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# POTENTIAL TANNING AGENTS FROM INDIGENOUS FLORA

**BUCIŞCANU Ingrid-Ioana[1], PRUNEANU Melinda[1], CREȚESCU Igor[2], BĂLĂU-MÎNDRU Tudorel[1]**

[1]"Gheorghe Asachi" Technical University of Iași, Faculty of Textiles, Leather and Industrial Management, Department of Textile and Leather Chemical Technology, 29 Prof. dr. docent Dimitrie Mangeron Blvd, 700050, Iaşi, Romania, E-Mail: decanat@tex.tuiasi.ro

[2] "Gheorghe Asachi" Technical University of Iaşi, Faculty of Chemical Engineering and Environmental Protection, Department of Environmental Engineering and Management, 73 Prof. dr. docent Dimitrie Mangeron Blvd, 700050, Iaşi, Romania, E-Mail: decanat@ch.tuiasi.ro

Corresponding author: Pruneanu, Melinda, E-mail: melindap@ch.tuiasi.ro, pruneanu_melinda@yahoo.com

***Abstract:*** *At present, about 90% of global leather is tanned with basic chromium salts, but public and authorities concern on the chromium toxicity forces tanneries to produce chrome-less or chrome-free leather. Lately, the old craft of vegetable tanning, which claims to be the most environmentally-friendly, has enjoyed a noticeable revival. At the same time, there is increasing interest in finding new vegetable sources for tannins from the European flora, as susatinable and cheaper alternatives to the expensive exotic species such as mimosa, quebracho or myrobalan. Along time, tanners have used barks and woods of different plants, but their properties are seldom documented. In this paper, some qualitative and quantitative tests on water extracts of* Alnus glutinosa*, (the black alder, Betulaceae family) and* Prunus spinosa *((blackthorn, Rosaceae family) were performed, in order to evaluate their tannin contents and potential tanning ability. Extraction was performed at a plant material:water mass ratio of 1:10, at 60°C, with extraction yield of 22.5% for adler extract (AE) and 8.9 % for blackthorn extract (BE). Spot tests were performed with positive responses. The total polyphenol contents (TPC) was determined by the Folin-Ciocalteu method, and expressed in terms of gallic acid equivalents(GAE). The TPC was 502.1 mg GAE/g AE and 249.72 mg GAE /g BE. The tannin content was estimated by a modified hide powder method, and was 155.43 mg GAE/g AE and 199.91 mg GAE/g BE. Given the working conditions, the extraction yield and tannin content of both species can be considered as satisfactory. Further leather tanning experiments are required to confirm their possible use as tanning agents.*

***Key words:*** *vegetable tannage, leather, tannin extract, Prunus spinosa, Alnus glutinosa, renewable resources*

## 1. INTRODUCTION

Leather has been used by mankind from the ancient times to nowadays, due to its unique qualities: thermal insulation, breathability, durability, pleasant sensory and aesthetic characteristics. The core of the leather-making process is the tanning operation. Today, almost 90% percent of the leather on the market is tanned with basic chromium (III) salts, but concerns regarding chromium toxicity are growing and pressure is put on tanneries both from environmental legislation and aware consumers, to apply environmentally-friendly alternative tanning processes. [1].

Lately, vegetable tanning has been particularly paid attention as a cleaner alternative to chrome tanning. Vegetable tanning is the oldest, the most natural, and claims to be the most environmental-friendly, even if the long duration of the process, high water consumption, and high concentration of slowly biodegradable phenolics of the spent floats are still issues of debate [2].

The actual interest in vegetable tanning is proved by the increasing number of small vegetable tanneries across Europe [3], and notable research on finding new plant sources for high quality tannins [4]. The drive for reconsidering the tanning potential of European flora is the intensive exploitation and depletion danger of exotic species with high tannin content, and the need to find cheaper sources, which are renewable resources at the same time. The limiting factor is the extraction yield and the tannin content of the extract. On the tannins market, it is generally accepted that species with more than 10% tannin content are suitable for commercial exploitation [5].

Extraction with hot water, in a temperature range of 40–90°C, is the most common method for producing tannins for leather industry, as the water solubles are the compounds of interest. Disadvantages are the lower yield of extraction and the co-extraction of non-tannin compounds.

Along time, apart from commercial tannins from exotic species, tannery practice have reported the use of different barks and woods, even if on limited small scale, and seldom documented. Amongst these, are the bark of alder tree (*Alnus glutinosa*) and branches of blackthorne (*Prunus Spinosa*), species from the European flora.

Historical use of alnus bark as a tanning agent in small tanneries in southeastern Europe was reported, due to its high tannin content of 9 to 16%, but the usage was limited because it imparts an objectionable reddish-brown color to the leather, and also tends to make the leather brittle. When used in combination with exotic tannins, the results were satisfactory [6] Blackthorn bark and branches have been traditionally used to tan leather and to prepare a dark color ink [7].

This study aims to draw attention upon the potential tanning ability of two indigenous plant species. In this respect, qualitative and quantitative analysis of polyphenolic and tannin phytoconstituents present in aqueous extracts of *Prunus spinosa* thorns and *Alnus glutinosa* bark were performed.

## 2. EXPERIMENTAL

### 2.1. Plant materials and chemical reagents

Fresh thorns of *Prunus spinosa* and bark of *Alnus glutinosa* were collected from Barnova Forest near Iasi. The plant species were identified with help of Botanical Atlas [8]. The wooden materials were first shade dried and then oven dried for 72 hours at 60 - 65°C, and grinded with an electric grinder. The 0.4 – 0.6 mm fraction was collected by sieving and stored in an exsicator. All the reagents and chemicals – Folin-Ciocalteu's phenol reagent and gallic acid monohydrate (Scharlau, Spain), sodium carbonate, ferric chloride, sodium chloride (Chemipar, Romania), slightly chromated hide powder and gelatin powder from (Merck, Germany) were of analytical grade.

### 2.2. Extracts preparation

Extracts were obtained by a three-stage, batch extraction method, with hot water as solvent. The plant material:water mass ratio was 1:10. A quantity of 10 g sieved vegetable material was mixed with 40 g distilled water (DW) and mixed for 60 min at 60°C. After that, the liquid was drained and the procedure was repeated two more times, with equal amounts of 30 g DW. The total recovered liquid of 80 mL was first concentrated on a water bath at 60-70°C and finally freeze-dried.

### 2.3. Spot tests

For tannin extracts, several common qualitative tests were performed, as follows [9]:

*Lead acetate test*: To 3ml of of 1:10 extract solution, 3 ml/a few drops of 10% lead acetate solution is added. The occurrence of white precipitates indicates the presence of tannins and phenols.

*Ferric chloride test.* To 3 ml of 1:10 extract solution, a few drops of 5% w/v ferric chloride solution are added. The blue – black color indicates the presence of hydrolysable tannins. A brownish green precipitate indicates the presence of condensed tannins. If the extract contains both types of tannins, a blue color is produced, which changes to olive-green as more ferric chloride is added.
*Fehling's* test*:* is used for the detection of reducing carbohydrates Fehling A and Fehling B reagents are mixed in equal volume and few drops of extract is added and boiled. Appearance of a brick red colored precipitate of cuprous oxide cofirms the presence of carbohydrates.
*Gelatin precipitation test.* A few drops of 1% tannin extract solution are added to a 1% gelatin in 10% sodium chloride solution. A white-yellowish color precipitate indicates the presence of tannins.

### 2.4. Determination of total extract, total phenol (TPC) and tannin material

*The total extract* or extraction yield was determined gravimetrically, and calculated as the ratio between the extract mass and the mass of the starting plant material (eq.1.):

$$Yield\,(\%) = (\,mass\ of\ \ solid\ extract / mass\ of\ \ plant\ material\ ) \times 100 \qquad \mathbf{(1)}$$

*The total phenolic content (TPC)* was quantified by the the Folin-Ciocalteu photocolorimetric method, using gallic acid (GA) as standard [10]. Fresh solutions of 400 mg/L AE and BE were prepared.The procedure was as follows: 1 mL of extract solution was mixed with 9 mL DW and 1 mL Folin-Ciocalteu reagent; after 5 min, 3 mL of 20% $Na_2CO_3$ was added and the volume was completed to 25 mL, in a volumetric flask. After 60 min incubation at 20°C, the solutions absorbance were measured on a UV VIS HACH DR/2010 spectrophotometer, at maximum absorption wavelength of 760 nm. Calibration curve was determined over a concentration range 0 - 500 $mgL^{-1}$ GA. The TPC in the plant extract was expressed as mg of gallic acid equivalents (GAE)/ g of freeze-dried extract, and was calculated using the formula (eq.2):

$$TPC\ =\ \left(C\ \times\ V\right) /\ M \qquad mgGAE\ /\ g\ extract \qquad \mathbf{(2)}$$

where: C = concentration of gallic acid established from the calibration curve, mg/L; V = volume of extract, L; M = weight of water extract of the plant, g.
*Tannin contents.* The tannin contents was determined by a modified hide powder method, based on measuring the TPC in the tannin solution, before and after the adsorbtion on hide powder, by the Folin-Ciocalteu method [11] Solutions of 5 g/L *AE* and 6 g/L *PE* were prepared. An equivalent of 3.2 g dry substance of slightly chromated hide powder was mixed with 100 ml tannin solution and agitated in a reciprocal mixer for 1 h at 20°C. The suspensions were filtered through a white band Whatman filter paper and the absorbance of filtrate was measured at 760 nm The tannin content of the extract was determined as the difference between the TPC of initial extract solutions and filtrate.

## 3. RESULTS AND DISCUSIONS

### 3.1. Extraction of water-soluble matter

The starting plant materials and final extracts of alder and blackthorn are given in Fig. 1. The alnus extract shows the characteristic reddish color, is very hygroscopic and has a sticky feel, due to the presence of sugars that were detected by the spot tests. The extraction yield at the working temperature and using water as solvent are high enough, namely 22.5% for adler extract (AE) and 8.9 % for blackthorn extract (BE), as given in Table 1 The temperature of 60°C was chosen to avoid the degradation of active compounds and to provide a convenient extraction yield. [12]. Water was chosen as solvent, as it was proved that the maximum yield of tannin was in water and the alcohol solvents have a negative influence upon the hydrolysable tannins - ellagitannins and gallotannins, which play the main role in the leather tanning process [13].

***Fig. 1**: Alnus glutinosa* bark and extract (left) and *Prunus spinosa* thorns and extract (right)*

### 3.2. Spot tests

Spot tests are simple qualitative chemical procedures which uniquely identify a substance. Results of spot tests for studied tannins are given in **Fig.2**. The lead acetate showed a positive result for phenolics and tannins. The ferric chloride test showed an intense dark blue coloration, which changed rapidly to green and precipitate formation. The gelatin test showed intense precipitation in

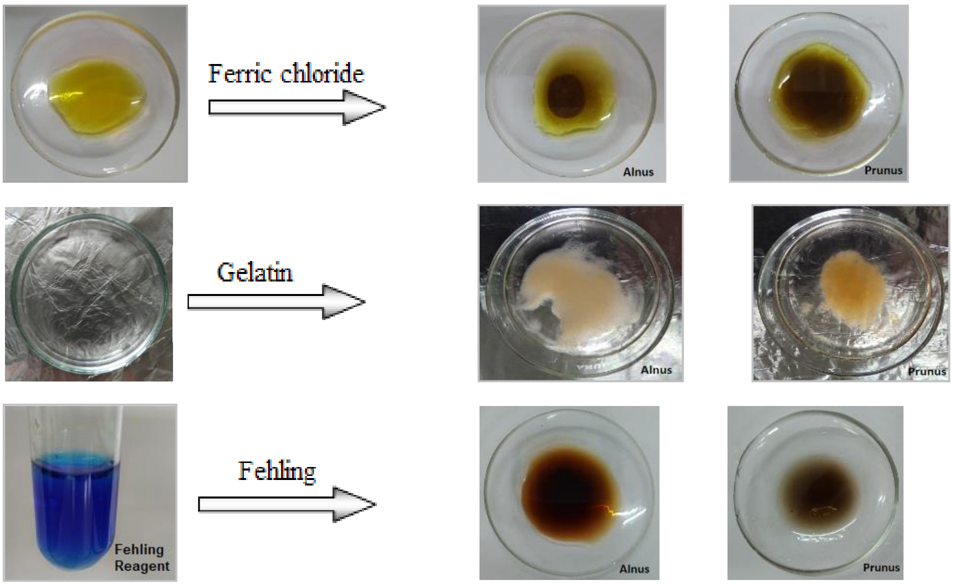


***Fig. 2**: Spot tests on* Alnus glutinosa *and* Prunus spinosa *water extracts*

both cases. Hence, it can be safely concluded that the phenolics also contain tannins. The presence of coloring matter is obvious in the *Prunus* extract. The Fehling test indicated a high content of reducing sugars in the *Alnus* extract and was negative for the *Prunus* extract. The presence of reducing sugars can impair the extract properties and hinder the tanning process, for instance our experimental observations showed that it was much more difficult to solubilize the alnus extract than the blackthorn extract.

### 3.1. Phenol contens and estimation of tannin contents

The experimental points and linear regression equation of the standard curve are given in **Fig. 1.** The experimental values of total phenols and tannins are given in **Table 1.** Available literature does not provide values of TPC of alder, but the high value of 502.07 mg GAE/g AE was expected, given the reported high contents of tannins [6]. The TPC of blackthorn is lower than those of 499.23 reported elswhere [14], but the differencence may be due to the fact that and in the present paper extraction was performed from thorns and not from the entire branches, using water instead of water-acetone mixture for extraction.

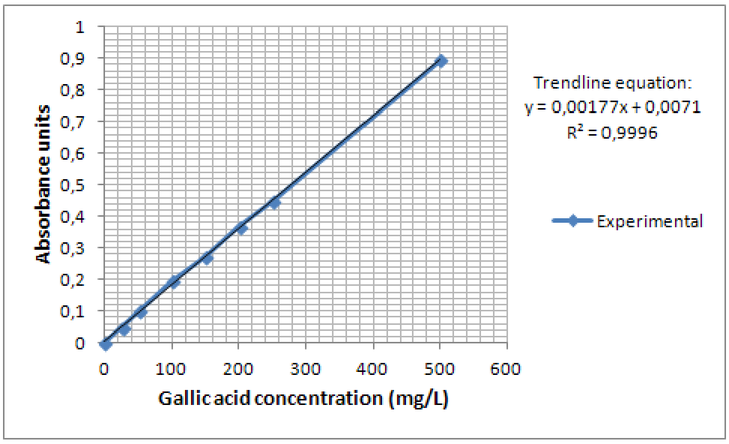


***Fig. 3**: The gallic acid standard curve for Total Phenolic Content by Folin-Ciocalteu method*

The tannin content of the alder extract is common for bark extracts, while the tannin content of blackthorn is unusualy high. This could be related to the presence in the total extract of coloring matter, which might be adsorbed by the hide powder substrate, and to the interferences that affect the photocolorimetric measuring method. Gravimetric determination of hide-binding power, i.e. the tannin fraction that is ireversibly binded to the hide powder, is further required.

***Table 1:** Characteristics of tannin extracts*

| Vegetable Species | $FeCl_3$ test | Gelatin test | Fehling test | Extraction yield % | TPC mg GAE/ g extract | Tannins mg GAE/ g extract | Tannins % of TPC |
|---|---|---|---|---|---|---|---|
| *Alnus glutinosa* | + | + | + | 22.5 | 502.07 | 155,43 | 30.95% |
| *Prunus spinosa* | + | + | - | 8.9 | 249.72 | 199.91 | 80.05% |

## 5. CONCLUSIONS

The revival of vegetable tannage determined an increasing interest in finding or reconsidering vegetable species from the European flora with high tannin content, as cheaper and sustainable alternative to conventional tannins, extracted from exotic plants.

Two indigenous species, *Alnus glutinosa* and *Prunus spinosa*, historically used for leather tanning, were assessed regarding the extraction yield, and phenolic and tannin content.

The extraction yield was 22.5% for the *Alnus* bark and 8.9% for the *Prunus* thorns, which can be satisfactory, taking in account the mild extraction working conditions.

The tannin content of the *Alnus* extract was 30.95%, while *Prunus* extract exhibited a very high measured value of 80%, which might include interfering compounds.

Practical leather tanning experiments are required, to confirm possible use of these tannins in leather-making processes.

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