bioactive compounds, which if properly and extensively studied could provide source of biologically active drug candidates and it also shows its great importance as therapeutic agent in preventing or curing the diseases caused due to oxidative stress.

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