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Hide-power and combined methods for characterization of vegetable tannin in plant

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Abstract

This current research shows different analytical techniques used for describing vegetable tannin materials in various plant species grown in Sudan. It illustrates the evaluation of tannin content by both hide powder and combined techniques, total phenolic content by Combined, Folin Denis, and Hagerman and Butler methods. The result showed that of the six parts studied; five had over 10% tannin content and were thus suitable for commercial exploitation. Chromatographic techniques (Paper and Thin layer) designated and confirmed the present of mixed kind of tannin (gallo-catechol) except *Acacia mearnsii* which is of catechol type meaning contain condensed tannins only. The advantage of using this analytical technique, had similar yield of polyphenols attained with a lesser solvent feeding and a shorter removal time.

Keywords: Phenolic Compounds; Extraction; Gallo tannins; Catechol; Stringency

1. Introduction

The phenolics compound (tannins) was introduced first time in 1796 [1] and came from the use of these compounds in tanneries. Tannins containing plant extracts have been used to process animal skin into leather since ancient times. Tannins can therefore be defined as a unique group of phenolic metabolites of relatively high mass having the power to complex strongly with carbohydrates and proteins [2]. Tannins is divided either by their chemical structure or by their solubility and extractability. Concerning the chemical structure, tannins is divided into four major groups, looking on the structure of the monomer: proanthocyanins or condensed tannins, hydrolysable tannins, phlorotannin found in marine algae [3] and complicated tannins. Since earlier period, this property of vegetable tannins has been empirically discovered to convert animal skins, a proteinaceous biomaterial, into leather [4,5]. The method, termed vegetable tanning, is one amongst the oldest known leathers making processes and it often succinctly described as a treatment of hides/skins with powdered barks, leaves, wood, fruits, pods or galls, or their extracts, obtained from different vegetable sources [6]. With this treatment, traditionally performed in pits, a chemical interaction between collagen protein (the main constituent of dermis) and tannins present in vegetable materials is slowly established, generating awfully useful and remarkably non-putrescible material under moist and warm conditions, termed vegetable tanned leather [5,7]. In certain positions proanthocyanins may sometimes be esterified with acid or exceptionally with sugars. Unlike hydrolysable tannins, proanthocyanins react to insoluble phlobaphene and red-colored anthocyanidins when treated with acid, hence the name “proanthocyanins” [8,9].

Hydrolysable tannins are polyesters of non-aromatic polyhydroxy compounds (sugar moiety) and organic acids. The designation “hydrolysable tannin” is thanks to the actual fact that these compounds undergo hydrolytic cleavage to the respective sugar and acid moiety upon treatment with diluted acids. In most cases the sugar component is glucose, but fructose, xylose, saccharose and infrequently structures like hamamelose also are found. If the acid component is gallic acid, the compounds are called gall tannins. Esters with hexahydroxy diphenic acid (forming ellagic acid when

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hydrolysed through elimination of water) are called ellagitannins. Most ellagitannins are mixed esters both with hexahydroxy diphenic acid and gallic acid.

The objective of this study was therefore to expand the knowledge about polyphenolic profiles in tanning process that had significant concentrations of those compounds. This information is vital to acknowledge tannin structure, recognize, to settle on suitable techniques for determination of tannin materials.

2. Material and methods

2.1. Collection and Preparation of Sample

Fresh plant tissue (bark) (0.5–3.0 kg) from different species growing in numerous parts of Sudan, were used for this study (Table 1). Soba Forestry Research Facility Herbarium confirmed the identity of the plant tissues species. The samples tissues were then freeze dried and transformed into a fine homogeneous powder by crushing with an electrical crusher (star mill). Then the powders were kept in a desiccator until starting of the analysis.

Table 1 Collection data for the investigated tannin plant species

Species	Part	Age	Collection site	Air-dried Material
Acacia mearnsii	Bark	25	Jebel Marra	2.5
Anogessus leiocarpu	Bark	25	Dalang natural Forest	1.5
	Leaves	25		1.5
Azadirachta indica	Bark	20	El Obeid area	2.5
Casuarina equistifolia	Bark	15	Blue Nile	1.0
Combretum hartmannianum	Bark	18	Blue Nile	1.5

2.2. Extraction of Tannins

The tannin materials were extracted with normal water (2 litres) using the strategy of American leather Chemist Association (ALCA) [10]. Gelatin salt test was used for determined the present of tannin within the extract, and iron-alum and formaldehyde-HCl test for identification the kinds either condensed or hydrolysable tannin [11].

2.3. Qualitative Analysis

2.3.1. Thin layer chromatography

Sheets of size (20 × 20 cm) and (thickness 0.2 mm) with polyamide precoated was used for thin layer chromatography, acetone-propanol-water with ratio of 5:4:1 was used as solvent system [12]. 5 grams of raw materials were used for preparation of samples by hydrolyze with 2M HCl using reflux extractor for 30 min. The solution was then cooled and filtered; then ethyl acetate was used to extracts the produced filtrate. The aqueous layer was heated to get rid of any trace of solvent and extracted with a little volume of amyl alcohol. The extracted solvent then were concentrated to thick syrup under vacuum [13]. The standard compounds with (Rf ×100) utilized in the analysis are fisetin, gallic acid, catechin, Tannic acid, robinetin, epicatechin, and dihydrofisetin.

2.3.2. Paper chromatography

Whatman No. 1 paper with forestall as solvent system of ratio 10:3:30 composed of concentrated acetic acid: HCl: water respectively was used in paper chromatography [13]. Techniques of ascending chromatograms were used for development of chromatography to 8-14 cm in height at room temperature. Detection of developed chromatograms were done firstly under UV light (254 nm) and so by spraying with ferric chloride reagent (2 g $FeCl_3$ in 98 ml methanol) or exposing to ammonia vapor [12].

2.4. Quantitative Analysis

Tannin, non-tannins, total solids, and soluble solids were determined by using official hide powder techniques (hider-powder batch C29) [14]. A combined techniques which is a modification of the hide-powder techniques was also used for determination [15]. Folin-Denis techniques was used for measuring the entire phenolic compound within the extract

[16]. 3.0 grams of hide powder (oven-dried) was prepared, which was already hydrated and chromated. Tannin was then allowed to grip onto the hide powder (hydrated and chromated), after which the remaining phenolic materials were determined. Techniques of Yazaki and Hillis [17] was used for determination of Stiasny number (catechin number) [16].

3. Results and discussion

Tannins is a unique group of phenolic metabolites of relatively high mass having the flexibility to complex strongly with carbohydrates and proteins [2].

Tannins will be divided either by their:

- Chemical structure, or
- Solubility and extractability.

Regarding the structure of the monomer (chemical structure), tannins can be classified into four major groups [3]:

- Condensed tannins (Proanthocyanins)
- Hydrolysable tannins,
- Phlorotannin found in marine algae, and
- Complex tannins.

Hydrolysable tannins are divided into gall tannins and ellagitannins on basis of the character of the phenolic carboxylic acid. Therefore, on hydrolysis ellagitannins yields hexahydroxy diphenic acid which is isolated as ellagic acid while gallo tannins yield gallic acid [18]. Hydrolysable tannin is organized to create complexes with reactive metals, avoiding atom generation which ends in oxidative damage of cellular membranes and DNA [19]. Additionally, to it hydrolysable tannins' clean free radicals within the body by neutralizing them before cellular damage occurs [13, 20].

3.1. Thin-layer and paper Chromatography

The presence of catechin, gallic acid, tannic acid, fisetin, epicatechin and a few unknown phenolics were confirmed by the chromatographic techniques (Thin-layer and paper chromatography) in numerous solvent systems. However, dihydro fisetin and robinetin, which were used as standards, weren't detected (Table 2).

3.2. Ferrous Aluminum Sulphate & Formaldehyde-HCl Analysis

The six parts which screened were of the Hydrolysable-condensed variety of tannins (Gallo-catechol) except *Acacia mearnsii* bark, which was of condensed type only (Catechol type). The Stiasny number (Catechin number) and gallic acid examination outcomes supported these course work (Table 2).

The measure data indicated that the full parts (bark & leaves) of 5 species, when extracted, contained quite 10% (oven-dry basis) of tannins, the extent of business interest. All investigated species had an appropriate extraction ratio (tannin to non-tannin) of 1.1-4.5. The tannin purity or the ratio of tannin/soluble solids was good >0.5, for five parts of the six parts studied (Table 3). However, the sort of tannin present and therefore the part extracted are important.

Dissimilar parts of species bark and leaves, had the identical form of tannin but in several proportions. Generally, the tannin content was higher within the barks (*Acacia mearnsii*, *Anogessus leiocarpus*, *Azadirachta indica*, *Casuarina equistifolia*, and *Combretum hartmannianum*) (Table 3). The Stiasny number (Catchin numbers) showed that every one of the investigated species contained condensed tannin in variable quantities (4.6-45.7), while the existence of both catechin and gallic acid implies that the tannin is of mixed type (hydrolysable-condensed) (gallo-catechol) (Table 3).

3.3. Methods of Determination of Tannins

The tannin content determined by the hide-powder method was highest (39.8%) for *Acacia mearnsii* followed by (20%) for *Anogessus leiocarpus* leaves, and (16%) for *Azadirachta indica* bark and (14.5%) & (14.2%) for *Anogessus leiocarpus* and *Combretum hartmannianum* bark respectively, and at last 10% for *Casuarina equistifolia* bark (Table 2). The hide powder results were compared with the total phenolic results technique done by [17,21] and Swain and Goldstein spectroscopic techniques [22] (Table 4). within the first instant the correlation between tannin content and total phenolics was high (r^2 = 98.7%, n = 24, $p < 0.01$). within the second comparison, the phenolic content by the Hagerman and Butler method [23,24] was approximately half that of Folin-Denis's assay, but the correlation between the two

assays was still high (r^2 = 70.9%, n = 24, $p < 0.01$). The combined method also gave slightly lower values of tannin content and extraction rates compared to hide powder technique (Table 4). Attention should be taken when comparing tannin content determined by different methods because the isolation procedures may affect the proportion and kinds of phenolic present (this thanks to different method have other ways of determination and isolation) [25]. The relative astringency values for many of those tannins were quite near to that of *A. mearnsii* tannin, but higher values were obtained for *Azadirachta indica*. Nevertheless, *Azadirachta indica* bark has low tannin contents (16.8%) (Table 4).

Table 2 Thin layer (TLC) and Paper Chromatography (PC) of Hydrolyzed Parts Extract

Species	Part	Extracted with	Gallic acid		Tannic acid		Catechin		Epicatechin		Fiseten		Unknown	
			TLC	PC	TLC	PC	TLC	PC	TLC	PC	TLC	PC	TLC	PC
			82	63	56	32	78	64	66	64	66	15		
Acacia mearnsii	Bark	Amy alcohol	-	-	-	-	77	67	66	66	-	-	-	-
		Ethyl acetate	-	-	-	-	-	-	-	-	-	-	-	-
Anogessus leiocarpus	Bark	Amy alcohol	-	-	-	-	77	67	-	-	-	-	-	-
		Ethyl acetate	-	62	-	-	-	-	-	-	-	-	-	-
	leaves	Amy alcohol	-	-	-	-	-	-	66	-	-	-	-	-
		Ethyl acetate	81	62	56	32	77	67	-	-	-	-	-	-
Azadirachta indica	Bark	Amy alcohol	-	-	-	-	77	67	67	-	-	-	-	-
		Ethyl acetate	-	62	-	-	78	-	-	-	-	-	-	-
Casuarina equistifolia	Bark	Amy alcohol	-	-	-	-	78	64	-	-	-	-	-	-
		Ethyl acetate	-	62	-	-	-	-	-	-	-	-	-	-
Combretum hartmannianum	Bark	Amy alcohol	-	-	-	-	78	64	-	-	-	-	-	-
		Ethyl acetate	-	62	-	-	-	-	-	-	-	-	-	-

Table 3 Analysis of the Tannin Cold Aqueous Extract (% oven dry weight)

Species	Part	Tannin type	Catechin number	Tot al solids (TS) %	Soluble solids (SS)%	Tannins, (T) %	Non Tannins (NT)%	Extraction Ratio (T/NT)	Gallic acid	pH	Purity (T/SS) %
Acacia mearnsii	Bark	C	45.7	51.8	48.7	39.8	8.9	4.5	-	6	0.8
Anogessus leiocarpus	Bark	HC	5.7	23.0	21.9	14.5	7.4	2.0	+	6	0.7
	leaves	HC	7.2	36.2	34.4	20.8	13.6	1.5	+	6	0.6
Azadirachta indica	Bark	HC	22.4	25.1	24.9	16.8	7.6	2.2	+	6	0.7
Casuarina equistifolia	Bark	HC	12.3	16.7	14.9	10.2	4.7	2.2	+	6	0.7
Combretum hartmannianum	Bark	HC	4.6	27.1	27.0	14.2	12.8	1.1	+	6	0.5

Table 4 Determination of phenolics content & relative stringency in tannin extract by different techniques

Species	**Part**	**Tannin content, % in oven-dry part extracted**		**Extraction Ratio (Tannin/non-tannin)**		**Total phenols, % in oven-dry part extracted**			
		Hide Powder Method	**Combined Method**	**Hide Powder Method**	**Combined Method**	**Combined Method**	**Folin Denis Method**	**Hagerman Butler Method**	**Relative Stringency**
Acacia mearnsii	Bark	39.5	38.1	4.5	2.7	72.8	35.6	17.8	0.16
Anogessus leiocarpus	Bark	14.5	14.3	2.0	0.2	69.6	14.2	4.1	0.12
	leaves	20.8	19.9	1.5	0.3	72.0	19.9	10.9	0.10
Azadirachta indica	Bark	16.8	15.5	2.2	0.6	46.8	16.0	0.8	0.18
Casuarina equistifolia	Bark	10.2	10.2	2.2	1.0	47.1	10.0	5.1	0.12
Combretum hartmannianum	Bark	14.2	14.3	1.1	1.2	60.0	13.8	7.0	0.12

3.4. Stringency Factor

Astringency values displays that the *Azadirachta indica* (0.18), *Anogessus leiocarpus* bark (0.12), *Casuarina equistifolia* bark (0.12), and *Combretum hartmannianum* bark (0.12), may be utilized in place of *A. mearnsii* (international commercial tannin materials) (0.16) since the degree of relative astringency or the ability of their tannin to mix with protein is near to that of *Acacia mearnsii*; in other wards these four species can give leather with characteristics comparable to that of *Acacia mearnsii.*

4. Conclusion

This research paper investigates the characterization of hide powder and combined techniques for determination of tannins in several plant parts. Each diagnostic methods gives dissimilar information of nature, which is helpful for the knowledge and research of vegetable tannin materials formerly used for leather production. Of the six parts of indigenous and exotic woody plant species studied, the entire plant parts contained over 10% tannin needed for commercial exploitation. The amusing exotic species, but of limited distribution, was *Acacia mearnsii* bark (wattle), followed by the indigenous *Anogessus leiocarpus* leaves (20%), *Azadirachta indica* bark (16.8%), *Anogessus leiocarpus* bark (14.5%), *Combretum hartmannianum* bark (14.2%), and *Casuarina equistifolia* bark (10%). the whole studied tannin species are of mixed tannin types (gallo-catechol) except *Acacia mearnsii* which is of catechol type that mean contain condensed tannins only. The advantage of using this analytical technique, had similar yield of polyphenols attained with a lesser solvent feeding and a shorter removal time.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no conflict of interests exists.

References

[1] Evans, W. C., (Ed.) Pharmacognosy, London, Balliere Tindall, 1989, p. 386 – 393.

[2] Porter, J. L., in: Harborne, J. B., Dey, P. M., (Eds.), Methods in Plant Biochemistry, London, Academic Press, 1989, p. 389 – 419.

[3] Ragan, M. A., Glombitza, K., Phlorotannins: Brown algal polyphenols, Prog. Phycol. Res. 1986, 4: 177 – 241.

[4] Goffer, Z. Archaeological chemistry, Hoboken, Wiley-Intercedence, 2007.

[5] Covington, A.D. Tanning chemistry: the science of leather, Cambridge, The Royal Society of Chemistry, 2009.

[6] Thomson, R. Leather, in: E. May, M. Jones (Eds.), Conservation science: heritage materials, Cambridge, The Royal Society of Chemistry, 2006, p. 92–119.

[7] Haslam, E. Vegetable tannage – where do the tannins go? J. Soc. Leather Tech. Chem. 1997, 81: 45–51.

[8] Bruneton, J., Pharmacognosy, Phytochemistry, Medicinal Plants, London, New York, Lavoisier, 1995, p 313.

[9] Tang, H., Covington, A., Hancock, R. Use of DSC to detect the heterogeneity of hydrothermal stability in the polyphenol-treated collagen matrix. J. Agric. Food Chem. 2003, 51:6652-6656.

[10] Schroepfer, M., Meyer, M., Investigations towards the binding mechanisms of vegetable tanning agents to collagen. Res. J. Phytochem. 2016, 10:58-66.

[11] SLTC. Official Methods of Analysis. 4th edition. Redbourne. Society of Leather Trade Chemists, 1965.

[12] Stahl, E. Thin-Layer Chromatography. New York, Springer Verlag, 1969, p. 20.

[13] Harborne, J. B. A Guide to Modern Techniques of Plant Analysis. 2ndedition. London, Chapman and Hall, 1998.

[14] Blainski, A., Lopes, G.C., De Mello, J.C.P., Application and analysis of the folin method for the determination of the total phenolic content from Limonium Brasiliense L. Molecules2013, 18: 6852-6865

[15] Swain, T. & Goldstein, J. L. Methods of Polyphenol Chemistry. New York, Macmillan, 1964.

[16] Folin, O. & Denis, W. A colorimetric method for the determination of phenols (and phenol derivatives) in urine. Journal of Biological Chemistry,1915, 22: 305–308.

[17] Yazaki, Y. &Hillis, W. E. Molecular size distribution of radiate pine bark extracts and its effect on properties. Hozforschung, 1998, 34: 125–130.

[18] Pizzi, A. Tannins: Major Sources, Properties and Applications Monomers, Polymers and Composites from Renewable Resources, oxford, Elsevier, 2008, p. 179-199.

[19] Lacoste, Clément, et al. "Bioresource pine tannin/furanic foams with glyoxal and." Industrial Crops and Products 2013, 45: 401-405.

[20] Gülcin, I., Huyut, Z., Elmastak,, M., Aboul-Enein, H.Y., Radical scavenging and antioxidant activity of tannic acid. Arab. J. Chem. 2010. 3: 43–53. 6.

[21] Hagerman, A. E. & Butler, L. G. Protein precipitation method for the quantitative determination of tannins. Journal of Agricultural and Food Chemistry,1978, 26: 808–812.

[22] Hagerman, A.E., Tannin Chemistry. http://www.http://www.users.muohio. edu/hagermae/tannins.pdfusers.muohio.edu/hagermae/tannins.pdf. 1998.

[23] Judd, W.S., Campbell, C.S., Kellogg, E.A., Stevens, P.F., Donoghue, M.J., Plant Systematics: A Phylogenetic Approach, 3rd.ed., Sunderland, Sinauer Association, MA. 2007.

[24] Talhouk, R.S., Karam, C., Fostok, S., El-Jouni, W., Barbour, E.K., Anti-inflammatory bioactivities in plant extracts. Journal of Medicinal Food, 2007, 10:1–10.

[25] Yazaki, Y., Collins, P.J.wood adhesive based on tannin extracts from bark of and spruce species some pine, Werkst, HolzRoh, 1994, 52: 307-310.