SCIENTIFIC SECTION

A STUDY OF PRECIPITATION IN FLUIDEXTRACT OF UVA URSI II.*[1]

THE EFFECT OF HEAT AND SELECTIVE SOLVENTS UPON THE FORMATION OF A PRECIPITATE IN FLUIDEXTRACTS OF UVA URSI.

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INTRODUCTION.

In a previous study of this problem, Tisher (8) showed that the precipitate which appears in the official fluidextracts of uva ursi is invariably crystalline. He described the crystals in considerable detail and suggested that their appearance could be prevented by percolating the drug with menstruums high in alcohol and glycerol content. Tisher also made extensive assays of tannin and arbutin in uva ursi, in fluidextract of uva ursi, and in the precipitate from the latter. He obtained urson and an unnamed gum-like substance from uva ursi but did not study them in detail.

The present paper is a report of our attempts to find acceptable ways of preventing the formation of a precipitate in fluidextracts of uva ursi.

EXPERIMENTAL—EXTRACTION OF UVA URSI WITH SELECTIVE SOLVENTS.

In accord with the suggestion of Lloyd (2), and Kelly and Krantz (4) an attempt was made to find a solvent that would extract the principle, or principles, responsible for the precipitation in fluidextract of uva ursi, but which would not, at the same time, remove the constituent, arbutin.

QUANTITATIVE EXTRACTION OF UVA URSI.

A series of small percolators was set up, and carefully weighed amounts of the drug percolated. The weight of each percolator was found, and the weight before and after extraction was taken. The percentage extracted was calculated from these values. The results of this experiment are shown in Table I.

TABLE I.

Sample Number.	Solvent.	Sample (Gm.).	Residue (Gm.).	Extract (Gm.).	Per Cent of Extractive.
1	Pyridine	1.7254	1.5514	0.1740	10.0
2	Ethyl Acetate	2.0430	1.7894	0.2536	12.4
3	Acetic Acid (99%)	2.7293	2.0519	0.6774	24.8
4	Acetic Acid (50%)	2.1821	1.2689	0.9332	42.7
5	Acetic Acid (10%)	2.1670	1.2553	0.9117	42.1
6	Xylol	2.7238	2.5071	0.2167	7.9
7	Amyl Alcohol	3.0732	2.7368	0.3364	10.9
8	*n*-Butanol	2.6653	2.3065	0.3588	13.5
9	Benzyl Alcohol	3.1024	..	..	..
10	Hexane	1.8815	1.7628	0.1187	6.3

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It will be seen that no entries have been made for residue, loss and percentage extracted for Sample 9. The percolator did not lose weight but on the contrary gained weight, though dried in a vacuum desiccator over sulfuric acid for five days before the final weighing. Our explanation for this is that addition or esterification products of benzyl alcohol were formed which were insoluble and therefore held back.

TESTS FOR ARBUTIN AND TANNIN.

A portion of each percolate obtained in Table I, was evaporated to dryness and a water extract made of the residue. This extract was freed from tannin, when necessary, by the addition of lead subacetate and subsequently freeing the solution of excess lead with hydrogen sulfide. The Jungmann Test (1) was run on the lead free-hydrogen sulfide free filtrate. The results of this experiment are shown in Table II.

TABLE II.

Sample Number.	Solvent.	Tannin.	Arbutin.	Character of Residue.	Color of Extract.
1	Pyridine	+	+	Resinous	Brown
2	Ethyl Acetate	−	−	Amorphous	Olive-green
3	Acetic Acid (99%)	+	+	Soft	Green-brown
4	Acetic Acid (50%)	+	+	Soft	Dark brown
5	Acetic Acid (10%)	+	+	Soft	Dark brown
6	Xylol	−	−	Resinous	Green
7	Amyl Alcohol	−	+	Resinous	Yellow-green
8	*n*-Butanol	−	−	Resinous	Brown
9	Benzyl Alcohol	−	+	Semi-liquid	Brown
10	Hexane	−	−	Resinous	Green-yellow

+ Present.
− Absent.

It was found that four of the solvents used—ethyl acetate, xylol, *n*-butanol and hexane, gave residues that were free from arbutin.

MODIFIED PROCESS FOR MAKING FLUIDEXTRACT OF UVA URSI.

Two hundred and fifty Gm. of Uva Ursi were extracted with ethyl acetate. The marc was dried, ground and aired. When all odor of ethyl acetate had been dispelled from the drug it was made into a fluidextract by the U. S. P. process.

This fluidextract showed no crystalline deposit, as do those made by the official method, for ten days after manufacture. However, a flocculent precipitate was thrown down. At the end of fifty-four days this sediment amounted to about 6% of the total volume. This fluidextract and its precipitate deserve further study.

STUDY OF A FLUIDEXTRACT MADE FROM A HEAT-TREATED DRUG.

Description and Method of Preparation.—The object of this experiment was to see whether heating the drug, as a preliminary phase to the regular manufacturing process, had any effect upon the amount or character of the precipitate subsequently deposited in the fluidextract.

To this end three fluidextracts were prepared, designated as lots X, Y, Z. In each case 500 Gm. of drug were used.

For fluidextract X the drug was macerated with 200 cc. of water for one hour. The beaker and contents were then placed in an autoclave and heat applied for 15 minutes at 17 pounds pressure. The drug was then removed and spread out on a drying screen and allowed to dry for two

days. The color and general appearance of the drug were not materially affected. When moist, the drug was slightly more brown than originally, but, when dry, the color was nearly the same shade of green as that of the untreated drug. When thoroughly dry the treated material was run through a drug mill to make it uniform for percolation. The treated material was then made into a fluidextract by the regular U. S. P. process.

Fluidextracts Y and Z were prepared in the same manner, except that different pressures were used. Table III gives the amount of drug, water used for maceration, pounds pressure, corresponding temperature and time of exposure for each of the three lots of drug.

TABLE III.

Sample.	Amount of Drug.	Volume of Water Used for Maceration.	Pounds Pressure Applied.	Corresponding Temperature.	Exposure.
X	500 Gm.	200 cc.	17	125	15 min.
Y	500 Gm.	500 cc.	25	130	15 min.
Z	500 Gm.	500 cc.	25	130	15 min.

Comments.—1. Heating the drug previous to the preparation of the fluidextract changes the character of the sediment. This change is from a decidedly crystalline structure to a more or less amorphous form, the quantity being much less.

COLORIMETRIC DETERMINATION OF ARBUTIN.

Jungmann (1) reported a color reaction with arbutin and phosphomolybdic acid. The color, a deep azure blue, could, according to Jungmann, be detected in dilutions of 1:140,000. It was thought, therefore, that a quantitative colorimetric determination of arbutin should be possible.

This idea was stimulated by the fact that our study required that a number of arbutin determinations be made upon uva ursi and its preparations. The best method available for this assay is, so far as we know, that of Zechner (6), a long and tedious process. A method for the colorimetric assay of arbutin, which we found to check satisfactorily with the Zechner process, is outlined here.

A. ARBUTIN ASSAY OF UVA URSI.

A sample, 0.5 Gm. to 2.5 Gm., of the drug is macerated in boiling water for about one hour. When cool the liquid is filtered off and the residue washed, into the filtrate, which is made alkaline with ammonia water. About 20 cc. of a 10% lead acetate solution, recently filtered, is added to the alkaline infusion which is then heated for about fifteen minutes upon a water-bath and allowed to stand for two hours, previous to filtering through a Büchner filter. The filtrate is saturated with hydrogen sulfide, in the usual way, and the lead removed by filtering. The lead-free filtrate is then boiled to get rid of the hydrogen sulfide and refiltered if necessary. This solution is tested colorimetrically against the Standard Arbutin Solution.

B. ARBUTIN ASSAY OF THE FLUIDEXTRACT OF UVA URSI.

A sample of 1 to 5 cc. of the preparation is diluted to 200 cc., filtered and the residue washed. The filtrate is made alkaline with ammonia water. The procedure from this point is the same as for the infusion in A above. It is tested against the Standard Arbutin Solution.

C. PREPARATION OF A STANDARD ARBUTIN SOLUTION.

A 15- to 20-Gm. sample of arbutin is dissolved in water in a 100-cc. volumetric flask. This is the standard arbutin solution expressed as milligrams per cc.

D. CALCULATING THE ARBUTIN COLORIMETRICALLY.

Procedure: An unknown filtrate such as is described under A and B above is made alkaline with ammonia water. One cubic centimeter of a 10% phosphomolybdic acid solution is then added and the volume made up to 500 cc. volumetrically.

One cc. of the Standard Arbutin Solution, described under C above, is placed in a 100 cc. flask and diluted to about 70 cc. The solution is made alkaline by the addition of 10 cc. of ammonia water. One cc. of 10% phosphomolybdic acid is then added and the solution made up to volume.

A colorimetric reading of an unknown arbutin solution is then compared with that of the standard and the quantity of arbutin in the unknown calculated by the following formula.

$$\frac{\text{Standard Solution reading}}{\text{Unknown Solution reading}} \times 5 \times F = \text{mg. arbutin}$$

"F" represents the mg. of arbutin per cc. in the standard solution.

If a 1-cc. liquid sample is used the answer may be expressed directly as mg. per cc. When a dry drug sample weight is used, the percentage of arbutin may be calculated in the usual way.

QUANTITATIVE DETERMINATION OF TANNIN IN UVA URSI.

Tannin is present in uva ursi in rather high percentages. For this reason it seemed advisable to make extensive studies of it in the drug and its preparations.

There are numerous reports, in the literature, concerning the technique of tannin determination by the method of Löwenthall. Some of the problems discussed are: rate of addition of permanganate, speed and uniformity of agitation, and the volume to be titrated. There also seems to be some confusion as to the correct indicator.

In the numerous tannin assays, which we are reporting in part, use was made of an air-driven agitator to accomplish uniform stirring. Titrations were made in three-liter beakers using 735 to 750 cc. of solution at a time. A sample of indigocarmine, prepared by the Nelson Baker Co., was used as the indicator.

The results of our tannin assays are given in the following tables.

Table IV. I Uva Ursi Lot 44865 (Lilly); II Uva Ursi Lot 21839 (Lilly); III Tannin (Merck's, Lot 23485).

TABLE IV.

	I.*	II.*	III.[1]
Sample weight	5.283 Gm.	4.978 Gm.	1.060 Gm.
Volume of infusion	500.00 cc.	500.00 cc.	500.00 cc.
Volume of $KMnO_4$ (A)	20.39 cc.	13.04 cc.	29.05 cc.
Volume of $KMnO_4$ (B)	16.36 cc.	10.61 cc.	15.29 cc.
A minus B	4.03 cc.	2.43 cc.	13.76 cc.
Normality of $KMnO_4$	0.0455	0.0731	0.0318
Tannin equivalents	6.28%	7.99%	86.85%

* Average of three determinations.

[1] Average of two determinations.

The assays of the tannin samples indicate a considerable loss during the process. However, a number of them were made and the results were consistent and for that reason are offered.

Table V gives the averages of several tannin assays upon fluidextract of uva ursi Lot 848847 (Lilly). Attempts were made to determine the effects of filtering, not filtering, and of the addition of varying amounts of glycerin in the process of assay. The tannin equivalent is given in mg. per cc. since the sample is a liquid.

From the results indicated in Table V we are inclined to believe that a tannin infusion, even though cloudy, should not be filtered during the assay procedure. It would seem that the filtering, in this process, removes some of the reducing activity of the infusion. The addition of glycerin to the tannin infusion seems to have increased the tannin equivalent.

TABLE V.

	I.	II.	III.	IV.	V.
Volume of sample	5.00 cc.	5.00 cc.	5.00 cc.	5.00 cc.	5.00 cc.
Volume diluted to	500.00 cc.	500.00 cc.	500.00 cc.	500.00 cc.	500.00 cc.
Normality of $KMnO_4$	0.0731	0.0731	0.0692	0.0692	0.0692
Volume of $KMnO_4$ (A)	11.30	11.32	13.00	13.32	13.35
Volume of $KMnO_4$ (B)	10.10	10.70	11.19	11.35	11.79
A minus B	1.4	0.62	1.81	1.77	1.76
Tannin equivalents	42.9 mg./cc.	19.0 mg./cc.	52.4 mg./cc.	51.4 mg./cc.	41.4 mg./cc.
Alterations in procedure	Not filtered	Filtered	10 cc. glycerin added	25 cc. glycerin added	100 cc. glycerin added

SUMMARY.

1. Arbutin is not extracted from uva ursi by ethyl acetate, xylol, *n*-butanol or hexane.

2. It was found that the drug, which had been treated in the autoclave previous to percolation, yielded a fluidextract whose precipitate was not only less in quantity, but amorphous rather than crystalline in character. This suggests that enzymes may be the primary cause of precipitation in this preparation.

3. A colorimetric assay for arbutin has been devised which was found to give results comparable to the Zechner process.

4. Filtering a turbid infusion of the crude drug, previous to the determination of the tannin, gives low values of the latter and should be avoided.

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A STUDY OF PRECIPITATION IN FLUIDEXTRACT OF UVA URSI III.*[1]

IDENTIFICATION OF THE CRYSTALLINE PRECIPITATE IN FLUIDEXTRACT OF UVA URSI.

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INTRODUCTION.

The manufacture and storage of fluidextract of uva ursi has been a troublesome problem due to the fact that it continues to develop a sediment over a long

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