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***Tecomella undulata*-Phenolic Compounds and Antioxidant Activities**

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ABSTRACT

The pharmacological actions of phenolic antioxidants stem mainly from their free radical scavenging properties. The present study was carried out to evaluate the antioxidant activity and phytochemical constituents of *Tecomella undulata* (Bignoniaceae) from methanolic extracts of different plant parts. Methanolic extracts of leaves, stems, roots and bark were prepared and screened for *in vitro* antioxidant activities by using peroxidase assay, Ferric Reducing Ability of Plasma (FRAP), lipid peroxidation assay (LPO), ABTS and the quantitative estimation of the total phenolics as Gallic Acid Equivalent (GAE) per gram dry weight and total flavonoid as Quercetin Equivalent (QE) per gram dry weight. Maximum total phenolic content was recorded in stems (13.75 ± 0.125 mg GAE/g DW). While maximum total flavonoid content was found in leaves (71.875 ± 18.393 mg QE/g DW), Leaves showed maximum radical scavenging activity for FRAP for methanolic extracts (96.664 ± 0.675 Mm L⁻¹ g⁻¹), LPO (26.66 ± 0.846 MDA/g DW) and peroxidase (0.383 Mm min⁻¹ g⁻¹ DW). While maximum free radical scavenging activity for ABTS assay found in stem (55.36 ± 3.53 Mmol min⁻¹ g⁻¹). It signifies that the plant-derived phenolics and flavonoids represent good sources of natural antioxidants. A good correlation with the total phenolic and flavonoidal content of plant is seen. It is seen that this plant exhibits significant antioxidant activity.

Key words: ABTS, LPO, FRAP, peroxidase 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, dadimachhada and dadimapuspaka (Sanskrit). Distribution of *Tecomella undulata* is restricted to the drier parts of the Arabia, southern Pakistan and northwest India. In Pakistan in Attock kala chita mountain it is found in Baluchistan and Sindh (Jain *et al.*, 2012a). 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 (Ahmad *et al.*, 1994; Almeida *et al.*, 2001). It was observed that the traditional medicinal practitioners use a total of seven Bignoniaceae species for treatment of pharmacological activities like antiproliferative activity, primarily used in the treatment of syphilis, painful swellings and cancer by traditional healers. Also, it is claimed to be useful in treating urinary discharges, enlargement of spleen, leucorrhoea, leukoderma, tumors, liver disorders, gonorrhoea, gout and promotes wound healing, snake bite, skin disorders, gastrointestinal disorders, respiratory tract disorders, gynecological disorders, malaria,

heart problems and sexually transmitted diseases in Indian traditional system of medicine (Rahmatullah *et al.*, 2010; Ravi *et al.*, 2011). *Tecomella undulata* stem bark reports hepatoprotective potential partially due to the presence of betulinic acid (Jain *et al.*, 2012b).

Reactive Oxygen Species (ROS) or Activated Oxygen Species (AOS) or free radicals are produced as byproduct of normal metabolism also by xenobiotic compounds, drugs or ionizing radiations (Freeman and Crapo, 1982). Excessive amounts of ROS may arise either from excessive stimulation of NAD(P)H oxidases. In mitochondria, ROS are generated as undesirable side products of the oxidative energy metabolism. An excessive and/or sustained increase in ROS production has been implicated in the pathogenesis of cancer, diabetes mellitus, atherosclerosis, neurodegenerative diseases, rheumatoid arthritis, ischemia/reperfusion injury, obstructive sleep apnea and other diseases. In addition, free radicals have been implicated in the mechanism of senescence (Droge, 2002). Medicinal plant parts are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant activity (Packer *et al.*, 1999).

Antioxidants terminate the chain reactions by removing free radical intermediates and inhibit other oxidation reactions by getting oxidized themselves (Lee, 1992). Plants also generate ROS as signaling molecules to control various processes such as programmed cell death, pathogen defence and stomatal behavior (Apel and Hirt, 2004). The ROS or free radicals are highly toxic, cause damage to genetic material and lipid peroxidation also inactivate membrane bound enzymes (Florence, 1995). They also cause chronic and degenerative disease like alzheimers, ageing, pulmonary disease, cardiovascular disease, cancer, rheumatoid arthritis (Pham-Huy *et al.*, 2008). Dysfunction induced by free radicals may thus be a major component of ischemic diseases of the heart, bowel, liver, kidney and brain (McCord, 1985). Many medicinal plants have great antioxidant potential as antioxidants reduce oxidative stress in cells and therefore are useful in treatment of disease like cancer, cardiovascular and inflammatory diseases (Krishnaiah *et al.*, 2011).

MATERIALS AND METHODS

Plant material: The different plant parts (roots, stems, leaves and bark) of *Tecomella undulata* were collected in month of October to 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 flavanoid content

Plant extraction: Two gram each of the dry material (leaves, stems, roots and bark) was extracted with 25 mL of methanol at room temperature for 48 h, filtered through Whatman No. 1 filter paper, stored and used for quantification.

Total phenolic content: Total phenolic contents were determined by the Folin-Ciocalteu method (McDonald *et al.*, 2001; Ebrahimzadeh *et al.*, 2008a, b; Nabavi *et al.*, 2008). 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 Na₂CO₃ (4 mL, 1 M) was 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⁻¹ solutions of gallic acid in methanol. Total phenol values are expressed in terms of gallic acid equivalent (mg g⁻¹ of dry mass), which is a common reference compound. Total phenolic content can be calculated from the equation:

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

Where:

T = Total phenolic concentration

C = Concentration of gallic acid from calibration curve (mg mL⁻¹)

V = Volume of extract (mL)

M = Wt. of methanol plant extract

Total flavanoidal content: Total flavonoid contents were determined by using aluminium chloride colorimetric method (AlCl₃) according to the known method (Dewanto *et al.*, 2002; Sakanaka *et al.*, 2005) with slight modifications using quercetin as standard. One milliliter of test material was added to 10 mL volumetric flask containing 4 mL of water. To above mixture, 0.3 mL of 5% NaNO₂ was added. After 5 min, 0.3 mL of 10% AlCl₃ was added. After 6 min, 2 mL of 1 M NaOH was added and the total volume was made up to 10 mL with distilled water. Then, the solutions were mixed well and absorbance was measured against blank at 510 nm. Total flavanoidal contents of the extracts were expressed in milligram of quercetin equivalents/g DW. Total flavanoidal content can be calculated from the equation:

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

Where:

T = Total flavanoidal concentration

C = Concentration of quercetin from calibration curve (mg mL⁻¹)

V = Volume of extract (mL)

M = Wt. of methanol plant extract

Statistical analysis: Experimental results are expressed as Means±Standard Deviation (SD). All measurements were replicated three times. The IC₅₀ values were also calculated by linear regression analysis. Experimental results were further analyzed for Pearson correlation coefficient (r) between total phenolic, flavanoid and free radical scavenging assay using the Microsoft Excel 2007 software.

Determination of antioxidant activity

Reducing ability (FRAP assay): The determination of the total antioxidant activity (FRAP assay) in the extract is a modified method of Benzie and Strain (1996). The stock solutions included 300 mM acetate buffer (0.3 M acetic acid and 0.3 M sodium acetate), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM ferric chloride (FeCl₃.6H₂O) solution. The

fresh solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ and 2.5 mL $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. The temperature of the solution was raised to 37°C before use. Plant extracts (100 μL each of methanolic ethyl acetate and hexane) were allowed to react with 2900 μL of the FRAP solution for 30 min in the dark condition. Readings of the coloured product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 100 and 1000 μM FeSO_4 . Results are expressed in mM Fe (II)/g dry mass and compared with that of BHT, ascorbic acid, quercetin and catechin.

Peroxidase assay: The method of assay measures the oxidation of pyrogallol to purpurogallin by peroxidase when catalyzed by peroxidase at 420 nm and at 20°C. Plant sample (200 mg) was homogenized with 10 mL of phosphate buffer, refrigerated and centrifuged at 10000 rpm for 20 min. The clear supernatant was taken as the enzyme extract. The activity was assayed after the method of Chance and Maehly (1955) with following modifications. The 2.4 mL of phosphate buffer, 0.3 mL pyrogallol (50 μM) and 0.2 mL of H_2O_2 (30%) were added. The amount of purpurogallin formed was determined by taking the absorbance at 420 nm immediately after adding 0.1 mL enzyme extract. The extinction coefficient of 2.8 mM cm^{-1} was used in calculating the enzyme activity that was expressed in terms of millimole per minute per gram dry weight.

Lipid peroxidation assay (LPO): About 0.5 g of dry material was homogenized with 10 mL of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged for 5 min (15000 g, 4°C). Supernatant was collected and 1 mL of supernatant was mixed with 4 mL of 0.5% (w/v) TBA (Thiobarbituric acid) in 20% (w/v) TCA and then incubated in water bath at 95°C for 30 min. Reaction was quickly ended by incubating on an ice bath. In case the solution is not clear, centrifuge at 10000 g for 10 min and the absorbance was measured at 532 and 600 nm. OD_{600} values were subtracted from MDA-TBA complex values at 532 nm and MDA concentration was calculated using the Lambert-Beer law with an extinction coefficient $\epsilon\text{M} = 155 \text{ mM cm}^{-1}$. Results were presented as μM MDA/g.

ABTS radical scavenging assay: To determine ABTS radical scavenging assay, the method of Re *et al.* (1999) was adopted. The stock solutions included 0.002 M ABTS solution and 0.07 M potassium persulphate solution. The working solution was then prepared by mixing the 25 mL of ABTS stock and 0.1 mL of potassium persulphate stock and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing ABTS solution with ethanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (1 mL) at varying concentration were allowed to react with 3 mL of the ABTS solution and the absorbance was taken at 734 nm after 6 min using the spectrophotometer. The ABTS scavenging capacity of the extract was compared with that of BHT and percentage inhibition calculated as:

$$\text{Inhibition (\%)} = \left[1 - \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right] \times 100$$

where, $\text{Abs}_{\text{control}}$ is the absorbance of ABTS radical+methanol, $\text{Abs}_{\text{sample}}$ is the absorbance of ABTS radical+sample extract/standard.

RESULTS AND DISCUSSION

In the present study we have investigated the antioxidant activity of *Tecomella undulata* by different assays and found their scavenging activity. Table 1 shows the total phenolic content maximum in stem (13.75±0.125 mg GAE/g DW) while minimum in roots (7±0.946 mg GAE/g DW) and total flavonoid content was seen maximum in leaves (71.875±18.393 mg QE/g DW) while minimum in roots (11.25±0.721 mg QE/g DW). Table 2 shows FRAP activity of ethyl acetate, hexane and methanol extracts. In ethyl acetate extract maximum activity is seen in bark (64.664±0.403 mM L⁻¹ g⁻¹) while minimum in roots (21.334±0.850 mM L⁻¹ g⁻¹). In hexane extract maximum activity is seen in bark (14.66±1.084 mM L⁻¹ g⁻¹) while minimum in stems (11.332±1.520 mM L⁻¹ g⁻¹), In methanol extract maximum activity is seen in leaves (96.664±0.675 mM L⁻¹ g⁻¹) while minimum in roots (58.09±1.317 mM L⁻¹ g⁻¹). Table 3 shows the peroxidase activity maximum in leaves (0.382±0.014 mM min⁻¹ g⁻¹ DW) while minimum in leaves and roots (leaves 0.1024±0.001 mM min⁻¹ g⁻¹ DW roots 0.103±0.003 mM min⁻¹ g⁻¹ DW). Table 4 shows LPO activity maximum in leaves (26.66±0.846 mg QE/g DW) while minimum in stem (2.79±0.407 mg QE/g DW). Table 5 shows the IC₅₀ of methanolic extracts of which stems shows highest activity for ABTS assay (55.36±3.534 Mmol min⁻¹ g⁻¹) while leaves show minimum activity (425.84±18.362 Mmol min⁻¹ g⁻¹). Table 6 shows correlation values of Total Phenolic Content (TPC) and Total Flavonoidal Content (TFC) with the different free radical scavenging assays performed.

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

Plant part	Total phenolic content (mg GAE/g DW)	Total flavonoidal content (mg QE/g DW)
Leaves	12.70±0.288	71.875±18.393
Stem	13.75±0.125	38.750±1.895
Root	7.00±0.946	11.250±0.721
Bark	10.25±1.063	15.625±1.644

Table 2: Total antioxidant (FRAP) activity of methanol, ethyl acetate and hexane extract of different plant parts of *Tecomella undulata*

Plant part	Methanol	Ethyl acetate	Hexane
Leaves	96.664±0.675	33.332±0.428	12.932±2.270
Stem	66.030±0.639	36.660±0.686	11.332±1.520
Root	58.090±1.317	21.334±0.850	13.330±0.178
Bark	78.090±1.543	64.660±0.403	14.660±1.084

Table 3: Peroxidase activity in different plant parts of *Tecomella undulata*

Plant part	Methanolic extract
Leaves	0.3820±0.014
Stem	0.1024±0.001
Root	0.1030±0.003
Bark	0.1560±0.011

Table 4: LPO activity in different plant parts of *Tecomella undulata*

Plant part	Methanolic extract
Leaves	26.66±0.846
Stem	2.79±0.407
Root	6.77±0.776
Bark	6.97±0.501

Table 5: IC₅₀ values of different plant parts of *Tecomella undulata* of ABTS radical scavenging assay

Plant part	IC ₅₀ values (µg mL ⁻¹)
Leaves	425.84±18.36258
Stem	55.36±3.534592
Root	320.33±20.015
Bark	308.46±22.35918

Table 6: Correlation

Antioxidant activity of different plant parts	Total phenolic content	Total flavonoid content
ABTS		
Leaves	-0.9434	-0.9824
Stem	-0.7636	0.7635
Root	0.8769	-0.7785
Bark	0.6557	0.3318
LPO		
Leaves	0.9922	0.9996
Stem	0.60054	-0.6005
Root	0.5361	-0.9847
Bark	-0.7932	-0.1369
Peroxidase		
Leaves	-0.3711	-0.5058
Stem	0	0
Root	-0.7395	0.9042
Bark	-0.4888	0.9999

For ABTS we find a positive correlation with the flavonoidal content of the plant in stem while a negative correlation with the phenolic content, this may be a reason for the highest activity of the stem methanolic extracts for the ABTS assay. While in leaves both flavonoidal and phenolic content show a negative correlation with the ABTS activity assay, this may prove the minimum activity by the leaf extract. For the LPO activity assay we see a positive correlation with both phenolic and flavonoidal content which may explains its highest activity, but in contrast to LPO, for peroxidase assay highest activity is seen in leaves but correlation with the phenolic and flavonoidal content is negative. Phenolic and flavonoidal content have shown a good correlation with antioxidant activity, this may be due to structural differences. According to the Singleton and Rossi Jr. (1965) various phenolic compounds have different responses in this assay. The molar response of this method is roughly proportional to the number of phenolic hydroxyl groups in a given substrate but the reducing capacity is enhanced when two phenolic hydroxyl groups are oriented at ortho or para positions. Since, these structural features of phenolic compounds are reportedly also responsible for antioxidant activity, measurement of phenols in infusions may be related to their antioxidant properties (Katalinic *et al.*, 2006). Phenolic compounds, such as flavonoids, phenolic acid and tannins, possess anti-inflammatory, anti-carcinogenic, anti-atherosclerotic and other properties that may be related to their antioxidant activities (Chung *et al.*, 1998; Wong *et al.*, 2006). Flavonoids and flavonols are two polyphenolic compounds that play an important role in stabilizing lipid oxidation and are associated with antioxidant activity (Yen *et al.*, 1993). Phenolic compounds may contribute directly to antioxidative action (Duh *et al.*, 1999). Polyphenolic compounds may have an inhibitory effect on mutagenesis and carcinogenesis in humans when as much as 1.0 g is ingested daily from a diet rich in fruits and vegetables (Tanaka *et al.*, 1998).

CONCLUSION

With the above study we conclude that the investigated plant *T. undulata* shows a very good antioxidant activity for different assays tested, this plant forms a good base for the further research on the antioxidant ability of this plant and its use as the commercial medicine for the free radicals related disease. This study shows that the phenolic and flavonoidal content also have a remarkable effect on the antioxidant activity.

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