

Research Article

**Pharmacognostic and Phytochemical Evaluation of *Tamarindus indica* Linn. Leaves.**

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**ABSTRACT**

*Tamarindus indica* Linn (Caesalpiniaceae) is a tropical evergreen tree, extensively used as traditional medicine in all countries. *T. indica* is commonly found in fertile areas throughout the Africa and Southern Asia. Even though this plant has gained scientific importance recently, there is a need for the pharmacognostic standardization. Hence, in the present work the leaf of the plant were subjected to various microscopical and physical evaluations. In the microscopical studies, the different cell structures and arrangements were studied and in physical evaluation the ash values and extractive values were studied. Pharmacognostic standardization of leaves of *T. indica* is necessary as it is highly potent commercially. The present study established macro and microscopic characteristics, physicochemical values and phytochemical screening of leaves of *T. indica*. The various pharmacognostic constants were obtained which could help in the development of a suitable monograph for the plant.

**KEYWORDS**

*Tamarindus indica*, Caesalpiniaceae, Pharmacognosy, Leaf constant, Proximal analysis, Phytochemistry.

## 1. INTRODUCTION

*Tamarindus indica* family: Fabaceae, subfamily: Caesalpiniaceae is a tropical evergreen tree native to fertile areas throughout the Africa and Southern Asia<sup>1</sup>. It is distributed throughout India, particularly in the south, often cultivated. It is commonly called tamarind, the botanical name is *T. indica* and it is variously called Tsamiya (Hausa), Ajagbon (Yoruba), Iekeku-oyibo (Igbo) and Dara (Nupe) commonly called as tamarind and widely cultivated as an ornamental tree. Due to its acidic fruits it is used in making drinks and a popular component of many decoctions used as health remedies. *Tamarindus* is a monotypic genus distributed throughout much of the tropics. Different parts of the plant such as leaves, fruits and seeds have been extensively used in traditional Indian and African medicines<sup>2</sup>. It used as an ingredient in *Ayurvedic* medicinal formulations which indicates it high medicinal value traditionally<sup>3,4</sup>.

The aqueous extract of seed reduced blood sugar level showed hypolipidemic effect, reduced 14-17% of plasma lipid, total lipid, cholesterol, lipoprotein and triglycerides. The seed coat extract has strong antioxidant property, used as additive to food, in cosmetics and pharmaceutical preparations<sup>5, 6</sup>. The fruit also has antimicrobial and antibiotic activity<sup>7</sup>. The plant having promising analgesic<sup>8</sup>, antidiabetic<sup>9</sup>, hypolipidemic<sup>10</sup>, anti-inflammatory<sup>11, 12</sup>, immunomodulatory<sup>13</sup>, carcinogenic<sup>14</sup>, antimicrobial<sup>15, 16</sup> and antioxidant activity<sup>17</sup>. Many important phytoconstituents responsible for the activity were isolated. On literature survey it was revealed that a variety of secondary metabolites have been reported from tamarind.

Herbs show a number of problems in when quality aspect is considered. This is because of nature of the herbal ingredients & different secondary metabolites present therein. It is also due to variation in the chemical profile of herbs due to intrinsic & extrinsic factors like growth, harvesting, geographical source, storage & drying etc.<sup>18</sup> Majority of the crude drugs come from wild sources and it is collected by poor, illiterate tribal without any attention to botanical identification and authentication. Standardization of natural products is a complex task due to their heterogeneous composition, which is in the form of whole plant. To ensure reproducible quality of herbal medicines, proper control of starting material is almost essential the first step towards ensuring quality of starting material is authentication followed by creating numerical values of standards for comparison<sup>19</sup>. Pharmacognostic parameters for easy identification like leaf constants, microscopy & physico chemical analyses are few of the basic protocol for standardization of herbals.

In the present study, leaves of *Tamarindus indica* Linn, was studied to evaluate its macroscopic and microscopic characters, physico-chemical analysis and phytochemical screening were also analyzed for the standardization of drug.

## 2. MATERIALS AND METHODS

### 2.1. Plant Material

Fresh sample of leaves of *T. indica* Linn were collected from Ahmednagar district (Maharashtra), and dried in the shade at room temperature. The plant were authenticated by P.G. Diwakar, Joint Director, Botanical Survey of India, Pune by comparing morphological features, with the Voucher specimen number VVD-3 (Ref. No.BSI/WRC/Tech/2009/497 Dated 11 Sep 2009).

## 2.2. Chemicals and reagents

All the chemicals and reagents used were of laboratory grade.

## 2.3. Organoleptic characters

Leaves were evaluated for its organoleptic characters like texture, taste, odour and colour etc.

## 2.4. Microscopical studies<sup>20, 21</sup>

### 2.4.1. Transverse section of leaf

Free hand sectioning was done for fresh leaf to obtain a thin section. Phloroglucinol and hydrochloric acid in the ratio 1:1 was used as a stain and mounted on a glass slide and focused under a microscope at different objectives.

### 2.4.2. Powder microscopy

Shade dried leaf and roots were powdered with the help of an electric grinder till a fine powder was obtained. This fine powder of the leaf and root were subjected to powder microscopy, as per standard procedures mentioned.

### 2.4.3. Determination of leaf constants

Leaf constant parameters like stomatal number, stomatal index, vein islet number and vein termination number was determined as per standard procedure.

## 2.5. Proximate analysis<sup>22</sup>

The various physicochemical parameters like foreign organic matter, moisture content (LOD), ash values and extractive values were performed as per the standard procedures.

## 2.6. Preparation of extracts<sup>23, 24</sup>

The mature leaves of *T. indica* collected locally were washed with water, shade dried and grounded into fine powder. 100g of the dried leaf powder was extracted using solvent petroleum ether, chloroform, ethanol, ethyl acetate and water in succession using soxhlet apparatus. Each extract obtained following successive extraction was filtered, dried to a semisolid mass using water bath and stored in a refrigerator at 4°C till further use.

## 2.7. Phytochemical analysis<sup>20, 21, 22, 24</sup>

A stock concentration of 1 % (w/ v) of each successive extract obtained using petroleum ether, chloroform, ethanol, ethyl acetate and water was prepared using the respective solvent. These extracts were tested for the presences of phytochemicals were detected by usual prescribed methods.

## 3. RESULTS AND DISCUSSION

### 3.1. External features

The plant is a large tree with wide girth of the trunk. The leaves are unipinnate and even pinnate. The leaflets are 15-17 in pairs, narrow oblong, 1.5 × 0.7 cm in size, glabrous or puberulous base and apex is obtuse; margin is entire. (Fig. 1)

### 3.2. Organoleptic characters

The plant leaves organoleptic characters observed and showed in Table 1

### 3.3. Microscopical studies

#### 3.3.1. Transverse section of isobilateral leaf

Thin transverse section of the leaf showed dorsiventral nature. (Fig. 2)

#### 3.3.2. Microscopic features of the Leaflets

The leaflets are uniformly thick with fairly prominent midrib. The midrib part is 600 µm thick and the lamina is 400 µm thick. The midrib is flat on the adaxial side and slightly convex on the abaxial side. The adaxial epidermis of the midrib is similar to the epidermal layer of the lamina. The abaxial epidermis consists of smaller slightly papillate cells. The palisade tissue of the lamina continues across the adaxial part of the midrib.

The vascular strand of the midrib is single, large circular and centrally placed. (Figure 3) It consists of thick cylindrical sclerenchymatous bundle sheath, which is thicker on the adaxial side. The vascular tissues consist of 5 or 6 in number, short. Parallel lines of xylem element which are wide, angular and thin walled. Along the abaxial part of xylem occur about 5 prominent discrete masses of phloem units. Calcium oxalate crystals are prismatic type occur along the outer periphery of the sclerenchyma (Fig. 6). The crystals are cuboidal, rhomboidal and double pyramidal in shape.

### *3.3.3. Microscopic features of the Lamina*

The lamina has even surface. It is 150 µm thick. The adaxial epidermis is thick comprising wide and dilated thin walled cells. The cells are 15 µm thick. The abaxial epidermis is comparatively thin and comprises narrow cylindrical cells. The mesophyll is differentiated into adaxial broad zone of palisade tissue and abaxial spongy parenchyma. The palisade cells are in two layers; they are narrow, cylindrical and 50 µm in height. The spongy parenchyma cells are 7-8 layers; they are highly lobed and interconnected with each other forming aerenchymatous tissue (Fig. 4).

### *3.3.4. Microscopic features of the Leaf margin*

The marginal part of leaflet is semicircular and slightly narrow. It is 140 µm thick. The epidermal layer become thin along the margin and the cells are cylindrical or barrel shaped and thick walled. There is wide circular vascular strand situated at the sub marginal part of the lamina. The vascular strand consists of 2 or 3 layer of thick walled lignified sclerenchyma cells of bundle sheath. Within the bundle sheath occur a small cluster of xylem and a thin band of phloem element (Fig. 5).

### *3.3.5. Powder microscopy*

The powder of the leaflet shows following conclusion;

#### **1. Adaxial epidermal peeling** (Fig. 7)

The adaxial epidermal fragments are seen in powder. The epidermal peeling is seen in surface view. The epidermal cells are polyhedral in outline; their anticlinal walls are fairly thick. The walls are beaded due to dense simple pits on the walls.

#### **2. Abaxial epidermal peeling** (Figure 8)

The fragments of lower epidermal layer of the leaflet are also seen in the powder. The abaxial layer is stomatiferous.

#### **3. Stomata** (Fig. 9)

The stomata are paracytic in type. A stoma has two lateral subsidiary cells lying parallel to the guard cells. The two subsidiary cells may be equal or unequal in size. The epidermal cells have thin, slightly wavy anticlinal walls.

#### **4. Venation of the leaflet** (Fig. 10)

Broken pieces of the leaflets are more fragments in the powder. These fragments exhibit the venation pattern. The secondary and tertiary veins are thick and prominent forming

dense reticulate venation. The vein-islets are wide and distinct. The vein terminations are developed. They are mostly branched. Some of them are repeatedly branched forming dendroid out line.

### 3.3.6. Determination of leaf constants

Results obtained are tabulated in Table 2 (Average reading)

### 3.7. Proximate analysis

The results obtained for the leaf are tabulated in Table 3

### 3.8. Preparation of successive extracts and their Preliminary Phytochemical analysis

Basic phytoinvestigations of the extracts for their major components is vital as the active principles of many drugs are these secondary metabolites found in plants. The yield obtained for each successive extracts of the leaves of *T. indica* in present study using petroleum ether, chloroform, ethanol, ethyl acetate and water (aqueous) is recorded to be highest in the case of ethanol. (Table.4) The all extracts were screened for the presence of various constituents. The result of this preliminary phytochemical evaluation is shown in table no. 5. The result revealed that presence of glycosides, alkaloids, steroids, flavonoids and tannins.

*T. indica* is used for the treatment of various physiological conditions. But so far the plant has not been standardized Pharmacognostically. The detailed pharmacognostic studies like microscopical studies, determination of leaf constants and proximate analysis reported in this work would be a useful for compilation of a suitable monograph for its proper identification and will help in establishing some biological indices. The present information thus would be of help to isolate and characterize the diverse pharmacologically active principles from tamarind leaves for their varied biological activities and the medicinal values.

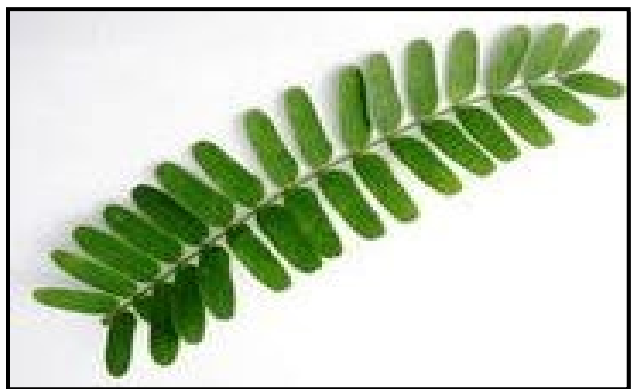
As a part of standardization study, the macroscopical examination of *T. indica* leaves was studied. Macroscopical evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of drugs. The ash value, extractive value, moisture content, foreign matter and crude fiber content of powdered leaves extracts have been carried out. The results showed greater extractive values in hot extraction, indicating the effect of elevated temperature on extraction. Percentages of the extractive values were calculated with reference to air-dried drug. The percent extractives in different solvents indicate the quantity and nature of constituents in the extracts. The extractive values are also helpful in estimation of specific constituents soluble in particular solvent. The preliminary phytochemical analysis of crude *T. indica* leaves extract gives idea about phytocomponent present in it. It may be useful for further studies.

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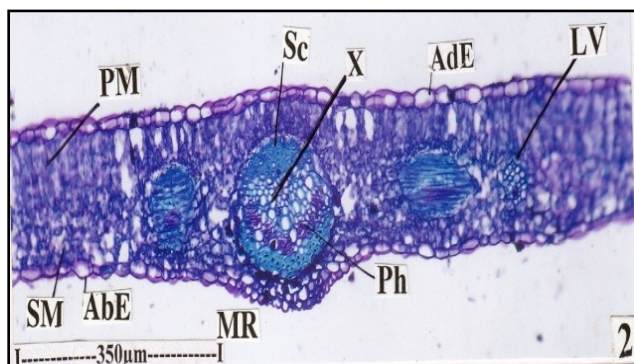
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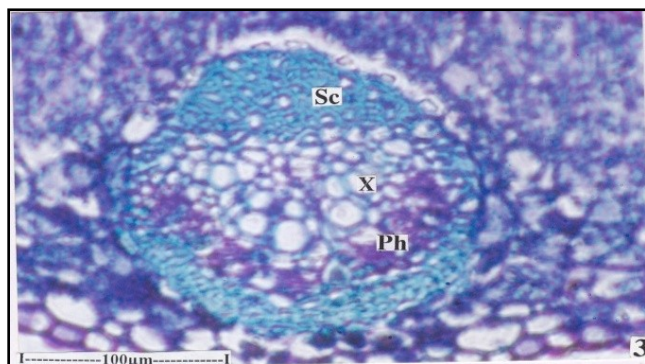
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**Fig. 1:** *T. indica* leaves.

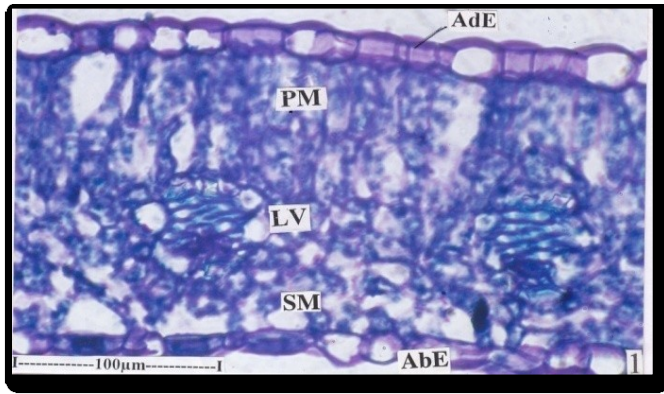


**Fig. 2:** Transverse section of *T. indica* Leaflet.

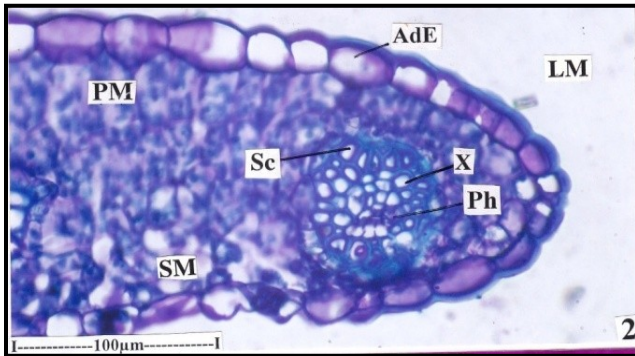


**Fig. 3:** Transverse section of *T. indica* Leaflet having midrib.

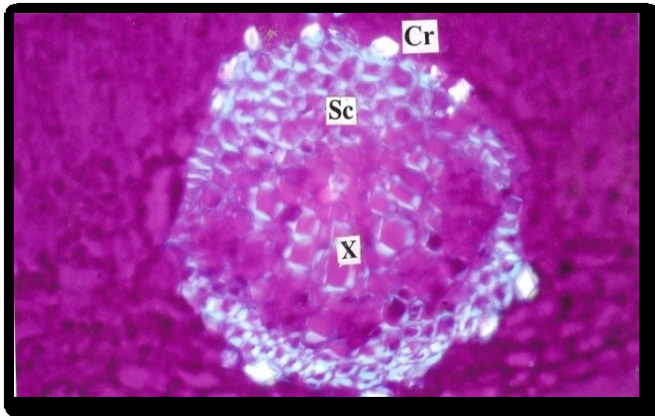




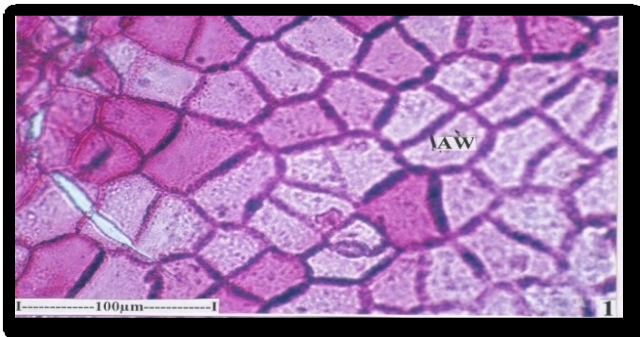
**Fig. 4:** Transverse section of Leaflet lamina.



**Fig. 5:** Transverse section of lamina through marginal portion.



**Fig. 6:** Calcium oxalate crystals.



**Fig. 7:** Adaxial epidermis peeling with anticlinal wall.





Fig. 8: Abaxial epidermis peeling with stomata.

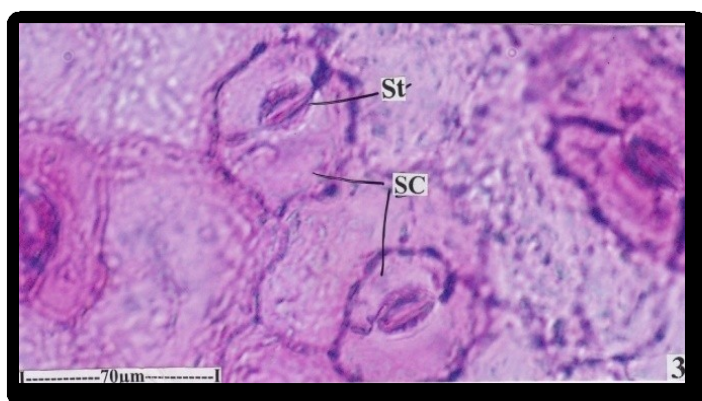


Fig. 9: Stomata with parasitic subsidiary cells.

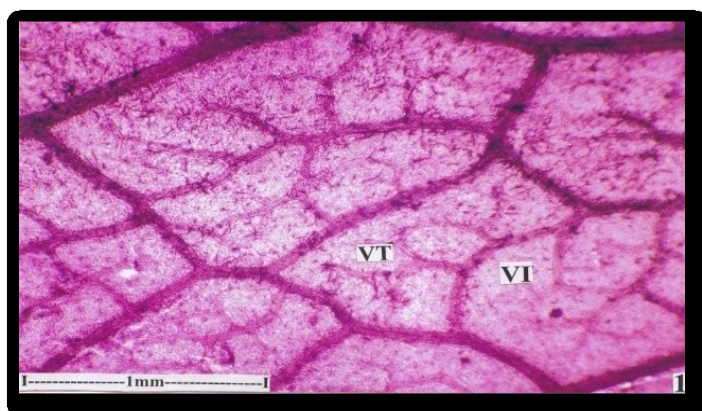


Fig.10: Venation pattern of the leaflet.

Table 1: Organoleptic characters of leaf of *T. indica*.

Sr. No.	Parameters	Characters
1	Texture	Soft and moist
2	Colour	Reddish-brown
3	Taste	Acidic and sweet

4	Odour	Characteristic, sour
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**Table 2:** Showing leaf constants of *T. indica* leaf.

Sr. No.	Leaf constant	Numbers (Average value)
1	Stomatal number	140
2	Stomatal index	29.16
3	Vein termination number	14-16
4	Vein islet number	8-11

**Table 3:** Proximate analysis leaf powder.

Parameter	Values obtained % w/w (Mean + SEM)
Moisture content	6.5 + 1.1
Foreign organic matter	0.5 + 0.1
Total ash	4 + 0.5
Acid insoluble ash	0.6 + 0.1
Water soluble ash	0.7 + 0.2
Sulphated ash	0.3 + 0.1
Crude fiber content	62 + 8
Ethanol soluble extractive values	12 + 2
Water soluble extractive values	10 + 2

**Table 4:** The yield and colour of the extracts of *T. indica* leaves obtained.

Solvent	Sample (Gram)	Total Hrs of Extraction	Yield %	Colour of final extract
Petroleum Ether	100	3.5hrs	8.58	Dark green
Chloroform	86	6 hrs	5.47	Yellow brown
Ethanol	81	7 hrs	18.24	Dark green
Ethyl acetate	74	6	6.22	Dark green
Aqueous	55	3 hrs	6.48	Dark green
Residue	48			

**Table 5:** Preliminary Phytochemical Screening of All Extracts of *T. indica*.

Extracts	Petroleum ether	Chloroform	Ethanol	Ethyl acetate	Aqueous
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<b>Tests for carbohydrates</b>					
<b>Molish Test</b>	-	-	+	-	-
<b>Fehling Test</b>	-	-	+	+	-
<b>Benedict Test</b>	-	-	+	+	-
<b>Test for Monosaccharide</b>					
<b>Barfoed's Test</b>	+	-	-	-	-
<b>Test for Non-reducing polysaccharides</b>					
<b>Iodine Test</b>	-	-	-	-	-
<b>Test for Proteins</b>					
<b>Biuret test</b>	-	-	-	-	-
<b>Millions test</b>	-	-	-	-	-
<b>Tests for Steroids</b>					
<b>Salkowaski reaction</b>	-	+	+	+	-
<b>Libermann Burchard reaction</b>	-	-	-	-	-
<b>Libermann reaction</b>	+	-	-	-	-
<b>Test for Glycosides</b>					
<b>Borntrager's Test</b>	+	-	-	+	-
<b>Killer- Killani Test</b>	-	+	+	-	-
<b>Test for Saponin</b>					
<b>Foam test</b>	+	-	-	-	+
<b>Tests for Flavonoids</b>					
<b>Shinoda test</b>	-	+	+	-	-
<b>Lead acetate Test</b>	+	+	+	-	-
<b>Sod-hydroxide Test</b>	+	+	+	-	-
<b>Tests for Alkaloids</b>					
<b>Meyers Test</b>	+	-	+	-	-
<b>Wagner's Test</b>	+	-	-	-	+
<b>Hager's Test</b>	-	-	-	-	-
<b>Dragendorff Test</b>	+	+	+	+	-
<b>Test for Tannins &amp; Phenolic compounds</b>					
<b>FeCl<sub>3</sub></b>	-	-	+	-	-
<b>Lead acetate</b>	-	-	+	+	-

**Note:** + Indicates presence of phytoconstituents, - Indicates absence of phytoconstituents