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X-RAY DIFFRACTION ANALYSIS OF TANNIN EXTRACTION FROM CAESALPINIA CORIARIA

KEYWORDS: Tannins, Extraction, Caesalpinia coriaria.

Dr.N.Lokeshwari

Assistant Professor, Department of Biotechnology, Dr.B.R.Ambedkhar University,Srikakulam - 532410

ABSTRACT *XRD is an extremely valuable tool for chemists interested in studying the makeup or structure of the potentially new compounds they have created. Drug companies frequently use XRD to analyze their new products because, if they can find even a slightly unique variation present, they are eligible to apply for a patent.*

Quantitative analysis of diffraction data usually refers to the determination of amounts of different phases in multi-phase samples. Quantitative analysis may also be thought of in terms of the determination of particular characteristics of single phases including precise determination of crystal structure or crystallite size and shape.

The subject of the present work is the study on X-ray diffraction analysis of tannins from caesalpinia coriaria by pressure autoclaving method. The extractable material is derived from a blend of raw materials with different particle characteristics. Preliminary studies indicated the advantage of employing small particle size for achieving high extraction efficiency.

Introduction:
There, the aim of this study was to examine the variation in different particle size of Divi Divi pod tannin extracted from a *caesalpinia* species using pressure autoclaving, and to determine the relative influence of particle size, temperature, and time on their contents.

***Caesalpinia coriaria* as a substrate for gallic acid production :**
Caesalpinia coriaria (Jacq.) Willd. -- *Caesalpiniaceae.*
Status of plant in nature: Cultivated ornamental.
Flowering & fruiting period: September-November.
Common name: The American Sumach.
Economic importance: Ornamental & also used in tanning industries.

The present study has been taken up with a view of exploring the possibilities of using Dividivin (***Caesalpinia coriaria***) as a substrate for tannin extraction.

It is a small thorn less tree or shrub of tropical America and West Indies and introduced into India, more than a century ago, whose seed pods are a rich source of tannin. It has been grown successfully in different parts of the country, particularly Madras state and Andhra Pradesh state, and is important as source of tanning materials. The pods of these species are used for the extraction of tannin and the tannin content is as high as 40 to 42 %. It prefers a well drained soil, becoming stiff in hard soils with high clay content, tolerating moderate to high temperatures. Flowers blossom between July and August and fruiting takes place between Octobers to December. The seeds are small and germination percentage is about 30%.

Tannin or tannic acid is found in some quantity in most plants, with concentrations in the wood, bark, roots, leaves, fruits and galls. In Divi Divi the major concentration is in the pods. Estimates of the tannin content of Divi Divi pods run from 25 to 45 % up to 40 to 55 %, with a mean of about 35 to 47 %. These variations in content estimates represent differences in methods of analysis, and also actual differences of content that have been conditioned by climatic, edaphic and other environmental factors. In any case, Divi Divi provides a cheap source of material with comparatively high tannin content. Examples of other average yields are: Babul pods 20 to 42 %; American chestnut bark 13 to 31 %, Gambier leaves 5 to 8 %, Vilonia acorns 17 to 40 %, tan bark oak 10 to 29 %, sumac 10 to 29 % and myrobalans 12 to 40 %.

Tannins are high molecular weight polyphenolic compounds that exist in a variety of plant species. Tannase (tannin acyl hydrolase, E C 3.1.1.20) is the enzyme responsible for the decomposition of hydrolysable tannins, especially gallotannins, to glucose and gallic acid (Ibuchi et al. 1972).

Tannins belong to the phenolic class of secondary metabolites formed via Shikmic acid path way leading to the formation of other phenolics such as flavones, coumarin, lignins and aromatic amino acids. They have been reported to be the fourth abundant plant constituents after cellulose, hemi cellulose and lignin. Tannins are widely distributed in the plant kingdom. They are common both in Gymnosperms and Angiosperms.

The plant materials are crushed or ground prior to extraction of tannins. Water is preferred as a solvent in view of its high saturation limit of the dissolved solids, inherent safety and ease of separation. However the water used for extraction (leaching) should be soft and should not contain iron. The extraction efficiency depends on process factors like solvent-solid ratio, temperature, leaching cycle, extractor type etc. Regerat, et. al., (1989), reported extraction of tannins from gall nuts from the Oak (*Quercus infectoria*) was hydrolyzed by the tannase of a selected strain of *A.niger.*

The tannins are broadly classified into two groups based on the complexity of their chemical structures. (i) Hydrolysable Tannins and (ii) Condensed Tannins. The hydrolys-

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able tannins are usually compounds containing a central core of glucose or other polyhydric alcohol esterifies with gallic acid (gallotannins) or hexahydroxydiphemic acid (ellagitannins), depending on the nature of phenolic moiety liberated upon hydrolysis (Gayon, 1972). These hydrolysable tannins give blue-black color with aqueous solutions of iron salts.

Materials and Method

Samples

The samples used in this study were Divi Divi pods (*caesalpinia coriaria)* were collected from coastal areas with in A.P. especially from Visakhapatnam with are located nearer to A.U. Campus.

Raw Material Characterization

The percentage of tannins present in raw stock varies from 10 - 40% depending on the type of material chosen. A proper blend of the raw materials is essential to maintain a high percentage of tannin in the extract. The physical characterization of the raw materials in the fixed bed extractor in terms of bulk density, void age, porosity, surface area etc., is essential. Appropriate measurement technique has been employed for the purpose. Tannins are used in a broad range of fields' e.g.-processing of leather, food processing etc. Tannins are used in preservation treatments of wood. They can fix biocides because of their excellent chelating properties.

Determination of moisture content

Moisture content of the sample was determined by the direct oven method of association of official Agricultural chemist (AOAC, 1960).

One gram of the Divi Divi pods were taken in a porcelain crucible and kept in an electric oven at 90°C for about 4 – 5 hours (until constant weight), cooled in desiccators and weighed. From the loss in the weight of the sample, moisture content was determined.

$$\%\ \text{moisture content (W/W)} = \frac{\text{loss in weight}}{\text{Total weight}} \times 100$$

Extraction of Tannins:

The Divi Divi pods were collected from the Costal area of Andhra Pradesh, India. The pods were dried and milled to get the particle size below 5mm. Small particle size achieve high extraction efficiency. Water is preferred as solvent in view of its high saturation limit of the dissolved solids, inherent safety and ease of separation. However the water used for extraction (leaching) should be soft and should not contain iron. The extraction efficiency depends on process factors like temperature, time and pressure. The material is extracted under pressure on autoclaving process at 10 PSI for 30 min and obtained extract was evaporated with vacuum filter and the obtained powder form was used for the entire experimentation. This process generally yields more extract than ordinary open vat extraction. It is generally accepted; that pressure extraction yields a higher amount of "extract" than is obtained by the open leach method, but the product is darker in color and contains a higher proportion of non-tannin substances.

Braemer's test:

For the identification of tannins present, to 2ml of aqueous extract 2ml of 5% FeCl3 was added. Formation of yellow brown or greenish black precipitate indicates that tannins are present.

XRD- Analysis for Tannin Extract:

Before performing XRD analysis, the steps extraction of tannins and Braemer's test was performed and obtained tannin extract was investigated by X-Ray diffraction (XRD) instrument (PAN analytical, X'Pert PRO), equipped with Cu Kα radiation (1.54060 Aº, 40mA 45kV). The X-Ray diffraction unit was a Philips Electronics high angle goniometer model equipped with a copper target tube. Scanning rate was 2º(2θ)/sec for diffractrogram.

The order degree was evaluated by computing the inter planer distances, D, using the Bragg relation

$$2D\sin\theta = n\lambda$$

Where
n is an integer,

θ is the incidence angle of X-rays,

λ is the wavelength of diffracted X-rays and

D is the interplanar distance of sample.

The Nano crystallite domain size was calculated from the width of the XRD peaks using Scherrer formula, assuming they are free from non-uniform strains.

$$D=0.94\lambda / \beta\cos\theta$$

where
D is the average crystallite domain size perpendicular to the reflecting planes,

λ is the wavelength of X-ray,

β is the full width at half maximum (FWHM) and

θ is the diffraction angle.

Results and Discussion

Raw material studies

Divi Divi pods are collected carefully, every day, after they drop from the tree and dried before storage. The Moisture content was observed before and after dries of pods. The moisture content was determined by direct hot air oven method and was found to be 4 to 5%. Fine powder substrate gives less tannin extract. The particle size 0.5mm substrate gives high tannin content. As the particle size decreases, more surface area will be available for tannin extraction and increase in tannin extraction was obtained. Optimum particle size is found to be 0.5mm

However, from grinding energy and product purification cost points of view, fine grinding is not favored. Hence a particle size of 0.5mm has chosen for further studies.

Extracted tannin extract was used for the further analysis of tannins by X-Ray Diffraction analysis.

X-RAY Diffraction analysis (XRD):

The XRD analysis of extracted tannin extract was studied; the results were tabulated in **Table 1.1**. The XRD process was sensitive enough to identify small differences both in terms of trace compounds and in terms of the relative percent composition of the phases. Differences in tannin structure can occur ever between similar plant species.

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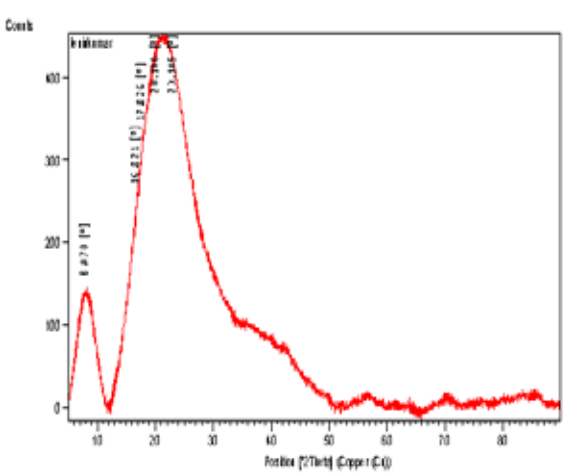
Table 1.1: XRD analysis for major peak list of tannin extract.

2θ	Height(cts)	d-spacing(A°)	FWHM 2θ	Rel.Int.%
6.711	88.669	13.160	1.108	29.72
7.497	132.238	11.782	0.118	44.32
8.070	134.860	10.947	0.316	45.20
9.110	105.727	9.698	0.396	35.44
16.821	170.995	5.266	0.316	57.31
17.826	229.958	4.971	0.316	77.07
20.100	298.364	4.414	0.792	100.00
23.104	237.926	3.846	0.950	79.74

The extracted tannin possess certain degree of crystalline, the most intense maximum being at 2θ = 20.100, for which D = 4.414 A°.

Bragg reflections (or lines), where the sum of intensities over an angular region (peak heights or integrated peak areas) is expressed as a linear combination of the intensities calculated from the crystal structure of the phases present (Ortiz *et.al.* 2001).

Graph .1: X- Ray diffractogram of tannin extract



Graph .1 shows X-Ray diffractogram of tannin extract. X-axis represents Intensity counts and Y-axis shows degrees (2θ). The XRD pattern showed two distinct diffraction peaks at 8.07° and 20.1°. The 100% intensity was found at 2θ value with 20.1° (**Table 1.1**). The average Nano crystallite size calculated from Scherrer formula was found to be 1.755. Detailed peak list was showed below in the **Table 1.2**

Table 1.2: Detailed peak list of the tannin extract.

Pos.[°2Th.]	Height [cts]	FWHMLeft[°2Th.]	d-spacing [Å]	Rel. Int. [%]	TipWidth	Matched by
5.563870	25.883130	0.475200	15.89960	8.68	0.5702	
6.711079	88.669310	1.108800	13.16037	29.72	1.3306	
7.497265	132.238900	0.118800	11.78200	44.32	0.1426	
8.070020	134.860400	0.316800	10.94703	45.20	0.3802	
9.110799	105.727300	0.396000	9.69869	35.44	0.4752	
10.863240	20.043350	0.237600	8.14520	6.72	0.2851	
12.537330	4.465591	0.237600	7.05462	1.50	0.2851	
14.221650	43.203360	0.079200	6.22268	14.48	0.0950	
16.821400	170.995000	0.316800	5.26636	57.31	0.3802	
17.826280	229.958100	0.316800	4.97169	77.07	0.3802	
20.100220	298.364100	0.792000	4.41408	100.00	0.9504	
23.104630	237.926800	0.950400	3.84644	79.74	1.1405	
31.442860	1.759354	0.237600	2.84285	0.59	0.2851	
31.997750	2.631073	0.158400	2.79480	0.88	0.1901	
35.740110	12.931610	0.158400	2.51027	4.33	0.1901	
40.024020	15.557950	0.950400	2.25091	5.21	1.1405	
43.017340	15.777930	1.425600	2.10096	5.29	1.7107	
45.455140	3.466442	0.118800	1.99379	1.16	0.1426	
46.005080	3.554039	0.158400	1.97122	1.19	0.1901	
46.547650	5.648676	0.118800	1.94950	1.89	0.1426	
47.439950	2.543888	0.475200	1.91489	0.85	0.5702	
48.393620	1.487135	0.158400	1.87936	0.50	0.1901	
49.623910	4.233190	0.316800	1.83561	1.42	0.3802	
50.219960	2.819816	0.158400	1.81521	0.95	0.1901	
51.054810	2.257818	0.079200	1.78747	0.76	0.0950	
52.348660	2.394843	0.475200	1.74630	0.80	0.5702	
52.958780	1.191396	0.118800	1.72761	0.40	0.1426	
53.471680	6.058814	0.118800	1.71224	2.03	0.1426	
54.035740	5.189769	1.267200	1.69569	1.74	1.5206	
55.928710	9.468502	0.633600	1.64270	3.17	0.7603	
56.620010	14.007550	0.316800	1.62427	4.69	0.3802	
57.404000	10.020800	0.475200	1.60394	3.36	0.5702	
59.286250	5.490507	0.158400	1.55743	1.84	0.1901	
60.319160	5.534938	0.237600	1.53321	1.86	0.2851	
61.094470	4.750265	0.237600	1.51560	1.59	0.2851	
61.536410	2.719461	0.237600	1.50577	0.91	0.2851	
62.305290	0.783308	0.079200	1.48902	0.26	0.0950	
62.638440	1.768710	0.158400	1.48190	0.59	0.1901	
63.040440	3.965536	0.118800	1.47341	1.33	0.1426	
64.016480	2.068428	0.237600	1.45328	0.69	0.2851	
64.463580	0.261535	0.079200	1.44428	0.09	0.0950	
67.182390	0.905953	0.158400	1.39228	0.30	0.1901	

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67.799430	3.989719	0.237600	1.38111	1.34	0.2851
68.176010	3.648329	0.237600	1.37439	1.22	0.2851
70.469940	9.407530	1.108800	1.33517	3.15	1.3306
71.548510	6.753367	0.237600	1.31767	2.26	0.2851
71.885520	6.197517	0.079200	1.31232	2.08	0.0950
72.137000	4.141409	0.158400	1.30836	1.39	0.1901
72.661370	1.806613	0.237600	1.30020	0.61	0.2851
72.942200	2.552546	0.079200	1.29589	0.86	0.0950
73.136120	4.692817	0.079200	1.29293	1.57	0.0950
73.594170	1.609982	0.158400	1.28601	0.54	0.1901
74.307270	7.712761	0.237600	1.27543	2.59	0.2851
75.636780	11.993520	0.118800	1.25628	4.02	0.1426
75.935330	8.155946	0.118800	1.25208	2.73	0.1426
76.610520	7.885957	0.237600	1.24272	2.64	0.2851
77.260930	6.233211	0.237600	1.23387	2.09	0.2851
77.681220	3.239326	0.158400	1.22824	1.09	0.1901
78.344870	4.383472	0.158400	1.21949	1.47	0.1901
78.734670	5.648154	0.237600	1.21443	1.89	0.2851
79.812180	9.774461	0.237600	1.20072	3.28	0.2851
80.220640	6.308907	0.118800	1.19563	2.11	0.1426
80.649570	14.792920	0.118800	1.19035	4.96	0.1426
82.466100	14.020360	0.079200	1.16867	4.70	0.0950
83.334070	14.824140	0.118800	1.15869	4.97	0.1426
84.110760	16.818560	0.079200	1.14996	5.64	0.0950
86.133800	12.810780	0.316800	1.12806	4.29	0.3802
86.551410	10.948470	0.158400	1.12369	3.67	0.1901
86.796460	14.818390	0.118800	1.12114	4.97	0.1426
87.295170	7.316256	0.316800	1.11602	2.45	0.3802
87.815890	6.920637	0.118800	1.11074	2.32	0.1426
88.473670	4.654794	0.396000	1.10417	1.56	0.4752
88.896680	5.196532	0.079200	1.10001	1.74	0.0950
89.236940	2.899957	0.079200	1.09669	0.97	0.0950

Conclusions

Extract was obtained from the dried pods with water and the extract was used as substrate for the submerged studies. However, from pressure drop, grinding energy and product purification cost points of view, fine grinding is not favored. A particle size range of 5-15 mm is favored as tannin extraction. The results suggest that a decrease/ degradation of these compounds is less noticeable at low temperature (40° C) on the other hand the effect of time and substrate concentration on the extraction and evolution of the analyzed compounds that is tannins seemed to be less important than temperature.

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