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Evaluation of *Tamarindus indica* L. Seed Tannins

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Article

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ABSTRACT

Tannins are water-soluble polyphenols mostly found in vascular plants and are important raw materials in many industries like leather, pharmaceuticals, and food and beverages. Tannins protect the plant and help its growth. In this study, *Tamarindus indica* L. seeds were extracted for the determination of tannin content and the tanning strength by the hide powder method. Earlier studies showed that *Tamarindus indica* L. seeds varied in the amount of tannins present. *Mimosa* was used as a control. The tanning strength was 1.73 for the tamarind seed extract and 2.06 for mimosa. The tannin content was >10% for both the tamarind seed extract and mimosa. It was concluded that both tamarind seed extract and mimosa had sufficient tanning strength exceeding (1.5) the minimum recommended for a tanning agent hence providing insights as a feasible material for the tanning process.

KEYWORDS

Tamarindus indica L. seed, tannins, mimosa, leather

INTRODUCTION

Tannins are polyphenolic astringent materials that bind and precipitate proteins, amino acids and alkaloids. Tannins are the fourth most abundant plant constituents after cellulose, hemicellulose and lignin. They are present in the bark of trees, roots, leaves, fruits, fruit pods and the galls of the plant [1,2]. They are naturally occurring water-soluble polyphenol chemicals with phenolic hydroxyl groups of molecular weight between 500-3000 Daltons (Da) [3,4]. The term "Tannin" derives from the Latin *tannāre* from *tannum* meaning oak bark, from Anglo-Norman *tanner*, referring to the use of oak and other bark in tanning hide from animals into leather [5]. It is achieved by creating insoluble complexes with collagen proteins and other macromolecules [6,7]. Tannins are widely used in dyes and surface coating, pharmaceuticals, food and beverages, water purification, leather industries and adhesive manufacture. In the leather industry, vegetable tanning materials dominating the market are from wattle, quebracho, *Caesalpinia spinosa* (tara), *Rhus coriaria* (sumac), *Castanea vesca* (Chestnut), and *Terminalia chebula* (myrobalan). In Kenya, the commercially available mimosa extract is derived from wattle (*Acacia mearnsii*) an indigenous tree that is valuable among many communities. *Acacia mearnsii* is considered for use as an eco-friendly source of tannins. Deforestation of acacia trees in search of vegetable tannins led to the depletion and extinction of the species. This has escalated the need to

search for other sources of tannins to compensate for the demand of the global market for these organic tannins.

The four categories of tannins include phlorotannins, complex tannins, condensed tannins, and hydrolysable tannins [8]. Based on molecular weight, complex tannins are a blend of both condensed and hydrolysable tannins. Phlorotannins are mostly found in the sea as weeds [9]. Hydrolysable tannins are made up of glucose or other polyhydric alcohols which are esterified with gallic acid (gallo tannins) or hexahydroxydiphenic acid (ellagitannins) [10,11]. Equally, condensed tannins are polymers of flavan-3-ols (catechin) or flavan-3, 4 diols, which are flavolans (leucoanthrocyanidins) [10].

Tannin content depends on the species of plant, habitat, harvest time and the extraction method [12]. However, some of the methods used for both qualitative and quantitative exploration in the detection of tannins in various industries amongst food and beverage are; Folin-Denis, Nuclear Magnetic Resonance (NMR), hide powder method, Circular dichroism (CD) and Reverse-Phase High-Pressure Liquid Chromatography (HPLC) with UV detection. Research by Elgailani and Ishak (2014) analysed and compared vegetable tannins in the bark, leaves and fruits of mature and immature species of three Sudanese Acacia trees [13,29].

The tannin content in mature and immature *Acacia nilotica* fruits was found to be 22.15%; 22.10% respectively. *Acacia nilotica* and *Acacia seyal* had 11.80%; 6.30% of tannins in the leaves while the barks of the said trees contained 10.47% and 12.15% respectively. *Acacia Senegal* contained only 3.49% tannins in its bark.

The Tamarind tree is a large monotypic tree having a genus with only *T. Indica*. It is an evergreen tree, in the family Leguminosae with fruits mainly rich in potassium, carbohydrates, tartaric acid and vitamin C. Different parts from the tamarind tree are adapted for use in industries such as food, pharmaceutical, chemical and textile. Fruits can either be consumed fresh or the pulp can be processed into juices to add value [14]. The delicate pods are used to season cooked rice, meat, and fish in Indian cuisines, while the pulp is a main component for souring curries, sauces, chutneys and definite beverages [15]. Powdered tamarind kernel which is mostly used in the food business as additives and gelling agents is made from seeds. The seeds are also used in the textile industry as a sizing substance as well as a source of polysaccharide, glue, oil, and tannin for different industrial applications [16]. The primary purified product of tamarind kernel powder (TKP), is a potential drug carrier polymer used in a variety of sustained pharmaceutical release formulations and bioadhesives [17].

Earlier research done was on the use of tamarind seed testa extract as a mordant in the eco-dyeing of textiles. According to additional research, the seed contained 23% tannins and was suggested for use to convert raw hides and skins into leather if blended appropriately [18,19]. However, little has been explored in this area to determine whether the tamarind seeds extract contained tannins with properties required for organic tanning materials. Therefore, this study is aimed at the extraction and

characterization of the selected *Tamarindus indica* L. seeds as a source of tannins for leather processing.

EXPERIMENTAL

Materials and methods

The chemicals used were ferric III chloride (Kobian), potassium hydroxide (Kobian), copper II sulphate (Kobian), Gallic acid (Sigma Aldrich), Chromium potassium Sulphate (LobaChemie), Kaolin (Sigma Aldrich), lead acetate (LobaChemie), Folin-Ciocalteu reagent (Sigma Aldrich) while mimosa was purchased from Sagana tanneries in Kenya.

Samples of mature tamarind fruits were collected from a farm in Kathagara, Igamba-Ngo'mbe sub-county, Tharaka Nithi County, Kenya. The pods of the fruit were crushed manually to separate them from the fresh pulp. The fresh pulp was soaked in water for 5 hours with continuous agitation to aid the separation of tamarind pulp from the fibrous network and seeds (Figure 1a). Oven drying of seeds was done to attain constant weight at 100 °C for 30 minutes. The seeds were ground into powder using a kitchen blender (Figure 1b) [20]. Samples were sieved on 300 and 150 micron sieves after which all that passed through were collected and stored in self-zipping plastic bags for further analysis.



a)



b)

Figure 1. Tamarind a) seeds; b) seed powder

Tannins extraction

The decoction method was used for extraction as outlined in the literature [21,22]. 10 grams of the ground samples were placed in approximately 250 mL of distilled water used as a solvent. The extraction process was run for approximately 40 minutes at 50 °C. Afterwards, the obtained mixture was cooled and filtered, using a cheesecloth. The residue obtained was further re-extracted two more

times as the first one to enrich the completion of the extraction of the tannins. The entire extracted solution was heated to a boil after which it was kept overnight and re-filtered the following day. A water bath was used to concentrate the filtrate to determine the extract yield (by weight) of the dry powdered material used [22,23]. The above experiment trials were performed three times.

Phytochemical analysis of extracts

Exactly 0.5 g sample extract was put in a small beaker containing 10 mL distilled water, stirred to solubilize and filtered before subsequent analysis. A few drops of 5% solution were used as outlined in the literature to confirm the presence and type of tannins [24-26].

Total phenol quantification

Folin- Ciocalteu (F.C) reagent which is a modified version of Folin-Denis reagent was used for total phenol quantification in accordance with method 2017.13 [27,45]. Approximately 100 mg of the extract sample material was weighed and put into a 100 mL volume flask (V.F) having 75 mL of distilled water in it. The content was mixed to aid in the dissolving of the extract after which the flask was filled up to the mark with distilled water. A series of seven test tubes were placed on the rack and each was filled with 15 mL of distilled water followed by 1 mL F.C reagent. In the first test tube (blank), 1 mL of water was added. Into the second to the sixth test tube, 1 mL of calibration standards was added in concentrations series as described and finally into the seventh 1mL of sample solution respectively. Proper mixing was done for each test tube after which they were put on a bench to rest for 6 minutes. 3 mL of twenty per cent aqueous sodium carbonate was added into each for all of the seven test tubes, shaken to mix the content and kept for 120 minutes at room temperature before reading absorbance at 765 nm.

A blank solution was run first, followed by calibration standards (different concentrations) and finally the samples. The experiments were performed in triplicate using UV-Vis Spectrophotometer (UV-model 16 S/N UED 1204004) and the calibration curve was plotted using a different range of calibration standards as outlined in the procedure. The phenolic contents of the extract were determined as gallic acid equivalents (GAE) using the formula below:

$$\text{Total phenol (\% by w/w)} = \left[\frac{A-b}{m} \right] \times \left[\frac{V \times D}{W \times 1000} \right] \times 100 \quad (1)$$

Where:

A = Sample test solution absorbance at 765nm;

b = Calibration curve y-intercept; m = slope of calibration curve;

W = weight in milligrams of the test material; V = volume of sample test solution;

D = dilution factor; 1000 = mL conversion to litre

NB. Conversion 0.1% = 1 mg/g

Determination of tannins using hide powder method

This is a gravimetric filter method that involved; the determination of total soluble solids (TSS) in sample extract, absorption of tannins onto the hide powder and determination of non-tannins (NT) materials left after absorption of tannins as described in [28]. The powder was prepared from pre-treated hide up to the pickling stage, after which it was treated with a solution of acetone, dried and ground [29]. 1.25L of tannin solution was prepared using 50 g tamarind seed powder in the ratio of 1:25 (w/v) [30].

A suitable amount of pickled dry hide powder (6.25 g) was taken for each analysis and mixed with 62.5 mL distilled water (10 times its weight) and stirred for 60 minutes. It was followed by an addition same weight as the dry matter of Chrome alum solution (62.5 mL), stirred for 120 minutes and left overnight. The chromed powder was subsequently separated with the help of clean muslin fabric and rinsed 15 times the dry powdered weight using distilled water. The content was homogenized for about 15 minutes by mixing. Afterwards, the cloth was removed and squeezed to retain about 75% of the moisture. The resulting powder was rinsed three more times with deionized water after which the weight of the chromed hide powder was finally determined [24].

Determination of moisture and total solids (TS)

About 2.5 g of powdered tanning samples were weighed in a dry crucible and recorded. Samples were oven dried for 4 hours at 100 ± 2 °C, and later cooled down in a desiccator containing silica gel beads for 20 minutes before re-weighing and recording. It was ensured that the difference in weight of samples did not exceed two milligrams [31]. The percentage moisture content and total solids were determined using Equations 2 and 3 respectively.

$$\text{Percentage Moisture (\%)} \text{ by weight} = \frac{W_1 - W_2}{W_1} \times 100 \quad (2)$$

$$\text{Percentage Solids (\%)} \text{ by weight} = \frac{W_2}{W_1} \times 100 \quad (3)$$

Where W1 = Initial weight of sample, W2 = Final weight of dried sample

Determination of total soluble solids (TSS)

For the determination of TSS in the extracts, un-filtered 200 mL tannin solutions prepared as earlier described were mixed with 1 g of kaolin and filtered to afford a clear filtrate which was collected. The residue weights were then calculated after 50 mL of the filtrate was pipetted out and put in a crucible for evaporation and drying. A constant dry weight is attained by repeating the procedure [39]. The total soluble solids were calculated as a percentage by weight using (Equation 4) outlined below;

$$\text{Total soluble solids (TSS\%)} = \frac{W_2}{W_1} \times \frac{V_1}{V_2} \times 100 \quad (4)$$

Where; W_2 = weight of the dry residual, W_1 = Weight of the extract tannin material used, V_1 = Volume of test solution in mL made, V_2 = Volume in mL of pipetted out solution

Determination of non-tannins (NT)

The chrome hide powder made (as previously described) was added to a 250 mL flask along with 100 mL of an unfiltered tamarind tannin infusion and 20 mL of distilled water to create the sample solution. Similarly, the operation was done for the control agent. The flask was stoppered and shaken ferociously by hand for 15 seconds, followed by 15 minutes on a rotary shaker set at 50–65 rpm. The solution was filtered and the filtrate (clear) was collected in a separate beaker. 20 mL of the filtrate was pipetted out and evaporated to dryness at 100 °C after which weighing was done until a constant weight was attained. The residual weight was multiplied by 1.2 to account for the wet hide powder and 20 mL water dilution in the 100 mL of tannin solution [29]. The percentage by weight of non-tannins was calculated using below (Equation 5) [29].

$$\text{Percentage of non – tans (\%)} \text{ by weight} = \frac{W_2}{W_1} \times \frac{V_1}{V_2} \times 100 \quad (5)$$

Where V_1 = original volume made up (mL) and V_2 = volume of test solution (mL) taken, W_1 = starting weight of tanning material (g), W_2 = final weight of residue after drying (g)

Determination of tannins

Calculation of the tannins was done as the difference between TSS and the NT as described in the literature [40].

Determination of pH

Hanna pH meter (Model 2221) was used to measure the pH of the extract solution. A two-point calibration was done using 25 mL of two buffers for each [28,32]. Afterwards, the pH of the tannin extract solution was measured by inserting the electrode probe into it until a stable value was recorded [32].

Spectroscopic analysis of tannins

Fourier transform infrared spectroscopic technique using a Jasco FT-IR model 4700 was done to aid in the identification of functional groups of the samples. This was performed according to the literature [24,41,42].

RESULTS AND DISCUSSION

The extract yield obtained from the tamarind seed using a water medium was $18.53 \pm 0.89\%$. From the literature, Singh et al. (2011) reported a yield of 18.39% w/w for the extraction of gum from tamarind seed using a water-based method. However, selecting the right extraction methods, the right solvent, and adjusting variables like time and temperature are crucial for maximizing the extraction yield [33]. To maximize the plant materials' "bioactive chemicals," cutting-edge, unconventional extraction techniques including irradiation and ultrasound-assisted extraction have been successfully used [34]. From the phytochemical screening (qualitative analysis), sample extract and control results are presented in (Table 1) below. The reactions for Tamarind seed extract (TSE) and control tannins samples solutions were similar to those determined in the literature [24].

Table 1. Phytochemical analysis of tamarind seed extract and control

Phytochemical	Test	Colour	TSE	Mimosa (control)
Tannins	Ferric III chloride	Green/blue black	+	+
Tannins	Copper II Sulphate	Green colour	+	+
Flavonoids	Lead acetate	Pink/Yellow	+	+
Condensed Tannins	Potassium Hydroxide	Red	+	+
Hydrolysable Tannins	Potassium Hydroxide	No Change	-	-

KEY: + Present, - Absent

From the results, it was observed both the sample extract solution and control confirmed the presence of tannins [24]. Upon addition of aqueous copper sulphate to sample extract and control, greenish precipitates of tannins were observed. This further confirmed the presence of tannins as the copper sulphate solution faded into a green solution. In addition to aqueous lead acetate to sample extract

and control, pinkish-yellow precipitates in the solution were observed [46]. This indicated the presence of flavonoids that are phytochemical and are precipitated by lead acetate solution [19,31].

To differentiate the type of tannins (condensed or hydrolysable), drops of aqueous Potassium Hydroxide were used. Red precipitates were formed by TSE which was similar to mimosa and hence confirmed to be condensed tannins [35].

Total phenol content

Folin-Ciocalteu is an improved reagent over the Folin-Denis method of total phenol quantification. The reduction of the reagent causes phenolic ions to turn the colour blue [33,36,45]. The higher the phenolic compounds in an extract, the darker the colour would be and hence higher absorbance [37]. From the results, the tamarind seed in the study had total phenolic content of $18.79 \pm 0.14\%$ which was equivalent to 187.9 mg/g Gallic acid equivalent (GAE), while the control had a total phenolic content of $59.45 \pm 0.93\%$ (594.5 mg/g GAE). This was higher than earlier research that showed the tamarind seeds contained phenolic content of 120.63 mg per gram of dry matter of tamarind seed husk. In another study, it was found that *Tamarindus indica* L. had 365.12 mg/g GAE [19]. Lower levels of phenolic contents were found in the tamarind fruit; with seed levels of 19.21 ± 0.29 g GAE/100 g and fruit levels of 2.14 ± 0.05 g GAE/100 g [22]. The differences could be a result of variations in the geographical habitat of plants, harvest time, climatic changes from season to season as well as the extraction method [38].

Quantitative analysis

To determine tannin content and tanning strength; moisture content, total solids, and total soluble solids amongst other parameters, were quantitatively examined in accordance with the method outlined in the literature [29]. Determination of tannins by filter method (hide powder) had been earlier studied and proved to be suitable for tannin quantification before use for conversion of raw hides/skins into leather [39,40]. The results for tamarind seed extract and control are depicted in Table 2 below.

Table 2. Quantitative analysis of tamarind seed extract and control

Parameters	Tamarind seed extract	Mimosa
Powdered Moisture content (%)	8.40 ± 0.33	7.14 ± 0.24
Powdered Total Solids (TS) %	91.60 ± 0.33	92.86 ± 0.24
Total Soluble Solids (TSS) %	23.89 ± 0.14	87.41 ± 0.17
Non-Tannins (NT) %	8.75 ± 0.10	28.50 ± 0.16
Tannins (T) % = (TSS – NT)	15.14 ± 0.93	58.91 ± 0.16
Tanning Strength = (T/NT)	1.73	2.06

Parameters	Tamarind seed extract	Mimosa
Purity Ratio = (T/TS)	0.63	0.67
pH	4.57	4.58
Total phenol (%)	18.79 ± 0.14	59.45 ± 0.93

The moisture content of tamarind seed powder and control were $8.40 \pm 0.33\%$ and $7.14 \pm 0.24\%$ respectively. The Tannin content for TSE was established to be $15.14 \pm 0.93\%$ while the non-tans were 8.75 ± 0.10 . Mimosa showed a higher tannin content of 58.91 ± 0.16 and non-tans of 28.5 ± 0.16 . Non-tans are important as they help in the solubilisation of the tans within the tan liquor. The ratio of tannin to non-tan was 1.73 and 2.06 for TSE and mimosa respectively. This means that the tannins were more than non-tannins since it was > 1.5 required value [29]. According to earlier research, the tannin content of *A. nilotica* and *A. seyal* leaves were 11.80% and 6.30% respectively, while those of *A. senegal*, *A. nilotica*, and *A. seyal* bark were 12.15%, 10.47% and 3.49% respectively [13]. The purity ratio of tamarind seed extract and control were 0.63 and 0.67 respectively. The pH of TSE extract was 4.57 while that of the mimosa was pH 4.58. This was within the range of an ideal vegetable tanning agent [3]. However, the pH of the tannins affects the tan liquor directly i.e. Lower pH below 4 results in rapid fixation of tannins on the leather surface. This is so-called surface tanning and is due to the formation of phlobaphenes [21]. The iso-electric point of leather is when the positive charges and negative charges are at equilibrium. This allows tannins to have better penetration i.e. above pH 4 and less than pH 6 [29].

Fourier transformation infrared spectroscopic study

In the study, the FT-IR spectrum was able to identify the functional groups that would cross-link with the collagen. Figure 2 below is the FT-IR spectrum obtained from *Tamarindus indica L.* seed tannin performed in the outlined range [24].

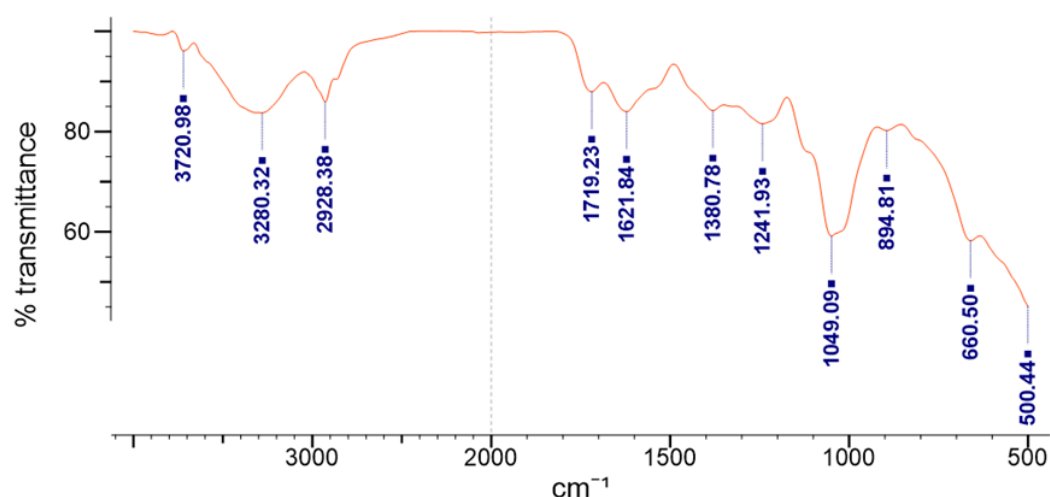


Figure 2. FT-IR Spectrum of *Tamarindus indica* L. seed tannins extract

The FT-IR spectrum obtained showed intensified spectra between the ranges of 1800 cm^{-1} to 1000 cm^{-1} . These are wave numbers of the phenolic molecules' absorbance [33]. Broad Peaks positioned at 3280.32 cm^{-1} and narrow peaks at 3720.98 cm^{-1} could be associated with stretching vibrations of O-H which are physical characteristics of tannins due to their poly-phenolic structure [41]. The peak at 2928.38 can be assigned to C-H₂ stretching vibration of aliphatic hydrocarbon structures which represents carbohydrates and sugar derivatives [33,41,42]. Ester groups associated with C=O stretching in the tamarind seed extract are at absorption band 1719.23 cm^{-1} .

Due to the high degree of polymerization, the aromatic rings' C=C stretching vibration can be attributed to the band at 1621.84 cm^{-1} [43]. The bands at 1380.78 and 1241.93 could be assigned to condensed tannins due to C-O asymmetric stretching ester [42,44]. The absorption bands present at region 894.81 cm^{-1} and 1049.09 cm^{-1} were cyclic ethers caused by stretching vibrations [42].

CONCLUSION

From the study, the selected tamarind seeds from Kathagara farm in Tharaka-Nithi County were of a condensed type and with a tannin content greater than 10%. The tanning strength was 1.73 for tamarind seed extract and 2.06 for mimosa. This was above the standard value (> 1.5) and both had a purity ratio (> 0.5). These findings provide insights into the likely use of these tannins for leather processing. This would ultimately add value to the seeds which are rendered as waste within Kenyan communities and by-products of pulp industries.

Further studies should be carried out to determine the variation in tannin content of tamarind species at different maturity stages to determine their suitability as a source of tannins to alternate mimosa tanning material.

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