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IN VITRO EVALUATION OF ANTI OXIDANT ACTIVITY, ESTIMATION OF TOTAL PHENOLIC AND FLAVONOID CONTENT OF DIFFERENT EXTRACTS OF *TECOMA STANS* (L.)

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ABSTRACT

Tecoma stans is a flowering perennial plant characterized by the presence of yellow colored trumpet-shaped flowers. The various parts of the plant possess several medicinal properties due to the presence of diversified group of biologically/ pharmacologically active phytochemicals and are used therapeutically for the treatment of various diseases such as diabetes, cancer, yeast infections etc. The plant is reported to hold the medicinal value as a powerful diuretic, vermifuge, cardiotonic, anti-spasmodic agent apart from its use as an ornamental, fuel, timber, shade/shelter, and barrier. Based on the consideration of these facts, we engaged to study in-vitro antioxidant activity, determination of total phenolic and flavonoid content of different extracts of T.stans. The antioxidant activity was observed to be high in stem methanolic extract. The leaf methanolic extract tends to contain more phenolic content (60.24 mg GAE/ gr. Ext.). The total flavonoid content was observed to be high in leaf methanolic extract (324.80 mg QE/gr. Ext.) and leaf aq. methanolic extract (127.18 mg CE/gr.Ext.) in terms of quercetin and catechin equivalents respectively.

KEY WORDS

Phytochemicals, Vermifuge, Cardiotonic, Anti-spasmodic, Catechin, Quercetin.

1. INTRODUCTION

The ***Tecoma stans*** plant is a speedy growing evergreen medicinal plant belonging to the family Bignoniaceae which grows to a height of about 20-30 feet and it is characterized by the presence of trumpet-shaped yellow colored flowers [1]. The plant is spread throughout the tropical and subtropical regions of India, South America, Florida, Bahamas, Trinidad, Africa, Asia and Australia [2]. The entire plant possesses medicinal value and the roots are used as diuretic, tonic, anti-syphilitic and vermifuge [3]. The decoction of flowers and bark are used for stomach pains. The plant is used in the treatment of diabetes [4]. The bark and flowers possess antimicrobial activity [5] and also used for the treatment of various cancers [6]. The bark shows smooth muscle relaxant [7] and mild cardiotonic activity. The root of the plant is reported to be a powerful diuretic, vermifuge, and tonic [8]. A grinding of the root and lemon juice is reportedly used as a remedy for snake and rat bites. It is also reported to have cardioprotective action [9]. The whole plant has promising activities like Wound Healing Potential [10], Antispasmodic Effect [11], Antimicrobial Activity [12], Cytotoxic Activity [13,14], Antifungal Activity [15], Anti-Inflammatory Activity [16] and Anti-diabetic Activities due to the presence of various phytoconstituents like Sugars, Terpenoids, Phenolics and Alkaloids [17,18]. The alkaloids Tecomine, Tecostanine, 5 Beta-Hydroxyskitanthine and Boschniakine isolated from *T. stans* plant exhibited good

anti-diabetic activity [19]. Apart from the medicinal usage, this plant was being widely used as an ornamental, fuel, timber, shade/shelter, barrier [20].

The plants rich in anti-oxidants are helpful for the prevention as well as the decrease in the oxidative stress and damage caused by the free radicals. The anti-oxidants inhibit the initiation and propagation of oxidative chain reactions. In view of the varied pharmacological and phytochemical reports of T. *stans*, the study on *in vitro* antioxidant activity and estimation of total phenolic and flavonoidal content of leaf and stem of *T. stans* was taken as part of our continuing phytochemistry work.

2. MATERIALS AND METHODS

2.1. Plant collection:

The leaf and stem parts of the plant *T. stans* were collected from CSIR-CIMAP, Research Centre, Hyderabad, Telangana, India. The plant was identified taxonomically and it was authenticated by Dr.A.Sabitha Rani, Assistant Professor, Department of Botany, Osmania University, Hyderabad, India. A voucher specimen (CIMAP-TS/18) was stored at CSIR-CIMAP Research Centre, Hyderabad.

2.2. Extraction:

The *T. stans* leaf and stem material (each 100 gm) were shade dried, powdered and successively extracted by the solvents with increasing order of polarity i.e Hexane, Ethyl acetate, Methanol by sonication equipment and also extracted with aq.methanol by Maceration method. Later, the extract solutions were filtered and distilled under reduced pressure using Rota vapor to obtain the crude extracts. These extracts were used for determination of Anti-oxidant activity and estimation of total phenolic & flavonoidal content.

2.3. Determination of *In-Vitro* Anti-Oxidant Activity:

The ability of the different extracts of *T. stans* leaf and stem to scavenge DPPH radical was determined by using DPPH radical scavenging method [21]. DPPH is a dark-colored crystalline, nitrogen-centered substance composed of stable free radicals. The principle involves reduction of DPPH (purple color) in the presence of anti-oxidant in the sample which results in the formation of non-radical DPPH (yellow color). The color transformation of purple to yellow is measured spectrometrically at 517nm. The decrease in absorbance indicates greater inhibition of DPPH free radicals which is an indication of higher antioxidant content in the sample.

The sample extract stock solutions (1mg/mL) were diluted to the final concentrations of 5, 10, 15, 20, 25, 50 µg/mL using methanol. To 5 mL of different extract solutions, 2 mL of 0.3 mM DPPH in methanol solution was added and allowed to react for 30 minutes at room temperature in a dark chamber. The reduction of DPPH radicals was measured by checking the absorbance in UV-VIS Spectrophotometer at 517nm.

Methanol (5mL) was used as the blank. DPPH solution (2mL) plus methanol (5mL) was used as negative control. The standard solutions of ascorbic acid 5, 10, 15, 20, 25, 50µg/mL were used as positive control.

The percentage inhibition of DPPH radicals by the anti-oxidant is calculated by using the formula-

$$\text{Percentage of Inhibition (\%)} = \frac{\text{Blank absorbance - Sample absorbance}}{\text{Blank absorbance}} \text{ X } 100$$

2.4. Estimation of Total Phenolic content:

The total phenolic content of different extracts of *T. stans* leaf and stem was determined by Folin-Ciocalteus assay method [22]. The folin – cioaclteau reagent comprising of phosphotunstat–phosphomolybdenum complex (yellow) gets reduced by phenolate ion in the sample, changing its color to blue in the presence of a base. The color transformation is measured spectrometrically and the greater phenolic ion content in the sample is indicated by the increased absorbance.

An aliquot (1 mL) of different extracts or standard solution of Gallic acid (50, 100, 150, 200 and 250 mg/lit) was added to 25 ml volumetric flask. To this flask, 9 mL of distilled water was added. Further 1 mL of Folin-Ciocalteu phenol reagent was added to this flask and shaken for few minutes. To the mixture, 10 mL of 7 % sodium carbonate (Na_2CO_3) solution was added after 5 minutes. The solution mixture was diluted to 25 mL with distilled water. The solution was placed aside for incubation for about 90 minutes at room temperature. Similarly, a reagent blank solution was prepared by omitting the samples to be analyzed. The absorbance of the sample extract and standard solutions against the prepared reagent blank was observed at 750 nm with the UV-Visible spectrophotometer. The phenolic content expressed as mg Gallic Acid Equivalents (GAE)

per gram extract weight. The total phenolic content was calculated by using the formula-

$$\text{Total Phenolic Content} = \text{Concentration} \times \frac{\text{Vol. of the Sample}}{\text{Weight of the Sample}}$$

2.5. Estimation of Total Flavonoidal content:

The total flavonoidal content of different extracts of *T. stans* leaf and stem were determined by aluminium chloride colorimetric assay using Quercetin and Catechin as the standards. The principle is based on the formation of acid stable complexes by aluminium chloride with the keto group and hydroxyl group of flavonoids.

Colorimetric assay (Quercetin)

The total flavonoid content was measured by the aluminium chloride colorimetric assay using quercitin as standard [23]. An aliquot of 2.5 mL of extracts or standard solution of Quercitin (150, 200, 300, 400 and 500 mg/lit) was added to 25 mL volumetric flask. To this flask, 10 mL of distilled water was added. Further 0.75 mL of 5 % sodium nitrite ($NaNO_2$) was added. To this solution mixture, 0.75 mL of 10 % aluminium chloride ($AlCl_3$) was added after 5 minutes. At the 6th min, 2 mL of 1 M sodium hydroxide (NaOH) was added and the total volume was made up to 25 mL with distilled water.

Similarly, the reagent blank was prepared by omitting the samples to be analyzed. The solutions were mixed well, and the absorbance was measured against the prepared reagent blank at 510 nm in UV-VIS spectrophotometer. The flavonoid content was expressed as mg Quercetin equivalents/gr. weight of extract.

Colorimetric assay (Catechin):

The total flavonoid content was measured by the aluminium chloride colorimetric assay using catechin as standard [24]. An aliquot of 2.5 mL of extracts or standard solution of Catechin (150, 200, 300, 400 and 500 mg/lit) was added to 25 mL volumetric flask. To this flask, 10 mL of distilled water was added. Further 0.75 mL of 5 % sodium nitrite (NaNO2) was added. To this solution mixture, 0.75 mL of 10 % aluminium chloride ($AlCl_3$) was added after 5 minutes. At the 6th min, 2 mL of 1 M sodium hydroxide (NaOH) was added and the total volume was made up to 25 mL with distilled water.

Similarly, the reagent blank was prepared by omitting the samples to be analysed. The solutions were mixed well, and the absorbance was measured against prepared reagent blank at 510 nm in UV-VIS spectrophotometer. The flavonoid content expressed as mg Catechin equivalents/gr. weight of extract.

The total flavoniodal content was calculated by using the formula

$$\text{Total Flavonoid content} = \text{Concentration} \times \frac{\text{Vol. of the Sample}}{\text{Weight of the Sample}}$$

3. RESULTS AND DISCUSSION

3.1 *In-Vitro* Antioxidant Activity

The DPPH assay establishes the capability of extracts (Ethyl acetate, Methanol and aq. methanol) to reduce DPPH radical to the hydrazine by changing the unpaired electrons to the paired electrons. In the present study, dose- dependent inhibition of DPPH radical indicates that the extracts caused the reduction of DPPH radical. Percentage scavenging of DPPH radical by the extracts (Ethyl acetate, Methanol and aq. Methanol extracts) examined at different concentrations is depicted in Table-1. Fig-1 illustrates the effect of Ethyl acetate, Methanol and aq. Methanol extracts on DPPH radicals.

Table 1: Effect of Ethyl acetate, Methanol, and 30 % aq. methanol extracts of T. stans leaf and stem on DPPH radicals.

S. No	Concentration (µg/ml)	Ascorbic Acid	% Inhibition of DPPH by anti- oxidant in the extracts					
			Leaf Extracts			Stem Extracts		
			Leaf Ethyl acetate	Leaf Methanol	Leaf Aq. Methanol	Stem Ethyl acetate	Stem Methanol	Stem Aq.Methanol
1	5	38.23	0.18	8.35	0.90	1.81	18.79	3.09
2	10	58.56	6.81	14.45	11.36	2.81	34.16	3.09
3	15	74.58	8.90	27.81	18.62	10.31	39.68	7.93
4	20	93.92	10.89	34.50	26.64	11.71	49.03	11.69
5	25	95.02	18.52	39.59	35.42	20.25	57.22	14.95
6	50	97.23	39.87	76.52	60.56	37.42	60.31	37.67

The Anti-oxidant activity of the *T.stans* leaf and stem extracts using DPPH method was observed to be more in stem methanolic extract when compared to other extracts. The order of Anti-oxidant activity of plant extracts by DPPH assay method is-

Stem Methanol> Leaf Methanol > Leaf Aq.Methanol > Stem Ethyl acetate > Leaf Ethylacetate > Stem Aq.Methanol

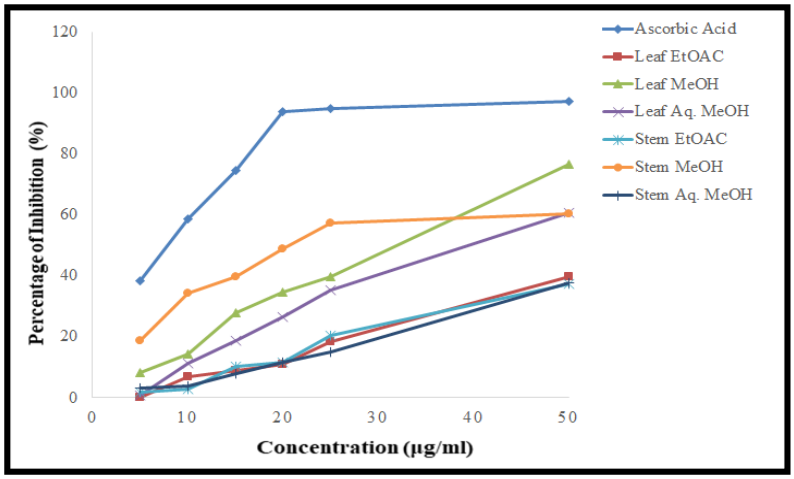

Figure 1: Graph of free radical scavenging activity (Anti-oxidant activity) of *T. stans* leaf and stem ethyl acetate, methanol and aqueous methanolic extracts on DPPH free radicals.

3.2. Total Phenolic Content

The total phenolic content of *T. stans* leaf and stem extracts were estimated by Folin-Ciocalteu's assay method. The results of the phenolic content of *T. stans* leaf and stem extracts (Hexane, Ethyl acetate, Methanol and 30% aq. Methanolic extracts) equivalent to 7.77, 54.61, 60.24, 58.34, 7.27, 54.12, 56.28 and 41.56 mg Gallic acid equivalents/gram of extract respectively were detected and the same were depicted in Table-3. The Standard graph of gallic acid is depicted in Table 2, Fig-2

Table 2: Absorbance values of Standard Gallic Acid

Concentration (µg/ml)	Absorbance
0	0
50	0.258
100	0.519
150	0.761
200	1.034
250	1.31

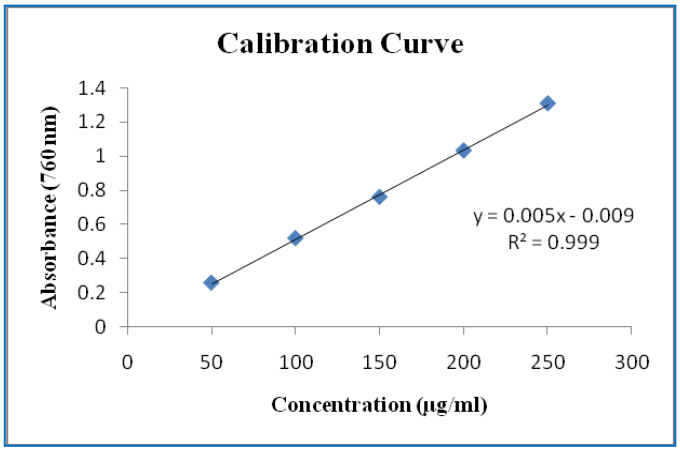

Figure 2: Standard graph of Gallic acid

Table 3: The phenolic content of Hexane, Ethyl acetate, Methanol and 30%aq. methanol extracts of T. stans leaf and stem in terms of mg/gm gallic acid equivalents.

S. No	Extract	Unknown Concentration (µg/ml)	mg/gm gallic acid equivalents
1	Leaf Hexane	77.78	7.77
2	Leaf Ethyl acetate	546.19	54.61
3	Leaf Methanol	602.44	60.24
4	Leaf Aq.Methanol	583.43	58.34
5	Stem Hexane	72.79	7.27
6	Stem Ethyl acetate	541.20	54.12
7	Stem Methanol	562.89	56.28
8	Stem Aq.Methanol	415.65	41.56

Based on the results, it is observed that in *T. Stans* the leaf Methanolic extract contains more phenolic content (60.24 mg GAE/ gr. Ext.) compared to other extracts. The order of Phenolic content of plant *T. stans* leaf and stem extracts-

Leaf Methanol > Leaf Aq.Methanol > Stem Methanol > Leaf Ethyl acetate > Stem Ethyl acetate > Stem Aq.Methanol > Leaf Hexane > Stem Hexane

3.3. Total Flavonoid Content

The total flavonoid content using quercetin and catechin as standards:

The total flavonoid content of *T. stans* leaf and stem extracts were estimated by aluminum chloride colorimetric assay method using Quercetin and Catechin as standards. The leaf and stem extracts such as Hexane, Ethyl acetate, Methanol and aq. Methanolic extracts showed the results equivalent to 62.92, 189.51, 324.80, 271.01, 109.05, 221.98, 241.17 and 124.91 mg/100-gram equivalents respectively of Quercetin and equivalent to 26.69, 65.87, 123.90, 127.18, 44.38, 69.47, 95.53 and 42.90 mg/100gram equivalents respectively of catechin. The results are depicted in table- 5. The standard graphs of quercetin and catechin were depicted in Table-4, Fig-3.

Table 4: Absorbance values of flavonoid content using Quercetin and Catechin as standards

Concentration (µg/mL)	Absorbance	
	Quercetin	Catechin
0	0	0
100	0.200	0.336
200	0.380	0.407
300	0.509	0.509
400	0.622	0.600
500	0.814	0.814
600	0.972	1.071

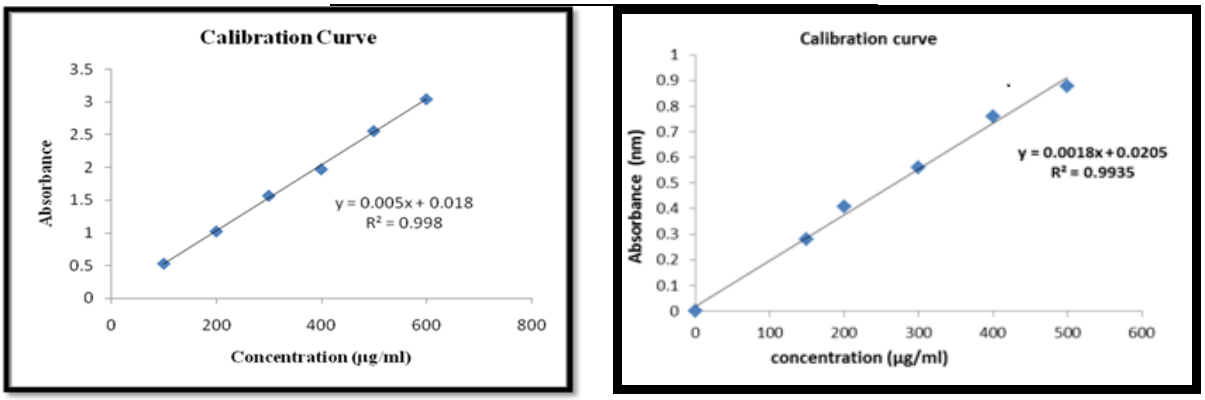

Figure 3: Standard graphs of Quercetin and Catechin

The Total Flavonoid content of extracts using quercetin as standard is observed to be more in leaf methanol extract (324.80 mg QE/gr. Ext.) when compared with the other extracts. The order of Flavonoid content of plant extracts using quercetin as the standard -
Leaf Methanol > Leaf Aq. Methanol > Stem Methanol > Stem Ethyl acetate > Leaf Ethyl acetate > Stem Aq. Methanol > Stem Hexane > Leaf Hexane

The Total Flavonoid content of *T. stans* leaf and stem extract using catechin as standard was observed to be more in leaf aq. methanol extract (127.18 mg CE/gr. Ext.) when compared with the other extracts. The Order of Flavonoid content of plant extracts using catechin as standard-
Leaf Aq. Methanol > Leaf Methanol > Stem Methanol > Stem Ethyl acetate > Leaf Ethyl acetate > Stem Hexane > Stem Aq. Methanol > Leaf Hexane

Table 5: The total flavonoidal content of Hexane, Ethyl acetate, Methanol, and 30% Aq. Methanol extracts of Tecoma stans leaf and stem in terms of mg/gm quercetin and catechin equivalents.

		Catechin		Quercetin	
Plant Part	**Extract**	**Unknown Concentration (µg/ml)**	**Flavonoid Content mg Catechin Equivalent/gr. Extract**	**Unknown Concentration (µg/ml)**	**Flavonoid Content mg Quercetin Equivalent/gr. Extract**
T. stans Leaf	Hexane	266.96	26.69	629.27	62.92
	Ethyl acetate	658.75	65.87	1895.19	189.51
	Methanol	1239.04	123.90	3248.01	324.80
	Aq. MeOH	1271.85	127.18	2710.16	271.01
T. stans Stem	Hexane	443.88	44.38	1090.05	109.05
	Ethyl acetate	694.78	69.47	2219.87	221.98
	Methanol	955.33	95.53	2411.72	241.17
	Aq. MeOH	429.08	42.90	1249.11	124.91

4. SUMMARY AND CONCLUSION

The extraction of *T. stans* leaf and stem using different solvents such as hexane, ethyl acetate, methanol, and aq. methanol was carried out. The antioxidant activity performed using DPPH method showed the stem methanolic extract as the most active one exhibiting free radical scavenging property with the percentage value of 57.22 µg/ml at 25 µg/mL concentration. The stem methanolic extract contains most effective anti-oxidant phytochemicals. Similarly, the total phenolic content was performed by Folin-Ciocalteu's assay method using Gallic acid as a standard. The leaf methanolic extract contained more amount of phenolic content (60.24 mg GAE/gr. Ext.). Likewise, the total flavonoidal content was performed by aluminium chloride colorimetric assay method using Quercetin and Catechin as the standards. The leaf methanolic extract showed a high amount of flavonoidal content in terms of quercetin equivalents (324.80 mg QE/gr. Ext.) whereas the leaf aq.methanol extract showed a high amount of flavonoid content (127.18 mg CE/ gr. Ext) in terms of catechin equivalents.

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