Studies on Tannins from Barks of *Pinus roxburghii*

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Summary: Extraction of tannin was carried out at different temperatures with different solvent systems. Moisture content, pH, viscosity and total dissolved solids of the extracts were measured. Hide powder shake method was used for % yield of tannins and nontannins. The analysis by chemical techniques revealed that mostly the condensed tannins are present in the extracts. The high value of staisny number indicates that the extracts contain large amount of reactable tannins.

Introduction

Tannins are composed of different polyphenols [1]. The type of phenolic constituents affects the quality of tannin extract. There are two types of tannins, the hydrolyzable tannins and the condensed tannins. The hydrolyzable tannins are mixture of simple phenols such as pyrogallol [2], ellagic acid [3] and esters of sugars mainly glucose with gallic [4] and diagallic acid [5]. Condensed tannins constituting more than 90% of the total world production of commercial tannins, are both chemically and economically more interesting for the preparation of adhesives and resins. Condensed tannins and their flavanoid precursors are known for their wide distribution in nature and particularly for their substantial concentration in the wood and bark of various trees [5]. Amongst the most important sources of tannins are Heartwood of Quebracho *(Schinopsis lorentazii)*, the bark of Black Wattle (*Rhizophora* and *Bruguiera* species) the Heartwood and bark of wandoos (*Eucalyptus wandoo* and *E. accedens)* and the bark of brown Mallet *(Eucalyptus astringens)*. The tannins obtained from these sources are used as raw material for the preparation of adhesives for plywood and chipboard industries.

The chipboard and plywood industries are growing rapidly in Pakistan and so is the demand of adhesives. A considerable amount of foreign exchange is being spent on the import of this item. No systematic study has been carried out in this country to explore the possibility of obtaining raw material from the renewable sources. Only one source of tannin (Babul bark) has been reported in the Literature [6]. Since a sizeable area of the northern part of this country is covered by chir pine *(Pinus roxburghii)* it is desirable to carry out a thorough investigation on the tannins of the barks.

Suitable solvent systems are being reported for economical extraction of tannins. The yield and quality of the tannins are promising.

Experimental

The barks of *Pinus roxburghii* were collected from Karaker in district Swat of NWFP. These samples were taken from trees of different ages. The age of the trees were measured by counting of annual rings on the wooden rod. The wooden rod was obtained by making a bore at breast hight upto

the pith of tree with increment borer. In some cases when rings were not clear the rod was stained in ethanol. Each ring on the rod corresponds to one year growth [7] (Table 2) Moisture content of each powdered sample was determined by standard technique.

Extraction of Tannins

The powdered material of standard particle size (40/60 mesh) was used for extraction. The extraction of tannins were carried out at two temperatures in two different solvent systems.

(a) The extraction at elevated temperature was carried out in soxhlet apparatus by using 1400 ml of water-ethanol mixture (1:1) for 50 g of solid material. The tannin solution extracted with water-ethanol mixture was reduced to 455 ml.

(b) The extraction at elevated temperature [8] was also carried out from 25 g of solid material in 1400 ml of water but the volume of extract was reduced to 228 ml.

The extraction at ambient temperature was carried out by keeping 20 g of solid material in 800 ml of water at $\pm$ 35°C for 10 days. The volume of the extract was reduced to 182 ml. pH and viscosity of the extract were measured on a pH meter (precision pH meter type : OP-205/1) and a viscometer (BS/IP/RE) at 25°C. The results obtained are shown in Table 1. Total dissolved solids of the extracts were also measured.

Estimation of tannins and nontannis [9]

2.0 ml extract was diluted to 10 ml by using the same solvent. This solution was poured to a conical flask (250 ml) containing 6.25 g hide powder. It was shaken for 15 minutes, filtered and to the filterate china clay (1.0 g) was added. After shaking, it was filtered and to a small portion of the filterate aqueous gelatin solution (1%) was added to check the absence of tannins. The filterate was dried to a constant weight which gave the weight of nontannins. The difference in the weight of total soluble solids and nontannins gave the weight of tannins present (Table 2).

Staisny number [23]

In a 250 ml falsk fitted with a reflux condenser, 50 ml of extract, 5 ml of concentrated hydrochloric acid and 10 ml of formaldehyde solution (37% by wt.) were mixed. The mixture was boiled for 30 minutes and filtered while hot through a preweighed cintered glass. The precipitate was washed with hot water and dried to a constant weight at 60°C under reduced pressure. The staisny number was calculated and the results are given in Table 2.

Identificatin of Tannins by Chemical Techniques

The identification of tannins by chemical techniques involved the colour and precipitation tests. The results are summarized in Table 3.

Results and Discussion

The reported percent yield of tannin, obtained at elevated temperature with water from the bark of various species are *Rhyzopora stylosa* 20% *Bruguiera parviflora* [10] 12%, Bakauan *(Rhizophora Sp.)* [11] 7.5% and Ipil-Ipil *(Leucaena leucocephla)* [1] 11.3%, while the present results obtained with water at elevated temperature ranges from 9-13% (Table 2). This shows that our finding is comparable with the reported value. The amount of tannin obtained with water at ambient temperature is smaller than that obtained at elevated temperature. So we can say that extraction at elevated temperature is more effective. A similar conclusion can be drawn from the results of water-ethanol system (Table 2). The comparison of the results from the two solvent systems suggests that water-ethanol mixture (1:1) is better than water alone. When one looks at the result (Table 2) of tannins extracted from water at ambient temperature it appears that tannin content of the bark decreases with increase in age of the tree

Age:	45,	49,	54,	62
% of tannin:	12.06,	6.21,	4.35,	3.16

However this trend is not observed in other solvent systems at elevated temperature.

Table 1:

Samples No.	Age of trees (Years)	Moisture content	At elevated temperature				At ambient temp.	
			Water-Ethanol Extract		Water Extract		Water Extract	
			pH	Viscosity	pH	Viscosity	pH	Viscosity
1	45	4.6	3.64	1.20	5.63	0.98	5.59	1.43
2	54	3.8	3.92	1.09	5.37	0.93	5.96	1.14
3	49	7.7	4.36	1.11	4.89	0.92	4.67	1.25
4	62	8.2	4.62	1.19	4.80	0.93	5.26	1.22

Table 2:

Sample	Total dissolved solid %	Tannin %	Nontannin %	Staisny number
1.	29.06	19.50	9.57	133 a
	21.00	9.33	11.67	97 b
	18.75	12.06	6.70	52 c
2.	27.58	18.10	9.48	88 a
	24.99	12.20	12.56	83 b
	9.46	4.35	5.11	38 c
3.	27.62	17.76	9.86	119 a
	23.71	13.39	10.32	89 b
	14.36	6.21	8.16	25 c
4.	27.76	17.84	9.91	109 a
	25.05	12.89	12.17	54 b
	9.91	3.16	6.25	76 c

(a) Extraction from water and ethanol mixture (1:1) at elevated temperature.

(b) Extraction from water at elevated temperature.

(c) Extraction from water at ambient temperture (35±2°C). The results are on moisture free basis.

Viscosity

The reported [12] extract viscosity from loblolly pine bark ranges from 2.5-0.53 poise for 15% soluble solids, while in the present investigation the range of relative viscosity is 1.4-0.91 for 29-9% soluble solids at 25°C (Table 1).

The rate of gellation varies with the viscosity of the tannin extract. The reduction of extract viscosity [13] improves the strength and water resistance of unfortified tannin formaldehyde adhesives. The viscosity of the tannin extract is very high and this may be due to the water solubility of hemicelulose in the extract [14]. Another reason is that the high molecular weight polyphenols develop intermolecular association in concentrated solutions which lead to a higher viscosity. In this study the viscosity of tannin extract increases with increase of concentration of tannin in the extract but in some cases where the concentration is more or less the same for different extracts, the variation in the viscosity of the extract may be due to the pH of the extract. The viscosity of the extract increases with increase of pH of the extract. This may be due to the fact that at low pH the high molecular weight polyphenols are more solvated as a result a decrease in the intermolecular association of polyphenols takes place and a consequent decrease in the viscosity occurs.

Condensed Tannin

In most pine bark [2] tannins, only phloroglucinol forms the flavonoid A-ring, while B-ring may be of two types either of pyrogallol or catechol. Both types of flavonoid structure are condensed tannins. The presence of condensed tannins [4,5] and their constituents are determined by various chemical techniques. The presence of tannin is shown by aqueous gelatin solution test (Table 3) which is a standard test for tannins [15,17]. It has

been suggested that the reaction between catechol and $FeCl_3$ proceeds as follows:-

$$C_6H_4(OH)_2 + FeCl_3 \quad Cl_2Fe\text{-}O\text{-}C_6H_4\text{-}OH + HCl$$

Weinland and Binder [18,19] also isolated the etherate of Cl_2Fe- O-C_6H_4-OH from the reaction, between ferric chloride and catechol.

The precipitation with aqueous lead acetate (Table 3) shows the presence of condensed tannins in chirpine bark, catechol forms lead salt as $(C_6H_4O_2)_2PbO_2.H.$, and pyrogallol [21] forms $(HO)_2C_6H_3.OPb.O_2.H.$

The potassium carbonate test (Table 3) is another evidence for the presence of condensed tannins. Condensed tannins can be precipitated from their aqueous solution on addition of potassium carbonate solution. According to E.H. Rodd, [21] Pyrogallol and phloroglucinol forms Pyrogallol-4-carboxylic acid and phloroglucinol forms phloroglucinol carboxylic acid, with aqueous potassium carbonate according to the following equation.

$$C_6H_3(OH)_3 + 2\,K_2\,CO_3 \quad C_6H_2\,(OH)_3\,OC.H + H + CO_2 + 4K + O_2$$

Pyrogallol Pyrogallol-4-carboxylic acid

$$C_6H_3(OH)_3) + 2K_2CO_3 \quad C_6H_2(OH)_3.COOH + CO_2 + 4K + O_2$$

Phloroglucinol Phloroglucinol carboxylic acid

Ammonium molybdate gives coloured solution with condensed tannins [15] which is also observed in the present investigation. The treatment of extract with urea and pyridine gives coloured solution (Table 3) which may be due to the presence of condensed tannins [15].

It has been shown [15] that catechol tannins give brown precipitates with formaldehyde in presence of hydrochloric acid. Bulky white precipitates are due to pyrogallol on addition of urea in the centrifugate.

The pH of the extract effects the gelation-time so it must be known prior to the tannin formaldehyde reaction. The reported [15] pH for condensed tannins is 3.5 to 5.3 while from the present results the range is from 3.6-5.9, which is another evidence for the presence of condensed tannins.

In the light of the above discussion it may be concluded that tannins from *Pinus roxburghii* bark are condensed tannins.

Staisny Number

Since all the tannins in the extract do not react with formaldehyde, one has to find out the staisny number [22] which is a measure of potential of tannins to react with formaldehyde. The staisny number of tannin extracts from *Pinus roxburghii*

Table 3:

S.No.	Experiments	Observation	Inference
1	$FeCl_3$ sol. (alcoholic) + Extract	Dark green colour	Catechol Tannin
2	$(CH_3COO)_2Pb$ (aq.sol) + Extract	Brownish ppt.	Catechol Tannin
3	Extract + HCHO (aq.sol.35%) + HCl(Conc)	Brownish ppt.	Catechol Tannin
4	Centifuge of the above ppt + Urea	Bulky white ppt.	Pyrogallol Tannin
5	$(NH_4)_6MO_7O_{24}$ $4H_2O$ + Extract	Pale green colour ppt.	Condensed Tannin
6	K_2CO_3 + Extract	Red ppt.	Condensed Tannin
7	Extract + Urea	Light yellow colour solution	Condensed Tannin
8	C_6H_5N (aq.sol) + Extract	Purple yellow colour	Condensed Tannin
9	Gelatin (aq.sol.1%) + Extract	Brown ppt.	Tannin

were determined and the results are reported in (Table 2). Since there is no economic method by which condensed tannins can be separated from other type of tannins as well as from nontannins so the tannins extract is used as such which contains all dissolved solids. The variation of staisny number for the same sample with different solvents and at different temperatures is not unexpected. The staisny number decreases with the decrease of total dissolved solid in the extract [23]. Exception in the last sample (Table 2) may be due to the difference in pH of the extract. At high pH there is greater association of phenolic molecules and hence more suspended particles are there in the extract which increase staisny number.

Finally it can be said that chir pine barks are a potential source of tannins which may be utilised for different industries in this country. Investigation is being carried out in this lab. on the use of these tannins for the development of adhesives for our plywood and chipboard industries.

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