

(2) When the cats were anesthetized with the barbituric acid compounds, it required more digitalis to kill than with ether.

(3) The size of the cat unit for digitalis varies with the anesthetic agent used.

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A CRITICAL STUDY OF THE TANNIN-BEARING GALENICALS.*¹

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This study was undertaken for the purpose of finding answers to several problems which have been raised concerning the official tannin-bearing galenicals. The objectives for this investigation briefly were as follows:

1. To determine the effects of the changes in menstrua, as specified in the recent revisions of the United States Pharmacopœia and National Formulary, upon the permanency, astringency and total extractive of the official tannin-bearing galenicals.
2. To determine the effect of precipitation upon the astringency of these galenicals.
3. To find out whether there is any correlation between the astringency, total extractive and precipitation in such preparations.
4. To determine whether precipitation in such galenicals can be prevented by the use of selective menstrua or other means.
5. Most important of all, perhaps, to determine whether these products can be reliably assayed for tannin.

THE ESTIMATION OF THE PRECIPITATE.

The sixteen galenicals which were studied are given in Table I and are arranged in the increasing order of the weights of their precipitates. The amounts shown here were obtained from 50-cc. sealed ampul samples of each of the preparations which had stood for five months. At the end of this period the ampuls were opened, the precipitate collected in Gooch crucibles and dried to constant weight.

An attempt was made to estimate the relative amounts of precipitates which formed in these preparations by examining liter portions of them after they had stood in Erlenmeyer flasks for about four months. Variations in the densities, and other physical differences in the precipitates, makes such readings difficult, especially when so many different drugs are involved.

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TABLE I.

Galenical.	Process.	Weight of Precipitate in Gm.
Tincture of <i>Krameria</i>	Special	0.0011 Gm.
Tincture of <i>Kino</i>	U. S. P. XI	0.0034 Gm.
Tincture of <i>Krameria</i>	U. S. P. X	0.0064 Gm.
Tincture of Nutgall	N. F. VI	0.0129 Gm.
Compound Tincture of Gambir	U. S. P. X	0.0134 Gm.
Tincture of <i>Kino</i>	U. S. P. X	0.0226 Gm.
Fluidextract of <i>Hamamelis</i> Leaves	N. F. V	0.0268 Gm.
Compound Tincture of Gambir	N. F. VI	0.0442 Gm.
Fluidextract of <i>Krameria</i>	N. F. VI	0.0466 Gm.
Fluidextract of <i>Krameria</i>	N. F. V	0.0466 Gm.
Fluidextract of <i>Hamamelis</i> Leaves	N. F. VI	0.0543 Gm.
Fluidextract of <i>Castanea</i>	N. F. V	0.0610 Gm.
Fluidextract of <i>Castanea</i>	N. F. VI	0.1051 Gm.
Fluidextract of <i>Rose</i>	N. F. VI	0.1221 Gm.
Fluidextract of <i>Uva Ursi</i>	N. F. VI	0.1574 Gm.
Fluidextract of <i>Uva Ursi</i>	U. S. P. X	0.2191 Gm.

COMMENTS CONCERNING THE PREPARATIONS.

Compound Tincture of Gambir.—The preparation official in the U. S. P. X provided a stable product in which slight precipitation occurred. The N. F. VI galenical precipitated badly. It should be stated that the latter formula specifies four times the amount of drug for extraction as does the U. S. P. X formula.

The N. F. VI tincture precipitates immediately after the percolation is completed and continues at a decreasing rate for an indefinite period of time. The deposit appears to be crystalline by macroscopic observation but gives no evidence of a definite structure under the microscope.

Tincture of *Kino*.—The U. S. P. XI product is an improvement over the one formerly official inasmuch as it is more stable, more limpid, and less liable to gelatinization.

Fluidextract of *Hamamelis* Leaves.—There is no question but that the new official product is superior from the standpoint of stability.

Fluidextract of *Castanea*.—There were more notable improvements manifested in the N. F. VI product over the N. F. V preparation than in any other galenical under consideration. The amount of precipitate was greatly reduced in the new official fluidextract.

Tincture of *Krameria*.—This galenical was unquestionably the most suitable preparation from the standpoint of stability. The tincture official in the U. S. P. X gave evidence of only a trace of sediment. The Tincture of *Krameria* Special (made according to the directions specified for Tincture of *Kino* U. S. P. XI) was the only tincture entirely free from sediment at the conclusion of this investigation.

From the point of view of stability, Tincture of *Krameria* Special proved to be the most desirable astringent galenical in this group.

Tincture of Nutgall.—Precipitation began in this tincture before the percolation was complete. The precipitation appears to be rather complete after a month or two of standing after which time there is little additional sedimentation. This galenical does not precipitate badly but tends to darken considerably upon standing.

Fluidextract of Rose.—The deposit of a precipitate in this galenical does not occur immediately upon the completion of the product and makes its appearance very gradually. The fluidextract is very viscous which may, in part, account for this.

Fluidextract of Krameria.—Here again the N. F. VI product shows marked improvement over the one formerly official in the N. F. V. The new official product was the only fluidextract under observation which showed no tendency toward sedimentation. The N. F. VI fluidextract is a more limpid fluid and has the general property of an improved physical appearance.

Fluidextract of Uva Ursi.—The N. F. VI galenical gave no visible signs of improvement over the U. S. P. X product. Both fluidextracts precipitated badly upon standing. The precipitate when examined under the microscope proved to be non-homogenous in nature and showed the presence of a distinct crystalline structure which has been identified as ellagic acid by Parks (17). This was the only precipitate that was definitely crystalline in structure. There was also present a large amount of amorphous matter in the precipitate.

DETERMINATION OF TOTAL EXTRACTIVE IN THE TANNIN-BEARING GALENICALS.

Total extractive determinations were made upon the sixteen galenicals immediately upon the completion of their manufacture, again after one month had elapsed, and a final determination at the completion of the investigation which represented a period of three to five months.

The procedure employed in making these total extractive determinations is as follows: Ten milliliters of the galenical were carefully pipetted on to a four-inch watch glass and the pipette allowed to drain until dropping had ceased. The watch glass was then placed in an electric oven and dried to constant weight at 105°. The weight of the residue multiplied by ten equals the percentage of total extractive, weight/volume. The results are shown in Table II.

TABLE II.—TOTAL EXTRACTIVES (PERCENTAGE, WEIGHT/VOLUME).

Galenical.	Determination.		Final.
	First.	Second.	
Compound Tincture of Gambir, U. S. P. X	5.1	5.0	5.1
Compound Tincture of Gambir, N. F. VI	15.3	20.2	19.7
Tincture of Kino, U. S. P. X	13.7	14.0	14.9
Fluidextract of Hamamelis Leaves, N. F. V	22.7	22.7	23.4
Tincture of Kino, U. S. P. XI	10.2	9.2	9.8
Fluidextract of Hamamelis Leaves, N. F. VI	10.3	11.5	11.3
Fluidextract of Castanea, N. F. V	15.9	15.4	15.1
Fluidextract of Castanea, N. F. VI	15.1	15.6	15.7
Tincture of Krameria, U. S. P. X	7.1	7.1	6.8
Tincture of Krameria, Special	8.6	8.4	7.7
Tincture of Nutgall, N. F. VI	10.2	9.5	9.5
Fluidextract of Rose, N. F. VI	34.4	41.4	42.3
Fluidextract of Krameria, N. F. V	33.5	33.6	33.7
Fluidextract of Krameria, N. F. VI	35.7	33.8	32.4
Fluidextract of Uva Ursi, U. S. P. X	37.9	36.9	36.3
Fluidextract of Uva Ursi, N. F. VI	35.2	35.1	33.5

Comments.—It was hoped that the increased alcoholic strength of the menstrua and solvents might cause a reduction in the amount of total extractive in these galenicals without appreciably affecting the astringency. Fluidextract of Hamamelis Leaves was the only preparation to show any marked improvement of this sort. Its total extractive dropped about 50% while its tannin content increased materially.

There was little noticeable change in the total extractives of the other preparations. Compound Tincture of Gambir was the only one to give any evidence of an

increased amount of extractive. This would be expected because the strength of the N. F. VI galenical is four times that of the U. S. P. X.

The fluidextracts of Rose, *Krameria* and *Uva Ursi* have maintained their high extractive content.

THE ESTIMATION OF TANNIN.

A modification of the Lowenthal Method of tannin analysis was used for the determination of tannin in this study (14). The method involves permanganate titration and gelatin precipitation.

Preliminary to applying the assay to the galenicals, a series of ten tannin solutions were prepared and assayed. Water was the solvent for one, a solution of alcohol 9 and glycerin 1 being the solvent for the other nine solutions. To seven of these reasonable amounts of presumably interfering substances were added. These were, gallic acid, resorcinol, resin, cholesterol, sucrose, glucose and chlorophyll. These solutions were assayed when freshly prepared, again at the end of ten days, and the third time at the end of twenty-four days.

From the results obtained it was concluded that tannin can be assayed reliably and reproducibly within about 1%. It was also shown that these tannin solutions are reasonably stable and that the substances added did not interfere with the tannin analysis.

The galenicals under study were each assayed five times by the method which has been mentioned. The intervals and results of these are shown in Table III.

TABLE III.

Galenical.	Process.	At Time of Completion.	After 10 Days.	After 20 Days.	After 30 Days.	Final 3-5 Months.
Compound Tincture of Gambir						
	U. S. P. X	1.0	1.3	1.6	1.7	2.0
	N. F. VI	2.8	2.8	2.4	5.6	5.8
Tincture of Kino						
	U. S. P. X	8.3	8.5	9.4	8.6	8.5
	U. S. P. XI	5.2	6.0	5.6	5.3	5.2
Fluidextract of Hamamelis Leaves						
	N. F. V	1.9	2.9	2.3	2.3	3.0
	N. F. VI	4.2	3.8	4.3	3.4	3.6
Fluidextract of Castanea						
	N. F. V	1.0	1.1	0.9	0.9	1.0
	N. F. VI	1.7	1.9	1.7	1.4	1.2
Tincture of <i>Krameria</i>						
	U. S. P. X	3.6	3.3	3.1	2.7	2.8
	Special	2.9	2.9	2.9	2.4	2.9
Tincture of Nutgall						
	N. F. VI	7.3	6.9	6.6	7.6	7.1
Fluidextract of Rose						
	N. F. VI	9.1	11.2	13.7	13.5	11.7
Fluidextract of <i>Krameria</i>						
	N. F. V	7.5	9.2	12.5	11.5	12.0
	N. F. VI	7.2	6.8	9.9	9.6	9.9
Fluidextract of <i>Uva Ursi</i>						
	U. S. P. X	7.43	6.6	7.1	6.7	6.7
	N. F. VI	6.2	6.8	9.5	9.6	8.6

COMMENTS UPON THE ESTIMATION OF TANNIN IN THE GALENICAL PREPARATIONS.

The value of any definite conclusions which might be drawn from the information tabulated in Table III is problematical. Reliable results seem to be evident in the instances of Tincture of Nutgall, Tincture of Krameria, Tincture of Kino, Fluidextract of Hamamelis Leaves and Fluidextract of Castanea. The galenicals, possessing high total extractive, namely: Fluidextract of Rose, Fluidextract of Krameria and Fluidextract of Uva Ursi, did not give concordant assay values. They showed a tendency toward increased permanganate values upon aging. Compound Tincture of Gambir also manifested this tendency.

Why these preparations showed an increase in tannin content remains an unsolved problem. This phenomenon cannot be explained upon the basis of evaporation of the highly volatile solvent. If such were the case, the occurrence would be common to all of the preparations. Neither can this discrepancy be attributed to faulty technique. It would seem that some of these preparations show a marked increase in permanganate value caused by some change within the galenical itself. It is difficult to say just what rôle the high total extractives play in the explanation of this definite trend toward increased tannin values.

It will be observed that there has been no marked decrease in the permanganate values, as would be expected if these preparations lost much of their astringency with age. If tannin were being thrown out as a part of the precipitate the assay should indicate it. Furthermore, it would seem that the assay figures for the freshly prepared galenicals should represent their tannin content and astringency rather well.

ATTEMPTS AT REDUCING THE FORMATION OF PRECIPITATES.

In 1933 Ball (15) showed that the precipitate which appears in Fluidextract of Uva Ursi could be greatly diminished by subjecting the crude drug, previous to percolation, to the action of steam under pressure. Parks (17) in 1936 followed up the work of Ball. By subjecting the crude drug to steam under fifteen pounds of pressure for thirty minutes, he was able to obtain fluidextracts which remained virtually free from precipitates for indefinite periods of time. He attributed the cause of precipitation in this particular preparation to the action of enzymes which were rendered inactive by the heat treatment.

The crude drugs, which are indicated in Table IV, were placed in porcelain evaporating dishes and exposed to twenty pounds of steam pressure for one hour on one day only, then used to make their respective fluidextracts.

Tannin assays were made upon these preparations and compared to controls. The effect of the heat treatment, upon precipitation is also shown in the following table.

TABLE IV.

Galenical.	Tannin Assay.		Effect upon Precipitation.
	Autoclave Sample.	Control Sample.	
Fluidextract of Rose	10.4%	9.8%	Decreased
Fluidextract of Hamamelis Leaves	3.8%	3.3%	Decreased
Fluidextract of Uva Ursi	7.7%	8.9%	Decreased
Fluidextract of Krameria	3.7%	3.8%	Undetermined

SUMMARY AND CONCLUSIONS.

1. The following galenicals, when made according to the new official formulas showed improvement as to permanency over those manufactured by the process formerly official:

- (a) Tincture of Kino
- (b) Fluidextract of Hamamelis Leaves
- (c) Fluidextract of Castanea
- (d) Fluidextract of Krameria

2. The U. S. P. X formula for Compound Tincture of Gambir provided a more stable galenical than the process now official in the N. F. VI. The marked increase in strength of the latter preparation probably explains this increased precipitation.

3. Tincture of Krameria, now non-official, provides a most desirable galenical if made by type process M using a menstruum of alcohol 9, glycerin 1 as solvent. No precipitation occurred in this preparation during four months' storage.

4. The Fluidextracts of Uva Ursi, Krameria and Rose contain a very high percentage of total extractive. This is objectionable from the standpoint of permanency.

5. Changes seem to occur in galenical preparations upon standing, especially those with high total extractive values. This change results in a marked increase of reducing substances.

6. An accurate assay for tannin in galenical preparations cannot be accomplished with any of the existing methods of analysis. This is not likely to be achieved until the chemistry of tannins is more fully understood and a method of isolation devised.

7. Precipitation in tannin-bearing galenicals does not seem to materially influence their astringency.

8. Autoclaving of the crude drug prior to the process of galenical manufacture, proved to be of definite value in reducing precipitation in the Fluidextracts of Rose, Uva Ursi, and Hamamelis Leaves.

9. Light is not an influencing factor in causing precipitation in the astringent galenicals.

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