Trop J Nat Prod Res, November 2024; 8(11): 9086 - 9096 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Methods for Quantification of Tannins and Other Polyphenols in *Syzygium cumini* (L.) Bark for Potential Use in Leather Tanning Colouration

Gilmar K. Henar[1], Olayinka Oderinde[2]*, Gerda Wesenhagen[1]*, Nykieta A. James[1], Fauz Sawirjo[3], Dayo A. Ayeni[2], Olubunmi A. Ogundiran[2,4].

[1]*Department of Chemistry, Anton De Kom University, Paramaribo, Suriname.*
[2]*Department of Chemistry. Faculty of Natural and Applied Sciences, Lead City University, Ibadan, Nigeria.*
[3]*Central (Chemical) Laboratory, Bureau of Public Health, Ministry of Health, Paramaribo, Suriname.*
[4]*Department of Chemical Sciences, Faculty of Sciences, Taraba State University, PMB 1167, Jalingo, Nigeria.*

ARTICLE INFO

Article history:
Received 02 October 2024
Revised 12 October 2024
Accepted 07 November 2024
Published online 01 December 2024

ABSTRACT

Most tanneries still use chromium as a tanning agent, specifically Cr(III), because of the their hydrothermal stability and excellent physical properties. However, the use of these synthetic dyes generates waste that is hazardous to the environment, especially the oxyanions of chromate and some Cr(VI) salts. Due to this, there is a growing interest in natural dyes that produce environment-friendly products, hence the search for vegetable tannins. This study attempts to determine whether the tanning parameters of *Syzygium cumini* (L.) (*S. cumini*) bark are sufficient to add to the list of the known vegetable tanning sources. To assess this, a methanolic extract was analysed for the total polyphenol content (TPC), tannin content, and tannin-to-non-tannin ratio. For the measurement of TPC, a colourimetric method utilising Folin-Ciocalteu reagent and pyrogallic acid reference solution was employed. Quantification was done using UV-Vis at 760 nm, and this revealed a TPC of 48.6%. Also, the method validation was tested and proven suitable, with an acceptable linearity from 12.81 – 27.45 µg/mL, and a LOD of 0.44 µg/mL. Moreover, the tanning strength based on the tannins/non-tannin ratio was determined via the total soluble content (TSC) and Stiasny number of the extract, with the assay showing an acceptable tanning strength of 3.21 for *S. cumini* (L.), with high non-tannin content. These results indicate the potential viability of using the *S. cumini* (L.) bark extracts for tanning purposes, hence useful as a viable vegetable tanning material for the potential production of chromium-free leathers from sustainable resources.

***Keywords*:** Vegetable tanning, Total polyphenol content, *Syzygium cumini*, Non-tanning content, Stiasny Index, Natural dye.

Introduction

The imperative for progress in chemical-, agricultural-, textile-, and other industries has precipitated significant environmental challenges. The extensive use of synthetic chemicals has resulted in the contamination of water and soil, with dyes, employed for colour processing and diverse industrial applications, emerging as substantial contributors to water pollution.[1,2] Approximately 90% of global leather production relies on basic chromium sulfate as the primary tanning agent.[3–5] However, the adverse environmental repercussions associated with its tanning byproduct, hexavalent chromium, Cr(VI), underscore the need for sustainable alternatives. Cr(VI), a non-biodegradable and persistent environmental pollutant, is recognized for its carcinogenic-, mutagenic-, and allergenic properties, posing significant health risks to living organisms.[6] According to Agency for Toxic Substances and Disease Registry of the U.S. Department of Health and Human Services, an exposure limit of 0.5 mg/m^3 chromium as chromium metal and Cr(II) and Cr(III) compounds averaged over an 8-hr work day is recommended, while a limit of 0.001 mg/m^3 for Cr(VI) compounds averaged over 10-hr work day.[7]

In response to these environmental concerns, vegetable tannins have emerged as a highly recommended alternative.[8] Plant extracts, deemed safe and renewable, offer promising natural chemical reagents, making them ideal for application as industrial chemicals.[9] These sustainable green tannins present an eco-friendly option, particularly when sourced and extracted from plants using green chemical processes.

Polyphenols represent a broader category of secondary plant metabolites with diverse subgroups, such as tannins, flavonoids, and phenolic acids.[10,11] They are naturally synthesised through plant secondary metabolism and are identified in human nutrition. These compounds stand out as the most prevalent phytochemicals derived from plants.[12–14] Polyphenols gained prominence for their antioxidant capabilities, which play an significant role in protecting cells against oxidative stress and mitigating the risk of chronic diseases.[15] Tannins, on another hand, classified as polyphenolic compounds, renowned for their astringency and protein-binding capabilities, have long been a staple in the leather industry for treating hides and imparting colour.[16] Their phenolic structure opens avenues for diverse applications such as wood adhesives,[17] insulating materials,[18] wastewater treatment,[19] mineral processing, [20] and green biochemical exploration.[21,22] For example, China et al.[23], condensed tannins were extracted from *Tessmannia burttii's* stem and root barks, presenting a promising environmentally friendly alternative to chromium salt, and contributing to sustainable leather manufacturing. Carlqvist et al.[24] on another hand,

*Corresponding author(s). E mail: yinkaoderinde@yahoo.com; wes10gerda@gmail.com,
Tel: +2348032801872; +597-7614425

Citation: Henar GK, Oderinde O, Wesenhagen G, James NA, Sawirjo F, Ayeni DA, Ogundiran OA. Methods for Quantification of Tannins and Other Polyphenols in *Syzygium cumini* (L.) Bark for Potential Use in Leather Tanning Colouration. Trop J Nat Prod Res. 2024; 8(11): 9086 – 9096 https://doi.org/10.26538/tjnpr/v8i11.17

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

developed cationised tannins from Norway spruce bark, which were adopted as renewable flocculants in wastewater treatment.

Condensed tannins, predominantly sourced from mangrove species, wattle, and quebracho, particularly from their bark, stems, roots, and leaves, are the primary commercial components extensively employed in leather tanning within tanneries.[25,26] Most locally used vegetable tannins lack comprehensive characterisation, hindering scientific insights critical for identifying and commercialising potential green tannin sources to boost their supply. This study focuses on *Syzygium cumini* (L.) (*S. cumini*), colloquially known as Jamun, a tropical evergreen tree indigenous to the Indian subcontinent and Southeast Asia, with widespread dispersion into numerous countries in Africa and Latin America. Beyond its cultural and culinary significance, the bark of *S. cumini* has drawn attention for its rich bioactive compounds, including tannins and polyphenols.[27] Scientific investigations into the traditional use of *S. cumini*, particularly in diabetes and inflammation, have highlighted its potential medicinal properties.[28]

Tannins are natural dyes which are easily obtained from vegetables and other plants and have found applications in various fields. For example, Surana et al.[29] extracted natural dye from the fruit of *S. cumini* after which its optical and electrochemical properties for the development of dye sensitised solar cell were studied, while Periyasamy, A.[30] utilised natural dye extracted from *S. cumini* fruits for the dyeing of cotton fabric and Mariselvam et al.[31] extracted natural dyes (tannins) from *S. cumini (L.)*, which showed good antibacterial activity, and thereafter used in developing antimicrobial fabric. Furthermore, Shamsheer et al.[32] extracted ecofriendly dyes from bark of *S. cumini* and applied it directly for the dying of leather, however, there is no report of the quantification and characterisation of the tannins and other polyphenols in *S. cumini* for potential application in these leather tanneries. While literature suggests the bark's richness in tannins and polyphenols, quantification and characterisation of these components are essential for unlocking its full potential. This work employs various analytical techniques to quantify and characterise tannins and other polyphenols in *S. cumini* bark, providing valuable insights into its suitability as a source of vegetable tannins for traditional and contemporary applications, which will thereafter be added to the growing number of renewable bioresources of dye and derivatives which are used in leather and other colouration purposes.

Materials and Methods

Chemicals

All solvents were of chromatographic grade (> 99%) and all chemical standards were of analytical grade (> 95%), while double distilled water was used. Methanol (≥ 99.9 % ACS reagent was procured from Merck, USA, and Folin-Ciocalteu's phenol (FC) reagent obtained from Merck, KGaA, while formaldehyde (37 % ACS reagent, 10-15% methanol) and pyrogallol solution (≥ 98 % HPLC grade) were both purchased from Sigma-Aldrich. Moreover, hydrochloric acid, HCl, (38 % ACS reagent) was obtained from Mallinckrodt Pharmaceuticals, USA, as sodium carbonate, Na_2CO_3 (ACS grade) was procured from *Fisher Chemicals*, USA.

Syzygium cumini (L.) (*S. cumini*) *Bark Sampling*

The bark samples of *S. cumini* were collected at a nearby forest of Paramaribo City located at latitude 5.82 and longitude -55.21, on December 12, 2022, while the climate and observations of the surroundings during the collection were noted. Identification of the plant species was done by Dr. Iwan Molgo and a voucher specimen (number UVS19822) was deposited in the the National Herbarium van Suriname (BBS), Paramaribo, Suriname. The outer bark of *S. cumini* was removed to reveal the dark brown and slightly red inner bark, which was then stored inside air-tight plastic bags to prevent polyphenol degradation from oxygen and light. Thereafter, the inner bark was thoroughly cleaned and then air dried using a dehumidifier, according to previous reports.[26,33] Afterwards, extraction using methanol from the ground bark was carried out according to a previous study, [34] followed by analysis for total solids (TSC), Stiasny index (SI), total polyphenols (TP), and analyte concentration.

Crude Extract (CE) Test Sample

To prepare test samples, a volume of CE (3 mL) was diluted to 100 mL in distilled water, and used as the stock solution, from where an aliquot (1 mL) was used to prepare working solutions similar to the SWS.

Determination of the Total solids (TS), Stiasny Index (SI) and Total polyphenols (TPs) and Analyte concentration

For the total solids content, three 10 mL (V_1) samples were taken from the crude extract and subsequently evaporated in a water bath until a solid residue was obtained (W_2), followed by drying to a constant weigh. The TSC (%) of the CE was determined using the Equation 1.

$$\frac{W_2}{W_1} \times \frac{V_1}{V_2} \times 100\ \% \qquad \text{Eqn. 1}$$

Where; W_1 = sample weight (g); W_2 = weight residue after drying (g); V_1 = volume of extract fraction. This procedure was done in five replicates using separate extracts.

For the SI, 5 mL of three samples each of the CE were taken and concentrated to about 1 mL. According to Sousa et al.,[34] the Stiasny index can be determined by firstly reacting the concentrated extract with 10 mL of distilled water, 4 mL of formaldehyde, and 2 mL of 38 % hydrochloric acid (which acts a catalyst) for the precipitation reaction. Thereafter, the mixtures were heated for 35 min under reflux, resulting in the formation of red-brown precipitate which was subsequently collected in a crucible of known weight under vacuum filtration. The solid residue obtained (tannin) was dried in an oven at 105 °C until mass stabilisation. At this point, the red-brown precipitate has turned into black flakes. The Stiasny Index (%) was then determined by calculating the ratio between tannin mass and the mass of total extractives, converted to a percentage according to the Equation 2.

$$S\% = \frac{A}{B} \times 100 \qquad \text{Eqn. 2.}$$

Where; S (%) = Stiasny index; A = dry weight of the solid; B = dry weight of extracts in 5 mL of the filtrate. This procedure was conducted alongside the total solid measurements by taking separate fractions of each prepared extract.

For the TPs, this was determined from a different CE prepared in five replicates, using the procedure as previously described.[35]

Furthermore, for the analyte concentration, a preliminary estimate in the test sample was obtained by preparing two calibration standards. All standards working solutions (SWS) were prepared from an analytical grade pyrogallol solution (49.1 mg dissolved in 100 mL). The concentration range of the calibration curve was determined by first identifying the expected concentration of polyphenols with a two-point linear regression equation. The marginal concentration values selected were 1 µg/mL and 10 µg/mL.[36,37] Each SWS was prepared from independent stocks, while, aliquots (0.5 and 5 mL) of pyrogallol solution were diluted to 10 mL in distilled water. To prepare the working solutions, Folin-Ciocalteu's phenol (FC) reagent (5 mL, 10% v/v) was added to an aliquot of stock (1 mL) after which it was thoroughly mixed, to form a clear green mixture. Afterwards, sodium carbonate was added (4 mL, 7.5 % w/v) [26] and the mixture was diluted to 25 mL (pH ≈ 13) in distilled water, followed by an incubation time of 30 min.[36] The addition of sodium carbonate turned the green mixture to dark blue almost immediately, and the quantification was done using UV-Vis spectrophotometer (Fischer Scientific Genesys 10S, USA) at 760 nm. Finally, the regression equation $y = ax + b$ obtained from the polyphenol concentration (x in µg/mL) and the absorbance (y) plotted in Excel were used to obtain the estimated analyte concentration. From the estimated analyte concentration, another series of five SWS were prepared so as to construct a multiple-point fitted calibration curve. To prepare these SWS, different volumes of pyrogallol solution (aliquots of 0.80 mL, 0.96 mL, 1.87 mL, 2.68 mL, and 2.90 mL) were diluted to 10 mL distilled water to make independent stock solutions. Of these stock solutions, an aliquot (1 mL) was used to prepare the SWS. The addition of Folin-Cioclateu reagent resulted into the formation of a dark green-blue solution even before the alkali was being added. The final concentrations were 1.60 µg/mL, 1.92 µg/mL, 3.75 µg/mL, 5.37 µg/mL, and 5.81 µg/mL. These standards were measured 18 h after preparation,

and storage was done at 25±1℃ in the absence of light. The total polyphenol (%) expressed as pyrogallol equivalents were calculated according to the Equation 3.

$$\text{Total Polyphenols (\%)} = \frac{c \times V}{m} \quad \text{Eqn. 3}$$

Where; *c* is the average concentration obtained from substituting test sample absorbance values into the calibration curve equation, V is the volume of sample solution (mL), and m is the mass of sample (g).

Method validation for linearity, precision, accuracy and limits of detection and quantification

The linearity was assessed across the working range of results produced by the Folin-Ciocalteu method with acceptable certainty. A series of test samples spanning the range from 0.7 to 1.5 times the expected analyte concentration was prepared to establish the linearity. The volumes of stock samples measured are 0.7 mL, 0.9 mL, 1 mL, 1.2 mL, 1.5 mL for which the working solutions are prepared similarly to the procedure previously described. The final concentrations according to the dry weight of the stock were equal to 11.35 µg/mL, 14.60 µg/mL, 16.22 µg/mL, 19.46 µg/mL, and 24.33 µg/mL respectively, with only the 16.22 µg/mL being prepared in triplicates. Visual inspection of the plotted absorbance signals as a function of the analyte concentration following the statistical analyses was used to evaluate the linearity of the proposed method. To confirm this, the use of ordinary least square method was justified by testing: (i) the normality (ii) the homoscedacity (iii) the autocorrelation of the residuals.

According to the Committee for Human Medicinal Products,[38] the precision of the analytical procedure is determined by using quality control (QC) samples at different concentration levels that quantify the extent of agreement of the replicate measurements with one another. QCs are independent from the calibration standard samples, meaning a different ES solution was prepared. Aliquots of ES analysed are 0.7 mL, 0.8 mL, 0.9 mL, 1 mL, 1.2 mL and are measured similarly to procedures previously described. The final weight of solid extractives equated to 18.7 mg, 21.4 mg, 24.1 mg, 26.8 mg, 32.1 mg respectively. The intra-day precision (repeatability) was assessed by measuring these 5 QCs on the same day twice and the inter-day precision was determined by analysing them in 3 analytical runs over two days. The coefficient of variation (CV) for each concentration level was determined according to the Equation 4.

$$\text{CV}(\%) = \frac{\text{SD}}{\text{Mean}} \times 100 \quad \text{Eqn. 4}$$

A CV over 15 % is considered unacceptable.[36]

Moreover, the analytical procedure's accuracy was tested through statistical analysis of QCs that mimics the study samples. These was prepared by spiking the matrix with a known quantity of analyte and was independent from the calibration curve standard samples. However, because the matrix and analyte are not exactly known, the preparation of a separated matrix is not possible. Thus, the ES itself can be utilised as QC samples. Different concentration levels across the range of the analytical procedure were evaluated, i.e. a solution without spike, low concentration (LC, 1 mL of spike solution) and high concentration (HC, 3 mL spike). The spike solution (40 µg/mL) was prepared from pyrogallol solution and added to aliquots (1 mL) of ES, as triplicates at each concentration level were prepared. Similar to the previously stated, the addition of FC and sodium carbonate solution following measurement at 757 nm allowed for the absorbances of the QCs was used to calculate the percentage (%) recovery of the analytical procedure, according to the Equation 5.

$$\text{\% recovery} = \frac{A \cdot 100}{A_T} \quad \text{Eqn. 5}$$

Where; A = absorbance of sample after addition of the standard; A_T = theoretical absorbance calculated. The method is considered accurate if the recovery percentages are between 85% and 115%.[36]

Furthermore, the limits of detection (LOD) and quantification (LOQ) were calculated from the standard deviations of the y-intercept (σ) and the slope (S) of the curve constructed from the linearity and the slope using Equations 6 and 7, with a confidence interval of 95% according to the ICH Q2 (R1) method validation guideline.[37]

$$\text{LOD } \left(\frac{\mu g}{mL}\right) = \frac{3.3\sigma}{S} \quad \text{Eqn. 6}$$

$$\text{LOQ } \left(\frac{\mu g}{mL}\right) = \frac{10\sigma}{S} \quad \text{Eqn. 7}$$

Results and Discussion

Moisture Levels of Dried Bark

After collecting the bark, the moisture content of the samples was determined gravimetrically by drying. At an average relative humidity (RH %) of 69 % ±7 % and temperature of 55 ℃ ±3 ℃ with variation based on the weather reading, the bark samples were observed to have lost an average of 11.39 g of moisture which is equal to an average moisture content of 51.47 % (± 3 %) over a drying period of 48 h.

Determination of Tannin and Non-tannins

Total Soluble Content (TSC) and Stiasny Number (SN)

Since the tanning strength of a vegetable tanning materials is judged based on the tannins/ non-tannins ratio of the tanning liquor, the determination of TSC in *S. cumini* bark was done by evaporating the extract until the solid residue was obtained. Table 1 shows how the mass of the residue along with the aliquot taken from CE (mL) and the volume of solvent used for the extraction (mL) were to determine TSC (%) (Eqn. 1), while the SN was calculated using the weight of phlobaphenes along with the TSC (%) of the sample analysed (Eqn. 2). The results were then plotted to inspect the data as well as identify the possible outliers. Possible outliers were detected and removed by median absolute deviation (constant *b* = 1.4826, threshold = 3) or MAD outlier test, as shown in Fig. 1 (a) and (b). Values outside the control limits (CL) warranted the inspection of the results obtained from other fractions on the same day. For the TSC data, two observations (observations 11 and 12, highlighted in red) were identified as outliers while the others did not exceed the limits. Further inspection of the observations showed that there were no anomalies in the extraction phase, thus can be assumed to some form of human error during weighing which played an important role. On the other hand, the SN data shows an example where the extract of a specific day (observations 1–3) all revealed significant deviation from the mean. Although only one of those observations exceeded the limit, it is therefore safe to assume the errors occurred during extraction phase and all the three observations have to be removed. The variability of the data can be attributed to the multiple factors. First, the temperature of the environment has a significant impact on the completion of the phenol-formaldehyde condensation reaction, also known as the formation of phenolic resins. This reaction is highly temperature-dependent due to its mechanism and the thermodynamics involved in its chemical kinetics.[39] Also, the exposure to light during the experiment and subsequent degradation of soluble polyphenols also play a critical role,[40] as the sample fractions taken for test are relatively small. Hence, this indicated that the most likely influence would be random error. The distribution of the TSC data was plotted in a graph by frequency (Fig. 2(a) and (b)). This depicts a right skewing or asymmetrical distribution of the data. A right skew indicates a mean (20.86 %) larger than the median (16.74 %). Similar findings of non-normality were observed in the SN data. When presented with non-normal continuous data, transformations are often applied to increase the suitability of the statistical analysis.[41] The log transformed data as shown in (Fig. 2(a) and (b)) reduced the spread (log transformed SD = 0.1) of the data but not skewness. Furthermore, the mean for the log-transformed data gives statements about medians for the original data. This validates the use of median and range to present the centre and spread of the TSC (median 16.7 % and values with a range from 14.9 % – 29.7 %) and SN (median 73.7 and values with a range from 61.3 − 99.40 %), being illustrated in terms of the first and third quartile as shown in Fig. 2(c).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

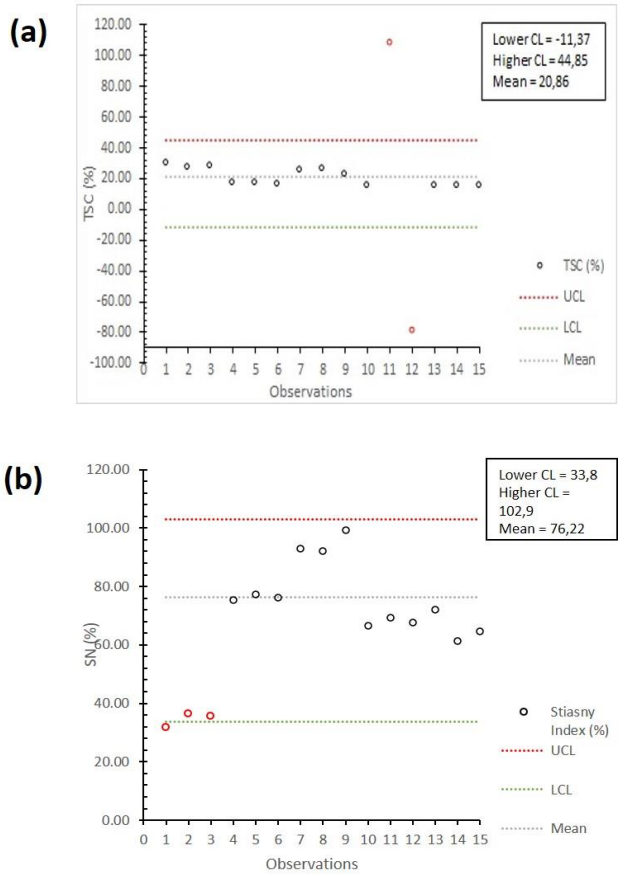

Figure 1: Identification and confirmation of outliers in *S. cumini* (L.) for (a) TSC data using MAD outlier test; (b) for SN data using MAD outlier test.

Evaluation of the TSC and SN Data

Stiasny indices (SI) were found varying between 61.3 % and 99.4 % with a median value of 73.7 %, while the TSC values obtained have a range of 14.9 % to 29.7 % with a median value of 16.7 %. An experiment using similar procedures found extractive yields in other common tannin producing trees ranging from 33.7 % to 50.2 % and a Stiasny value ranging from 70.4−88.0 %,[42] while SI for tannin containing plant extracts ranging from 65.4−102 % was also previously reported.[43] The literature indicates, as outlined in Table 2, that the TSC of *S. cumini* bark was found to be lower in comparison to other high polyphenol containing plants. Conversely, the SI values was shown to be within the ranges found for other plant species.

Determination of TPC and Validation of the Method

TPC Calibration Curve Using Pyrogallol

Evaluation of the observations

The concentration of TPC was determined using a calibration curve (Fig. 3a) and a linear plot with an R^2 value of 0.9906 was obtained. Before this calibration was used to determine the TPC, the linearity was assessed through visual examination of the *x-y* plot, visual inspection of the residuals of the calibration curve, and testing different assumptions corresponding to the linearity of the residuals. To identify the points of influence as well as issues with the experimental design, the residuals were plotted and inspected visually (Fig. 3b and 3c). A point of influence can cause either a leverage or bias effect on the line, while an uneven distribution of the calibration levels could lead to an increased bias effect from the center point, or leverage effect from the extremes. A disproportionate effect from one point due to uneven distribution can cause relatively small errors in the observed response to propagate into more significant errors in the position of the regression line.[44,45] Visual inspection of the calibration curve suggests that the levels weigh more heavily on the left part of the line, resulting in a leverage effect from the right extreme. A solution to this would be to have added 1 more level at around 7.5 µg/mL.

Conducting residual analysis of the linear model in Rstudio allow problems in the visual inspection to be confirmed (Fig. 3c). Although it is more difficult to identify randomness in small data sets, the residual versus fits plot can detect non-linearity, unequal variances, and outliers. The plot shows the deviation from the fitted line being relatively small, with the largest standardised residual being 1.5 from the highest concentration, as shown in Fig. 3c. Normally, an observation with a standardised residual larger than 3 is deemed to be an outlier,[46,47] thus indicating that there are no outliers. However, the residuals are not able to confirm assumptions such as linearity or normality. To accomplish this, elaborate statistical tests must be utilised such as the quantile-quantile normality plot.[48,49]

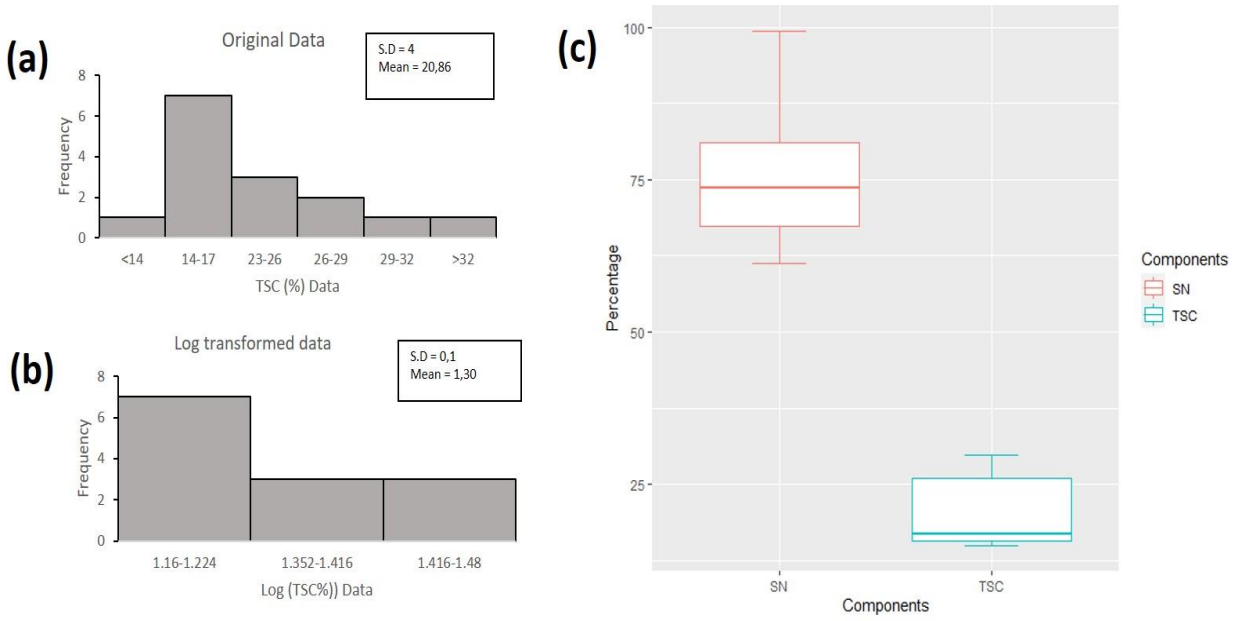

Figure 2: (a)-(b) Skewed frequency distribution of *S. cumini* (*L.*) TSC data; **(c)** Presentation of median and range *S. cumini* (L.) TSC and SN data in terms of the first and third quartile

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Table 1: TSC (%) and SN (%) determined for *S. cumini* bark methanolic extract

Parameter	Day 1			Day 2			Day 3			Day 4			Day 5		
Sample grams (W1)	10.11			10.01			10.20			10.01			10.02		
Residue grams (W2)	0.2003	0.1831	0.1922	0.1114	0.1117	0.1108	0.0856	0.1765	0.1568	0.0997	0.7204	-0.5225	0.1037	0.1037	0.1041
(W2/W1)	0.01980	0.01810	0.01900	0.01113	0.01116	0.01107	0.00839	0.01731	0.01538	0.009963	0.07199	-0.05221	0.01035	0.01035	0.01039
TSC % (W2/W1)*(V1/V2) (%)	29.71	27.16	28.51	16.70	16.74	16.61	25.18	25.96	23.06	14.94	107.99	-78.32	15.52	15.52	15.58
Phlobaphenes (g)	0.0305	0.0350	0.0343	0.0419	0.043	0.0424	0.0648	0.0644	0.0694	0.0332	0.0345	0.0337	0.0374	0.0318	0.0336
Stiasny Index (%)	31.79	36.48	35.75	75.29	77.27	76.19	92.81	92.24	99.40	66.60	69.21	67.60	72.04	61.25	64.72

Table 2: Comparison of TSC (%) & SN (%) results to literature

Species	TSC (%)	SN (%)	Ref.
Acacia	13 - 49.7	-	[59]
Acacia	33.7 - 50.2	70.4 – 88	[42]
R. mucronata, Quebracho	-	65.4, 102	[43]
S. cumini	14.9 - 29.7	61.3 – 99.4	This work

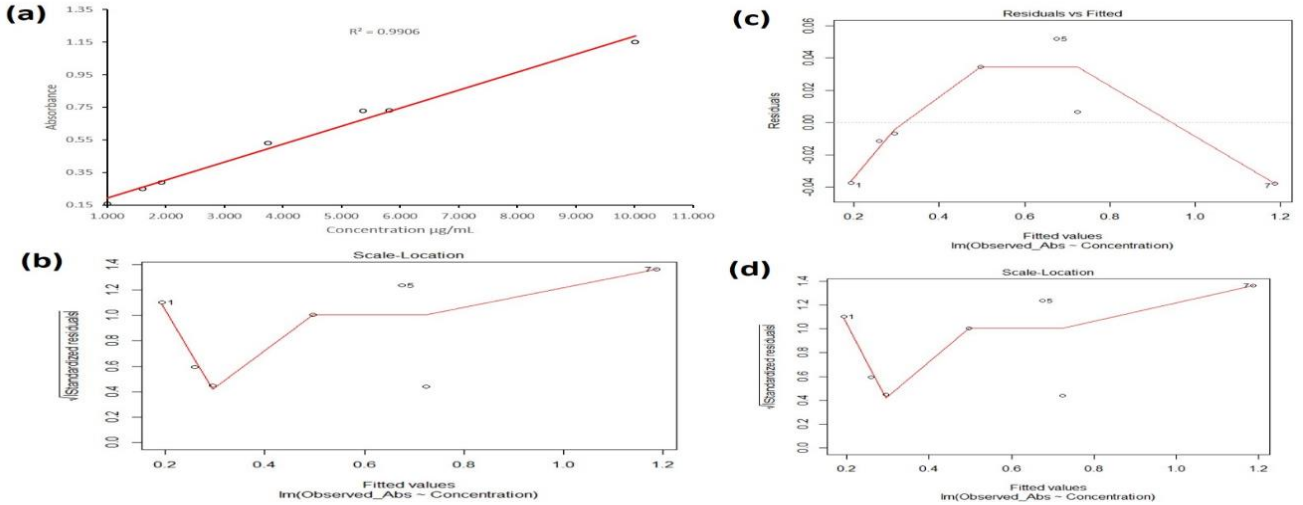

Figure 3: (a) Visual inspection of calibration curve (absorbance vs concentration); **(b)** Residuals analysis plot against fitted observations for calibration standards; **(c)** Standardized residuals analysis plot against fitted observations for calibration standards; **(d)** Residuals analysis of the normal quantile-quantile plot to assess linearity.

If the normal quantile-quantile (QQ plot) plot of the standardised residuals (Fig. 3d) is analysed, the assumption of normality can be confirmed based on its approximately linear relationship. If a normal probability plot of the residuals is approximately linear, we proceed assuming that the error terms are normally distributed.[46,47] Fig. 3d shows a clear linear relationship between the observations, thus confirming the assumption of normality in the data. Furthermore, ordinary least squares method relies on a number of assumptions besides normality, namely, the homoscedasticity and independency of the error terms. The results of these tests, as indicated in Table 3, confirm the assumption of homoscedasticity ($p > 0.05$) but suggests dependency in the residuals ($p < 0.05$). This implies that a generalised least squares method must be used in order to obtain the linear regression model. This method is a modification of the ordinary least squares' method and considers the equality of variance in the observations. Using Rstudio, the covariance matrix $\boldsymbol{V}$ is computed, followed by the generalised lest square estimate β,[50,51] which shows the slope and intercept of the calibration curve for the statistical data for regression line of the standard calibration curve and linearity test of TPC in *S. cumini* (L) (Table 3).

TPC results

Using the linear regression equation as shown in Table 6, a calculated average bark concentration of 7.80 µg/mL was obtained using the mean absorbance for the working solutions from *S. cumini* (L.) (0.653 ± 0.004). This was calculated using Equation 4 to a TPC expressed as pyrogallol of 48.6 % (9753 ± 42 mg % pyrogallic acid equivalents). In an attempt to evaluate the obtained TPC, it was found that these results are much higher than those obtained by Blainski and De Mello,[52] with a TPC of 23.5 % in *L. brasiliense* root extract. Also, the TPC in *S. cumini* (L.) fruit determined by Migliato and coworkers[37] showed a TPC of 27.7 % while Chamnansila et al.[53] found 268 mg.% gallic acid equivalents, with the TP levels for the bark extracts being similar to TP in fruit extract. There are two main factors that influence such a high TPC content. Firstly, the use of generalised least squares (GLS) method changes the slope and intercept of the linear regression equation drastically (ordinary least squares method OLSM $y = 0.120x + 0.0630$ while GLS $y = 0969x - 0.1031$). Furthermore, the leverage effect caused by the uneven spread of the concentration levels influences the slope of the curve, which is also suggested by the position of the observation in Fig. 4(a).

Comparing the extract with commercial tanning agents

The properties of extracts traditionally used in the leather tanning field, quebracho and mimosa, were compared to those obtained in this experiment. The properties compared are the total polyphenols %, condensed tannins as %, non-tannins as %, and the tannin to non-tannin (T/NT) ratio. This assists to indicate whether or not the proposed method and plant extract could possibly be used as an alternative to traditional vegetable tanning agents. For the comparison, the mean values were used without the spread, as comparing between median and mean is not advised. The TPC for *S. cumini* was much higher compared to quebracho and mimosa plant (Fig. 4(b)), while the condensed tannin levels are a little lower when compared to mimosa, but possess significantly higher values for the non-tannin content, in comparison to the both traditionally used plants which caused the drastic difference of the T/NT ratio. Although these results differ with traditional tanning agents. *S. cumini* still exhibited good potentials as its T/NT ratio exceeds 1.5.[26] This suggests that although the results obtained for the tannin % are lower to quebracho and mimosa, the extract from *S. cumini* bark is still suitable as an alternative tanning agent. Moreover, the abundance of the *S. cumini* plants and the relative ease with which it grows also make it appealing. Besides this, optimisations to the experimental design to prevent degradation of polyphenols could cause higher levels of tannins % to be detected. Although polymerisation of condensed tannin assays is a common method used to determine the concentration of tannins, it only provides an estimation, as the reaction is never complete depending on the conformation of its structure, as well as the type of linkage (A-type, B-type, or C-type) which have different levels of resistance to polymerisation reactions.[54] Furthermore, the bark also contains a reasonable amount (less than 5 %) of non-tannins which contain sugary substances, gums, soluble mineral salts and acids that are extracted together with the tannins, which are beneficial for producing a soft leather.[55]

Table 4: Residual analysis of linearity data for the identification of possible outliers

Observation	Predicted Y	Residuals	Standard Residuals
1	0.4728	-0.01481	-0.5912
2	0.5847	-0.01767	-0.7051
3	0.6406	0.008398	0.3350
4	0.6406	0.01540	0.6143
5	0.6406	-0.005602	-0.2235
6	0.7525	0.04454	1.777
7	0.9202	-0.03024	-1.207

Table 5: Precision for the used method of TPC determination in *S. cumini* (*L.*) samples in terms of CV(%)

Concentration level[a] (µg/mL)	Intra-day			Interday		
	Mean	Std. Dev	CV (%)	Mean	Std. Dev	CV (%)
12.8	0.471	0.0007	0.2	0.491	0.02	3
14.6	0.539	0.001	0.3	0.561	0.02	3
16.4	0.615	0.004	0.6	0.601	0.02	3
18.3	0.625	0.004	0.6	0.670	0.02	3
22.0	0.758	0.004	0.5	0.735	0.03	4

[a]Concentration determined from the volume of sample and the dry weight of extractives in CE

Linearity

It is important that the concentration range for the sample to be analysed has a linear relationship with the response. Having this range to be linear means results that are obtained will be relatively accurate and precise. To establish the linearity of the proposed method, a series of sample solutions were analysed with one being done in triplicates for use in the lack-of-fit test (Fig. 5). A linear plot was obtained with an R^2 value of 0.969. Similar to the calibration curve, in an attempt to evaluate the linearity, the following procedure was followed: (i) visual inspection of the *x-y* plot; (ii) visual inspection of the residual plot; (iii) testing different assumptions to validate the use of OLSM.

Prior to the statistical analysis, a scatter plot of the observations was visually inspected to identify possible points of influence. A clear leverage effect is caused by the upper extreme of the regression line

(Fig. 5), altering the slope. Furthermore, an examination of the change in correlation coefficients (r^2) indicates this extreme as a point of influence. Replicates of each concentration give information on the variability of the response measurements.[56,57]

The Cook distance has been used previously for the detection and removal of values with large error. Inspection of this plot (Fig. 6) confirms the upper extreme of the regression line as an influential point, but in order to be deemed as an outlier, the effect on the residual variance is further examined. To accomplish this, points of standardised residuals that are larger than 3 are subjected to outlier testing, as indicated in Table 4. Similar to the Cook's distance test, the residuals of the observations (Figure 7(a)) showed the upper extreme to be a source of error. Additionally, the 6th observation also indicates this but cannot be considered an outlier as it does not exceed the value of 3 as a standardised residual.

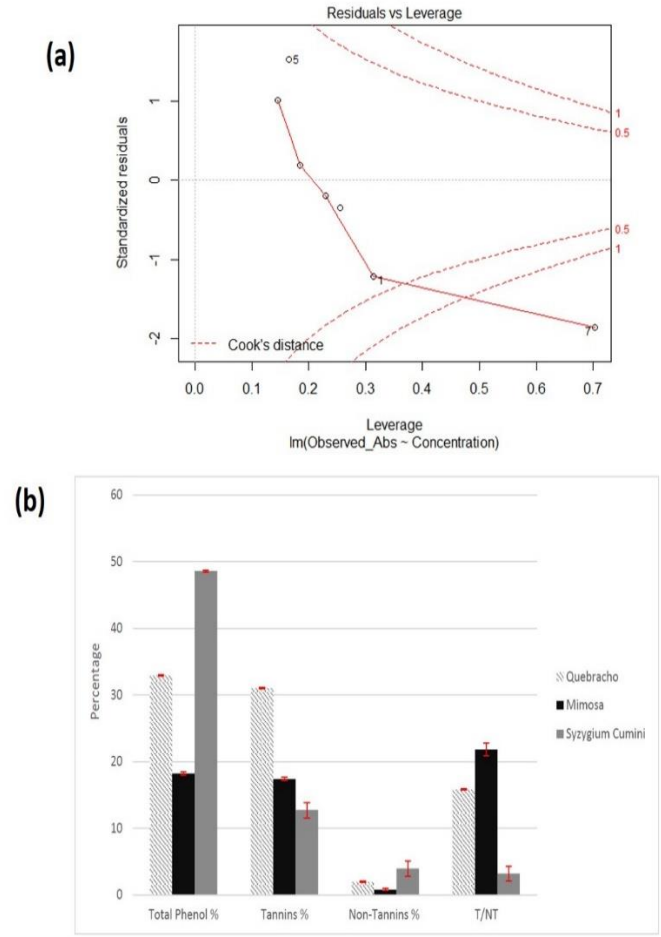

Figure 4: (a) Confirming leverage effect of observations using Cook's distance; **(b)** Comparison of tannin, non-tannin, and T/NT ratio to traditionally used vegetable tanning agents, as obtained from Moursi A. I. N.[60]

Linearity Assessment

Through the testing of different residual assumptions, the use of ordinary least square method (OLSM) can be validated.[59] These tests include (i) the normality (ii) the homoscedasticity and (iii) the autocorrelation or independency of the residuals. Furthermore, the null hypothesis of linearity for the model will be accepted or rejected based on the significance of the F lack-of-fit test. The normality at $\alpha = 0.05$ in the residual plots was confirmed by the Shapiro-Wilk's test and by visual inspection, and also the quantile-quantile plot (QQ plot), which portrays an approximate straight line. The QQ plot is shown in Fig. 7(b) along with the W statistic and the obtained p-value. The normal distribution of the residuals indicates random scattering that can also be confirmed for the residuals of the standard calibration curve (Fig. 6). The assumption of homoscedasticity at $\alpha = 0.05$ for the residuals is confirmed ($p > 0.05$) by the Breuch-Pagan test, being derived from the Lagrange multiplier (LM) test principle, which tests the variability in the residuals (Table 3). The null hypothesis for this test assumes homoscedasticity for the data, indicating that the residual variability across the concentration levels is significantly different. If a conditional heteroscedasticity is indicated, the weighted least squares method can be used for the validation of linearity.[36,58] The null hypothesis of the Durbin-Watson (DW) test at $\alpha = 0.05$ assumes that there is no serial correlation in the residuals (independency of the data). When nearing the value of 2, the DW statistic (*d*) indicates no autocorrelation and independency of the data. In the case of autocorrelated data, the generalised least square method is used to validate linearity. Since the homoscedasticity and independency assumptions residual have been confirmed ($p > 0.05$), the fit of the linear model can be tested by the F lack-of-fit test at $\alpha = 0.05$. A significant lack-of-fit ($p < 0.05$) would indicate that the linear model is inadequate and a different model would have to be used.[47] The results of the test indicate a non-significant lack-of-fit ($p > 0.05$), thus the concentration range 12.81 µg/mL to 27.45 µg/mL of *S. cumini* (L.) bark extract is linear (Table 3).

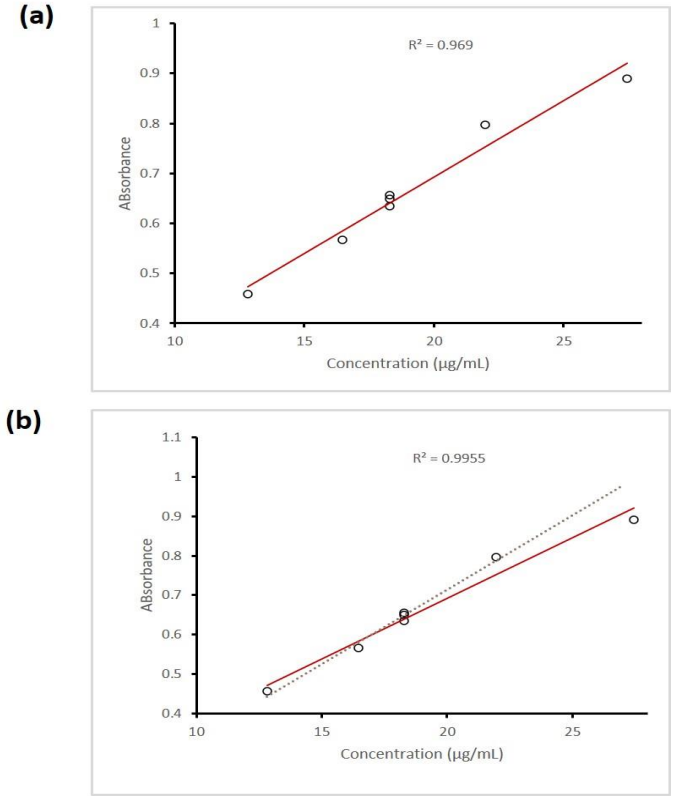

Figure 5: Leverage effect on the linearity regression line by the upper extreme: R^2, correlation coefficient

Precision

The precision is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings.[38] This can be quantified using the coefficient of variation (CV) otherwise known as the relative standard deviation, which is a measurement of the variability of the standard deviation with respect to the mean of the data. The CV quantifies how much values vary relative to the mean value and is calculated according to Equation 4. Generally, if the CV values for inter- and intraassay should exceed 15 %,[36] it means there are errors in the analysis. The repeatability and intermediate precision for the samples were measured at a concentration range of 12.8 µg/mL – 22.0 µg/ and show a CV ranging from 0.2 % to 4 % (Table 5) suggesting the analysis is precise. On top of this, the data analysis showed a significant difference between the means of the two days ($t_4 = -0.8428$, $p < .05$) indicating a rapid fading of the blue colour, although the blue colour that developed from the reduction of FC is relatively stable.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

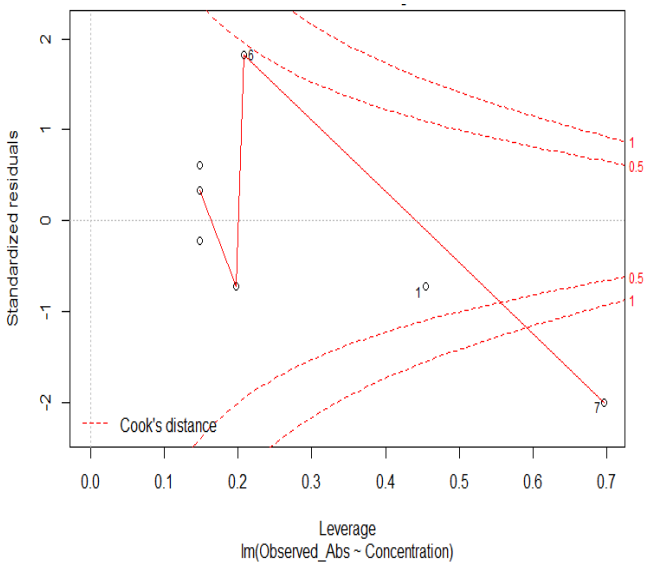

Figure 6: Linearity standardized residuals vs leverage plot showing influential points past Cook's Distance

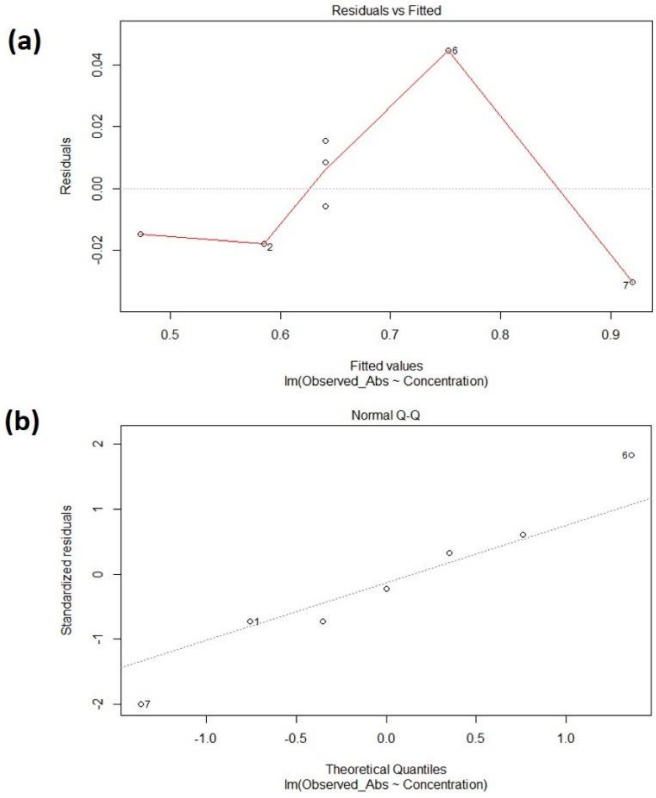

Figure 7: (a) Linearity residuals plot for detection of possible outliers, (b) Residuals analysis using normal quantile-quantile plot to asses normality: *W*, Shapiro-Wilk's statistics

Accuracy

Using the linear regression equation (Table 6), the theoretical absorbance at low spike and high spike was calculated, and the ratio between the actual absorbance and theoretical absorbance was expressed as a percentage recovery according to Equation 5. The percentage recovery varies from 103 % to 113 % with mean values being 111 % and 105 % for low spike and high spike, respectively (Table 6). Both levels fall within a range of 85-115 %, which has been reported to be the lower and upper limits for acceptable error (tolerance interval). Outside this interval, the method used is considered inaccurate. As it was observed that the addition of FC reagent caused blue-coloured oxides to form prior to alkalination, the possible causes of error are other reducing compounds such as sugars present in the sample solution. Other causes of error could be due to calibration drift, as well as the sensitivity of the spectrophotometer.

Limits of detection and quantification (LOD & LOQ)

The LOD and LOQ are parameters in method validation that indicate the degree of impurity in samples. The LOD is the lowest concentration of an analyte in a sample that can be detected, but not quantified as an exact value. The LOQ is the lowest concentration of analyte in a sample that can be quantified with suitable accuracy and precision as an exact value.[57] Although these parameters can be calculated by different methods, the method that uses the standard deviation of the response and the slope of the linearity regression line was used in this analysis. Using Equations 6 and 7 as well as the standard deviations of the slope (0.0306) and standard deviation of the intercept ($0.002 \times \sqrt{5}$) as shown in **Error! Reference source not found.** 6, the detection limit was calculated to be 0.44 µg/mL, while the LOQ was 1.46 µg/mL. These results are in line with Migliato et al.[37] who reported a LOQ of 0.64 µg/mL for the analysis of extract of *S. cumini* fruits.

Conclusion

This study explores Syzygium cumini bark extract as a potential vegetable tanning agent for leather, due to its high polyphenol content. The total polyphenol content (TPC) and tannin-to-non-tannin ratios were determined, and the described method was validated analytically, with TPC reaching higher values than commercially used quebracho (32%) and mimosa (18%). All parameters analysed for the analytical validation method using Folin-Ciocalteu reagent showed acceptable results, indicating the method's suitability for quantifying TPC in *Syzygium cumini* bark extract. The high TPC in *Syzygium cumini* suggest strong antioxidant and antimicrobial properties, which could find potential applications in other industries including cosmetics and pharmaceuticals. Furthermore, it is recommended to make methodological improvements and optimisation to obtain more consistent results for tannin content.

Table 6: Accuracy for the proposed TPC determination in *S. cumini (L.)* samples in terms of % recovery

Sample	No spike	Low spike (1.6 ug/mL)	% recovery	High spike (4.8 ug/mL)	% recovery
Unspike 1	0.656	0.76	108	1.093	108
Unspike 2	0.635	0.782	111	0.563*	55*
Unspike 3	0.669	0.799	113	1.047	103
Mean	0.653	0.780	111	0.901	105
± SD	0.02	0.02	2.8	0.03	2.95

*Indicates values statistically proven to be outliers at Q-test > 0.76

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors appreciate the National Herbarium of Suriname (BBS) for providing the literature on plant species in Suriname, as well as the Bureau Openbare Gezondheidszorg (BOG) for allowing the use of their UV-Vis spectrophotometer and Dr. J. Codrington from the Laboratory of Clinical Chemistry for the sourcing of chemical reagents used in this experiment.

References

1. Kishor R, Purchase D, Saratale G. D, Saratale R. G, Ferreira L. F. R, Bilal M, Chandra R, Bharagava R. N. Ecotoxicological and Health Concerns of Persistent Coloring Pollutants of Textile Industry Wastewater and Treatment Approaches for Environmental Safety. J. Environ. Chem. Eng. 2021; 9(2): 105012, https://doi.org/10.1016/j.jece.2020.105012.
2. Imron M. F, Kurniawan S. B, Soegianto A, Wahyudianto F. E. Phytoremediation of Methylene Blue Using Duckweed (*Lemna Minor*). Heliyon 2019; 5(8): e02206. https://doi.org/10.1016/j.heliyon.2019.e02206.
3. Ramesh R. R, Javid M. A, Ponnuvel M, Rathinam A. Sustainable Utilization of Agro-Industrial Waste for the Production of Chromium Tanning Agent through Redox Synthesis for Leather Industry: A Circular Economy Approach. Biomass Convers. Biorefinery 2022. https://doi.org/10.1007/s13399-022-03305-3.
4. Arellano-Sánchez M. G, Devouge-Boyer C, Hubert-Roux M, Afonso C, Mignot M. Chromium Determination in Leather and Other Matrices: A Review. Crit. Rev. Anal. Chem. 2022; 52(7): 1537–1556. https://doi.org/10.1080/10408347.2021.1890545.
5. Fei Y, Liu C. Detoxification and Resource Recovery of Chromium-Containing Wastes. In Environmental Materials and Waste; Elsevier, 2016; pp 265–284. https://doi.org/10.1016/B978-0-12-803837-6.00012-3.
6. Sharma P, Singh S. P, Parakh S. K, Tong Y. W. Health Hazards of Hexavalent Chromium (Cr (VI)) and Its Microbial Reduction. Bioengineered 2022; 13(3): 4923–4938. https://doi.org/10.1080/21655979.2022.2037273.
7. Wilbur S, Abadin H, Fay M. Toxicological Profile for Chromium; Atlanta Georgia, 2002. https://doi.org/10.1201/9781420061888_ch63.
8. China C. R, Maguta M. M, Nyandoro S. S, Hilonga A, Kanth, S. V, Njau K. N. Alternative Tanning Technologies and Their Suitability in Curbing Environmental Pollution from the Leather Industry: A Comprehensive Review. Chemosphere 2020; 254: 126804. https://doi.org/10.1016/j.chemosphere.2020.126804.
9. Ofosu F. K, Daliri E. B. M, Elahi F, Chelliah R, Lee B. H, Oh D. H. New Insights on the Use of Polyphenols as Natural Preservatives and Their Emerging Safety Concerns. Front. Sustain. Food Syst. 2020; 4(12): 525810. https://doi.org/10.3389/fsufs.2020.525810.
10. Rajha H. N, Paule A, Aragonès G, Barbosa M, Caddeo C, Debs E, Dinkova R, Eckert G. P, Fontana A, Gebrayel P, Maroun R. G, Napolitano A, Panzella L, Pasinetti G. M, Stevens J. F, Schieber A, Edeas M. Recent Advances in Research on Polyphenols: Effects on Microbiota, Metabolism, and Health. Mol. Nutr. Food Res. 2022; 66(1): 1–11. https://doi.org/10.1002/mnfr.202100670.
11. Kostić A, Milinčić D. D, Gašić U. M, Nedić N, Stanojević S. P, Tešić Ž. L, Pešić M. B. Polyphenolic Profile and Antioxidant Properties of Bee-Collected Pollen from Sunflower (*Helianthus Annuus* L.) Plant. LWT 2019; 112:108244. https://doi.org/10.1016/j.lwt.2019.06.011.
12. Musa A, Abu M. L, Lawal A. B, Uthman A. Soil Amendment with Graded Levels of Craseonycteris Thonglongyai Compost on the Concentrations of Some Phytotoxins in the Leaf of *Cnidoscolus Aconitifolius* (Tree Spinach). Trop. J. Nat. Prod. Res. 2022; 6(2): 265–269. https://doi.org/10.26538/tjnpr/v6i2.15.
13. Sari L.M, Wulandari D, Bustami A, Subita G. P, Auerkari E.I. Tannin Screening, Phenolic Compounds Analysis, and Antiproliferative Activity of Areca Nut Extract by Decreasing Ki-67 Protein in Oral *Squamous Carcinoma* Cell Lines. Trop. J. Nat. Prod. Res. 2020; 4(9): 563–570. https://doi.org/10.26538/tjnpr/v4i9.12.
14. Wan M. L. Y, Co V. A, El-Nezami H. Dietary Polyphenol Impact on Gut Health and Microbiota. Crit. Rev. Food Sci. Nutr. 2021; 61(4): 690–711. https://doi.org/10.1080/10408398.2020.1744512.
15. Sharifi-Rad M, Anil Kumar N. V, Zucca P, Varoni E. M, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou P. V, Azzini E, Peluso I, Prakash Mishra A, Nigam M, El Rayess Y, Beyrouthy M. E, Polito L, Iriti M, Martins N, Martorell M, Docea A. O, Setzer, W. N, Calina D, Cho W. C, Sharifi-Rad J. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. Front. Physiol. 2020; 11: 1–21. https://doi.org/10.3389/fphys.2020.00694.
16. Fraga-Corral M, García-Oliveira P, Pereira A. G, Lourenço-Lopes C, Jimenez-Lopez C, Prieto M. A, Simal-Gandara J. Technological Application of Tannin-Based Extracts. Molecules 2020; 25(3): 1–27. https://doi.org/10.3390/molecules25030614.
17. Dhawale P. V, Vineeth S. K, Gadhave R. V, Fatima M. J. J, Supekar M. V, Thakur V. K, Raghavan P. Tannin as a Renewable Raw Material for Adhesive Applications: A Review. Mater. Adv. 2022; 3(8): 3365–3388. https://doi.org/10.1039/D1MA00841B.
18. Eckardt J, Sepperer T, Cesprini E, Šket P, Tondi G. Comparing Condensed and Hydrolysable Tannins for Mechanical Foaming of Furanic Foams: Synthesis and Characterization. Molecules 2023; 28(6): 2799. https://doi.org/10.3390/molecules28062799.
19. Kavitha V. U, Kandasubramanian, B. Tannins for Wastewater Treatment. SN Appl. Sci. 2020; 2(6): 1081. https://doi.org/10.1007/s42452-020-2879-9.
20. Tangarfa M, Semlali Aouragh Hassani N, Alaoui A. Behavior and Mechanism of Tannic Acid Adsorption on the Calcite Surface: Isothermal, Kinetic, and Thermodynamic Studies. ACS Omega 2019; 4(22): 19647–19654. https://doi.org/10.1021/acsomega.9b02259.
21. Melo L. F. M. de, Aquino-Martins V. G. Q, Silva A. P, Oliveira Rocha H. A, Scortecci K. C. Biological and Pharmacological Aspects of Tannins and Potential Biotechnological Applications. Food Chem. 2023; 414: 135645. https://doi.org/10.1016/j.foodchem.2023.135645.
22. Wang A, Hou J, Xu Q, Wu J, Xing B. Green Synthesis of Zero Valent Iron Using Tannins to Activate Persulfate for Sulfamethoxazole Degradation. Environ. Pollut. 2023; 336: 122418. https://doi.org/10.1016/j.envpol.2023.122418.
23. China C. R, Nyandoro S. S, Munissi J. J. E, Maguta M. M, Meyer M, Schroepfer M. Tanning Capacity of Tessmannia Burttii Extracts: The Potential Eco-Friendly Tanning Agents for the Leather Industry. J. Leather Sci. Eng. 2021; 3(13): 1-9. https://doi.org/10.1186/s42825-021-00055-2.
24. Carlqvist K, Arshadi M, Mossing T, Östman U. B, Brännström H, Halmemies E, Nurmi J, Lidén G, Börjesson

P. Life-Cycle Assessment of the Production of Cationized Tannins from Norway Spruce Bark as Flocculants in Wastewater Treatment. Biofuels, Bioprod. Biorefining 2020; 14(6): 1270–1285. https://doi.org/10.1002/bbb.2139.
25. Kumar Das A, Nazrul Islam M, Omar Faruk M, Ashaduzzaman M, Dungani R, Rosamah E, Hartati S, Rumidatul A. Hardwood Tannin: Sources, Utilizations, and Prospects. In Tannins - Structural Properties, Biological Properties and Current Knowledge; IntechOpen, 2020. https://doi.org/10.5772/intechopen.86003.
26. China C. R, Hilonga A, Nyandoro S. S, Schroepfer M, Kanth S. V, Meyer M, Njau K. N. Suitability of Selected Vegetable Tannins Traditionally Used in Leather Making in Tanzania. J. Clean. Prod. 2020; 251: 119687. https://doi.org/10.1016/j.jclepro.2019.119687.
27. Kumari N, Kumar M, Chaudhary N, Zhang B, Radha, Chandran D, Joshi S, Singh D, Dey A, Rajalingam S, Natarajan K, Muthukumar M, Mohankumar P, Sheri V, Dhumal S, Lorenzo J. M. Exploring the Chemical and Biological Potential of Jamun (*Syzygium Cumini* (L.) Skeels) Leaves: A Comprehensive Review. Chem. Biodivers. 2023; 20(9): e202300479. https://doi.org/10.1002/cbdv.202300479.
28. Ahmed R, Tariq M, Hussain M, Andleeb A, Masoud M. S, Ali I, Mraiche F, Hasan A. Phenolic Contents-Based Assessment of Therapeutic Potential of *Syzygium Cumini* Leaves Extract. PLoS One 2019; 14(8): e0221318. https://doi.org/10.1371/journal.pone.0221318.
29. Surana K, Idris M. G, Bhattacharya B. Natural Dye Extraction from *Syzygium Cumini* and Its Potential Photovoltaic Application as Economical Sensitizer. Appl. Nanosci. 2020; 10(10): 3819–3825. https://doi.org/10.1007/s13204-020-01452-5.
30. Periyasamy A. P. Natural Dyeing of Cellulose Fibers Using *Syzygium Cumini* Fruit Extracts and a Bio-Mordant: A Step toward Sustainable Dyeing. Sustain. Mater. Technol. 2022; 33: e00472. https://doi.org/10.1016/j.susmat.2022.e00472.
31. Mariselvam R, Ranjitsingh A. J. A, Mosae Selvakumar P, Krishnamoorthy R, Alshatwi A. A. Eco Friendly Natural Dyes from *Syzygium Cumini* (L) (Jambolan) Fruit Seed Endosperm and to Preparation of Antimicrobial Fabric and Their Washing Properties. Fibers Polym. 2017; 18(3): 460–464. https://doi.org/10.1007/s12221-017-1097-6.
32. Shamsheer H. B, Mughal T. A, Ishaq A, Zaheer S, Zahid K. Extraction of Ecofriendly Leather Dyes from Plants Bark. Pakistan J. Sci. Ind. Res. Ser. A Phys. Sci. 2017; 60(2): 96–100. https://doi.org/10.52763/PJSIR.PHYS.SCI.60.2.2017.96.100.
33. Elgailani I. E. H, Ishak C. Y. Determination of Tannins of Three Common Acacia Species of Sudan. Adv. Chem. 2014; 2014: 1–5. https://doi.org/10.1155/2014/192708.
34. Sousa T. B, Souza S. G, Franco T. B. B, Silva de Jesus M, Mori F. A. Quantification of Tannins from Curupay Bark. Floresta e Ambient. 2019; 26(1): e20160082. https://doi.org/10.1590/2179-8087.008216.
35. Al-Ogaili N. A, Al-Jaboury I. S, Yaseen Mohammed Hasan Z. Qualitative and Quantitative Determination of Total Phenols in *Achillea Tenuifolia* Lam. Results Chem. 2023; 5: 100931. https://doi.org/10.1016/j.rechem.2023.100931.
36. Blainski A, Lopes G, de Mello J. Application and Analysis of the Folin Ciocalteu Method for the Determination of the Total Phenolic Content from *Limonium Brasiliense* L. Molecules 2013; 18(6): 6852–6865. https://doi.org/10.3390/molecules18066852.
37. Migliato K. F, Carvalho E. S. de, Sacramento L. V. S. do, Mello J. C. P. de, Baby A. R, Velasco M. V. R, Salgado H. R. N. Total Polyphenols from *Syzygium Cumini* (L.) Skeels Fruit Extract. Brazilian J. Pharm. Sci. 2009; 45(1): 121–126. https://doi.org/10.1590/S1984-82502009000100015.
38. International Conference Harmonisation. ICH Guideline M10 on Bioanalytical Method Validation Step 2B. Sci. Med. Heal. 2019; 44, 6/7-20/40-41/49-57.
39. Tychinkin I. V, Shishlov O. F, Glukhikh V. V, Stoyanov O. V, Kolpakova M. V. The Effect of Lignin on the Reactivity of Phenol-Formaldehyde Resin and Properties of a Thermal-Insulation Material Based on It. Polym. Sci. Ser. D 2023; 16(2): 239–244. https://doi.org/10.1134/S1995421223020454.
40. Antony A, Farid, M. Effect of Temperatures on Polyphenols during Extraction. Appl. Sci. 2022; 12(4): 2107. https://doi.org/10.3390/app12042107.
41. Akram M, Cerin E, Lamb K. E, White S. R. Modelling Count, Bounded and Skewed Continuous Outcomes in Physical Activity Research: Beyond Linear Regression Models. Int. J. Behav. Nutr. Phys. Act. 2023; 20(1): 57. https://doi.org/10.1186/s12966-023-01460-y.
42. Guangcheng Z, Yunlu L, Yazaki Y. Extractives Yields, Stiasny Values and Polyflavanoid Contents in Barks from Six Acacia Species in Australia. Aust. For. 1991; 54(3): 154–156. https://doi.org/10.1080/00049158.1991.10674572.
43. Tahir P. M, Musgrave O. C, Ashaari, Z. Determination of Polyphenolic Content of Bark Extracts for Wood Adhesives. Holzforschung 2002; 56(3): 267–272. https://doi.org/10.1515/HF.2002.044.
44. Stark P. *Errors In Regression*. University of California, Berkeley. https://www.stat.berkeley.edu/~stark/SticiGui/Text/regressionErrors.htm (accessed 2024-09-25).
45. Barwick V. *Preparation of Calibration Curves: A Guide to Best Practice*; 2003.
46. *OLS diagnostics: Influential data tests*. APTECH. https://www.aptech.com/resources/tutorials/econometrics/ols-diagnostics-influential-data-tests/ (accessed 2024-09-25).
47. The Pennsylvania State University. *Identifying Outliers (Unusual Y Values)*. The Pennsylvania State University. https://online.stat.psu.edu/stat462/node/172/ (accessed 2024-09-25).
48. Cleophas T. J, Zwinderman A. H. Quantile-Quantile Plots, a Good Start for Looking at Your Medical Data (50 Cholesterol Measurements and 58 Patients). In Machine Learning in Medicine – A Complete Overview; Ton J. Cleophas, A. H. Z., Ed.; Springer International Publishing: Cham, 2020; pp 319–327. https://doi.org/10.1007/978-3-030-33970-8_43.
49. Wang C.-C, Lee W.-C. Evaluation of the Normality Assumption in Meta-Analyses. Am. J. Epidemiol. 2020; 189(3): 235–242. https://doi.org/10.1093/aje/kwz261.
50. *Generalized Least Squares*; Madrid, Spain, 2012.
51. *Calculate Variance-Covariance Matrix for a Fitted Model Object*. https://rdrr.io/r/stats/vcov.html (accessed 2024-09-25).
52. Blainski A, Lopes G, De Mello J. Application and Analysis of the Folin Ciocalteu Method for the Determination of the Total Phenolic Content from *Limonium Brasiliense* L. Molecules 2013; 18(6): 6852–6865. https://doi.org/10.3390/molecules18066852.
53. Chamnansilpa N, Aksornchu P, Adisakwattana S, Thilavech T, Mäkynen K, Dahlan W, Ngamukote S. Anthocyanin-Rich Fraction from Thai Berries Interferes with the Key Steps of Lipid Digestion and Cholesterol Absorption. Heliyon 2020; 6(11): e05408. https://doi.org/10.1016/j.heliyon.2020.e05408.
54. Li X, Li W, Chen F, Xiao J, Liao X, Hu X, Ji J, Ma L. Guideline for Measurement of Condensed Tannin. Food Front. 2023; 4(1): 533–541. https://doi.org/10.1002/fft2.174.
55. Kuria N. A. Evaluation of Tanning Strength and Quality of Leathers, University of Kenya, Nairobi, Kenya, 2015.
56. Azadeh M, Gorovits B, Kamerud J, MacMannis S, Safavi A, Sailstad J, Sondag P. Calibration Curves in Quantitative Ligand Binding Assays: Recommendations and Best

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Practices for Preparation, Design, and Editing of Calibration Curves. AAPS J. 2018; 20(1): 22. https://doi.org/10.1208/s12248-017-0159-4.
57. Broekaert J. A. C, Harris D. C. Quantitative Chemical Analysis, 9th Ed. Anal. Bioanal. Chem. 2015; 407(30): 8943–8944. https://doi.org/10.1007/s00216-015-9059-6.
58. de Souza S. V. C, Junqueira R. G. A Procedure to Assess Linearity by Ordinary Least Squares Method. Anal. Chim. Acta 2005; 552(1–2): 25–35. https://doi.org/10.1016/j.aca.2005.07.043.
59. Mugedo J. Z. A, Waterman P. G. Sources of Tannin: Alternatives to Wattle (*Acacia Mearnsii*) among Indigenous Kenyan Species. Econ. Bot. 1992; 46(1): 55–63. https://doi.org/10.1007/BF02985254.
60. Moursi A. I. N. Using Some Plants and Their Crude Extracts In Leather Tanning, Alexandria University, Alexandria, Egypt, 2011, pp. 1-102