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Studies on phytochemical analysis and antioxidant activity of methanolic leaf Extract *Tecomela undulata*

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Abstract

Plants are being explored as a major source of medicinal compounds. The property of being used as a source of medicine is due to the presence of phytochemicals present in them. This present study was intended to explore the various phytochemicals present in the plant *Tecomella undulata* as it is well known for its medicinal value**.** Each and every plant phytochemical possesses its own character and so its dissolving ability varies. Thus this study was conducted to screen the presence of different phyochemicals by extracting the leaf powders with different solvents. The antioxidant property has shown very effective results which paves way to bring this plant chemical compound as a product for commercial use.

Keywords: Phytochemicals, solvents, medicine, *Tecomella undulata, antioxidant*

1. Introduction

The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Duraipandiyan *et al*., 2006).The plant kingdom has proven to be the most useful in the treatment of diseases and they provide an important source of all the world's pharmaceuticals. The most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. Plants in all facet of life have served as a valuable starting material for drug development. Antibiotics or antimicrobial substances like saponins,

glycosides, flavonoids, alkaloids, etc., are found to be distributed in plants, yet these compounds are not well established due to the lack of knowledge and techniques (Hafiza *et al.*, 2002). The phytoconstituents which are phenols, anthraquinones, alkaloids, glycosides, flavonoids and saponins are considered to be the antibiotic principles in plants.

Reactive oxygen species (ROS) including superoxide radical, hydroxyl radical, singlet oxygen and hydrogen peroxide are often generated as by-products of biological reactions or form exogenous factors and molecules are responsible for cellular injury and aging processes (Wu *et al*., 2008). Oxidative stress is mediated by reactive oxygen species (ROS) which are generated during the normal and aberrant cellular metabolism that utilizes molecular oxygen. The imbalance between production of ROS like O^{2-} , H_2O_2,OH^-, ROO^- and the capacity of the normal detoxification systems in favour of the oxidants leads to oxidative stress, which itself leads to cellular damage caused by the interaction of ROS with cellular constituents. Oxidative stress is involved in many acute and chronic diseases including cancer, cardiovascular troubles and neurodegenerative diseases. The balance between antioxidation and oxidation is believed to be critical in maintaining a healthy biological system (Hong and Liu, 2004; Judge *et al*., 2005; Katalinic *et al*., 2006; Montuschi *et al*., 2004).

2. Materials and Methods

Phytochemical Screening

The presence of different phytochemicals extracted in different solvents was confirmed by the following tests.

Collection of Plant Material

Fresh plants were collected from a place near Coimbatore, Tamilnadu, India. The leaves were separated, washed under running tap water and shade dried at room temperature. The dried leaves were ground to fine powder using a blender. The powder was preserved in an air tight bottle for further use.

Preparation of leaf extracts

10 g of the leaf powder was mixed with 100ml of solvent and kept shaking on a shaker at 10°C overnight at 200rpm. Different solvents used for extraction were, Methanol, The extracts kept overnight were centrifuged at 8000 rpm for 6 minutes and the supernatant was transfered to fresh vials. The collected supernatants were used for phytochemical screening.

Qualitative analysis of phytochemicals

Test for carbohydrates

Molishs test

To 2mL of extract 2 drops of molish's reagent was added and shaken well. 2mL of conc. H_2SO_4 was added on the sides of the tubes. A reddish violet ring appeared at the junction of two layers immediately indicating the presence of carbohydrates (Santhi *et al*., 2011).

Test for Tannins

5mL of extract was added to few drops of 1% lead acetate. A yellow precipitate indicated the presence of tannins (Thenmozhi *et al*., 2011).

Test for steroids (Khan *et al*., 2010)

Leaf extract was mixed with 1mL chloroform and later 2-3 drops of conc. H_2SO_4 was added. Pink or red colour formation indicated the presence of steroids (Khan *et al*., 2010).

Test for Terpenoids

Libbermann-Burchard reaction

To 200mg of plant material 10mL of chloroform were added and filtered using whattman filter paper no: 1 then 2mL acetic anhydride was added and a 3drops of concentrated H_2SO_4 along the sides of the tubes. Blue, green ring indicates the presence of steroids (Siddiqui *et al.*, 2009).

Triterpenoids detection

(Siddiqui *et al.*, 2009).In a test tube 2 (or) 3 granules of tin is added & dissolved in 2ml of thionide solution. Add test solution to it. Pink colour produced which indicates the presence of Triterpenoids

Test for Alkaloids

Mayers Test

To the extract added 1% Hydrochloric acid and 6 drops of Mayer's reagent (1.36g of mercury chloride, 5g of potassium iodide in 100ml of water) and Drangendroff's reagent was added. An organic precipitate indicated the presence of alkaloids in the sample (Santhi *et al*., 2011).

Detection of Flavanoids

Lead acetate test

The aqueous extract was treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavanoids (Beknal *et al.*, 2010).

Test for cardiac glycosides (Keller-Killiani test)

0.5g of extract diluted to 5mL of water then added 2mL of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1mL of concentrated sulphuric acid. A brown ring at the interface indicates the

presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer (Santhi *et al*., 2011).

Antioxidant Studies

DPPH free radical scavenging activity

Radical scavenging activity was determined according to Blois (1958). Different concentrations (300, 400, 500, 600 and 700 µg/mL) of test sample was dissolved in DMSO and mixed individually with 0.1 mM DPPH and 50 mM Tris-HCl buffer (pH 7.4). Reaction mixture was incubated at 37 ºC for 30 min and then absorbance was measured at 517 nm. The percentage of DPPH free radical scavenging activity was calculated using the following equation:

% Inhibition = $[(A_B - A_A)/A_B] \times 100$,

Where A_B, absorption of blank sample, A_A, absorption of test sample.

Metal chelating activity

Chelation of ferrous ions by methanolic extract was estimated by method of Dinis *et al*., (1994). Briefly, 2 mM $FeCl_2$ was added to different concentrations (500, 650, 700, 850 and 900 µg/mL) of test sample. Further, reaction was initiated by the addition of 5 mM ferrozine. The mixture was vigorously shaken and left to stand at room temperature for 10 min. Absorbance was measured at 562 nm after 10 min.

% Inhibition = $[(A_B - A_A)/A_B] \times 100$,

Where A_B, absorption of blank sample, A_A, absorption of test sample.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of the test compound was measured by the method of Zhao *et al*. (2006). Reaction mixture contained 0.5 ml of 7.5 mM $FeSO_4$, 0.5 ml of 7.5 mM 1, 10-phenanthroline, 2.5 ml of 0.2 M phosphate buffer (pH7.8), 0.5 ml of 30 mM H_2O_2 and test sample at different concentrations (50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 µg/ml). The reaction was started by adding H_2O_2. After incubation at room temperature for 5 min, the absorbance of the mixture at 536 nm was measured.

% Inhibition = $[(A_B - A_A)/A_B] \times 100$,

Where A_B, absorption of blank sample, A_A, absorption of test sample.

Reducing power assay

The reducing power of the test compound was determined by the method of Yildrim *et al*., (2001). Different concentration (200, 250, 300, 350, and 400µg/mL) of the test compound was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide. The reaction mixture was incubated at 50°C for 30 min. 2.5mL of 10% trichloroacetic acid (TCA) was added to the above mixture and centrifuged for 10 min at 3000 rpm. 2.5 mL of supernatant solution was mixed with 2.5 mL distilled water and 0.5 mL of 0.1% ferric chloride. Absorbance was measured at 700 nm.

3. Results and Discussion

The phytochemical analysis of methanolic leaf extract of *Tecomella undulata* was analysed (Table-1) for the compounds such as Alkaloids, Flavanoids, and Cardiac glycosides, Saponins, Steroids and Tannins. The preliminary phytochemical analysis revealed the presence of five compounds i.e. Alkaloids, Flavonoids, Saponins, Steroids, Tannins and absence of Cardiac glycosides. Various tests have been performed to find out the phytochemical constituents mentioned above.

Table 1: The Preliminary phytochemical analysis of methanolic leaf extract of *Tecomella undulates*.

Name of the Compound	Observation
Alkaloids	+
Flavonoids	+
Cardiac glycosides	-
Saponins	+
Steroids	+
Tannins	+

+ Present, - Absent

Antioxidant activity

The presence of various compounds may be the result for the potential activity of antioxidant property of *Tecomella undulata*. This has been reported by many workers and the activity is commonly found among the members of Bignoniaceae.

DPPH Radical Scavenging Activity

The DPPH radical scavenging effect was studied and the results are presented in the (**Table-2**.) The IC_{50} value for DPPH radical scavenging of the extract was found to be 226.55µg/ml. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. It is visually noticeable as a

discoloration from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants (Duh *et al*., 1999).

Table 2: The DPPH free radical scavenging activity of methanolic leaf extract of *Tecomella undulata*

Concentration (µg /µl)	Percentage of inhibition	IC_{50} (µg/mL)
50	56.3 ± 0.76	226.55
100	58.4 ± 1.65	
150	60.5 ± 1.38	
200	69.0 ± 3.01	
250	71.8 ± 2.98	
300	73.9 ± 1.21	
350	78.1 ± 0.62	
400	80.2 ± 0.51	
450	83.0 ± 2.01	
500	88.0 ± 1.01	

Metal chelating activity

It has been reported that the chelating agents, which forms σ bonds with the metal are effective as secondary antioxidants, because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Gordon, 1990). The *Tecomella undulata* was analysed for the metal chelation property. The concentration of the extract varied from 50 to 500 µg/ml. The chelation capacity of the extract was found to be increasing with the increase in extract concentration. The IC_{50} value was found to be 282.48µg/ml. The values obtained are tabulated in the Table 3. The results of our study demonstrate that our extract have an effective capacity for iron binding, suggesting their antioxidant potential.

Table 3: The Metal chelating activity of methanolic leaf extract of *Tecomella undulata*

Concentration (µg /µl)	Percentage of inhibition	IC_{50} (µg/mL)
50	41.6 ± 2.10	282.48
100	47.5 ± 1.63	
150	48.3 ± 0.21	
200	51.6 ± 0.74	
250	55.0 ± 3.01	
300	58.3 ± 0.40	
350	60.0 ± 0.26	
400	63.3 ± 3.60	
450	68.3 ± 1.29	
500	75.8 ± 2.63	

Reducing Power of *Tecomella undulata*

The reducing power assay was carried out with *Tecomella undulata* at the concentration range of 50µg/mL to 500µg/mL. The results obtained from the study are presented in the(**Table-4**.) It is observed that with the increase in concentration the absorbance also increases. The IC_{50} value obtained for reducing power 208.33 µg/ml. The reducing power property indicates that the phytochemical compounds are electron donors and can reduce the oxidized intermediates of lipid per oxidation processes, so that they can act as primary and secondary antioxidants.

Table 4: The Reducing power activity of methanolic leaf extract of *Tecomella undulate*

Concentration (µg /µl)	Percentage of inhibition	IC_{50} (µg/mL)
50	0.32 ± 0.060	208.33
100	0.42 ± 0.012	
150	0.59 ± 0.003	
200	0.69 ± 0.020	
250	0.72 ± 0.001	
300	0.80 ± 0.061	
350	0.91 ± 0.046	
400	0.94 ± 0.002	
450	0.98 ± 0.004	
500	0.99 ± 0.030	

Hydroxyl Radical scavenging activity

The hydroxyl radical scavenging activity of the extract was analysed and the results are presented in **Table-5.** The IC_{50} concentration is considered at which 50% of hydroxyl radicals are scavenged. The IC_{50} value obtained for hydroxyl radicals 355.61 µg/ml. The hydroxyl radical is one of the most toxic ROS, with the ability to damage biomolecules, the results indicate that *Tecomella undulata* has potent antioxidant property by curbing the hydroxyl radicals and thereby protecting DNA, lipids and proteins.

Table 5: The Hydroxyl radical scavenging activity of methanolic leaf extract of *Tecomella undulate***.**

Concentration (µg /µl)	Percentage of inhibition	IC_{50} (µg/mL)
50	18.0 ± 0.60	355.61
100	20.4 ± 1.07	
150	23.7 ± 1.21	
200	28.6 ± 0.63	
250	33.6 ± 0.51	
300	36.0 ± 0.74	
350	44.2 ± 3.01	
400	46.7 ± 1.5	
450	62.2 ± 2.4	
500	83.6 ± 0.61	

4. Conclusion

The phytochemical analysis of the leaf extract showed higher concentration of a phenolic compound followed by a furan and a furanosyl compound. Many other compounds such as stigmasterol, pyranol, etc., were also present in observable levels but lesser in concentration when comparing with the above said. This is evident that phenolic compound might be the compound having potential bioactivity.The antioxidant property has shown very effective results which paves way to bring this plant chemical compound as a product for commercial use.

5. References

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