Prilimnary Phytochemical Analysis and Pharmacognostical Studies on the Leaf Extracts of *Tecomella Undulata* (Sm.) Seem.

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ABSTRACT

The leaves of Tecomella undulata (Sm.) Seem., a members of Bignoniaceae family is reported to have good medicinal values in the traditional system of medicine for healing various diseases. The present study deals with pharmacognostical examination include the morphological characters and physical constants of Tecomell undulata leaves including determination of loss on drying, ash values and extractive values. The preliminary phytochemical screening of various leaf extracts like petroleum ether, methanol, chloroform, acetone, ethyl acetate and water was also carried out and it is revealed the presence of various phytoconstituents like alkaloids, anthroquinone, glycosides, carbohydrates, flavonoids, protein and amino acids, tannins, phytosterols and saponins. The determination of these characters will aid in future for pharmacological analysis of this plant species.

Keywords: *Tecomella undulata*, pharmacognostic, Physicochemical parameters, extracts phytochemical screening.

INTRODUCTION

Tecomella undulata (Sm.) Seem. is commonly known as Rohida is an important medicinal shrub or tree belongs to the family *Bignoniaceae,* is one of the co-dominant species of Drier parts of India in the desert of western Rajasthan and Gujarat. It is an important agro-forestry, deciduous or nearly evergreen tree. It has been used since ancient times for the treatment of human ailments. It is reported to have important properties like anticancer activity, hepatoprotective activity (Khatri *et al*., 2009), analgesic activity (Ahmed F *et al.,* 1994), antibacterial activity (Parekh *et al.,* 2005), mild relaxant, cardiotonic and chloretic activities etc. The bark obtained from the stem is contains certain secondary metabolites like tecomin, alkenes, alkanols, β-sitosterols, ester glycosides,(Pandey *et al.,* 1970),chromone glycosides (Gujral *et al.,* 1979), undulatoside, A and B, iridoid glucosides (Verma *et al*., 1986) , tecomelloside, tecoside, lapachol, veratric acid (Rastogi RP and Mehrotra BN, 2006; Joshi C, 1972; Nadkarni KM and Nadkarni AK, 2006) and is employed for the treatment of various diseases of skin, central nerves system, urinary disorders, enlargement of spleen, gonorrhoea, leucoderma, liver diseases, jaundice, diabetes, cancer and swellings. Seeds are used against abscess.

Leaves shows significant antimicrobial activity and contains certain chemical constituents like triacontanol, betulinic acid, oleanolic acid and ursolic acid. Triacontanol is an effective plant growth regulator while both betulinic acid and ursolic acid is potent antihuman immunodeficiency virus

(HIV) and are used in treatment of AIDS (Azam, M. M., 1999). The plant constituted reputed drug of Ayurveda and its multiple uses have also been made to avoid their excessive exploitation for their in situ conservation.The present study deals with the pharmacognostical and phytochemical screening of *Tecomella undulata* (Sm.) Seem., leaves.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

The leaves of *Tecomella undulata* (Sm.) Seem were collected from Balaram sanctuary of Banaskhantha District Gujarat (India) in the month of March 2011. The plant material were identified and authenticated by a taxonomist Dr. R.S. Patel senior scale lecturer of Maninagar science college Ahmadabad.

Drying of plant material

The leaves of *Tecomell undulata* (Sm.) Seem were first washed with water and subjected to shade drying for about 15 weeks. The shade dried leaf material was further milled into coarse powder using a mechanical grinder and stored in air tight container for further analysis.

Determination of physicochemical parameters: (Indian Pharmacopia, 1996;

Khandelwal KR *et al*., 1996 and Kokate CK , 2008)

The dried leaf material was subjected for determination of physicochemical parameters such as total ash value, acid insoluble ash value, water soluble ash value, loss on drying and extractive values like petroleum ether soluble extractive, methanol soluble extractive, chloroform soluble extractive, acetone soluble extractive, ethyl acetate soluble extractive and water soluble extractive values were calculated as per the Indian pharmacopoeia.

Ash Values

Total ash: 2g of accurately weighed dried leaf powdered of *T. undulata* was taken in a tarred silica crucible and incinerated at a temperature not exceeding 450^0C until free from carbon, cooled and weighed. Calculate the percentage of total ash with reference to dried leaf powder.

Acid-insoluble ash: Boiled the ash with 25 ml of dilute Hydrochloric acid for five minutes. The insoluble matter was collected on an ash-less filter paper, washed with hot water and ignited, cooled and weighed. The percentage of acid insoluble ash was calculated with reference to dried leaf powder.

Water-soluble ash : Boiled the for 5 minutes with 25ml of distilled water, collect the insoluble matter on an ash-less filter paper, washed with hot water and ignited for 15 minutes at temperature not exceeding 450^0C. Subtracted the weight of the insoluble ash. The percentage of water-soluble ash was calculated with reference to dried powder.

Loss on Drying

An accurately weighed quantity of the dried leaf powder of *Tabernaemontana undulata*. was taken in a tarred glass bottle and the initial weight was taken. The crude drug was heated at 105^0C in an oven and weighed. This procedure was repeated utill a constant weight was obtained. The moisture content of the sample was calculated as percentage with reference to the dried material.

Extractive Values

Organic solvent soluble extractive value: The dried leaf powder of *Tabernaemontana undulata* were subjected to sequential soxhlet extraction using the solvents of different polarity such as petroleum ether, methanol, chloroform, acetone and ethyl acetate for 48 hrs. The extracts were filtered individually, evaporated to dryness and the percent yields of all the extracts were determined.

Water soluble extractive value: 5g of Powder was macerated with 100 ml of water in a closed flask, shaking frequently during the first 6hrs and allowed to stand for 18hrs and filtered. Evaporated 25ml of filtrate to dryness in a tarred flat bottom shallow dish dried at 105^0C and weighed. Percentages of extractive values were calculated with reference to the dried powder.

Pharmacognostic studies: (Khandelwal KR *et al*., 1996)

Macroscopy: The following macroscopic characters for the fresh leaves were noted: size and shape, colour, venation, the apex, margin.

Powder analysis: powder of leaf of *T. undulata* was examined for colour, odour and taste. Powder of drug treated with various chemical reagents and then observed for change in colour.

Phytochemical investigation: (Kokate CK *et al*., 2008 and 2009)

Preparation of extracts: 20 g of powder leaf material was extracted with petroleum ether, methanol, chloroform, acetone, ethyl acetate and water successively in a soxhlel extractor.

Preliminary phytochemical analysis : It involves testing of all extracts of leaf powder for their contents of different classes of compounds. The methods used for detection of various phytochemicals were followed by qualitative chemical test to give general idea regarding the nature of constituents present in crude drug. The qualitative chemical tests for various phytoconstituents were carried out for all the extracts of *T. undulata* as explained below.

A.Test for Alkaloids (Harborne JB, 1998; Evans WC, 2002)

1.Mayer's test

Alkaloids give cream color precipitate with Mayer's reagent [Potassium mercuric iodide solution].

2.Dragendorff's test

Alkaloids give reddish brown precipitate with Dragendorff's reagent [Potassium bismuth iodide solution].

3.Wagner's test

Alkaloids give a reddish brown precipitate with Wagner's reagent [Solution of iodine in potassium iodide].

4.Hager's test

Alkaloids give yellow color precipitate with Hager's reagent [saturated solution of Picric acid].

B. Test for Glycosides 1.Raymond's test

Test solution when treated with dinitro- benzene in hot methanolic alkali, gives violet color. **2.Legal's test**

Treat the extract with pyridine and add alkaline sodium nitroprusside solution, blood red color appears. **3. Baljet's test**

Test solution when treated with sodium picrate formation of yellow to orange colour reveal the presence of glycosides.

4.Modified Borntrager's test

The extracts were treated with ferric chloride solution and heated on boiling water bath for about 5 mins. The mixture was cooled and shaken with volume of benzene. The benzene layer was separated and treated with half of its volume of ammonia solution. The formation of cherry red colour in the ammonical layer indicated the presence of anthranol glycosides.

c.Test for Cardiac Glycosides 1.Kedde's test

Extract the drug with chloroform, evaporate to dryness, add one drop of 90% alcohol and 2 drops of Kedde's reagent. Make alkaline with 20% sodium hydroxide solution. A purple color is produced.

2.Keller killiani test [test for Deoxy sugars]

Extract the drug with chloroform and evaporate it to dryness. Add 0.4ml of glacial acetic acid containing a trace amount of ferric chloride. Transfer to a small test tube; add carefully 0.5ml of concentrated sulphuric acid by the side of the test tube, blue color appears in the acetic acid layer.

D.Test for Saponin 1.Froth Test

Place 1ml solution of drug in water in a semi-micro tube and shaken well and noted for a stable froth.

2.Hemolysis test

Add 0.2ml solution of saponin (prepared in 1% normal saline) to 0.2ml of v/v blood in normal saline and mix well,centrifuge and note the red supernatant compare with control tube containing 0.2ml of 10% blood in normal saline diluted with 0.2ml of normal saline.

E. Test for Tannins & Phenolic Compounds 1.Gelatin test

Test solution with 1 % gelatin solution containing 10% sodium Chloride gives white precipitate.

2.Ferric chloride test

Test solution gives blue green color with ferric chloride.

3.Vanillin Hydrochloride test

Test solution when treated with few drops of vanillin hydrochloride reagent gives purplish red color. **4.Alkaline reagent test**

Test solution with sodium hydroxide solution gives yellow to red precipitate within short time.

F. Test for Flavonoids 1.Shinoda test (Magnesium Hydrochloride reduction test)

To the test Solution, add few fragments of Magnesium ribbon and add concentrated Hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes.

2.Zinc Hydrochloride reduction test

To the test solution add a mixture of Zinc dust and conc. Hydrochloric acid. It gives red color after few minutes. **3.Alkaline reagent test**

To the test solution add few drops of sodium hydroxide solution; formation of an intense yellow color, which turns to Colorless on addition of few drops of dil. acid, indicates presence of Flavonoids.

G. Test for Anthroquinone

The powder drug was heated in water containing 10% $FeCl_3$ solution and 1ml of conc. HCl. The aqueous layerwas separated and shaken with diethyl ether. The ether extract was further with strong ammonia. Pink or deep red colouration of aqueous layer indicated the presence of antroquinone. **H. Test for Proteins & Amino Acids**

1.Millons test

Test solution with 2ml of Millons reagent , white precipitate appears, which turns red upon gentle heating.

2. Xanthprotic test

Test solution treated with conc HNO_3 and boil yellow precipitate is formed. After cooling it, add 40% NaOH solution orange colour is formed indicates presence of protein.

3.Biuret test

Take 1ml of 40% NaOH solution and 2 drops of 1% $CuSO_4$ solution till a blue colour is produced , and then add to 1ml of test solution, formation of pinkish or purple violet colour indicates the presence of protein.

4.Ninhydrin test

The test solution boiled with 0.2% solution of Ninhydrin, purpul colour appears indicates presence of amino acid.

I. Test for Sterols & Triterpenoids 1.Libermann- Buchard test

Extract is treated with few drops of acetic anhydride, boil and cool, con. Sulfuric acid is added from the sides of the test tube, shows a brown ring at the junction of two layers and the upper layer turns green which shows the presence of Steroids and formation of deep red color indicates the presence of triterpenoids.

2.Salkowski test

Treat extract in Chloroform with few drops of cone. Sulfuric acid, shake well and allow standing for some time, red color appears at the lower layer indicates the presence of Steroids and formation of yellow colored lower layer indicates the presence of Triterpenoids.

J. Test for Carbohydrates 1.Molisch's test

Treat the test solution with few drops of alcoholic alpha napthol. Add 0.2ml of con. Sulfuric acid slowly through the sides of the test tube, a purple to violet color ring appears at the junction.

2.Benedict's test

Treat the test solution with few drops of Benedict's reagent and heated on boiling water bath, reddish brown precipitate forms if reducing sugars are present.

3.Fehling's test

Equal volume of Fehling's A and Fehling's B reagents are mixed and few drops of sample is added and Boiled, a brick red precipitate of cuprous oxide forms, if reducing sugars are present.

4.Barfoed's test

Treat the test solution with few drops of Barfoed's reagent heated on boiling water bath,red precipitate indicates the presence of sugar.

RESULT AND DISCUSSION

Morphological studies

The macroscopical examination of the leaves is presented in Table 1.

Physicochemical parameters

Different physicochemical parameters for the purpose of standardization such as loss on drying, ash value (total ash, water soluble ash, acid insoluble ash)and extractive values (petroleum ether, methanol, chloroform, acetone, ethyl acetate and water)for leaves of *T.*

undulata were determined and given in Table 2.

Powder Analysis

Treatment of Leaf powder of *T. undulata* with different chemical reagents has revealed that the presence of different phytocontituents are tabulated in Table 3.

Preliminary phytochemical screening

The extracts were subjected to preliminary phytochemical analysis to determine the presence of various phytoconstituents and results are tabulated in Table 4.

CONCLUSION

In the present study, the pharmacognostical investigations are helpful for the future identification and authentification of the plant in the herbal industry. The physical parameters will be useful to identify and authenticity of the drug even from the powder material and it can also serve as an important source of information to determine the quality and purity of the plant material in the future. The qualitative phytochemical screening is an essential step towards discovery of new drugs.

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Table-1 macroscopical examination of leaves of *tecomella undulata*

Sr.No.	Observation	Leaves
1	Size	Lengh 5-12cm, Width 1-3cm
2	Coluor Outer surface Inner surface	 Dark green Light green
3	Apex	Obtuse
4	Margins	Undulate
5	Venetion	Reticulate
6	Taste	Tasteless
7	Odour	Odourless

Table-2 physical evaluation of powdered drug of leaves of *tecomella undulata*

Sr.No.	Parametrs	Values (%)w/w
1	**Ash Values**	
	Total Ash	14.3
	Water soluble Ash	6.5
	Acid insoluble Ash	9.2
2	**Extractive Values**	
	Peroleum Ether Extractive	48.48
	Methanol Extractive	40.42
	Chloroform Extractive	42.54
	Acetone Extractive	49.75
	Ethyl Acetate Extractive	52.20
	Water Extractive	23.56
3.	**Loss on Drying**	8.5

Table-3 powdered drug analysis of leaves of *tecomella undulata* with different chemical reagents

Sr. No.	Treatment	Colour Observed
1	Powder such as	Green
2	Powder + Conc. H2SO4	Dark Brown
3	Powder + Conc. HNO3	Dark Green
4	Powder + Conc. HCl	Orange Yellow
5	Powder + Glacial acetic acid	Yellow
6	Powder + 1N NaOH in water	Green
7	Powder + NaOH in Methanol	Yellowish Green
8	Powder + 5% Ferric chloride	Greenish Yellow
9	Powder + 1N KOH	Light Brown
10	Powder + Picric acid	Yellow
11	Powder + Ammonia solution	Yellowish Green
12	Powder + HNO3 +Ammonia solution	Yellow

Table-4 qualitative phytochemical tests of extracts of leaves of *tecomella undulata*

Sr. No.	Name of the Test	P. E. extracts	MET. extracts	CHL. extracts	ACE. extracts	E. A. extracts	Water extracts
1	**Test for Alkaloids**						
A.	Dragendroff's Test	+	-	+	-	-	-
B.	Mayer's Tes	-	+	+	-	-	-
C.	Wagner's Test	+	+	+	-	-	-
D.	Hager's Test	-	-	-	-	-	-
2	**Test for Glycosides**						
A.	Legal's Test	+	-	+	+	+	-
B.	Baljet's Test	+	+	-	-	+	+
C.	Raymond's Test	-	-	-	-	-	-
D.	Modified Borntrager's test	+	-	+	-	+	+
4	**Test for Cardiac Glycosides**						
A.	Kedd's Test	-	+	-	-	+	-
B.	Keller-Killiani Test	+	+	-	-	+	-
5	**Test for Saponins**						
A.	Forth Test	-	+	+	-	-	-
B.	Hemolytic Test	-	+	+	+	-	-

6	**Test for Flavanoids**						
A.	Shinoda Test	-	+	-	+	-	-
B.	Zn-HCl Test	-	-	+	-	-	-
C.	Alkaline reagent Test	+	-	+	+	+	-
D.	Lead acetate test	+	+	-	+	-	-
E.	Ammonia Test	+	-	-	+	+	+
7	**Test for Tannins**						
A.	Gelatin Test	-	-	+	-	-	-
B.	Ferric Chloride Test	-	-	+	-	+	-
C.	Vanillin-HCl Test	+	-	+	-	-	+
D.	Lead acetate Test	+	+	+	-	+	+
8	**Test for Sterols**						
A.	Libermann- Buchard test	+	+	+	-	-	+
B.	Salkowaski Test	+	+	+	-	-	-
C.	Sulphur Test	-	+	+	+	-	+
9	**Test for Anthroquinone**	+	+	+	+	+	+
10	**Test for Triterpinoids**						
A.	Libermann- Buchard test	-	-	-	+	+	-
B.	Salkowaski Test	-	+	-	-	-	+
11	**Test for Proteins**						
A.	Xanthoproteic Test	-	-	-	+	+	+
B.	Millon's Test	-	+	-	-	-	-
C.	Biuret Test	+	-	+	-	-	-
D.	Ninhydrin Test	+	-	+	-	+	+
12	**Test for Charbohydrates**						
A.	Molisch's Test	-	+	+	-	-	+
B.	Barfoed's Test	-	+	-	-	-	-
C.	Benedict's Test	-	+	-	-	-	-
D.	Fehling's Test	-	-	-	-	-	-
E.	Phloroglucinol Test	+	-	-	-	-	-

(+) indicates presence (-) indicates absence

(P.E.- Petroleum ether, MET- Methanol, CHL- Chloroform, ACE- Acetone, E.A.- Ethyl acetae)

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