ORIGINAL ARTICLE

Comparative *in vitro* antioxidant activities of ethanolic extract, ethyl acetate extract (EAE), and hexane extracts (HE) of *Tecoma gaudichaudi* flowers

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Abstract

Background: Free radicals excessive production causes biological molecules direct damage to DNA, proteins, lipids, and carbohydrates and may cause the development of tumor and its progression. Phytochemically derived natural antioxidant molecules may directly inhibit the radical production or their propagation is limited or to protect the system they will be destroyed. **Objective**: In the present study, *in vitro* antioxidant activity of ethanolic extract (EE), ethyl acetate extract (EAE), and hexane extracts (HE) of *Tecoma gaudichaudi* flowers was investigated. **Materials and Methods**: *In vitro* antioxidant activity of EE, EAE, and HE of *T. gaudichaudi* flowers was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, reducing power method, phosphomolybdenum assay and hydroxyl radical scavenging assay and IC50 values were calculated. Ascorbic acid is used as standard drug. **Results:** EE, EAE, and HE of *T. gaudichaudi* exhibited a significant antioxidant status which is evident from their IC50 values 21.42, 44.77, and 71.44 ug/mL in DPPH method and 22.3, 57.27, and 95.49 ug/mL in hydroxyl radical. In reducing power method and phosphomolybdenum assay, there is concentration-dependent increase in absorbance. The results are significant when compared to the standard drug ascorbic acid. **Conclusion:** EE, EAE, and HE of *T. gaudichaudi* flowers and specifically the EE reveal several properties such as higher free radical scavenging properties, significant antioxidant capacities compared to other extracts.

Key words: 2,2-diphenyl-1-picrylhydrazyl scavenging, hydroxyl radical scavenging, phosphomolybdenum, reducing power, *Tecoma gaudichaudi*

INTRODUCTION

One or more unpaired electrons are present in free radicals due to which they are highly unstable and other molecules get damaged by extracting electrons to attain stability.[1] The body's normal metabolic process generates free radicals and these exhibit dual role in the body as both harmful and beneficial species. The tissue damage and different diseases result from reactive oxygen species (ROS) excess production and/or a decrease in antioxidant levels.[2] In protecting our body from disease by reducing the oxidative damage to cellular component caused by ROS antioxidant plays a key role.[3] Various research studies reveal that the plant origin antioxidants with free radical scavenging properties may have great therapeutic importance in free radical-mediated diseases such as diabetes, cancer, neurodegenerative disease, cardiovascular diseases, aging, gastrointestinal diseases, arthritis, and aging process. Many synthetic antioxidant compounds have shown toxic and/or mutagenic effects, while relatively plant-based medicines confer fewer side effects than the synthetic drug in some instances.[4] Oxidative stress leads to many diseases. Increased cell oxidation contributes to cardiovascular diseases, tumor growth, wrinkled skin, Alzheimer's disease, and even a decline in energy and endurance. Oxygen is an

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essential element for life to perform biological function such as catabolism of fats, proteins, and carbohydrates. However, a parallel role of oxygen as a toxic agent for living tissue has also been discovered. Oxygen is involved in the generation of the ROS. ROS interact with biomolecules and nucleic acids. That leads to an onset of degenerative diseases. "Antioxidant defense system" is highly useful from the protection against the free radicals. This "antioxidant defense system" is having the enzymes, compounds, and they remove the free radicals before they cause any of the tissue damage in body. Oxidation reactions are definitely occurring in the body; they can also be damaging the body. Multiple types of antioxidants such as Vitamin A, Vitamin E, and Vitamin C, glutathione and some enzymes such as superoxide dismutase, catalase, and various peroxidases are present in various plants and animals. Oxidation reactions produce the free radicals. Then, these radicals can start chain reactions. If the chain reactions occur in a cell, it may cause the damage or death of the cell. Antioxidants terminate the chain reactions by removing the free radicals and inhibits the oxidation reactions. The antioxidants oxidize themselves first, thus they are often reducing agents such as ascorbic acid, polyphenols, and thiols. So far, much pharmacological work has not been reported on this plant and aim of the present study to evaluate antioxidant activities of ethanolic extract (EE), ethyl acetate extract (EAE), and hexane extracts (HE) of *Tecoma gaudichaudi* flowers.

MATERIALS AND METHODS

Plant Material

T. gaudichaudi flowers were collected from Surampalem area of East Godavari district of Andhra Pradesh. The plant was authenticated by Dr. S.B. Padal, Associate Professor, Botany Department, Andhra University, and given voucher specimen number is 22204.

Preparation of Ethanolic, Ethyl acetate, and HE

Flowers of *T. gaudichaudi* were undergone shade drying at room temperature for 4–5 days. The dried flowers were then powdered in a mixture. The powder was taken and weighed. From the obtained fine powder, 300 g (each 100 g) powder was taken and it is macerated in 200 mL of ethanol for 3 days, ethyl acetate for 7 days, and hexane for 7 days. Then, hot percolation for 3 h. Then filtered and then extraction was concentrated through distillation. Selected solvents are non-toxic effect(s) or minor interfering efforts to the living cells, animals, and human beings. Selection of ethanol, ethyl acetate, and hexane as a solvent would extract high hydrophilic, moderate hydrophilic, and high lipophilic secondary metabolites from the plants, respectively. Individual extracts were collected and filtered. The filtrate of ethanol, ethyl acetate, and HE was concentrated through distillation individually. After distillation, crude extracts were collected individually and obtained extracts were weighed. The physical characteristics and percentage yield of various extracts were reported. The crude extracts were placed in desiccator for further studies. Output of extract, percentage yield, and physical characteristics was mentioned in the results.

Preliminary Phytochemical Testing

Preliminary phytochemical screening of *T. gaudichaudi* flower extracts was done to test the presence of the active chemical constituents such as alkaloids, flavonoids, tannins, phenolic compounds, saponins, fixed oils, and fats.[5]

In vitro Antioxidant Activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

In this method, 0.1 mM DPPH solution in methanol was prepared, and this solution (of 1 mL) was added into extracts (of 3 mL) solutions in methanol of various concentrations (50, 100, 300, and 500 µg/mL). After 30 min, the absorbance was measured at 517 nm. A blank was prepared without adding the extracts. Various concentrations of ascorbic acid (50, 100, 300, and 500 µg/mL) were used as the standard. The experiment was repeated in triplicate. Higher free radical scavenging activity is indicated by its low absorbance of scavenging the DPPH radical.[6,7]

Scavenging ability on DPPH radicals (%) = $[(A_o–A_1)/A_o] \times 100$

Where, A_o is the absorbance of the control reaction (containing all reagents except the sample extract), and A_1 is the absorbance of the sample extract. Ascorbic acid was used as positive controls.

Hydroxyl Radical Scavenging Assay

The scavenging ability of the five sample extracts on hydroxyl radicals was determined according to the method described by Smirnoff and Cumbes[8] with some modifications. Briefly, individual sample extracts (1 mL) at different concentrations (50, 100, 300, and 500 µg/mL) were added to the reagent containing 1 mL 1.5 mM FeSO4, 0.7 mL 6 mM H_2O_2, and 0.3 mL 20 mM sodium salicylate. After incubation for 1 h at 37°C, absorbance of the reaction mixture was read at 562 nm. The scavenging ability on hydroxyl radicals was calculated using the following equation:

Scavenging ability on hydroxyl radicals (%) $= [(A_o–A_1)/A_o] \times 100$

Where, A_o is the absorbance of the control reaction (containing all reagents except the sample extract), and A_1 is the absorbance of the sample extract. Ascorbic acid was used as positive controls.

Phosphomolybdenum Antioxidant Assay

The antioxidant activity of the extracts of *T. gaudichaudi* flower extracts was evaluated by the phosphomolybdenum method according to the procedure.[9] The assay is based on the reduction of Mo (VI)–Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. 0.3 mL of extracts (0.05, 0.1, 0.3, and 0.5 mg/mL) was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. The solution absorbance using spectrophotometer against blank after cooling to room temperature at 695 nm was measured.

Reducing Power Method

Different concentration of ethanolic, ethyl acetate, and HE of *T. gaudichaudi* (0.05, 0.1, 0.3, and 0.5 mg/mL) extracts in 1 mL of distilled water was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K 3 Fe(CN) 6] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. Trichloroacetic acid (10%) of 2.5 mL was added to the above mixture, which was later centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and FeCl 3 (0.5 mL, 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid was used as the reference material. All the tests were performed in triplicate and the results averaged. Increased absorbance of the reaction mixture indicates increase in reducing power.[10]

RESULTS

After extraction with different solvents such as ethanol, ethyl acetate, and hexane, the residues were dried and measured. The residue obtained was 12.1% w/w, 8.12% w/w, and 6.34% w/w for ethanol, ethyl acetate, and HE of *T. gaudichaudi* flower, respectively. The brownish-green, brownish-green, and green residues were obtained for ethanol, ethyl acetate, and HE of *T. gaudichaudi* flower, respectively. The ethanol extracts were sticky in nature. The other extracts were little gummy in nature.

The results of antioxidant activity were expressed in terms of IC50 values using different antioxidant methods. The calculated IC50 values using DPPH method for *T. gaudichaudi* are 21.42 µg/mL, 44.77 µg/mL, and 71.44 µg/ mL for ethanolic, ethyl acetate, and HE and for ascorbic acid it is 33.11 µg/mL. The IC50 values using hydroxyl radical scavenging method are 1.69 µg/mL for ascorbic acid and 22.3 µg/mL for EE, 57.27 µg/mL for EAE and 95.49 µg/mL for hexane flower extract, and the results are indicated in Table 1 and Figure 1.

Table 1: Effect of ethanolic, ethyl acetate, and hexane flower extracts of ***T. gaudichaudi*** on DPPH radical scavenging assay and hydroxyl radical scavenging assay

Tested material	Concentration (µg/mL)	DPPH method		Hydroxyl radical scavenging	
		% inhibition	IC50 (ug/mL)	% inhibition	IC50 (ug/mL)
Ethanolic extract of *T. gaudichaudi*	50	58.81±0.001	21.42	56.62±0.17	22.3
	100	73.36±0.002		76.32±0.18	
	300	85.21±0.005		88.78±0.012	
	500	90.23±0.005		89.72±0.08	
EAE of *T. gaudichaudi*	50	50.71±0.001	44.77	44.8±0.06	57.27
	100	62.22±0.005		62.76±0.16	
	300	75.41±0.004		80.52±0.04	
	500	83.74±0.007		85.36±0.15	
HE of *T. gaudichaudi*	50	43.56±0.002	71.44	38.51±0.06	95.49
	100	56.45±0.001		47.91±0.06	
	300	70.49±0.005		73.12±0.15	
	500	79.75±0.003		80.62±0.08	
Ascorbic acid	50	55.36±0.22	33.11	69.47±0.32	1.69
	100	60.32±0.27		82.55±0.26	
	300	70.85±0.13		83.46±0.12	
	500	80.32±0.12		87.65±0.16	

Values are expressed as mean±SEM; *n*=3 in each concentration, *T. gaudichaudi: Tecoma gaudichaudi*, EE: Ethanolic extract, EAE: Ethyl acetate extract, HE: Hexane extract

Table 2: Effect of ethanolic, ethyl acetate, and hexane flower extracts of ***T. gaudichaudi*** on phosphomolybdenum and reducing power antioxidant method

Tested material	Concentration (µg/mL)	Absorbance±SEM	
		Phosphomolybdenum method	Reducing power method
EE of *T. gaudichaudi*	50	0.012±0.0015	0.0083±0.00115
	100	0.027±0.0013	0.011±0.00113
	300	0.102±0.0009	0.018±0.00125
	500	0.198±0.0012	0.027±0.00163
EAE *T. gaudichaudi*	50	0.009±0.0005	0.007±0.0006
	100	0.022±0.0016	0.009±0.00121
	300	0.096±0.0011	0.015±0.00056
	500	0.113±0.0014	0.019±0.00242
HE of *T. gaudichaudi*	50	0.005±0.0003	0.005±0.00321
	100	0.019±0.0016	0.007±0.00125
	300	0.087±0.0009	0.012±0.00154
	500	0.099±0.0016	0.015±0.00143
Ascorbic acid	50	0.181±0.32	0.052±0.25
	100	0.381±0.18	0.129±0.15
	300	0.621±0.23	0.352±0.36
	500	0.973±0.36	0.386±0.12

Values are expressed as mean±SEM; *n*=3 in each concentration, *T. gaudichaudi: Tecoma gaudichaudi*, EE: Ethanolic extract, EAE: Ethyl acetate extract, HE: Hexane extract

DISCUSSION

The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability[11] and is a useful reagent for investigating the free radical scavenging activities of compounds.[12] From the results obtained, it may be postulated that *T. gaudichaudi* flower extracts reduce the radicals to the corresponding hydrazine when it reacts with the hydrogen donor in the antioxidant principles. Free radical scavenging activity of the ethanolic, ethyl acetate, and hexane flower extract of *T. gaudichaudi* is concentration dependent. Lower IC50 value reflects better protective action.

The reducing power assay measures the electron-donating ability of antioxidants using potassium ferricyanide reduction method. Antioxidants reduce the ferric ion/ferricyanide complex to the ferrous form, the Perl's Prussian blue complex.[13] The antioxidant activity of various flower extracts of *T. gaudichaudi* and ascorbic acid has been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity, and radical scavenging.[14,15] The reducing capacity of various flower extracts of *T. gaudichaudi* and ascorbic acid indicates their potential antioxidant activity. The reduction of Mo (IV)–Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 695 nm is the main principle involved in the phosphomolybdenum method. The antioxidant capacity

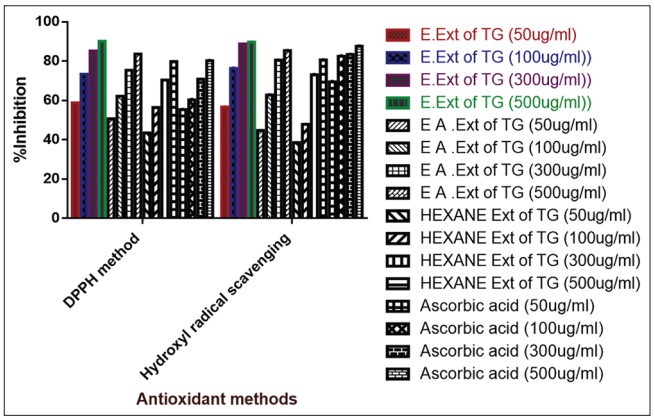

Figure 1: Effects of ethanolic, ethyl acetate, and hexane flower extracts of *Tecoma gaudichaudi* on DPPH radical scavenging assay and hydroxyl radical scavenging assay

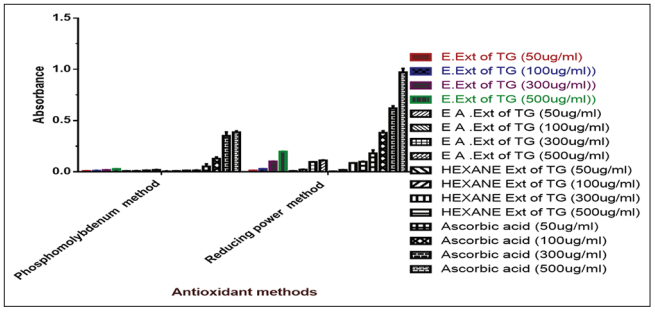

Figure 2: Effects of ethanolic, ethyl acetate, and hexane flower extracts of *Tecoma gaudichaudi* on phosphomolybdenum and reducing power antioxidant method

Table 3: Preliminary phytochemical analysis of ethanolic, ethyl acetate, and hexane flower extracts of *T. gaudichaudi*

Compounds	Tests	Results		
		EE	EAE	HE
Alkaloids	Dragendorff's	+++	-	-
	Mayer's test	+++	-	-
	Hager's test	+++	-	-
Glycosides	General test	+++	-	-
	Legal's test	+++	-	-
	Modified Borntrager's test	+++	-	-
Flavonoids	Lead acetate test	+++	++	-
	Zinc hydrochloride test	+++	++	-
	NaOH test	+++	++	-
Saponins	Froth formation test	+++	+++	+
Triterpenoids	Salkowski test	+++	++	+
	Liebermann-Burchard's test	+++	++	+
Tannins	Ferric chloride test	+++	++	-
Carbohydrates	Molisch's test	+++	+++	+
	Benedict's test	+++	+++	+
	Fehling's test	+++	+++	+
Proteins	Xanthoproteic test	+++	++	+
	Millon's test	+++	++	+
	Biuret test	+++	++	+
Phenolic compounds	Ferric cyanide test	+++	++	++
	Gelatin test	+++	++	++

+++: Highly present, ++: Moderately present, +Low, -: Absent, EE: Ethanolic extract, EAE: Ethyl acetate extract, HE: Hexane extract

of extracts was found to increase with increase in concentration [Table 2 and Figure 2].

Flavonoids, tannins,[16] alkaloids,[17] saponins,[18] triterpinoids,[19] and glycosides[20] have potential antioxidant properties. According to phytochemical studies [Table 3], all the above compounds are soluble in EE, but alkaloids and glycosides are not soluble in EAE. In HE, only saponins and triterpenoids are soluble. Hence, from the phytochemical investigation, we can say that EE may have potent antioxidant activity than the ethyl acetate and HE.

CONCLUSION

On the basis of the results obtained in the present study, it is concluded that an EE of *T. gaudichaudi* flowers, which contains large amounts of phytoconstituents (flavonoids, tannins, etc.) exhibits high scavenging and reducing power activities compared to ethyl acetate and HE. These *in vitro* assays indicate that this plant extract is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. However, the components responsible for the antioxidative activity are currently unclear. Therefore, further, investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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