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Analyzing vegetable tannins: Incorporating ultraviolet spectroscopy and hide powder techniques

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Abstract

The ultraviolet technique, known for its speed and cleanliness, proved to be highly advantageous in this study. It requires no reagents or pretreatment of the samples, making it a convenient method for analyzing different commercial vegetable tannins. By employing ultraviolet spectroscopy and Hide Powder techniques, valuable insights into the primary absorption bands were obtained, providing information about the chemical composition and structure of the extracts. In addition, specific absorptivity was determined, and the total polyphenols and tannin content of each extract were quantified using the filter and Folin Denis methods, respectively. The results showed that extracts of condensed tannins had the lowest values of specific absorptivity, while hydrolysable tannins exhibited the highest values. When analyzing the total polyphenols, condensed tannins, such as *Acacia mearnsii* and *Azadirachta indica*, displayed the highest percentages, with values of 73% and 60%, respectively. Hydrolysable tannins ranged from 51% to 36%. Furthermore, the evaluation of tanning percentage revealed that *Acacia mearnsii* and *Azadirachta indica* exhibited percentages around 40%, followed by *Acacia nilotica ssp Tomentos* pods, *Acacia seyal var. seyal*, and *Pithecellobium dulce*, with percentages of 29%, 25%, and 17%, respectively. These findings provide important information about the tanning potential of the different vegetable tannins studied.

Keywords: Ultraviolet; Vegetable tannins; Total phenols; Tannin content; Specific absorptivity

1. Introduction

Vegetable tannins, extracted with water from various plants, have long been utilized in the tanning of leather. These polyphenolic substances, ranging in molecular weight from 500 to 30,000 Da, form a diverse group of secondary metabolites found throughout the plant kingdom. Tannins are commonly present in bark, wood, fruits, fruit pods, leaves, roots, and plant galls, serving as natural protective agents [1-5]. Tannins can be categorized into two main types: hydrolysable tannins and condensed tannins [6]. Hydrolysable tannins consist of a polyol core that is acylated by gallic or ellagic acid units and their derivatives [3,5,6]. On the other hand, condensed tannins, also known as proanthocyanidins, are oligomers and polymers formed within the basic structure of flavan-3-ols [2-7].

These compounds have a wide range of applications, including but not limited to:

- Flocculants: They are used to promote the clumping together of suspended particles in a liquid, facilitating their removal [8,9].
- Anti-corrosion agents: These compounds help protect surfaces from corrosion and degradation caused by chemical reactions with the environment.

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- Tanning agents: They are utilized in the process of converting raw animal hides into leather, imparting desirable characteristics such as durability and flexibility
- Adhesives: These compounds are employed to bind materials together, providing strength and durability to various products [10,11].
- Pharmaceutical agents: They find application in the development of drugs and medical treatments, serving various purposes such as targeting specific diseases or enhancing drug delivery [12].
- Foaming agents: They are utilized to generate foams with specific properties, such as insulation or cushioning, in industries like construction and packaging [13,14].

These compounds demonstrate their versatility by being employed in a wide array of industries and applications, showcasing their importance in various fields.

The complex composition of vegetable tannins has posed challenges in identifying their nature. Advanced techniques, including mass analysis have been commonly employed for this purpose [3,5-6,15]. The spot test is a simple and cost-effective technique for identifying the nature of tannin extracts. Falcão and Araújo [16] introduced a methodology specifically designed for identifying extracts in historic vegetable leathers. By utilizing chemical spot tests, tannins in leather fibers can be characterized, providing indirect information about the approximate vegetable sources used in the production of leather, both in the past and present. These spot tests serve as a valuable tool for quickly and easily assessing the quality and origin of hides, making them a convenient and efficient solution [16]. The ATR-FTIR technique has been successfully employed for characterizing tannins in recent studies [1,2,7,17,].

The ultraviolet and visible spectrophotometer (UV-vis) is a popular technique widely used for characterizing tannins in analytical chemistry [18]. It provides a simple and robust method for analytical determinations in various fields. In organic chemistry, it is utilized for quantifying polyphenolic compounds in natural extracts [19,20]. Additionally, the UV-vis technique is employed for the qualitative analysis of these compounds in leathers [3]. Tannins exhibit absorption in the aromatic region, with the aromatic rings serving as the primary chromophore groups in these extracts [21,22]. The presence of conjugated electrons within an aromatic ring leads to characteristic absorption peaks of moderate intensity around 205 nm and a less pronounced band within the range of 250-275 nm.

This work presents a methodology that combines UV-vis techniques with Hide Powder techniques to determine the nature of vegetable tannins. UV-vis analysis is recognized as one of the rapid and widely used instrumental techniques. It does not require the use of reagents or pre-treatment of samples, providing information on the prominent absorption bands. Furthermore, the specific absorptivity, total polyphenol content, and tannin content of each extract were determined using the filter and Folin Denis methods, respectively.

2. Material and methods

2.1. Preparation of sample

Fresh different plant parts (bark & pods) (0.3–2.0 kg) from different species growing in Khartoum area, Blue Nile, and South Kordofan, were used for this study (Table 1). The conformation of the identity of the plant species is done by Soba Forestry Research Center Herbarium. The samples were air-dried and reduced to powder with a star mill. The fractions passing through 40-mesh and retained on 85-mesh sieve were collected, thoroughly mixed and kept in airtight containers.

Table 1 Collection data for the tannin plant species under the studies

Species	Part	Age	Collection site	Air-dried Material
Acacia mearnsii	Bark	25	Jebel Marra	2.0
Acacia nilotica ssp tomentosa	pods	10	Soba	1.0
Acacia seyal var. seyal	Bark	15	Soba	0.5
Azadirachta indica	Bark	20	Soba	0.5
Pithecellobium dulce	Bark	9	Blue Nile	1.0

2.2. Chemicals

The chemicals used in the experiment were of analytical-reagent grade and the water was distilled. The specific chemicals used were as follows: 2 N Folin-Denis reagent, sodium carbonate-tartrate, formaldehyde, hydrochloric acid from Synth (Diadema, Brazil), and gallic acid from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Extraction of samples

The extracts were subjected to quantitative analysis for total and soluble solids, non-tannins, and tannins using the official hide-powder method [23] (hide-powder batch C28). Additionally, a modified version of the hide-powder method, known as the combined method [24], was employed. The total phenolic content in the extract was determined using the Folin-Denis method [25]. Initially, freshly hydrated chromated hide-powder, equivalent to 3.0 g of oven-dried weight, was prepared. Subsequently, tannins were allowed to adsorb onto the hide powder, while the remaining phenolic materials were quantified. The catechin number (Stiasny number) was determined following the method outlined by Yazaki and Hillis [25]. For this purpose, a 100 ml extract was filtered through a glass fritted funnel (G4) and transferred into a conical flask. Stiasny reagent, consisting of 5 ml of HCl and 10 ml of 37% formaldehyde, was added to the flask. The mixture was then allowed to stand at room temperature (30–35 °C) for 24 hours. Subsequently, the precipitate was filtered using a tared crucible (G4) and dried to a constant weight at approximately 100 ± 5 °C to obtain the weight of catechin [25].

2.4. Ultraviolet Visible Spectroscopy (UV-vis)

Ultraviolet spectra were recorded using a double beam spectrophotometer equipped with ultraviolet-visible spectroscopy capabilities (PG Instruments, T80+ model) within the wavelength range of 190–380 nm. The measurements were conducted using 1 cm quartz cells. For analysis, the extracts were prepared in 100 mL volumetric flasks with a concentration of 0.01 g L^{-1}. The experiments were performed in triplicate using water as the solvent, and the average spectra were obtained.

2.5. Measurement of total phenols

Total polyphenols were determined using the Folin-Denis colorimetric method, as described in previous literature [20,26]. The Folin-Denis reagent contains a mixture of phosphotungstic and phosphomolybdic acids, which, in a basic medium, undergo reduction to oxidize polyphenols, resulting in the formation of blue oxides. The quantification of polyphenols is based on the measurement of their absorbance. The color development involved adding 1 mL of Folin-Denis reagent and 10 mL of sodium carbonate-tartrate reagent to a 50 mL sample. After allowing the mixture to rest for 30 minutes, the absorbance was measured. A calibration curve was constructed using a standard gallic acid, with seven data points ranging from 0.02 to 10 ppm. Absorbance measurements were performed at a wavelength of 725 nm.

2.6. Measurement of Specific Absorptivity

The specific absorptivity of an extract is calculated as the ratio between the maximal absorbance and the concentration of the solution. Since natural extracts are complex mixtures without defined molar concentrations, the concentrations of the solutions are typically expressed in g L^{-1}. The calculation involves considering the absorptivity observed at the wavelength of maximum absorption, along with the sample concentration and optical path length.

3. Results and discussion

Based on the formaldehyde-HCl and iron alum tests, it was found that out of the five species screened, two belonged to the condensed type (catechol), while the remaining three species were of the mixed, hydrolysable-condensed type (gallo catechol). These assignments were supported by the results of the gallic acid and catechin number tests, as shown in Table 2. The quantitative data revealed that all parts (bark and pods) out of the five species contained more than 10% tannins (oven-dry basis), which is of commercial interest. In addition to that all species exhibited an acceptable extraction ratio (tannin to non-tannin) ranging from 1.7 to 4.5. The tannin purity, indicated by the ratio of tannin to soluble solids, was good (>0.5) for all species (Table 2). However, it is important to consider the type of tannin present and the part of the plant that is extracted. If a bark has a high tannin content but is very thin, a substantial amount of bark would be required for economically feasible extraction, unless the bark is readily available as waste from other wood uses. The catechin numbers indicated that all the species studied contained varying amounts of condensed tannins ranging from 22.4 to 45.7(Table 2). The presence of both gallic acid and catechin indicated that the tannins were of a mixed nature.

Table 2 Analysis of the tannin cold aqueous extracts (% oven-dry part extracted)

Parameters	**Species**				
	Acacia mearnsii	***Azadirachta indica***	***Acacia nilotica ssp Tomentos***	***Acacia seyal var. seyal***	***Pithecellobium dulce***
Part	Bark	Bark	Pods	Bark	Bark
Total solids (TS)%	51.8	25.1	63.1	39.0	38.9
Soluble solids (SS)%	48.7	24.9	62.1	36.6	35.7
pH	6	6	6	6	6
Tannins, (T)%	39.8	16.8	39.4	24.8	28.8
Non-Tannins, (NT)%	8.9	7.6	22.7	11.7	6.9
Extraction Ratio (T/NT)	4.5	2.2	1.7	2.1	4.2
Catechin number	45.7	22.4	36	32.4	26.6
Gallic acid	-	-	+	+	+
Tannin type	Condensed	Condensed	Hydrolysable-Condensed	Hydrolysable-Condensed	Hydrolysable-Condensed
Purity(T/SS), %	0.8	0.7	0.6	0.7	0.8

H = Hydrolysable tannin, C = condensed tannin, + = Detected

Vegetable tannin extracts, despite having larger molecular weights of up to 3000 Da, exhibit absorbance at lower wavelengths compared to smaller molecules. This is attributed to the presence of aromatic ring structures, which are the primary chromophore groups in these extracts. The absorption spectrum of these extracts can be influenced by the auxochrome groups attached to the aromatic rings, causing a shift in the absorption profile. Due to the limited electronic displacement in aromatic rings, their absorption is shifted towards the ultraviolet region (Figure 1).

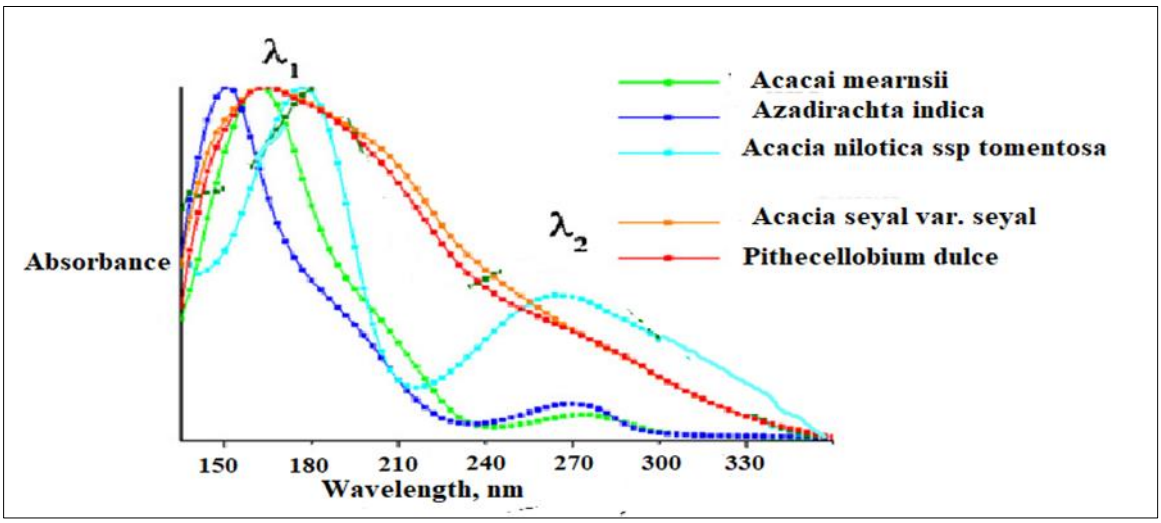

Figure 1 Ultraviolet absorption profiles of vegetable tannin extracts

When analyzing the absorption spectra of the extracts, namely *Acacia mearnsii, Azadirachta indica, Acacia nilotica ssp Tomentos pods, Acacia seyal var. seyal*, and *Pithecellobium dulce*, two distinct absorption bands were observed in *Acacia mearnsii* and *Azadirachta indica* extracts. On the other hand, *Acacia nilotica ssp Tomentos pods, Acacia seyal var. seyal*, and *Pithecellobium dulce* exhibited a single, broader absorption band. The first group of extracts displayed a maximum absorption peak ranging from 275 to 285 nm, with a more intense absorption observed between 150 and 180 nm. *Acacia nilotica ssp Tomentos* pods extracts, however, demonstrated a single absorption peak at 190 nm, extending up to 360

nm, possibly due to overlapping signals from different structures present in these extracts. The specific maximum absorption values for each vegetable extract are provided in Table 3. In the analysis of hydrolysable tannins, *Acacia nilotica ssp* Tomentos pods and *Acacia seyal var. seyal* exhibit striking similarities in their absorption spectrum (Figure1). The peak of highest intensity absorption (λ_1) is observed at longer wavelengths compared to the other extracts, specifically at 190 nm for *Acacia nilotica ssp Tomentos* pods and 170 nm for *Acacia seyal var. seyal*. Additionally, both extracts display a prominent absorption band at longer wavelengths (λ_2), centered around 270 nm, which is more pronounced than in the other extracts within this range. These findings align with the literature, which reports absorption peaks for tare at 190-170 nm (peak λ_1) and 270-274 nm (peak λ_2), confirming the consistency of our results with previous studies [3].

Table 3 Spectroscopic data for the absorption maxima obtained by ultraviolet spectroscopy

Polyphenolic	**λ_1**	**λ_2**
Extract	nm	nm
Acacia nilotica ssp Tomentos	190	270
Acacia mearnsii	180	280
Acacia seyal var. seyal	170	270
Azadirachta indica	150	270
Pithecellobium dulce	175	-

Table 4 presents the specific absorptivity and determination of active constituents in tannin extracts. Specific absorptivity refers to the intrinsic property of a substance to absorb light at a specific wavelength. While pure substances are typically measured in terms of molar absorptivity (mol L^{-1} cm^{-1}), complex substances, such as tannin extracts, are evaluated in terms of specific absorptivity, with concentrations expressed in g L^{-1} instead of mol L^{-1}. Among the condensed tannin extracts, the lowest specific absorptivity values were observed for *Acacia mearnsii* (7.9±0.65L g^{-1} cm^{-1}) and *Azadirachta indica* (11.8±0.20 L g^{-1} cm^{-1}). In the case of hydrolysable tannins, *Acacia seyal var. seyal* exhibited the lowest specific absorptivity (25.3±0.35 L g^{-1} cm^{-1}), followed by *Pithecellobium dulce* (39.8±0.56 L g^{-1} cm^{-1}). On the other hand, *Acacia nilotica ssp Tomentos* displayed the highest specific absorptivity value (59.5±0.65 L g^{-1} cm^{-1}). During the analysis of total polyphenols, it was found that condensed tannins exhibited the highest percentage, with *Acacia mearnsii* and *Azadirachta indica* showing 73% and 60%, respectively. Hydrolysable tannins, on the other hand, fell within the range of 51-36%, with the *Pithecellobium dulce* displaying the lowest results. Tannin content determination through filter analysis involves measuring the quantity of active material capable of interacting with skin collagen. Insoluble matter refers to the portion of the extract that cannot permeate the skin during the tanning process.

Table 4 Determination of specific absorptivity and active constituents in tannin extracts

Extracts	**Total polyphenols, %**	**Tannin content, %**	**Insoluble, %**	**Specific absorptivity, $Lg^{-1}cm^{-1}$**
Acacia nilotica ssp Tomentos	50.7±0.96	39.40±0.15	22.7±0.25	59.5±0.65
Acacia mearnsii	72.8±0.87	39.8±0.16	8.9±0.06	7.9±0.65
Acacia seyal var. seyal	40.8±0.68	24.8±0.9	11.7±0.3	25.3±0.35
Azadirachta indica	59.8±0.35	16.8±0.5	7.6±0.04	11.8±0.20
Pithecellobium dulce	35.6±0.39	28.8±0.12	6.9±0.02	39.8±0.56

This analysis is commonly employed in the tanning industry to assess the proportion of active material present in tanning extracts for the conversion of hides into leather. The insoluble content (non-tannin) of all extracts, except for *Acacia nilotica ssp Tomentos*, was found to be below 12%. However, *Acacia nilotica ssp Tomentos* exhibited percentages exceeding 21%. This disparity can be attributed to the fact that the pods of *Acacia nilotica ssp Tomentos* typically marketed in a micronized form rather than being extracted in an aqueous solution like the other extracts. Consequently, a higher proportion of insoluble material is observed in these cases. During the assessment of tannin percentage, *Acacia*

mearnsii and *Acacia nilotica ssp Tomentos* demonstrated approximately 40% tanning efficiency. *Pithecellobium dulce*, *Acacia seyal var. seyal*, and *Azadirachta indica* exhibited lower percentages, measuring at 29%, 25%, and 17%, respectively.

4. Conclusion

In this research, a total of 30 samples of vegetable tannin extracts, representing five commercially available types, were subjected to ultraviolet and hide powder analyses. This technique provided valuable insights into the primary absorption bands, which are indicative of the extracts' chemical composition and structure. Furthermore, specific absorptivity was determined, and the total polyphenols and tannin content of each extract were quantified using the Folin-Denis and filter methods, respectively. The specific absorptivity values for condensed tannin extracts were found to be the lowest, with *Acacia mearnsii* exhibiting 7.9±0.65 L g^{-1} cm^{-1} and *Azadirachta indica* displaying 11.8±0.20 L g^{-1} cm^{-1}. On the other hand, hydrolysable tannin extracts demonstrated higher specific absorptivity values for *Acacia nilotica ssp Tomentos* (59.5±0.65L g^{-1} cm^{-1}) and *Pithecellobium dulce* (39.8±0.56 L g^{-1} cm^{-1}), while *Acacia seyal var. seyal* (25.3±0.35L g^{-1} cm^{-1}) displayed the lower values. In terms of total polyphenol analysis, the condensed tannins exhibited the highest percentages, with *Acacia mearnsii* and *Azadirachta indica* displaying 73% and 60 % respectively. Hydrolysable tannins were within the range of 51-36%, with *Pithecellobium dulce* demonstrating the lowest results. During filter analysis, the insoluble content of all extracts except *Acacia nilotica ssp Tomentos* was found to be below 12%. However, *Acacia nilotica ssp Tomentos* exhibited percentages exceeding 21%. In terms of evaluating tannin percentage, *Acacia mearnsii* and *Acacia nilotica ssp Tomentos* demonstrated approximately 40% tanning efficiency. *Pithecellobium dulce*, *Acacia seyal var. seyal*, and *Azadirachta indica* exhibited lower percentages, measuring at 29%, 25%, and 17%, respectively. The utilization of the ultraviolet technique proved successful in discerning the characteristics of the polyphenolic extracts derived from *Acacia mearnsii*, *Azadirachta indica*, *Acacia nilotica ssp Tomentos*, *Acacia seyal var. seyal*, and *Pithecellobium dulce*, which were investigated in this study.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no conflict of interests exists.

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