TINCTURES OF KINO, KRAMERIA AND GAMBIR COMPOUND. A STUDY IN STABILITY AND ASSAY METHODS.—A PRELIMINARY REPORT.

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INTRODUCTORY.

It is generally conceded that the potency of Kino, Krameria, Gambir and their preparations depends on the tannin content. These tannins are subject to changes which materially reduce their usefulness. Accordingly, assay processes for these drugs and their preparations comprise methods which determine tannin content.

The number of methods of tannin assay is large. Allen states that 94 methods have been elaborated.[1] The choice of a particular assay process depends on the use to which a tannin material is put. A tanner evaluates tannin materials by their ability to combine with hide to form leather. For his purpose the hide powder method is very useful. For the dyer who is interested in the dyeing properties of tannins the hide powder results are of little value and a different method of evaluation is required. The physician on the other hand is interested in the astringent effect of tannins on body tissues. A method of assay is required, therefore, which will determine the amount of therapeutically active substance in the tannin material.

It is the purpose of this investigation to select a method or methods of assay which are applicable to the determinations of tannins in tannin-bearing drugs and their preparations which are used for their astringent effect; incidentally, the stability of the preparations is to be determined.

The experiments reported in this study were done on Kino, Krameria, Gambir and preparations (tinctures) of these drugs.

EXPERIMENTAL.

(A) The following tinctures were prepared:

Tincture of Kino U. S. P. X
Tincture of Krameria U. S. P. X
Compound Tincture of Gambir U. S. P. X

Methods and menstrua employed; all by percolation:

Group I—U. S. P. methods and menstrua
Group II—4 volumes of alcohol, 1 volume of water
Group III—4 volumes of alcohol, 1 volume of glycerin.

(B) Assay Methods:

The following methods were tried; Scoville suggested the use of I, a colorimetric method with an iron reagent to indicate total tannoid bodies and II, a precipitation method to indicate unhydrolized tannins. Thus gallic acid, for example, would be included in an assay by Method I but not by Method II. The stability of a tincture as well as tannin-content may be thus demonstrated.

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[1] Allen's "Organic Analysis," 5th Edition, Vol. V, page 107.

I. The Colorimetric Method.[1]

Technique and Reagents required:

1. Iron mixture—0.04 Gm. potassium ferricyanide in 500 cc. H_2O; add 1.5-cc. solution ferric chloride.
2. Tannin solution—0.04 Gm. purest obtainable tannin in 500 cc. H_2O.
3. Solution to be tested—0.8 Gm. of drug to 500 cc. H_2O.

Place six 50-cc. beakers on a white surface.

In No. 1 place 5 drops of drug extract.
In No. 2 place 4 drops tannin solution.
In No. 3 place 5 drops tannin solution.
In No. 4 place 6 drops tannin solution.
In No. 5 place 7 drops tannin solution.
In No. 6 place 8 drops tannin solution.

Place 5-cc. iron mixture in each beaker; after one minute add 20 cc. of water; examine within three minutes.

The test was tried as described with no satisfactory results. The tannin standard solution (being gallotannin) gives a blue color with iron salts while the tannins under test (being of the catechol group) yield green solutions with iron salts. Therefore, this is not a satisfactory method of color comparison. To overcome this, cinchotannic acid, which is a catechol tannin (prepared in the writer's laboratory) was tried as a standard for color comparison. Here, again, the tints could not be compared as the drug solutions were turbid and the colors formed by the addition of iron reagent were not clear. Accordingly, comparison was either difficult or impossible.

Another attempt was made employing the following technique.

Infusion of drug, 50 mg. to 250 cc., was prepared. This was shaken with two portions, each 25 cc. ethyl acetate. The ethyl acetate solutions were again shaken with two portions, each 25 cc. distilled water. These aqueous solutions were compared colorimetrically with solutions of cinchotannic acid by the addition of iron reagent. All solutions in this trial were clear but the drug solutions were not of the same shade of green as the standard. They were bluish green and, therefore, not comparable.

This matter was taken up with the laboratory of Hide and Leather Investigation, Department of Agriculture, Washington who wrote as follows:

"The development of a bluish green color to which you refer is the result, probably, of the presence, in small amounts, of the natural tannins and closely related non-tannins of the pyragallol or iron-bluing group, as distinct from the catechol tannins or those that produce green with iron salts. It is because of the occurrence of such admixture in natural products that we have given little consideration to colorimetric methods, and I believe that you have but little chance of developing a satisfactory method along such lines."

II. Precipitation Tests.

a. By Use of Gelatin and an Indicator.[2]

This test depends on the precipitation of tannin by gelatin in the presence of an aniline dye. The end-point is indicated by the decolorization of the solution.

[1] Allen's "Organic Analysis," 5th Edition, Vol. V, page 186.

[2] "Allen," 5th Edition, Vol. V, page 175.

Technique and solutions required:

1. A small quantity (few cc.) of 10 per cent solution of mercuric iodide in its own weight of potassium iodide.
2. *Tannin Solution.*—Five grams pure gallotannin in distilled water, add 0.5-cc. solution No. 1 and sufficient distilled water to make 1000 cc.
3. *Gelatin Solution.*—Five grams gelatin in about 1000 cc. hot distilled water. Boil the liquid, add sufficient white of egg to clarify it, cool, add 0.5-cc. solution No. 1 and NaOH solution to render slightly alkaline and distilled water sufficient to make 1000 cc.
4. *Calcium Acetate Solution.*—Fifty grams of pure dry calcium acetate in 1000 cc. H_2O, filter and add few drops solution No. 1.
5. Indicators—4 per cent Nicholson's blue BB, or 1 per cent Blue-Black NBI, or 1 per cent solution methylene blue for tannin solutions which are not colored.

Use 60-cc. flask with 3-cm. neck. Place 1-cc. gelatin solution, 2 drops indicator 5-cc. calcium acetate solution; fill with distilled water to neck at 75° to 80° C. by means, of small burette at 40 drops to a cc. Add small quantity of standard tannin solution a little at a time and shake after each addition until decolorized. Repeat the same with the unknown. Allow precipitate to rise in the neck of the bottle. The process is repeated with a solution of the unknown, which if acid should be cautiously neutralized.

The writer was unable to obtain either Nicholson's blue BB or Blue-Black NBI. Methylene blue was used as the indicator but the results were not satisfactory as it was difficult to ascertain the end-point. The precipitate settled instead of rising.

b. Precipitation with Quinine Sulphate.[1]

Technique and reagents used:

1. *Standard Tannin Solution* to represent 250 mg. tannin in 10-cc. solution, prepared as under II-*a*, solution No. 2.
2. *Unknown.*—Prepare solution to represent about 150 mg. tannin in 400 cc. of water and filter.
3. *Quinine Reagent.*—For each test use a solution of

Quinine Sulphate	1.0 Gm.
H_2SO_4, $N/_1$	2.5 cc.
Water to make	25.0 cc.

Dilute sample to 400 cc., add 25-cc. solution No. 3, allow to stand about 15 minutes, filter on a tared filter paper, dry, precipitate and weigh.

The standard tannin solution was used to check the accuracy of the method. Weight of precipitate times 0.75 equals weight of tannin.

This method was used for the nine tinctures, with results indicated in Table I.

III. The Lowenthal Permanganate Method.

Due to the high standing of this method and also due to the suggestions of the Drug Research Unit (L. E. Warren) and the Hide and Leather Investigation Laboratory (Mr. Frey) both of the Department of Agriculture, the writer attempted to adopt this method for use in assaying the nine tinctures.

Technique and solutions:

1. *Potassium Permanganate Solution* 1 : 1000.
2. *Indigo Carmine Solution.*

[1] "Allen," 5th Edition, Vol. V, page 180.

Indigo Carmine	5 Gm.
Concent. H_2SO_4	50 cc.
Dist. Water to make	1000 cc.

This solution must be titrated with the $KMnO_4$ solution so that 20-cc. indigo carmine solution is decolorized by about 14–16 cc. $^1/_{1000}$ $KMnO_4$ solution.

3. *Gelatin Solution.*—2 per cent gelatin solution prepared in the usual way and preserved with mercuric iodide-potassium iodide solution as under Method 2-*a*, solution No. 3.
4. *Salt Solution.*

Concent. H_2SO_4	50 cc.
Dist. H_2O to make	1000 cc.
NaCl to saturation.	

Use enough of the unknown drug so that it requires not over 10 cc. $^1/_{1000}$ $KMnO_4$ to oxidize it. Dilute with distilled water to about 750 cc. and add 20-cc. indigo carmine solution. To this mixture add enough $^1/_{1000}$ $KMnO_4$ solution from a burette, one cc. at a time to decolorize the 20 cc. of indicator, shaking the flask briskly after each addition. Continue adding $KMnO_4$ solution until contents of flask are decolorized, that is, turn yellow. Now subtract total number of cc. $KMnO_4$ from that required to decolorize 20 cc. of indicator and call it *a*.

Gelatin precipitation:

Use an amount of unknown equal to five times the quantity used in the first titration but not exceeding 50-cc. in volume. Add 25-cc. gelatin solution and 25-cc. salt solution and 10 Gm. Kaolin. Shake briskly several minutes and filter. Use 20 cc. of filtrate (equal to amount of drug in first titration), dilute to about 750 cc., add 20-cc. indigo carmine solution and titrate with $KMnO_4$ solution as above.

Let number of cc. $KMnO_4$ used in this titration = C. Therefore $a - C$ = cc. $^1/_{1000}$ $KMnO_4$ used in oxidation of tannins. C = number of mg. $KMnO_4$ required to titrate 63 mg. oxalic acid (10 cc. $N/10$ oxalic acid). C = 31. Therefore $C:(a - b) = 63+$.

Using this method and formula the tannin content of the drugs and tinctures was calculated and the results indicated in Table II.

The quantity of drug or tincture required in the titrations so as not to require more than 10 cc. $KMnO_4$ solution for oxidation was determined by repeated trial.

TABLE I.—INDICATING RESULTS OF PRECIPITATION BY QUININE SULPHATE.

Sample.	Name of Preparation.	Quantity Used for Test.	Quantity of Drug Represented Mgms.	Wt. of Ppt.	Wt. Tannin.	Per Cent Tannin.
1	Standard Tannin Solution	10 cc.	250	320 mg.	240.00 mg.	96.0%
2	Tr. Kino, U. S. P.	5 cc.	500	165 mg.	123.75 mg.	24.6%
3	Tr. Kino, 4 vol. Alc., 1 vol. H_2O	5 cc.	500	165 mg.	123.75 mg.	24.6%
4	Tr. Kino, 4 vol. Alc., 1 vol. Glyc.	5 cc.	500	165 mg.	123.75 mg.	24.6%
5	Tr. Krameria, U. S. P.	5 cc.	1000	180 mg.	120.00 mg.	12.0%
6	Tr. Krameria, 4 vol. Alc., 1 vol. H_2O	5 cc.	1000	180 mg.	120.00 mg.	12.0%
7	Tr. Krameria, 4 vol. Alc., 1 vol. Glyc.	5 cc.	1000	180 mg.	120.00 mg.	12.0%
8	Tr. Gambir Comp., U. S. P.	10 cc.	500	80 mg.	60.00 mg.	12.0%
9	Tr. Gambir Comp., 4 vol. Alc., 1 vol. H_2O	10 cc.	500	80 mg.	60.00 mg.	12.0%
10	Tr. Gambir Comp., 4 vol. Alc., 1 vol. Glyc.	10 cc.	500	80 mg.	60.00 mg.	12.0%

TABLE II.—INDICATING RESULTS OF LOWENTHAL'S METHOD.[1]

Sample.	Name of Preparation.	Quantity of Drug Represented.	Value of *a*.	Value of *b*.	Per Cent Tannin in Terms of Oxalic Acid.
1	Standard Tannin Solution	15 mg.	10.0 cc.	1.5 cc.	114.90
2	Tr. Kino, U. S. P.	20 mg.	7.0 cc.	3.0 cc.	40.65
3	Tr. Kino, 4 vol. Alc., 1 vol. H_2O	20 mg.	7.0 cc.	3.0 cc.	40.65

Sample.	Name of Preparation.	Quantity of Drug Represented.	Value of *a*.	Value of *b*.	Per Cent Tannin in Terms of Oxalic Acid.
4	Tr. Kino, 4 vol. Alc., 1 vol. Glyc.	20 mg.	6.0 cc.	3.0 cc.	30.40
5	Tr. Krameria, U. S. P.	80 mg.	10.0 cc.	4.5 cc.	13.90
6	Tr. Krameria, 4 vol. Alc., 1 vol. H_2O	80 mg.	10.0 cc.	4.5 cc.	13.90
7	Tr. Krameria, 4 vol. Alc., 1 vol. Glyc.	80 mg.	10.0 cc.	5.0 cc.	12.70
8	Tr. Gambir Co., U. S. P.	20 mg.	8.0 cc.	6.0 cc.	20.30
9	Tr. Gambir Co., 4 vol. Alc., 1 vol. H_2O	20 mg.	8.0 cc.	6.0 cc.	20.30
10	Tr. Gambir Co., 4 vol. Alc., 1 vol. Glyc.	20 mg.	8.0 cc.	6.0 cc.	20.30
11	Infusion Kino,[2] U. S. P., 1%	20 mg.	7.0 cc.	3.0 cc.	40.65
12	Infusion Krameria, U. S. P., 4%	80 mg.	8.0 cc.	2.0 cc.	15.25
13	Infusion Gambir, U. S. P., 0.5%	20 mg.	8.5 cc.	6.0 cc.	25.40

[1] Allen gives the following figures in terms of oxalic acid:

Pure Tannin	Botanical names not indicated	120–22
Kino		51–59
Krameria		19–20
Gambir		43–51

[2] The infusions were prepared by boiling the required quantity of the drug 1/2 hour in half the volume of water of the expected finished infusion, straining and adding sufficient water to the desired volume.

COMMENTS.

1. *Appearance of Tinctures.*—The tinctures were prepared early in September 1931. Judging from their appearance at the writing of this report it would seem that the best method for the preparation of Tincture of Kino is the present U. S. P. method; Tincture of Krameria is by employing a menstruum of 4 volumes of alcohol, 1 volume of glycerin; Tincture of Gambir Compound by using a menstruum of 4 volumes alcohol and 1 volume of glycerin.

2. In inspecting the figures quoted from Allen's in Table II it must be borne in mind that botanical names are not given. This is significant, for it will be found, for instance, that Kino has a tannin content of 30.3 per cent to 73.2 per cent depending on the botanical variety (see page 51, "Allen," Vol. 5, 5th Edition).

3. The Assay methods reported in this preliminary report reveal the quantity of material precipitated by quinine sulphate and gelatin. The amount of hydrolized material such as catechins or gallic acid present in the preparation is not indicated. Experimental work is being done with a view to elaborate the assay methods, so as to include in the process the determination of both hydrolyzed and unhydrolyzed material. Stronger alcoholic menstrua will be tried to observe their effects on the stability of the preparations.

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Volumetric Determination of Quinine.—D. Jatrides and G. Thomis, after a study of a number of methods for determination of quinine in pharmaceuticals, recommend the following: The alkaloid is extracted from an alkaline solution with chloroform; an aliquot portion is diluted with 95% alcohol and titrated with 0.2 normal hydrochloric acid, using lacmoid as indicator; the end-point is determined by colorimetric comparison with standard adjusted to p_H of 4.9 to 5.0.—*J. pharm. chim.*, 15 (1932), 230–242.