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**Research Article**

**Physicochemical and Phytochemical Investigation of  
Stem Bark of *Terminalia tomentosa* roxb (ex dc)  
Wight and Arn.**

**AB Joshi\*, M Bhohe and A Babu**

Department of Pharmacognosy, Goa College of Pharmacy, Panaji-Goa 403001, India.

**ABSTRACT**

The present study was undertaken for the development of physicochemical & phytochemical parameters of stem bark of *Terminalia tomentosa* Roxb (ex DC) Wight & Arn., belonging to family Combretaceae. The plant is known in Sanskrit as Asana, in Hindi as Asan, Saj, Sain and in Marathi as Ain. The physicochemical & phytochemical investigation confirms the purity & authenticity of *T.tomentosa* stem bark using standard methods. The physicochemical study revealed the presence of moisture content as 12.5% w/w, total ash as 19.95% w/w, acid insoluble ash as 16.35% w/w, water soluble ash as 0.9% w/w, alcohol soluble extractive as 1% w/w, water soluble extractive as 0.8% w/w, ether soluble extractive as 0.2% w/w, foaming index as less than 100 & swelling index as 1.14 cm. The fluorescence analysis in short wavelength, long wavelength & day light is also reported, which is a tool to determine the chemical nature of crude drug. Preliminary phytochemical screening of the ethanolic extract of the stem bark revealed the presence of carbohydrates, flavonoids, triterpenoids, steroids, tannins & saponins. All these methods will help in setting down pharmacopoeial standards in determining the quality & purity of the *T.tomentosa* stem bark.

**Keywords:** *Terminalia tomentosa*, Combretaceae, Fluorescence, Physicochemical.

**INTRODUCTION**

*Terminalia tomentosa* Roxb (ex DC) Wight & Arn. (Synonyms: *Terminalia alata* Heyne ex.Roth, *Terminalia crenulata* Roth, *Terminalia elliptica* Willd.) belonging to family Combretaceae<sup>1,2,3</sup>. *T. tomentosa* is a large deciduous tree, 20-35m high & 1m in diameter<sup>4</sup>. The bark is rough, dark grey to black in colour with deep vertical fissures & transverse cracks<sup>5</sup>. Leaves are simple, sub-opposite or the uppermost alternate, thick coriaceous, ovate-oblong or elliptic-oblong, rarely obovate, softly tomentose when young ;becoming more or less glabrous when mature, with 1-2 glands ( which are often turbinate or long stalked ) usually on the midrib but sometimes absent. Flowers are hermaphrodite and in axillary fulvous-pubescent spikes or terminal panicles. Fruits are 1<sup>1</sup>/<sub>2</sub> -2 inches long and ¾ inch wide with 5 broad, coriaceous, brown, glabrous wings striated with numerous straight lines running horizontally from the axis to the edges<sup>6</sup>. The plant is common in the forests, especially in the humid regions of India, including

the sub-Himalayan tracts of North West provinces, Nepal & Sikkim, also Southwards throughout the Peninsula<sup>7</sup>. It is a prominent part of both dry and moist deciduous forests in southern India up to 1000 m. The bark is bitter & stypic, useful in vitiated conditions of pitta, ulcers, vata, fractures, haemorrhages, bronchitis cardiopathy, strangury, wounds, haemoptysis, dysentery, cough, verminosis ,leucorrhoea, gonorrhoea & burning sensation (Ayurveda)<sup>8,9</sup>. Phytoconstituents such as tannins like arjunic acid, arjunolic acid, arjunetin, ellagic acid, gallic acid, triterpenoids like oleanolic acid, betulinic acid and steroid like β-sitosterol have been reported to be present in *T.tomentosa*<sup>10,11,12,13</sup>. The plant is known to possess many pharmacological properties like antifungal<sup>14,15,16</sup>, antioxidant<sup>17</sup>, anti-hyperglycaemic<sup>18</sup>, anti-diarrhoeal, anti leucorrhoeal<sup>19</sup>. From the literature survey, it is learnt that no substantial work has been carried out on the stem bark of *T.tomentosa* in terms of physicochemical and preliminary phytochemical screening of *T.tomentosa*. Hence an

attempt was made to perform an extensive study on the physicochemical and phytochemical screening of the stem bark of *T. tomentosa*.

## MATERIALS AND METHODS

### Authentication and Collection of the Plant Material<sup>6</sup>

The stem bark of *T. tomentosa* was collected from Darbandora, Ponda- Goa during October 2012. It was authenticated by Prof G. I. Hukkeri, Dept. of Botany, Dhempe College of Arts & Science, Miramar-Goa.

The stem bark was collected, washed thoroughly, dried in shade, powdered and then used for physicochemical evaluation, fluorescence analysis and preparation of ethanolic extract.

### Preparation of ethanolic extract<sup>20</sup>

The dried powdered stem bark was extracted by maceration with ethanol (95%) for 3 days. After 3 days ethanolic layer was decanted off. The process was repeated thrice. The solvent from the total extract was distilled off using rotary vacuum evaporator (Superfit) and the concentrate was evaporated to a syrupy consistency, evaporated to dryness (80g) and then used for the preliminary phytochemical investigation.

### Physico-chemical evaluation

#### Moisture content<sup>21</sup>

2g of stem bark powder was taken into glass dish which was previously weighed. This glass dish was kept into the hot air oven at 100-105°C. The weight was noted every hour till two successive readings remain constant. At last the weight of stem bark powder was determined and percent yield was calculated.

#### Ash values<sup>22</sup>

##### Determination of total ash

2g of the powdered material was accurately weighed into a previously, ignited and tarred silica crucible. The material was then spread in an even layer in the crucible, ignited by gradually increasing the heat to 500-600°C until free from carbon, cooled in a desiccator and weighed. The percentage of total ash was calculated with reference to the air dried drug.

##### Determination of acid insoluble ash

To the crucible containing the total ash, 25 ml of hydrochloric acid (approx. 70g/l) test solution was added, covered with a watch glass and boiled gently for 5min. The watch glass was rinsed with 5ml of hot water, which was then added to the crucible. The insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was then transferred to the

original crucible, dried on hot plate and ignited to constant weight. The residue was allowed to cool in suitable desiccator for 30 min and weighed without delay. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

##### Determination of water soluble ash

To the crucible containing the total ash, 25 ml of water was added and boiled for 5 minutes. The insoluble matter was collected on an ashless filter paper and washed with hot water & ignited for 15 minutes, at a temperature not exceeding 450°C. Subtract the weight of the residue obtained from the weight of total ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

#### Extractive values<sup>22</sup>

##### Method: Cold maceration

##### Determination of alcohol soluble extractive

About 4.0g of coarsely-powdered, air dried plant material was weighed accurately, in a glass-stoppered conical flask and macerated with 100ml of 90% alcohol for 6 hours shaking frequently and then allowed to stand for 18 hours. It was filtered rapidly taking care not to lose any solvent; 25ml of the filtrate was transferred to tarred flat bottomed dish and evaporated to dryness on water-bath. It was dried at 105°C for 6 hours and cooled in desiccator for 30 min and then weighed without delay. Percentage of alcohol soluble extractive was calculated with the reference to the air dried sample.

##### Determination of water soluble extractive

About 4.0g of coarsely-powdered, air dried plant material was weighed accurately in a glass-stoppered conical flask and macerated with 100ml of water for 6 hours shaking frequently, and then allowed to stand for 18 hours. It was filtered rapidly taking care not to lose any solvent; 25ml of the filtrate was transferred to tarred flat bottomed dish and evaporated to dryness on water-bath. It was dried at 105°C for 6 hours and cooled in desiccator for 30 min and then weighed without delay. Percentage of water soluble extractive was calculated with the reference to the air dried sample.

##### Determination of ether soluble extractive

About 4.0g of coarsely-powdered, air dried plant material was weighed accurately, in a glass-stoppered conical flask and macerated with 100ml ether for 6 hours shaking frequently and then allowed to stand for 18 hours. It was filtered rapidly taking care not to lose any solvent; 25ml of the filtrate was transferred to tarred flat bottomed dish and evaporated to dryness on water-bath. It was dried at 105°C for 6 hours and cooled in desiccator for 30 min and then weighed without

delay. Percentage of ether soluble extractive was calculated with the reference to the air dried sample.

#### Determination of foaming index<sup>23</sup>

About 1g of the plant material was reduced into a coarse powder (sieve size no. 1250), weighed accurately and transferred to a 500ml conical flask containing 100ml of boiling water which was maintained at moderate boiling for 30 min. Then it was cooled and filtered into a 100ml volumetric flask, sufficient water was added to the filtrate to dilute the volume to 100ml. The above decoction was placed into 10 stoppered test tubes (height 16cm, diameter 16mm) in a series of successive portions of 1, 2, 3, up to 10ml and the volume of the liquid was adjusted in each tube with water to 10ml. The tubes were stoppered and shaken in lengthwise motion for 15 seconds (2 frequencies per second). It was allowed to stand for 15 min and the height of foam was then measured.

- If the height of foam in every test tube was less than 1cm the foaming index was less than 100
- If in any tube the height of the foam of 1 cm is measured, the dilution of the plant material in this tube [a] is the index sought. If this tube is the 1<sup>st</sup> or 2<sup>nd</sup> test tube in series, it is necessary to have an intermediate dilution prepared in a similar manner to obtain a more precise result.
- If the height of the foam is more than 1 cm in every test tube the foaming index is over 1000. In this case the determination needs to be made on a new series of dilutions of the decoction in order to obtain results.

$$\text{Foaming index} = 1000/a$$

a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming is observed

If the height of the foam is more than 1 cm in every test tube the foaming index is over 1000. In this case the determination needs to be made on a new series of dilutions of the decoction in order to obtain results.

#### Swelling index<sup>23</sup>

1g of stem bark powder was weighed accurately into a 25ml glass stoppered measuring cylinder. The length of the graduated portion of the cylinder should be about 125mm, the internal diameter about 16mm subdivided in 0.2ml and marked from 0- 25ml in upward direction. 25ml of water was added to the cylinder and shaken thoroughly at intervals of 10 min. for 1 hour. It was then allowed to stand for 3 hours at room temperature. The volume in ml occupied by the plant material, including any sticky mucilage was measured. The

mean value of individual determinations, related to 1g of plant material was calculated.

#### Florescence Analysis<sup>24, 25, 26</sup>

Many herbs show fluorescence when the cut surface or powder is exposed to UV light and this can be useful in their identification. The fluorescence character of the stem bark powder of *T.tomentosa* was studied both in daylight and UV light (254 nm and 366 nm) and after treatment with different reagents.

#### Preliminary Phytochemical screening (Qualitative Analysis)<sup>27, 28</sup>

The preliminary phytochemical studies were performed for testing the different phyto-constituents present in the ethanolic extract of stem bark of *T.tomentosa* using standard procedures.

#### Alkaloids

##### Dragendorff's Test

To 2mg of the ethanolic extract, 5ml of distilled water was added; then 2M hydrochloric acid was added until an acid reaction occurred. To this 1ml of Dragendorff's reagent was added. Formation of orange or orange-red precipitate indicated the presence of alkaloids.

##### Mayer's Test

To 2mg of the ethanolic extract, a few drops of Mayer's reagent were added. Formation of white or yellow precipitate indicated the presence of alkaloids.

##### Wagner's Test

To 2mg of the ethanolic extract, 1ml of dilute hydrochloric acid was added along with few drops of Wagner's reagent. A yellow or brown precipitate indicated the presence of alkaloids.

##### Hager's Test

To 2mg of the Ethanolic extract, a few drops of Hager's reagent were added. Formation of yellow precipitate confirmed the presence of alkaloids.

#### Carbohydrates

##### Molisch's Test

In a test tube containing 2ml of extract, 2 drops of freshly prepared 20% alcoholic solution of  $\alpha$ -naphthol was added. 2ml of conc. sulphuric acid was added so as to form a layer below the mixture. Red- violet ring appeared, indicating presence of carbohydrates, which disappeared on the addition of excess of alkali.

##### Benedict's test

To 0.5ml of extract, 5ml of Benedict's solution was & boiled for 5 minutes. Formation of brick red coloured precipitate indicated the presence of carbohydrates.

**Fehling's Test**

To 2ml of extract, 1ml mixture of equal parts of Fehling's solution A & B were added & boiled for few minutes. Formation of red or brick red coloured precipitate indicated the presence of reducing sugars.

**Flavonoids****Shinoda Test**

In a test tube containing 0.5ml of the extract, 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish or brown colour indicated the presence of flavonoids.

**Lead acetate Test**

To 2mg of plant extract, 1ml of lead acetate solution was added. Formation of yellow precipitate indicated presence of flavonoids.

**Vanillin –hydrochloric acid Test**

Vanillin HCl was added to the alcoholic solution of drug, Formation of pink colour indicated presence of flavonoids.

**Triterpenoids****Libermann's–Burchard's Test**

2mg of the dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1ml of conc sulphuric acid was added along the sides of the test tube. Brown ring was formed at junction of two layers & Formation of deep red colour in the upper layer indicated the presence of triterpenoids.

**Steroids****Libermann's–Burchard's Test**

2mg of the dry extract was dissolved in acetic anhydride, heated to boiling, cooled & then 1ml of conc Sulphuric acid was added along the sides of the test tube. Brown ring was formed at junction of 2 layer Formation of green colour in the upper layer indicated the presence of steroids.

**Salkowski Test**

2mg of dry extract was shaken with chloroform. To the chloroform layer; sulphuric acid was added slowly by the sides of test tube. Formation of red colour indicated the presence of steroids.

**Tannins and Phenolic Compounds**

To 1-2ml of the extract, few drops of 5% FeCl<sub>3</sub> solution were added. A green colour indicated the presence of gallotannins, while brown colour indicated the presence of pseudotannins.

To 1-2ml of the extract, lead acetate was added. White precipitate indicated the presence of tannins & phenolic compounds.

**Resins**

1ml of extract was dissolved in acetone & the solution was poured in distilled water. Turbidity indicated the presence of resins.

**Proteins****Biuret's Test**

To 1ml of hot extract, 5-8 drops of 10% w/v sodium hydroxide solution, followed by 1 or 2 drops of 3% w/v copper sulphate solution were added. Formation of violet red colour indicated the presence of proteins.

**Millon's Test**

1ml of extract was dissolved in 1ml of distilled water & 5-6 drops of Millon's reagent were added. Formation of white precipitate, which turns red on heating, indicated the presence of proteins.

**Glycosides**

Free sugar content of the extract was determined and hydrolysed with mineral acids (dil. HCl/dil. H<sub>2</sub>SO<sub>4</sub>). The total sugar content of hydrolysed extract was again determined. Increase in the sugar content indicated the presence of glycosides in the extract.

**Test for Cardiac glycosides****Baljet's Test**

A thick section showed yellow to orange colour with sodium picrate.

**Legal's Test**

To aqueous or alcoholic extract, 1ml pyridine and 1ml sodium nitroprusside was added. Pink to red colour indicated presence of cardiac glycosides.

**Test for Deoxysugars (Killer–Killani Test)**

To 2ml extract, glacial acetic acid, one drop 5% FeCl<sub>3</sub> and conc. H<sub>2</sub>SO<sub>4</sub> were added. Reddish brown colour appeared at the junction of the two liquid layers and upper layer appeared bluish green indicated presence of deoxysugars.

**Liebermann's Test (Test for bufadienoloids)**

3ml extract with 3ml acetic anhydride was mixed. It was then heated and cooled. Few drops of conc. H<sub>2</sub>SO<sub>4</sub> were added. Blue colour indicated presence of bufadienoloids.

**Test for Anthraquinone Glycosides****Bortrager's Test for Anthraquinone Glycosides**

To 3ml extract, 5ml 5% dil. H<sub>2</sub>SO<sub>4</sub> was added. It was then boiled and filtered. To the cold filtrate; equal volume of benzene or chloroform was added and shaken well. The organic solvent was separated and then ammonia was added. Ammonical layer turned pink or red indicated the presence of anthraquinone glycosides.

**Modified Borntrager's Test for C-Glycosides**

To 3ml extract, 5ml 5% dil. HCl, few drops of FeCl<sub>3</sub> were added. It was then heated for 5 minutes in boiling water and cooled. To the cold filtrate equal volume of benzene or organic solvent was added. It was shaken well. The organic solvent was separated and ammonia was added. Ammonical layer turned pink or red indicated the presence of C-glycosides.

**Test for Saponins****Foam Test**

The drug extract or dry powder was shaken vigorously with water. Persistent foam observed indicated presence of saponins.

**Test for Coumarin glycosides**

Powder was moistened and taken in a test tube. The test tube was covered with filter paper soaked in dilute NaOH and kept in water bath. Later the filter paper was exposed to UV light. Yellowish-green fluorescence indicated the presence of coumarin glycosides.

**Starch**

0.01gms of Iodine and 0.075gms of KI were dissolved in 5ml of distilled water and 2-3ml of extract was added. Formation of blue colour indicated the presence of starch.

**RESULT AND DISCUSSION**

The stem bark of *T.tomentosa* was subjected to systematic physicochemical, fluorescence and preliminary photochemical analysis. The data generated is helpful in determining the quality and the purity of the crude drug, especially in the powdered form. In this study the parameters included for the evaluation of *T.tomentosa* stem bark were moisture content, ash values (total ash, water soluble ash and acid insoluble ash), extractive values using alcohol, water and ether as solvents, swelling index and foaming index (Table No. 1). The extractive values are, however,

moderate but will be useful for the further extraction of phytoconstituents from this plant. The alcohol soluble extractive indicated the presence of polar constituents like phenols, flavonoids<sup>[21]</sup> etc. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign matter such as metallic salts or silica<sup>21</sup>. The fluorescence analysis performed showed a wide range of fluorescent colours for the crude drug with different reagents<sup>23, 24, 25</sup> (Table No. 2). Fluorescence study of the stem bark powder helps in the qualitative evaluation which can be used for its identification. The preliminary phytochemical screening of the ethanolic extract of stem bark was performed and it was found to contain carbohydrates, flavonoids, triterpenoids, steroids, tannins and saponins (Table No. 3).

**CONCLUSION**

Recently there has been a shift in the universal trend from synthetic to herbals as a result there has been rapid increase in the standardization of the medicinal plant of potential therapeutic significance. Despite the modern techniques, identification of plant drug by pharmacognostic study is more reliable. The physicochemical parameters, fluorescence analysis and chemical tests performed in this study will further guide in pharmacological and therapeutical evaluation of the species and will assist in standardization for quality, purity and sample identification. In conclusion, the parameters reported in this study will be useful in the development of pharmacopoeial standards for future studies.

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**Table 1: Results of the physicochemical tests of powdered stem bark of *T.tomentosa***

S.No.	Physicochemical Test	Results
1.	Moisture Content	12.5%w/w
2.	Ash Values	
	➤ Total Ash	19.95%w/w
	➤ Acid Insoluble Ash	16.35%w/w
3.	➤ Water Soluble Ash	0.9%w/w
	Extractive Matter	
	➤ Alcohol Soluble Extractive	1.0%w/w
4.	➤ Water Soluble Extractive	0.8%w/w
	➤ Ether Soluble Extractive	0.2%w/w
5.	Foaming Index	Less than 100
5.	Swelling Index	1.14 cm

**Table 2: Results of the Florescence Analysis of powdered stem bark of *T. tomentosa***

S. No.	Drug +reagent	Day light	Short wavelength 254nm	Long wavelength 366nm
1.	Powder	Light brown	Brown	Dark brown
2.	Powder+50% (aq) NaOH	Brown	Dark brown	Brown
3.	Powder+50% (alc) NaOH	Pale yellow	Grey	Bluish green
4.	Powder +Ammonia	Reddish brown	Dark brown	Dark brown
5.	Powder+ Picric acid	Reddish brown	Brown	Yellowish brown
6.	Powder+ 10%HCL	Light brown	Brown	Brown
7.	Powder+ 10%H <sub>2</sub> SO <sub>4</sub>	Light brown	Brown	Brown
8.	Powder+ Conc HCL	Brown	Dark brown	Dark brown
9.	Powder+ Conc H <sub>2</sub> SO <sub>4</sub>	Brownish black	Brownish black	Brownish black
10.	Powder+ Conc HNO <sub>3</sub>	Orangish red	Orangish red	Orangish red
11.	Powder+ 10% NaOH	Brown	Brown	Brown
12.	Powder+ dist. H <sub>2</sub> O	Light brown	Brown	Brown
13.	Powder+ Methanol	Wine red	Reddish brown	Reddish brown
14.	Powder+ Pet. Ether	Colourless	Light brown	Violet
15.	Powder+ CHCl <sub>3</sub>	Light brown	Brown	Brown

**Table 3: Result of Qualitative Tests for phyto-Constituents isolated from the Ethanolic extract of the stem bark of *T.tomentosa***

S. NO.	CHEMICAL TEST	INFERENCE
1.	Alkaloids :	
	• Dragendorff's Test	-ve
	• Mayer's Test	-ve
	• Wagner's Test	-ve
2.	Carbohydrates:	
	• Molisch Test	+ve
	• Fehling's Test	+ve
	• Benedict's Test	+ve
3.	Flavonoids :	
	• Shinoda Test	+ve
	• Lead acetate Test	+ve
4.	Triterpenoids :	
	• Liebermann- Burchard's Test	+ve
	5.	Steroids :
• Liebermann- Burchard's Test		+ve
6.	• Salkowski Test	+ve
	6.	Tannins :
7.	Resins :	-ve
8.	Saponins :	+ve
9.	Proteins :	
	• Biuret Test	-ve
	• Millon's Test	-ve
10.	Starch	-ve

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