Potentilla Erecta (L.) Raeusch as an Alternative Source of Environmentally Friendly Polyphenols for Leather Tanning

by

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Abstract

High content of polyphenols in plant extracts explains their biological activitity. These extracts also can be used in antimicrobial therapy as an alternative to chemical drugs. A large number of hydroxyl and other functional groups in the polyphenols preconditions formation of strong cross-links with proteins and other macromolecules. The leather tanning technology, known as vegetable tanning, was the prevailing process for leather manufacturers over centuries until it has gradually been displaced by tanning with a use of inorganic chromium compounds by the end of the 19th century. Unfortunately, the vegetable sources with importance for leather tanning are limited in number of plant materials. *Potentilla* is considered as a tannin-rich plant. It is estimated that tannin content in *Potentilla erecta (L.) Raeusch* is approx. 15-22%. The results of the present study have shown that *Potentilla erecta (L.) Raeusch* can be characterized as a potential tannin rich plant source for leather tanning industry. The extracts of *Potentilla* mainly contain condensed tannins, but also some amounts of hydrolysable tannins are present. The yield of the extractives from *Potentilla* rootstocks was found to be 21.3%: 11.5% tannins and 9.8% non-tannins. The tannin content in *Potentilla* is comparable to the ones extracted from *Ouebracho* and *Chestnut* wood, tannins that are used widely in leather industry.

Introduction

Traditional medicine has been using the *Potentilla* species for long time. *Potentilla* genus has over 300 species mainly distributed in the temperate regions of the northern hemisphere.[1] *Potentilla erecta (L.) Raeusch* traditionally is used for the treatment of wounds, inflammations, bleeding, diarrhea, dysentery, inflammatory bowel disease, fungal, bacterial and viral infections, and certain forms of cancer, as well as mouth and throat antiseptic.[2-5] *Potentilla* is widespread in Baltic States.[6] In Lithuania it grows on moderately moist and moist mineral soils and peat.

High content of polyphenols in plant extracts explains their biological activities;[7] these extracts also can be used in antimicrobial therapy as an alternative to chemical drugs. *Potentilla* genus, due to its quantification of phyto-constituents as well as pharmacological profile, is very attractive as an alternative for synthetic antimicrobial agents. The results of recent clinical surveys have shown that extracts from *P. erecta* rhizome may be regarded as a safe bactericidal agent in treating acute toxicity in humans.[8] Its actions are attributed to the bacteriostatic and bactericidal activity against Gram-positive bacteria and inhibiting activity on the growth of some yeast strains.[9]

The leather tanning technology, known as vegetable tanning, was the prevailing process for leather manufacturers over centuries until it has gradually been displaced by tanning with a use of inorganic chromium compounds by the end of the 19th century.[10] On the other hand, until nowadays the vegetable tannins have been considered as a available and environmentally friendly choice to displace or diminish use of the chromium compounds in tanning process. Tannins are usually found in larger amount in the bark of trees where they act as a barrier for micro-organisms and protect the tree.[11] A large number of hydroxyl and other functional groups in the polyphenols preconditions formation of strong cross-links with proteins and other macromolecules. Unfortunately, the vegetable sources with importance for leather tanning are limited in number of plant materials.[12] *Potentilla* is considered as a tannin-rich plant.[13] It is estimated that tannin content in *Potentilla erecta (L.) Raeusch* is approx. 15-22%.[14] Therefore, its use for leather tanning could be very promising. Also, it could be presumed that leather tanned using tannins prepared from *Potentilla* could have some

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bacteriostatic or antibacterial properties. The previous research has shown that vegetable tanned leather is more resistant towards bacterial action.[15] Sung *et al.* have reported that tannins display bacteriostatic and bactericidal effect against harmful bacteria such as *Listeria*, *Escherichia*, *Staphylococcus*, *Pseudomonas*, *Streptococcus*, and *Salmonella*.[16]

The leather tanned with vegetable tannins displays as comfortable, compatible with human skin, and having high dimensional stability, which is very important for making shoes for people with special needs. Thus, the vegetable tanned leather is widely used for orthopedic footwear. Also, patients with diabetes mellitus (DM) have higher need for special and protective footwear.[17] Nowadays, diabetes is one of the main health system problems and a global public health threat. Unfortunately, patients with diabetes have inclination to various complications, and diabetic foot ulcer is one of most prevailing. It is known[18] that the course of the diabetic foot syndrome (diabetic podopathy) is conditioned by the footwear significantly. Recently, due to increasing awareness of material safety, the interest in natural substances has increased on account of their potentially higher compatibility and relatively low toxicity. Hence, the goods finished with environment friendly materials are preferred by the modern consumer.

The aim of the present study was characterization of *Potentilla erecta (L.) Raeusch* as a potential tannin rich plant source for leather tanning industry.

Experimental

Materials

Hide powder used for the determination of tannin content of the extracts was purchased from Sigma Aldrich. Domestic cow hides were used in the tanning process. All reagents used for research were of analytical grade.

Methods

Preparation of Plant Extracts

Potentilla erecta (L.) Raeusch rootstocks was purchased from "Svencioniu vaistazoles" (Lithuania). The *Potentilla* rootstocks (moisture content 8.8%) were milled to the powder using IKA A11 analytical mill. 100 grams of received powder mass was placed into a flask and extracted at 70°C (7 h) with 1000 ml of water, stirring at 150 rpm. The extract was filtered and dried at 70°C. The yield of the extractives was calculated according to the following equation:

Yeld (%) = [Extractives (g) / Amount of the absolutely dry *Potentilla* rootstocks (g)] x 100

Total Content of Phenolic Compounds

A determination of the content of phenolic compounds was carried out according to the method described by Damasius et al.[19] 1 ml of prepared extract solution was transferred to a volumetric flask with 5 ml of Folin–Ciocalteu reagent and 4 ml of 7.5% sodium carbonate. The absorbance was measured after 30 min incubation using a UV spectrophotometer at 765 nm and compared with a gallic acid calibration curve. The content of phenolic compounds was presented in equivalents of Gallic acid (GAE). All measurements were made in duplicate.

Determination of Total Flavonoid Content

For calibration curve quercetin was used. 10 mg of quercetin was dissolved in ethanol and then diluted to 6.25, 12.5, 25, 50, 80, and 100 mg/ml. A calibration curve was made by measuring with a spectrophotometer the absorbance of the dilutions at 415 nm (λ_{max} of quercetin). 1% aluminum chloride and potassium acetate solutions were prepared.[20, 21] 0.5ml of each extract stock solution, 1.5 ml of ethanol, 0.1 ml of aluminum chloride, 0.1 ml of potassium acetate solution and 2.8 ml of distilled water were added and mixed well. The aluminum chloride solution was replaced by distilled water for the preparation of blank sample. Accordingly, sample and blank sample of all three extracts were produced and their absorbance was measured.

DPPH Radical Scavenging Activity

Free radical scavenging activities of solutions of the tannins and synthetic antioxidant butylated hydroxytoluene (BHT) prepared in ethanol at 0.05 mg/mL were determined in accordance with the Shimada *et al.*[22] method, which is based on the principle of scavenging the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. DPPH was added to the solutions prepared with tannin solution and standard antioxidant substance BHT and stirred. Each mixture was kept in the dark for 30 min and the absorbance was measured at 517 nm against a blank.[22] A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in duplicate. The radical scavenging activities of the tested samples, expressed as percentage of inhibition, were calculated according to the following equation:[23]

Percentage of inhibition of DPPH activity (%) =
$[(A_B - A_A) / A_B] \times 100$

A_A and A_B are the absorbance values of the test and blank sample, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and represented as IC_{50} value for each of the test solutions.

Analysis of Tannin Content (Hide-powder Test)

The method consists of two main steps: low-chromed hide powder preparation and adsorption of tannins by low-chromed hide powder. The low-chromed hide powder used in analysis was

prepared from hide powder. 10 time's higher amount of dist. water was poured on the air-dried hide powder and stirred (100 rpm) for 1 hour at room temperature. 30 g/l chromium (III) potassium sulfate solution was (1 ml of solution to 1g of hide-powder) added and mixture was stirred for 1 h, then it was filtered through unbleached flax cloth and squeezed. The prepared low-chromed hide powder was washed several times with dist. water until filtrate reaction with barium chloride became negative and air dried.

100 ml of aqueous solution of extract (10 g/l) was added to 6.25 g of low-chromed air-dried hide powder. The suspension was stirred (100 rpm) for 10 min and filtered. Fifty milliliters of filtrate were evaporated to determine the non-tannin substance. The amount of tannins (%) was calculated as the difference between the soluble solids (%) and the non-tannins (%).

FTIR – Spectroscopy

The tannin (about 2 mg) was added into potassium bromide (100 mg), mixed and grinded to powder of 2 mm particle's diameter. Then 60 mg of the powder were pressed to form pellets. The spectra of samples were obtained on Perkin-Elmer FT-IR system *Spectrum GX* at a resolution of 1 cm^{-1}, scan rate 0.2 cm s^{-1} and scan quantity 16×.

Table I
Tanning parameters for pickled hide.

Process	Parameters of processes		
	Material, % from pelt weight	**Temperature, °C**	**Duration and regime**
Pre-tanning	water – 100; sodium chloride – 4.0;	20-25	15 min, run continuously
	Cromeco 33 Extra – 2.4		16 h, run continuously
Neutralization*	sodium carbonate – 0.5 sodium carbonate – 0.5 sodium carbonate – 0.5	20-25	30 min, run continuously 30 min, run continuously 23 h, run continuously
Vegetable tanning	water – 220; *tannin* – 20	36±2	96 h, run continuously
Washing	water – 150	25-30	1 h, run continuously
	water – 150		1 h, run continuously
Neutralization	water – 150; sodium hydrocarbonate – 1.0; sodium formate – 1.0	40±2	1 h, run continuously
Washing	water – 200 water – 200	60±2	0.5 h, run continuously 0.5 h, run continuously
Fatliqouring	water – 200 *Oleal 146* – 2.0 *Oleal 1946* – 4.0 *Fosfoliker 661* – 3.0 *Fosfoliker 6146* – 4.0	60±2	1 h, run continuously
	formic acid – 0.5 formic acid – 0.5		20 min., run continuously 20 min., run continuously
Washing	water – 200; *Fungicide FDE* – 0.2	20-25	20 min, run continuously
Drying		18–20	24 h

* Neutralization carried out in pre-tanning solution.

NMR Spectroscopy

The ^{1}H NMR spectra were obtained using DMSO-d_6 on a *Bruker Ascend* 400 (^{1}H 400 MHz) spectrometer. Chemical shifts (δ) are presented in parts per million (ppm) calibrated from TMS (0 ppm) as an internal standard for ^{1}H NMR.

Particle Size Distribution

The distribution of the nanoparticles was determined using *Zetasizer Nano-S* (Malvern Instruments) in aqueous solution at pH 7–8. The analysis was performed at a scattering angle of 173°C at 20°C. The four measurements were performed for each samples and average particle diameter was calculated.

Zeta Potential Measurements

Zeta potential measurements were performed using electroacoustic spectrometer *DT-300* (Dispersion Technology Inc.) in aqueous solution at pH 7–8 at 20°C.

Molecular Weight Distribution

One milligram of each tannin was dissolved in 1 ml of tetrahydrofuran, and a gel permeation chromatography (GPC) analysis was carried out for the solutions. The molecular weights (Mn – number average molecular weight; Mw – weight average molecular weight) were determined by GPC with *Malvern Viscotek* system containing GMHHR-M columns and Bischoff LAMBDA 1000 detector. Polystyrene standards were used for calibration of the columns. THF (tetrahydrofuran) was chosen as an eluent.

Leather Tanning

The pickled pelt was obtained from JSC "Kedainiu oda" (Lithuania). Pickled pelt was processed by conventional technology. The pickled pelt was tanned according to the method shown in Table I.

Cromeco 33 Extra is a basic chromium sulfate (contains 25% of chromium (III) oxide, 33% basicity) produced by Gruppo Chimico Dalton (Italy). Fatliqours as *Oleal 146*, *Oleal 1946*, *Fosfoliker 661* and *Fosfoliker 6146* are technical products produced by Codyeco (Italy). Fungicide FDE is a product of KEMCOLOR S.p.a. (Italy).

Results and Discussion

Plants are a valuable source of natural bioactive compounds. One of such bioactive compound groups are phenolics. The phenolics are important in defense against predators, parasites,

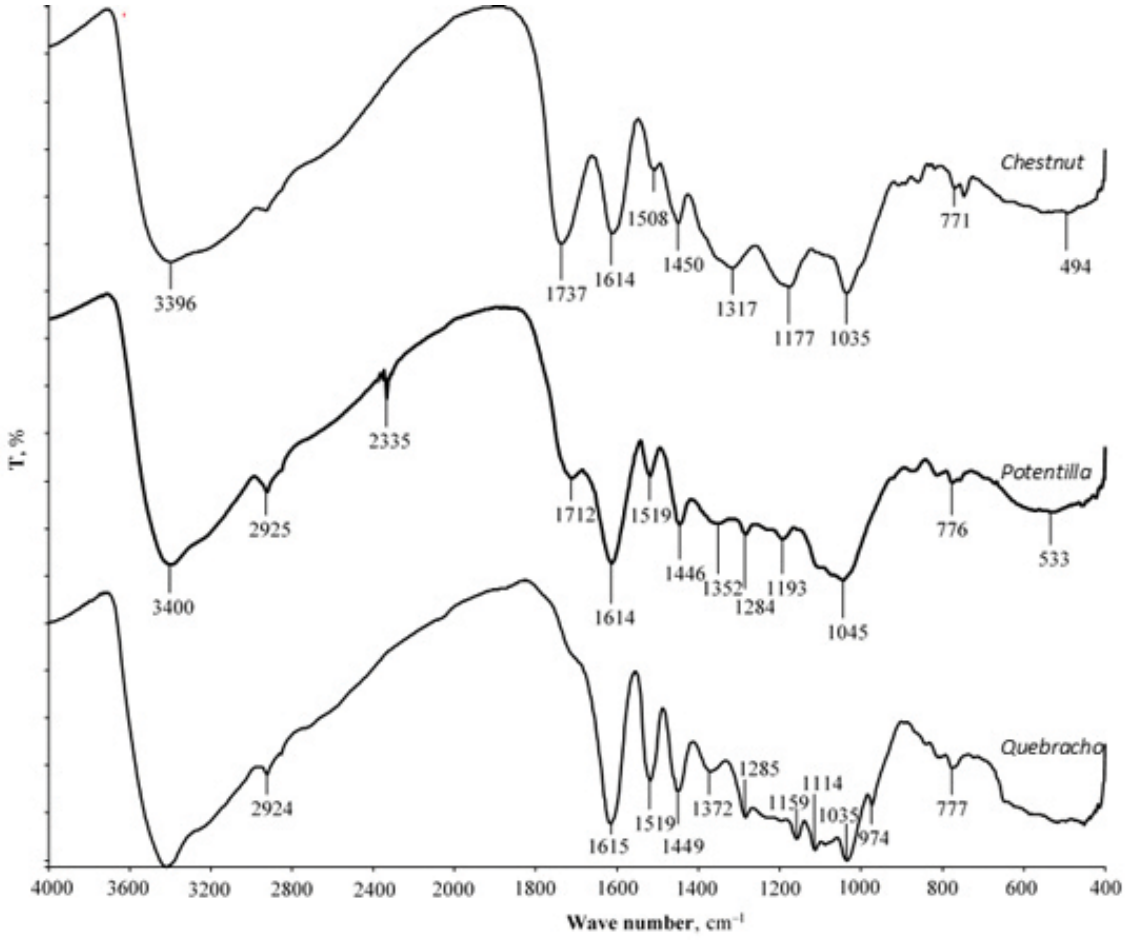

Figure 1. FTIR spectra of tannins.

and pathogens. They participate in the defense against ultraviolet radiation as well. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins.[24] Every tannin possesses its own characteristics: solubility, color, acidity, salt and carbohydrate content, average molecular weight and molecular weight distribution.[25]

FTIR Spectra

Plant polyphenols are classified by their structural characteristics into hydrolysable as and condensed. It is known that extracts of *Potentilla* mainly contain condensed tannins, but some amounts of hydrolysable tannins are present as well.[14] Commercial *Quebracho* and *Chestnut* tannins (Tanac S.A., Brasil) were used as control samples in this study.

The hydrolysable tannins have a carbonyl function, especially in the form of esters, and may be hydrolysed to acids in aqueous systems.[26,27] The C=O stretching of hydrolysable tannins' esters, particularly gallic acid derivatives, can be observed in the 1722–1702 cm^{-1} region.[28] The peak at 1718 cm^{-1} is characteristic to the tannic acid.[29] In our case, *Chestnut* tannin spectra show peak at 1737 cm^{-1} and the one for *Potentilla* displays a peak at 1712 cm^{-1} (Fig. 1). In the spectra of tannin obtained from *Quebracho* this peak is absent. This leads to the assumption that some amount of hydrolysable tannin is present in the prepared *Potentilla* extract.

The strong sharp band at 1520 cm^{-1}, observed in the *Potentilla* and *Quebracho* spectra, is attributed to simple catechin and is a recognition pattern for condensed tannins (Fig. 1). The band in the range of 1287-1285 cm^{-1} is attributed to flavonoid-based tannins and flavanols, and is not observed in the spectra of hydrolysable tannins such as the ones obtained from *Chestnut*.

FTIR Spectra of Tannins

Physical Characteristics

Tannin solutions are colloidal in nature and they contain tannin particles of different molecular weights.[12] The compounds of low molecular weight are just too small and do not act as effective cross-linking materials. The compounds of high molecular weight commonly are insoluble or just too large for crosslinking with proteins. Tannin which molecular weight is in the range of 500-3000 is suitable for linking with collagen active groups and forms stable cross-linked structures. The molecular weight, molecular weight distribution, Zeta potential and particle size of tannins were determined.

Since the content of phenolic acids is not high in the condensed tannin extract, and tannins themselves are not carboxylated, the

Table II

Tannins characteristics.

Tannin	Tannin class	pH of 10% solution	Mw, Da	Mn, Da	Mw/Mn	Particle size, nm	Zeta, mV
Potentilla	Condensed	4.7	907	613	1.5	407.7	-5.48
Quebracho	Condensed	4.6	711	636	1.8	452.6	-5.93
Chestnut	Hydrolysable	3.8	1154	406	1.8	461.4	-5.39

Table III

Bioactivity of tannins.

Index	Tannin		
	Potentilla	***Quebracho***	***Chestnut***
Total content of flavonoids, mg Quercetin equivalent /g	22.0	42.0	71.1
Total content of phenolic compounds, mg Gallic acid equivalent /g	167.9	294.1	167.7
DPPH free scavenging capacity (IC_{50}), μg/ml	15.74	12.74	12.08

Note: IC_{50} of BHT – 20.59μg/ml.

tan liquors have a relatively high pH.[30] The results have shown (Table II) that pH of *Potentilla* 10% tannin solution was similar (4.7) to the one of *Quebracho* (4.6) and higher than the one of *Chestnut* (3.8).

Proanthocyanidins are complexes of oligomers built up of flavan-3-ol monomer units and high molecular weight polymers.[31] Molecular weight of flavan-3-ol is 226. Therefore, based on the data presented in Table II the assumption could be made that dimers and trimers dominate in *Potentilla* and *Quebracho* extracts. In case of hydrolysable tannin, monomer units are gallic or ellagic acids. Therefore, dimers dominate in *Chestnut* extract if we consider the gallic acid as a monomeric unit with molecular weight 170. As seen from the data presented in Table II, polydispersity (Mw/Mn) of all tannins isn't high and varies from 1.5 up to1.8.

Teng *et al.*[32] have determined a dependence of tanning ability on the Mn: the higher Mn value of the tannin indicates the stronger tanning ability. From obtained data it can be assumed that tanning ability of *Potentilla* tannin is comparable to the ones of other two commercial tannins, because Mn, particle size and Zeta potential of all tannins are very similar.

NMR Spectra

NMR studies have shown that the majority of the signals of the condensed *Potentilla* and *Quebracho* tannins in the spectra are present in the region of 5.84 - 7.21 ppm (due to the aromatic moiety in condensed tannins). Signals in *Quebracho* tannins spectra in the range of 5.84-6.40 ppm and 6.40-7.21 ppm are attributed to the aromatic A ring and signals between 6.40 and 7.21 ppm correspond to the aromatic B ring. In case of the spectrum for *Potentilla* tannins, the multiplet attributed to A ring is shifted down-field to the region between 6.55 and 7.01 ppm. In the spectra for *Chestnut* and *Potentilla* tannins, singlets at 7.35 and 7.39 ppm are attributed to the gallolyl group which is typical of hydrolyzed tannins. The multiplet at 6.25 and 7.01 ppm in the *Chestnut* tannin spectrum could be attributed to the less conjugated aromatic system which presence has been confirmed by the estimated Mn value (Table II) which is lower than the one for *Chestnut* tannin (406 Da).

Table IV
Composition of plant extractives.

Indexes	Plant		
	***Potentilla erecta* rootstocks**	***Quebracho* wood [12]**	***Chestnut* wood [12]**
Amount of tannins, %	11.5	14-26	5-15
Amount of non-tannins, %	9.8	1-2	1-2

Table V
Tanning quality indexes.

Index	Tannin				
	Potentilla	***Chestnut***	***Quebracho***	***Potentilla/ Chestnut***	***Potentilla/ Quebracho***
Pretanned leather T_S, °C	74.0				
Vegetable tanned leather T_S, °C	80.0	86.5	93.0	91.0	85.0
Amount of tannins in leather, %	30.4	31.1	29.9	26.8	25.2
Tannin extract uptake, %	26.8	62.6	67.3	38.6	38.3
Tensile strength of leather, N/mm^2	14.6	18.3	19.6	14.6	18.7
Relative elongation of leather, %	79.3	73.0	78.3	70.4	78.6

Bioactivity

Phenolic compounds, including flavonoids, play a very important role in a plant due to their antioxidant activity. Accordingly, flavonoids are the phenolic derivatives, which exhibit effective antioxidant properties.[33] As presented in Table III, the total phenolic content of *Quebracho* is higher (294.1 mg GAE/g) compared to that obtained from *Potentilla* and *Chestnut* (167.9 mg GAE/g). The total flavonoid content was higher in *Chestnut* tannin (71.1 mg GAE/g). Herewith, extracts characterized by higher phenolic content did not definitely contain higher flavonoid content.[34] To estimate tannins antioxidant activity the IC_{50} values were evaluated. The concentration of prepared tannin solutions in ethanol were 0.05 g/l. Since a lower IC50 corresponds to a higher antioxidant activity, this parameter is widely applied to assess the free radical scavenging activity. The results have shown that the IC_{50} value was lowest for *Chestnut* tannin (12.08μg/ml). Accordingly, it could be concluded that the antioxidant activity of tannins correlates with the total content of flavonoids.

Tanning Ability

The most important tannin property, which is very important for tanners, is tanning ability which could be assumed as an amount of tannins in plant extract. The results obtained from the tannin analysis are provided in Table I. All comercial vegetable tannins (the solid extracted from plant materials) consist of tannins, sugars, salts, gums, lignin and other components of plant materials passed into extract.[35] Therefore, extracts prepared from plants contain tannins and non-tannins as well as soluble and insoluble compounds. The analysis of the prepared (according to conditions described in the EXPERIMENTAL) *Potentilla* aqueous extract had been carried out, and the yield of the extraction of *Potentilla* was found to be 21.3% (percentage is based on absolutely dry rootstocks' mass). Amounts of tannins and non-tannins in the extract were determined as well (Table IV).

The results obtained allows proposition that tannin content of *Potentilla* is comparable to the ones of the tannins such as of *Ouebracho* and *Chestnut* which are widely used in leather industry. Herewith, the amount of non-tannin substances is considerably higher (9.8%). Non-tannins don't react with collagen active groups; however they participate in tanning process indirectly: they fill leather derma and stimulate the tanning process.[36]

Leather Tanning

The process of conversion of raw hide/skins into leather is known as leather tanning. During the tanning, collagen as a major protein of hide/skin reacts with tannins, chromium, alum, or other chemical agents, thus becoming significantly more thermostable and resistant against action of various microorganisms. Chromium (III) compounds and vegetable tannins obtained by extraction of various tree barks or wood commonly are used as tanning materials. Although almost all plants contain tannins, only few species have sufficient amounts to be of commercial importance.[37] The main merits of vegetable tanning are compatibility with human body, comfort, and volume fixity. Lack of softness and lower shrinkage temperature are the main disadvantages of vegetable tanned leather.[12] Conventionally, tanning method which uses small amount of chromium salts and vegetable tannins in combination is applied.[38-39] The tanned leather quality indexes for various combinations are given in Table V. The amount of tannins in all tanned samples is very similar and equal to 30%, but the shrinkage temperature of the samples differs in range of 13°C. The highest T_S was determined for sample tanned with *Quebracho* (93°C) and the lowest one was for the sample tanned with *Potentilla* (80°C). The higher T_S of the *Quebracho* tanned samples could be explained by formation of higher number of links with active collagen groups what increase hydrothermal stability of the leather. The mixture of *Potentilla* tannin with *Quebracho* or *Chestnut* tannins increased tanned leather T_S by 5-11°C.

The tensile strength of the leather samples was in the range of 14.6-19.6 N/mm^2 (Table V). Leather tanned only with *Potentilla* tannin had the lowest tensile strength (14.6 N/mm^2) and the highest percentage extension (79.3%). It leads us to assumption that it formed less links between collagen active groups and tannin due to the low tannin uptake. It means that the parameters of the tanning process with *Potentilla* tannins do not coincide with the tanning process parameters when common commercial tannins are used and should be readjusted.

Conclusions

The results of the present study have shown that *Potentilla erecta (L.) Raeusch* could be characterized as a potential tannin rich plant source for leather tanning industry. The results have shown that extracts of *Potentilla* mainly contain condensed tannins, but also some amounts of hydrolysable tannins are present. The yield of the extraction of *Potentilla* was found to be 21.3%. The prepared *Potentilla* extract and extracts of tannins from *Ouebracho* and *Chestnut*, which are used widely in leather industry, have comparable tannin content. Leather tanned with *Potentilla* tannin had low tensile strength (14.6 N/mm^2) and high percentage extension (79.3%), but tannin content in leather was similar as after tanning with commercial products (*Quebracho* and *Chestnut*). The investigation results lead to assumption that leather tanning process needs to be further studied because *Potentilla* tannin extract uptake was low: 26.8%.

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