TANNIN SPOT-TESTS.*

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Of the many reagents recommended for the detection of tannins it is only reasonable to assume that a few are more sensitive than the others. To determine which reagents give positive results at great dilutions the following series of tests were made.

Four aqueous tannin solutions—gallotannic acid, gallic acid, catechutannic acid (prepared from Gambir), and the drug Kino—were freshly prepared with distilled water and were tested with thirty-three reagents.

In each case, five dilutions of the tannin solution were made—1:1000, 1:10,000, 1:100,000, 1:1,000,000, 1:10,000,000. One drop of each dilution was placed on a white porcelain spot-plate and a drop of the reagent was added to the test-drop.

Reagents 5, 19, 23, 24, 29, 33 gave no visible change in any case; reagents 20, 25, 30 being of limited value in dilutions as great as those used. The most sensitive general reagents for the different tannins seemed to be 2, 3, 7, 8, 15, 18, 26, 31.

The last-mentioned group of eight reagents was used in a series of tests on certain official, tannin-containing drugs. Each powdered drug (20 mg.) was macerated in cold distilled water (20 cc.) for 15 minutes, then filtered, and 1 cc. of the filtrate was diluted with 9 cc. water. A drop of this 1:10,000 dilution was treated on the spot-plate with a drop of the reagent. Results are tabulated in Table I.

The same reagents and the same dilutions (1:10,000) were employed in carrying out spot-tests on filter paper instead of the spot-plate. The results were for the most part similar to those obtained with the spot-plate, although the color changes were more difficult to interpret. In general the most satisfactory procedure was to permit the first drop to dry thoroughly before adding the second, thus reducing the degree of diffusion. Applying the reagent-drop to the dried test-drop was more satisfactory in some cases, *e. g.*, reagent 26; while in other cases, *e. g.*, reagent 18, best results were obtained by allowing the reagent-drop to thoroughly dry on the filter paper and then adding the test-drop.

List of Reagents:

1. 1% Ferric Sulphate
2. 5% Ferric Sulphate
3. 1% Ferric Acetate
4. 5% Ferric Acetate
5. 1% Mercuric Chloride
6. Saturated Potassium Dichromate
7. Saturated Potassium Dichromate plus trace of acetic acid
8. 1% Sodium Carbonate
9. 5% Sodium Carbonate
10. 1% Potassium Ferricyanide plus equal volume 1% NH_4OH
11. 1% Ammonium Molybdate in nearly saturated solution of NH_4Cl
12. 1% Lead Acetate (neutral)
13. 1% Lead Acetate (basic)
14. 1% Copper Acetate
15. 5% Copper Acetate
16. 1% Chromic Acid in dilute H_2SO_4
17. 1% Ferric Chloride (neutral)
18. Saturated Calcium Hydroxide
19. 1% Caffeine
20. 1% Antipyrine
21. 1:500,000 Methylene Blue
22. 1% Gelatin
23. 1% Amygdalin
24. Starch Test Solution, U. S. P. X
25. Saturated Quinine Sulphate
26. Nessler's Solution
27. 1% Iodine in 1.5% KI. Test-drop first made faintly alkaline (NH_4OH)
28. 10% Stannous Chloride in conc. HCl
29. Bromine Water
30. 1% Potassium Antimonyl Tartrate
31. 1% Potassium Cyanide
32. 1% Ferric Alum
33. 1% Albumin

* Scientific Section, A. PH. A., Toronto meeting, 1932.

TABLE I.

1:10,000 Dilution.	2.	3.	7.	8.	15.	18.	26.	31.
Galla	Dark blue ppt.	Dark brown ppt.	Orange-brown with slight ppt.	Yellowish brown; ppt. slight	Greenish brown ppt.; liquid blue-green	Brownish red ppt.	Greenish brown ppt.	Orange-brown color
Kino	Cadet gray ppt.	Brown ppt.	Orange-brown with ppt.	Rose color; ppt. slight	Greenish brown ppt.; liquid blue-green	Rose-red ppt.	Dark brown ppt.	Rose color; slight ppt.
Rhus Glabra	Pale bluish color	Brown; slight ppt.	.....	Very pale straw color	.....	Very pale pink color	Light yellow-brown; slight ppt.	.....
Castanea	Blue-violet; ppt. slight	Lighter brown color and ppt.	.....	Slight apricot color	Blue-green color; ppt. very slight	Slight rose ppt.	Ochre ppt.	Slight apricot color
Rosa	Bluish black ppt.	Brown ppt.	Brownish orange color (hardly significant)	Straw color	Blue-green color; ppt. slight	Reddish brown ppt.	Ochre ppt.	Straw color
Hamamelidis Folia	Light gray color	Light brown color; ppt. slight	.....	Pale straw color	.....	.....	Dark brown ppt. (slight)	.....
Gambir	Green-gray color and ppt.	Brown; ppt. slight	Brownish orange color; ppt. slight	Apricot color; to brownish orange	Green color; ppt. slight at first	Brownish orange ppt.	Dark brown ppt.	Brownish orange; slight ppt.

Experiments with greater dilutions of these drugs gave reactions with both the filter paper and spot-plate method; the depth of color depending on the concentration of the test-drop.

The simplicity of the tests and the facility with which they can be carried out render them worthy of consideration for inclusion in the revised monographs of the official drugs which are used primarily because of their tannin content. Because these spot-tests are indicative of the tannin content of the drugs in question we would suggest the determination of the most satisfactory reagent for each of them, as well as the limiting concentration of the respective drugs which will respond with a significant reaction, the data to be incorporated in a statement appended to the drug monograph.

Ferric sulphate was chosen, because of the marked color change yielded by its reaction with an aqueous nutgall solution, for the following experiments in which the influence of certain variable factors—temperature, time, adsorption—were investigated.

Four portions of powdered nutgall (200 mg. each) were placed in beakers and three were allowed to macerate for twenty minutes with 20 cc. of water. The temperatures of the portions were, respectively, 26.5° C. (chilled), 30.5° C. (room temperature), 53.0° C. (heated). The fourth portion was boiled for twenty minutes with 20 cc. of water, the volume being kept nearly constant during that time. All portions were then filtered through paper previously moistened with water, the volume of the fourth portion being made up to 20 cc. by slight washing of the filter. One cc. of each filtrate was then diluted with 99 cc. of water to make 1:10,000 dilutions. Subsequent testing on the spot-plate with the ferric sulphate reagent revealed no perceptible difference in the depth of color yielded by the various portions. Greater dilutions (up to 1:50,000) also showed no difference. Beyond this point no darkening could be distinguished.

To investigate the effect of the time of maceration, five portions (200 mg. each) of the powdered drug were macerated with water (20 cc.) at room temperature (29° C.) for periods of 5, 10, 15, 20 and 30 minutes, respectively. Dilutions of 1:10,000 were prepared as before and again no difference was noted upon testing. The end-point of the test was reached at 1:50,000 dilution.

The possibility of adsorption during the filtration after maceration was examined by preparing two 1:10,000 dilutions under the same conditions (200 mg. drug; 20 cc. water; 29° C.; 20 minutes), excepting only that filtration was omitted in one case and a dry filter was used in the other. Again there was no distinguishable difference in their reactions at any dilution, and again the end-point was found to be at 1:50,000 dilution.

Allowing the reagent and the drug dilutions to age from two to four days decreased the sensitivity of the reactions in every case.

The most satisfactory method of procedure was found to be: Carefully weigh 200 mg. of the very fine drug powder and place in a beaker containing 20 cc. of distilled water. Agitate occasionally, and after a minimum of five minutes has elapsed, filter through a paper previously moistened with distilled water. Prepare the dilution indicated by adding the requisite amount of distilled water to 1 cc. of the filtrate. After thorough mixing, place one drop of the dilution on a white, porcelain spot-plate and add a drop of the reagent solution.

Conclusions.—Various tannin reagents show a wide range of sensitivity when applied to dilute aqueous solutions of tannins in spot-tests.

Neither the time of maceration (within a 5- to 30-minute range), nor the temperature of the water used in maceration, nor the filtration of the extract are significant factors in altering the positiveness with which a dilute aqueous extract of a very fine drug powder will respond to selected tannin reagents.

Solutions must be freshly prepared with distilled water for best results.

SUMMARY.

Thirty-three reagents were tested to determine their sensitivities toward aqueous tannin solutions in spot-tests. The reactions of eight reagents on aqueous dilutions of seven official drug powders are tabulated. Spot-plate and filter paper methods are described and compared. Spot-tests are useful in evaluating tannin content and they are recommended for inclusion in the revised monographs of certain official drugs. After consideration of some variable factors, a method of procedure is described.

The National Pharmacy Week Prize-Winning Window.—The window shown above is the prize-winning window, and the successful contestant is John O'Brien Drug Store, Omaha, Neb. Only apparatus and material from the stock and laboratory were used—the background was built for the window and colored by an artist. The pharmacist shown in the prescription room is Kenneth O'Connor. The photograph presents its story very well for, evidently, it impressed the Committee. The chairman of the latter is Frank East, of Boston; the donor of the Prize Cup is the Federal Wholesale Drug Company.