ESTIMATION OF TANNIN IN PLANT MATERIALS.

PART I.—*Cassia auriculata.*

By N. Srinivasan.

A number of methods have been proposed for the estimation of tannin in plant materials and these differ in principle according to the object in view. The official method (Chemists' Year Book, 1933, 953) is a 'model of empirical accuracy' and is useful for commercial purposes particularly for the evaluation of different vegetable tannin materials. It is nevertheless, somewhat tedious and requires large samples for work. The method described in the present paper is an improvement on the official one in that it requires only small samples. The estimation is also carried out more rapidly. It is based on the precipitation of tannin from the extract (Nierenstein, *Chem. Ztg.*, 1911, **35**, 31 ; Spiers, *J. Agric. Sci.*, 1914, **6**, 77 ; Hartong, *Woch. Brau.*, 1929, **46**, 11) and obtaining the tannin equivalent from the difference in the concentration of the solution before and after detannisation as determined by the Pulfrich refractometer.

EXPERIMENTAL.

Extraction of Tannin.—It was observed that finely ground powder gave turbid extracts while incomplete leaching was the result of extracting coarse material. After a number of trials, it was found that particles passing a sieve of 25-30 meshes to the inch are the most suitable for the extraction. Boiling water was found to extract best. Repeated leaching with fresh quantities of boiling water was more effective than a single extraction with a large amount of water. Four extractions thus carried out in succession was found to remove the whole of the tannin as shown by the gelatin test. The time taken for a complete extraction was about one hour.

The extract was next filtered through paper pulp. The pressed pulp was soaked in water, well beaten with a large quantity of water and poured into a funnel with its opening closed by a small piece of muslin. After filtering the tannin extract the pulp was washed repeatedly with small quantities of boiling water to remove the tannin and other soluble materials.

The following would illustrate the procedure. The water (200 c.c.) was raised to boiling in a 500 c.c. conical flask and kept simmering. The powdered plant material was divided approximately into six equal portions each one of which was dropped into the water at intervals of 5 mins. After addition of the last portion, the boiling was continued for 5 mins. and then stopped. The hot extract was then decanted into the filter. To the residue 100 c.c. of boiling water were

added and the mixture kept simmering for 15 mins. and the extract again decanted out. This operation was then repeated twice with the residue, the time of boiling being 10 and 5 mins. respectively. The filtered solution and the washings were collected in a 500 c.c. measuring flask and made up to the mark. Aliquots (50 c.c.) in duplicates were evaporated on a water-bath (1½ hrs.) dried in the oven (102-104°C.) for three hours, cooled and weighed. From the weight thus obtained the total soluble matter in the bark was calculated in the usual way.

To determine the efficacy of the method, the total solid contents of extracts from three different samples of bark of *Cassia auriculata* (Tamil, *Avaram*; Canarese, *Thangadi*; Hindustani and Bengali, *Tarwar*; Cutch, *Avla*) were determined. The results have been presented in Table I.

TABLE I.

Weight of bark in grams (10% moisture basis)	Percentage of total solids as estimated		
	Specimen I	Specimen II	Specimen III
7	32·8	34·7	30·6
8	..	34·7	..
9	..	30·5	30·5
10	32·5	34·5	30·5
11	32·5	..	..
12	32·4	34·3	30·3
13	32·4	34·2	30·2

There was a slight fall in the efficiency of extraction as the quantity of bark powder was increased. For most practical purposes, however, the difference (about 0·5 per cent.) may be regarded as being negligible.

Detannisation.—Aliquots (100 c.c.) of the bark extract were shaken with 6 g. each of fat-free casein in narrow-mouthed stoppered bottles in a shaker (reciprocating type) for 15 mins. A further quantity (3 g.) of casein was then added and the shaking repeated for 10 mins. The suspension was immediately passed through filter paper (S. & S. 589). Aliquots (50 c.c.) of the filtrate were next evaporated on the water-bath, dried and weighed as previously described. From the figures thus obtained the quantities of tannin and soluble non-tannins were calculated (Table II).

TABLE II.

Weight of bark in grams (10 % moisture basis)	PERCENTAGES					
	Specimen I		Specimen II		Specimen III	
	Tans	Soluble non-tans	Tans	Soluble non-tans	Tans	Soluble non-tans
10	17·8	14·7	18·9	15·6	..	..
11	18·0	14·5	19·0	15·4	19·7	10·7
12	18·0	14·4	..	..	19·6	10·7
13	18·0	14·4	18·9	15·3	..	..

It may be seen from the above that there is fairly close agreement between the figures obtained for tannin or non-tannins in each case.

Total solids by the refractometer.—A few drops of the bark extract were introduced into the single cell of the Pulfrich refractometer. A sodium flame was used as the source of light and the angular position measured accurately at constant temperature (27°C.). The cell was then cleaned thoroughly, a few drops of distilled water introduced and the angular position determined as before.

Fig. 1 represents the relationship between the total solubles as

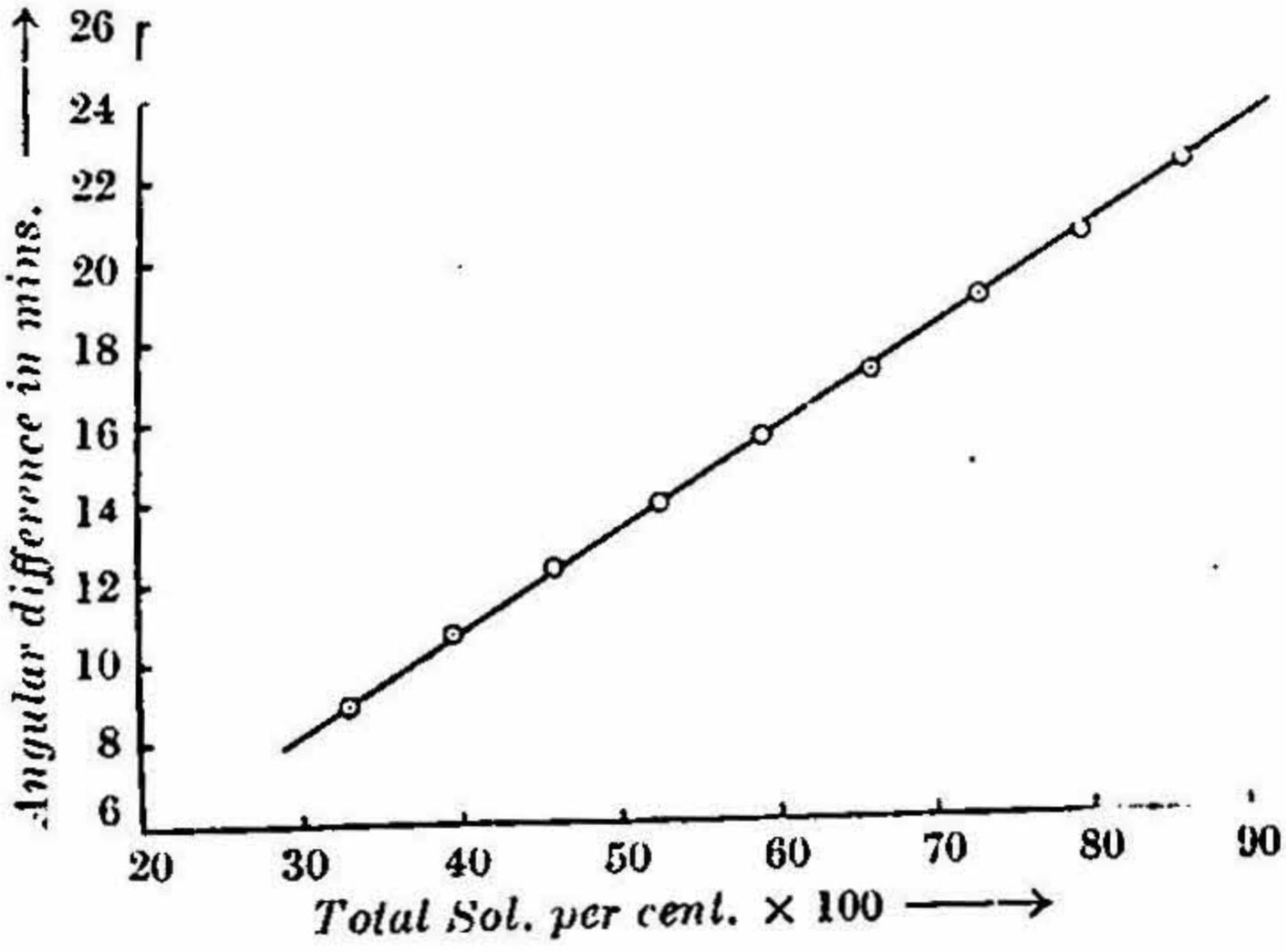

Fig. 1.

Relation between the Total Soluble Concentration of extract and difference in angular positions for the extract and distilled water.

Equation : $C = 4a - 2{\cdot}2$.

(C : Unknown concentration. a : Angular difference determined.)

determined by the evaporation method and the corresponding angular differences for distilled water and the extract.

The relationship was tested not only on extracts prepared from the same specimen but also on those from different localities. The results have been presented in Table III.

TABLE III.

Angle of deviation for distilled water : 66°33·2′

Angular difference in mins.	Concentration of the extract as read from Fig. 1	Concentration obtained by actual evaporation
	SPECIMEN I	
11·9	0·45	0·46
20·0	0·78	0·78
18·5	0·72	0·72
14·2	0·55	0·54
22·0	0·86	0·84
	SPECIMEN II	
15·2	0·59	0·56
19·1	0·74	0·72
12·4	0·47	0·44
16·5	0·64	0·61
	SPECIMEN III	
12·2	0·47	0·49
17·5	0·68	0·69
22·4	0·87	0·88

It may be seen from the above that there is a fair amount of agreement between values obtained from Fig. 1 and those by evaporation. The agreement is very close in the case of the particular specimen (No. I) of bark, extracts of which were used for the refractometer readings in Fig. 1. It is less marked in the case of the other two specimens, the values obtained from the refractometer readings being slightly higher or lower than those obtained by evaporation.

A straight line graph similar to Fig. 1 was obtained for the relation between the angular difference and the solid content of the solution of non-tans left after removal of tannin in the manner already described. The agreement between the figures expected from the readings and those actually found for specimen I were quite close. In the cases of specimens II and III, the difference between the figures obtained by actual evaporation and those read off from the curve were nearly of the same order as in that of total solids. These observations suggest that the slight discrepancy observed in cases of specimens II and III are due to some difference in the nature and proportion of non-tans; that the error for total solids and non-tans being of the same order, the difference representing the tannin would yield correct values irrespective of the origin of the specimen.

An attempt was made to trace the relation between the angular difference of the bark extract and the tannin content as determined by the usual evaporation method (Fig. 2). The results (Table IV)

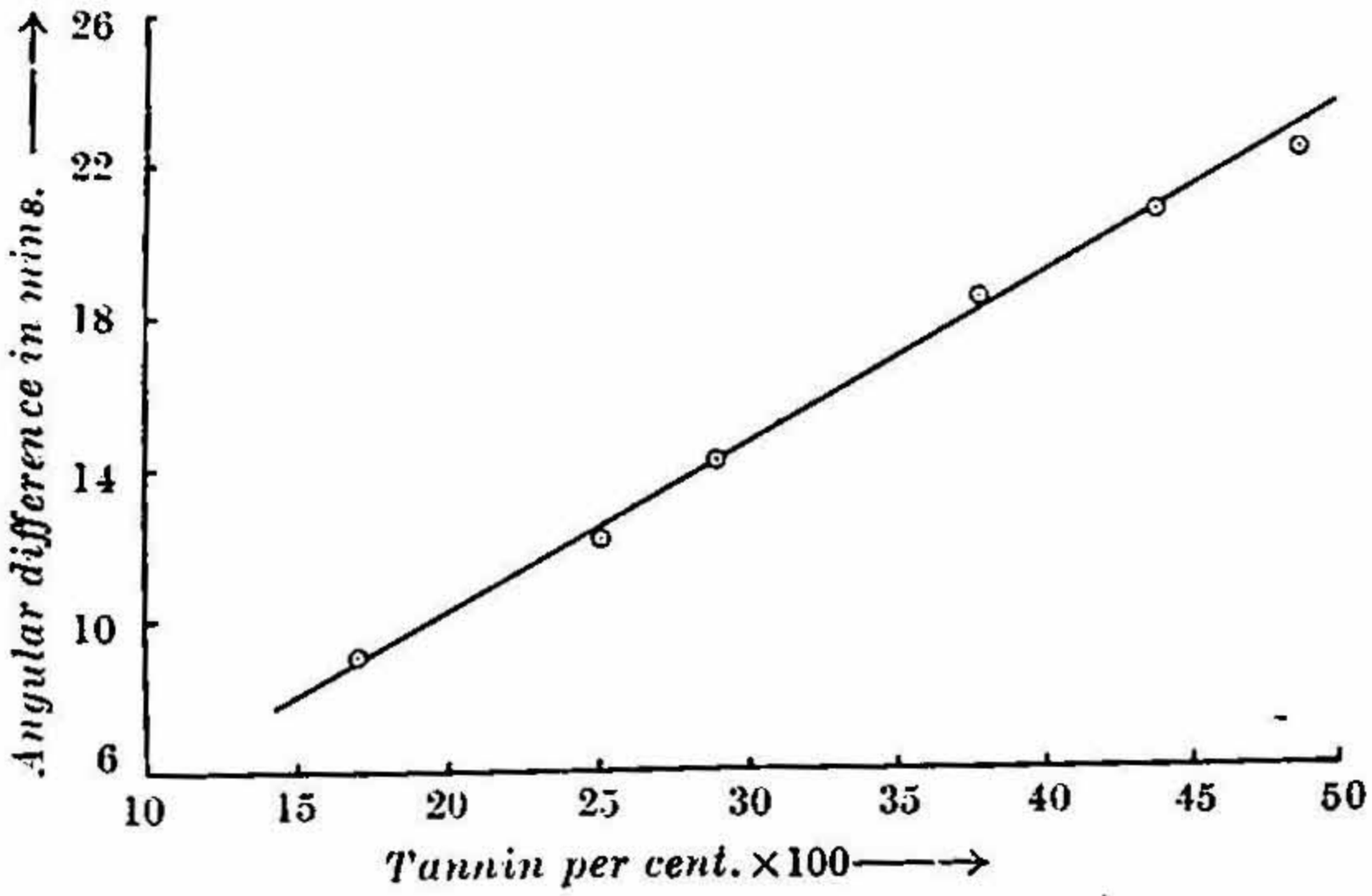

Fig. 2.

Relation between Tan strength of extract and difference in angular positions for the extract and distilled water.

Equation : $C = 2 \cdot 2a - 1 \cdot 76$.

(C : Unknown concentration. a : Angular difference determined.)

showed however that although there was some agreement in the case of the particular specimen (I) extracts of which were used for obtaining the data in Fig. 2, it was not close in the case of others (specimens II and III). This should be traced to the non-proportionality between the tannin and the non-tannins in the different extracts.

TABLE IV.

Angular difference in mins.	Percentage tannin concentration from graph	Percentage tannin concentration by evaporation
	SPECIMEN I	
11·9	0·24	0·23
20·0	0·42	0·43
18·5	0·39	0·40
14·2	0·30	0·29
22·0	0·47	0·47
	SPECIMENS II & III	
15·2	0·32	0·32
19·1	0·40	0·45
11·2	0·23	0·26
16·5	0·35	0·38

Since non-tans are primarily responsible for the discrepancy between the results calculated from the refractometric readings and those estimated by evaporation, some experiments were next carried out determining the angular deviations before and after detannisation and plotting the difference against the tannin content as obtained by the evaporation method. The results have been presented in Fig. 3.

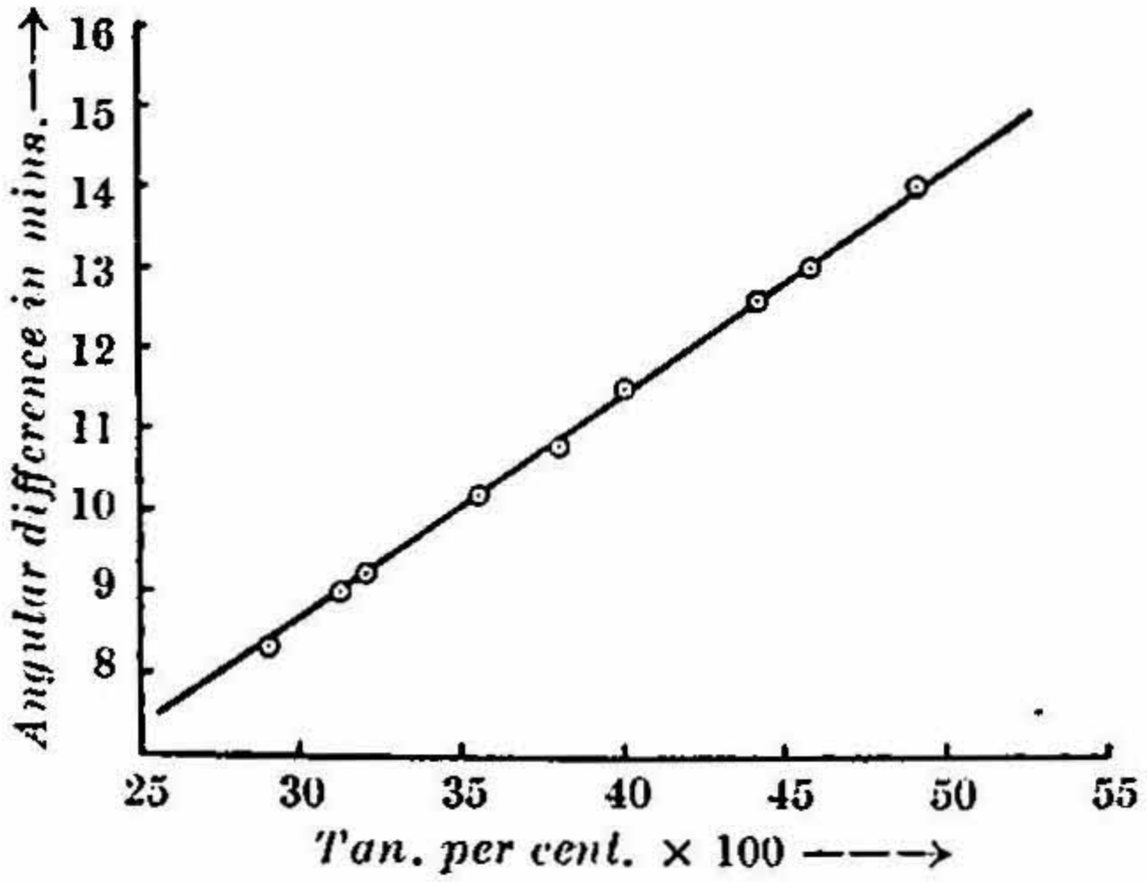

Fig. 3.

Relation between Tan strength of extract and difference in angular positions for the extract and the detannised solution.

Equation : $C = 3 \cdot 625a - 1 \cdot 375$.

(C : Unknown concentration. a : Angular difference determined.)

With a view to determining whether the relation between difference in angular deviation and tannin content holds true for all samples of bark irrespective of locality, some experiments were carried out comparing the estimates by the refractometric and the evaporation methods (Table V).

TABLE V.

Locality from which the sample was obtained	Weight of bark in grams (10% moisture basis)	Volume of extract in c.c.	Total solubles in 50 c.c. by evaporation	Non-tans in 50 c.c. by evaporation	Angular difference in mins.	Tannin per cent.	
						by evaporation	by refractometric method
Trichinopoly	10	500	0·346	0·157	10·8	18·9	18·9
Dindigul	10	500	0·335	0·157	10·3	17·8	18·0
Kopbal	10	500	0·357	0·160	11·4	19·7	20·0
Bellary	10	500	0·321	0·143	10·3	17·7	18·0
Pudukotah	9	500	0·311	0·142	9·7	18·8	18·8

It may be seen from the above that there is fairly close agreement between tannin contents as obtained by the two methods.

Estimation of tannin in small samples.— The quantity of extract required for refractometric measurements being very small, some experiments were next carried out to determine whether that method can be extended to the estimation of tannin in small quantities of bark. The trials were carried out with quantities corresponding to one-tenth of those used in the previous experiments and the results compared with those obtained in larger quantities by the evaporation method (Table VI).

The foregoing observations show that fairly accurate results can be obtained by the refractometric method though only small quantities of bark are taken. The angular positions can be read on the instrument upto one-tenth of a minute and this corresponds to an accuracy of 0·004 per cent. on the tannin concentration of the extract. The observations further suggest that in addition to its application in the analysis of commercial samples of bark or bark extract, the refractometric method can be successfully adopted in the study of biochemical problems relating to the origin and distribution of tannins in plants. The technique would render it possible to examine small samples of bark even from the living plant without appreciably affecting growth, so that it would also be of considerable assistance in the investigation of problems relating to tannin metabolism at various stages in the life of the plant.

TABLE VI.

Locality from which the sample was obtained	Weight of bark in grams (10% moisture basis)	Angular difference in mins.	Percentage of tannin in bark	
			by evaporation	by refractometric method
Bellary	1·2	12·2	17·7	17·9
	1·0	10·2		17·8
	1·3	13·3		18·0
Dindigul	1·2	12·0	17·8	17·6
	1·0	10·2		17·8
Kopbal	1·0	11·3	19·7	19·8
	1·2	13·7		20·1
Pudukotah	1·2	12·9	18·8	18·9
	1·0	10·7		18·7
	1·1	11·8		18·8
Trichinopoly	1·2	13·0	18·9	19·1
	0·9	9·9		19·2
	1·0	10·9		19·1

Although the present enquiry relates primarily to the examination of the tannin in *Cassia auriculata*, the results do yet suggest that the refractometric method can be extended for the estimation of tannin in other plant materials as well. It has already been shown that the relation between the tannin contents and the difference in refractometric readings for the whole and detannised *Cassia auriculata* bark extracts can be represented by a simple equation of the type $Y=a+bX$. If similar equations can be obtained for other plant materials as well, the tannin concentrations—within a useful range—can be calculated from refractometric readings without reference to any figure specially drawn for the purpose. It is proposed therefore to extend the present enquiry to include other tannin-bearing materials as well and to work out the equation in each case. It is also hoped that it would soon be possible to construct a simple direct reading instrument that would give fairly accurate estimates of tannin contents of commercial samples of different barks and bark-extracts.

SUMMARY.

1. A simple and rapid method for the estimation of tannin in the bark of *Cassia auriculata* has been described. It is based on the measurement of the angles of deviation on the refractometer before and after

detannising the extract and calculating the tannin content from the difference.

2. For the same sample of bark, there is close correlation between the tannin contents and refractometric readings for the whole extract. The relation is not so close when extracts from different specimens are compared. This is traceable to the varying proportions of non-tans present in the different cases. To obtain accurate estimates of tannin contents, it is necessary therefore to take readings before and after detannising the extracts.

3. The application of the method to the estimation of tannin in small samples of bark and its practical significance have been discussed.

4. The extension of the method to other tannin-bearing materials as also the possibility of the construction of a simple direct reading instrument for routine estimations have been indicated.

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Department of Biochemistry,
Indian Institute of Science,
Bangalore.

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