



## Foliar epidermal microscopy and preliminary phytochemical screening of the leaves and stem bark of *Tamarindus indica* Linn. (Family: Fabaceae/Caesalpinioideae)

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### ABSTRACT

The microscopy of the foliar epidermis and the preliminary phytochemical screening of the leaves and stem bark of *Tamarindus indica* Linn. were carried out. The foliar epidermal analysis revealed the presence of diacytic stomata, prismatic crystals as well as polygonal cell shape on the lower and upper surfaces. The stomata are amphistomatic (on both lower and upper surfaces). Trichomes were not observed on the leaf surfaces. The phytochemical screening of the water extracts of the leaves revealed the presence of carbohydrates, saponin and flavonoids and the absence of tannin, anthraquinone and phlobatanins; while the stem bark revealed the presence of tannins, saponins and flavonoids and the absence of anthraquinone and phlobatannins. The result from this study provides the finger print for the identification of *Tamarindus indica* and shows some of the chemical constituents of the plant.

**Key words:** Microscopy, Amphistomatic, Diacytic stomata, *Tamarindus indica*, Secondary metabolites

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### INTRODUCTION

*Tamarindus indica* L. belong to the family Fabaceae/Leguminosae, sub-family Caesalpinioideae. Synonyms of the plant include: *Tamarindus erythraea* Matt.; *T. occidentalis* Gaertn.; *T. somalensis* Matt. and *T. officinalis* Hook.

*Tamarindus indica* otherwise known as tamarind is indigenous to tropical Africa, particularly Sudan, where it continues to grow wild. It is also cultivated in Cameroon, Nigeria and Tanzania. It got to South Asia likely through human transportation and cultivation, several thousand years ago prior to the Common Era [1] and [2]. In the 16<sup>th</sup> century, it was introduced to Mexico and South America by Spanish and Portuguese colonist to the extent that it became a staple ingredient in the region's cuisine [3]. Today South Asian and Mexico remains the largest consumers and producers of Tamarind [3].

Tamarind is a long-lived, medium growth, bushy tree. The tree grows well in full sun, in clay, loamy, sandy and acidic soil types with a high drought and aerosol (wind-borne salt as found in coastal areas) resistance [4]. Leaves are ever green, bright-green in colors, elliptical, ovular; arrangement is alternate of the pinnate compound type with pinnate venation. The branches drop from a single, central trunk as the tree matures and is often pruned in human agriculture to optimize tree density and ease of fruit harvest. At night, the leaflets close up. Flowers are 5-petaled, borne on small racemes and yellow with orange or red streaks. Buds are pink and sepals are pink and are lost when the fruits mature. Fruits are an indehiscent legume, sometimes called a "pod" with a hard, brown shell [4]. The fruit

has a fleshy, juicy, acidulous pulp. It matures when the flesh turns brown or reddish brown. The fruit is best described as “sweet and sour” in taste and is high in acid, sugar, vitamins and oddly for a fruit calcium [4].

In Northern Nigeria, the stem bark and leaves are used as decoction mixed with potash for the treatment of stomach disorders, general body pains, jaundice and yellow fever, and as blood tonic as well as skin cleanser [5]. In Indonesia, Malaysia, Philippines and Japanese traditional medicine the leaves are used as herbal infusion for malaria fever, the fruit juice as an antiseptic and scurry and for the cure of cough. Throughout South-East Asia, the fruit of *T. indica* is used in Ayurvedic medicine for gastric and/or digestion problems and in cardio-protective activity [5]. Research has shown in Hens that *T. indica* has been found to lower cholesterol in their serum, but not in the yolk of the eggs they laid [6].

Based on human study, *T. indica* intake may delay the progression of skeletal fluorosis by enhancing excretion of fluoride. However, additional research is needed to confirm this result and excess consumption has been noted as a laxative [7].

## EXPERIMENTAL SECTION

### Collection of plant

The leaves and stem bark of *Tamarindus indica* was collected from Chaza, Suleja Local Government Area of Niger State, Nigeria, in October 2014. Identification and authentication were done at the herbarium, National Institute for Pharmaceutical Research and Development, Idu Abuja. A voucher specimen with number NIPRD/H/6709 was deposited at the same herbarium.

### Plant processing

The plant parts were air-dried at room temperature for several days and reduced to moderate coarse powder using electric blender. The powder sample was sieved using sieve number 40 (420 micrometre) and used for the phytochemical analysis.

### Microscopy

The method used by [8] was adopted. About 5mm– 1cm squared leaf fragments were obtained from the standard median portion of the leaf and macerated in concentrated Nitric acid in petri-dish for a period of 24 hrs. The appearance of bubbles on the surface of the leaf fragment indicated their suitability for separation. The fragments were transferred into water in a petri-dish with a pair of forceps. Both epidermises were carefully separated by teasing them apart and pulling each epidermis back at itself. The leaf epidermises were cleaned with the Carmel hair brush. These were rinsed in distilled water and later transferred into 50% ethanol to harden. They were then stained in Safranin O for 5 minutes and excess stain washed off in water. They were then mounted in glycerin on a slide with the edge of the cover slips ringed with nail varnish to prevent dehydration. The slides were labeled appropriately and examined under the light microscope while photographs were taken using NICON AFX-DX microscope with NICON FX-35DX camera attached at a magnification of x100 and x400.

### Phytochemical screening

#### Plant Preparations

The leaves and stem bark of *Tamarindus indica* were collected by Mall. Muazzam from Chaza Banburu, Suleja, Niger State on 27<sup>th</sup> May, 2013. The fresh plant materials (leaves and stem bark) were dried at room temperature ( $27 \pm 1^\circ\text{C}$ ) and then powdered using a mortar and pestle. The powdered leaf and stem bark samples were used for the phytochemical screening. Only the dried leaf was used for the microscopy.

#### Test for carbohydrates (General Test-Molisch's Test)

About 1g of the powdered material was boiled and extracted in 10mL of distilled water. The mixture was filtered and a few drops of Molisch's reagent were added. Small quantity of conc. Sulphuric acid was added and allowed to form a lower layer. A purple layer on the interface of the liquids indicates the presence of carbohydrate. The mixture was shaken and allowed to stand for 10 minutes and diluted with 5 mL of distilled water, a purple precipitate indicates the presence of carbohydrates [9].

**Fehling's test for free reducing sugars**

To 2mL of the water extract was added 5mL of the mixture of equal volumes of Fehling's solution A and B and boiled on a water bath for 2 minutes. A brick colour indicated the presence of reducing sugars [10].

**Test for tannins**

Ten millilitres (10mL) of distilled water was added to 1g of the powdered herb in a test tube and boiled for 3min in a water bath. The mixture was allowed to cool and then filtered with Watman No.1 filter paper. Then 1 mL of the filtrate was diluted with 4mL of distilled water and 2 drops of 10% Ferric Chloride were added. Instant formation of blue-black or greenish coloured solution indicates the presence of tannin [9].

**Test for Anthraquinone**

About 1g of the powdered material was extracted in 10 mL of benzene in test tube, filtered and 5mL of 10% ammonium solution added to the filtrate. The mixture was shaken and the presence of a pink, red or violet colour in the ammonia (aqueous) phase indicates the presence of free hydroxyl-anthraquinones [9].

**Test for Saponin (Froth test)**

Two grams of the powdered sample were placed in a test tube and 95% ethanol was added and boiled in water bath. The mixture was filtered and 2.5 mL of the filtrate was added to 10mL of distilled water in a test tube and shaken vigorously for 30 seconds. After shaking the mixture was allowed standing for 30 minutes. The presence of honey comb froth indicated the presence of saponin{[11]; [12]; [13]; [14]}.

**Test for Phlobatanin**

Two millilitres (2mL) of water extract was boiled in an equal volume of 1% aqueous hydrochloric acid. The deposition of a red precipitate indicates the presence of phlobatanin{[9]; [13]}.

**Test for Flavonoids**

Fivegrams (5g) of each of the powdered samples were completely detanned with acetone. The residue was extracted in warm water after evaporating the acetone in a water bath. The mixture was filtered. To the detanned water extract was added 10% lead acetate solution. A cream coloured precipitate indicates the presence of flavonoids{[11]; [12]}.

**Chlorogenic acid**

To 1mL of the water extract was added 2 drops of 10% ammonia solution. The mixture was heated over a flame and then exposed to the air. A green colour indicated the presence of chlorogenic acid [15].

**Barfoed's test for monosaccharides**

About 1mL of dilute solution of the water extract and 1mL of Barfoed's reagent were taken in a test tube and heated on a water bath for 2 minutes. A red precipitate indicated the presence of monosaccharides [10]. The test was repeated using glucose as standard.

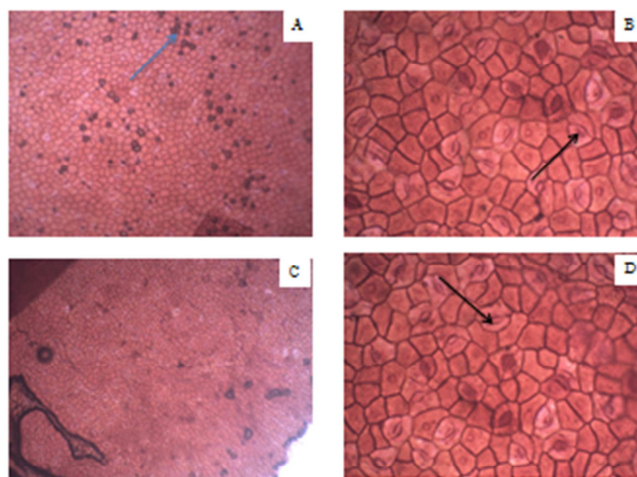
**Test for cardiac glycosides****Kedd's test (for free or combined cardenolidesaglycones)**

About 1g of the powder was boiled with 10 mL of 70% w/v alcohol for 2-3 minutes. The resulting mixture was filtered and cooled. The filtrate was diluted with water and three drops of a strong solution of lead sub-acetate was added. This was mixed thoroughly and filtered. The filtrate was treated with 1mL of 2% solution of 3,5 dinitrobenzoic acid in 95% alcohol and the solution basified with 5% Sodium hydroxide. A purple-blue colour indicates the presence of free or combined cardenolideaglycone [13].

## RESULTS AND DISCUSSION

**Microscopy**

The microscopy of the leaf epidermis revealed the stomata type to be diacytic (surrounded by two epidermal cells) and is found on both the lower and upper surfaces of the leaf, a term known as amphistomatic (Plate 1. A - D). Prismatic crystals are found scattered on the lower surface (Plate 1 A) and the epidermal cells shape are polygonal on both surfaces. No trichomes were observed on both surfaces.



**Plate 1. Photomicrographs of *Tamarindus indica* Leaf**  
 (A) Lower surface x100 (crystals blue arrow) with diacytic stomata  
 (B) Lower surface x400 (diacytic stomata, black arrow)  
 (C) Upper surface x100 with diacytic stomata  
 (D) Upper surface x400 (stoma black arrow surrounded by two epidermal cells)

### Phytochemical screening

The phytochemical screening (Table 1) revealed the presence of carbohydrates (reducing sugars), saponin, and flavonoids and the absence of monosaccharide, tannin, chlorogenic acid anthraquinone, cardiac glycosides and phlobatanins in the leaves. The stem bark revealed the presence of carbohydrates (reducing sugars), tannin, saponin, flavonoids, and absence of monosaccharide, chlorogenic acid, anthraquinone, cardiac glycosides and phlobatannin (Table 1).

**Table 1. Phytochemical screening of the leaf and stem bark of *Tamarindus indica***

Secondary metabolites	Leaf	Stem bark
Carbohydrates	+	+
Monosaccharides	-	-
Pentose sugars	+	+
Reducing sugars	+	+
Tannins	-	+
Saponins	+	+
Chlorogenic acid	-	-
Flavonoids	+	+
Anthraquinones	-	-
Cardiac glycosides	-	-
Phlobatannins	-	-

**Key:** + = presence - = absence

The secondary metabolites observed are the presence of carbohydrates, saponin and flavonoids and the absence of monosaccharides, chlorogenic acid, anthraquinones, cardiac glycosides and phlobatannins in the leaves and stem bark of *T. indica*. Tannin was present in the stem bark and absent in the leaves. Tannin and phlobatannins was observed in the leaves by [4] which are absent in this study. This corroborate with the fact that the environment is known to influence the expression of compounds in plants as observed by some researchers: {[16]; [17]; [18]}. It is evident that from the above result, the difference in the phytochemical profile of the leaf and stem bark is the absence of tannins in the leaf of the plant. Tannin is a well-known secondary metabolite with numerous and important pharmacological activities such as anti-oxidant, anti-microbial and anti-inflammatory activities as well as diuretic [9]. It has also been reported that certain tannins are able to inhibit HIV replication selectively. Plant tannins have been widely recognized for their pharmacological properties and are known to make trees and shrubs a difficult meal for many caterpillars [19]. Thus the absence of this metabolite (tannins) in the leaf may mark significant

variation in the activities of the different parts of the plant. Generally, secondary metabolites are relevant for different purposes for instance:

Saponin has been reported to have hypocholesterolemic properties and as such, consumers of saponin containing food may enjoy chemoprotection against heart diseases [20]. Saponin also relieves stress from body's vital immune system, thus enabling the body natural immune factors. Saponin has been reported to have anti-fungal properties [21] and inhibits  $\text{Na}^+/\text{Ca}^{2+}$  antiporter producing elevated cytosolic  $\text{Ca}^{2+}$  which strengthens the contractions of heart muscle and thereby reducing congestive heart failure [20]. Saponin also has anti-carcinogens' properties, immune modulatory activity and cholesterol lowering activity [22]. It is used as mild detergents and in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hypercholesterolaemia, hyperglycaemia, antioxidant, anti-cancer, anti-inflammatory and weight loss [23].

Flavonoids are highly diversified plant pigments that are present in a wide range of fruits, vegetables and nuts. Flavonoids suggest that the leaves might have anti-oxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-microbial and anti-allergic activities [19]. Plants flavonoid has been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergen, virus and carcinogens. They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities [19]. Many of these alleged effects of flavonoids are reported to be linked to their strong antioxidants, free radical scavenging and metal chelating properties [24].

Carbohydrates primary function in the human body is to supply energy. When carbohydrates are consumed, some exist as indigestible fibers which are needed to increase the bulk of stool and assist in regulation of bowel movement. It is believed that carbohydrates help lower cholesterol and decrease glucose level [13].

Microscopy of the leaves which shows the presence of numerous stomata on both surfaces of the leaves (Amphistomatic) implies that gaseous exchange takes place on both surfaces for photosynthesis and water loss (opening and closing of stomata). The straight cell walls indicate storage depot/organ for carbohydrate and structural support and protection. It also serves as pressure vessels as the major function is preventing over expansion (Turgor pressure) when water enters the cell. The presence of crystals mainly protects the plant from herbivores though not chemically poisonous but due to their shapes, they can prick soft tissues resulting in irritation and itching which are unpleasant to the skin of the herbivores.

## CONCLUSION

The finding in this study showed that tannins was absent from the leaf sample of the plant and this could serve as an authenticating parameter for the plant sample from the area. The study established the phytochemical profile and the foliar microscopic character of *T. indica* found in Chazza village, Niger State, North Central Nigeria. The information obtained in this study may be useful for sample authentication and monograph development.

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