TABLE VI.—CRUDE FIBER

Source of Leaves (1939 Crop Only)	Yield, Per Cent
Wild plants	7.85
Cultivated plants, without fertilizer, from seeds of wild plants	8.55
Cultivated plants, with fertilizer, from seeds of wild plants	8.90
Cultivated plants, without fertilizer, from seeds obtained from the U. S. D. A.	8.36
Cultivated plants, with fertilizer, from seeds obtained from the U. S. D. A.	8.64

SUMMARY

1. The alkaloidal content of wild Puerto Rican stramonium averages 0.29%, based on results for each of two successive years.

2. Cultivated plants grown from seeds of wild stramonium produced a higher percentage of total alkaloids than the wild plants even when no fertilizer was added.

3. Commercial fertilizer increased the yield of alkaloids but not as much as cultivation alone did.

4. Seeds from the continental United States produced a lower yield than native seeds. The application of fertilizer increased the yield but not up to that of the native plants.

5. The total ash content in the wild plants of both years' leaves was notably higher than in any of the cultivated plants.

6. Fertilized plants yielded more ash than the unfertilized plants, probably due to the salts of the fertilizer.

7. The wild plants contained a higher percentage of acid-insoluble ash than the cultivated plants in almost all cases.

8. The unfertilized plants produced a higher percentage of acid-insoluble ash than the fertilized plants in the majority of cases.

9. The moisture content of the wild plant leaves was much lower than that of any of the cultivated plants, which were uniform, presumably due to the irrigation that was necessary.

10. The wild plants produced the least amount of total ether-soluble extract and crude fiber.

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The Potency and Stability of a New Extract of *Convallaria majalis* Leaves*

By James R. Weeks,† Donald R. Mathieson and Harald G. O. Holck

During the past few years there has been a renewed interest in therapy using standardized extracts of *Convallaria majalis* leaves, at first in Europe (1, 2, 3), and then in the United States (4, 5). Our preparation[1] was made in May, 1940, by a method similar to that described by Straub (6). It consisted of an aqueous extract of the leaves, treated with ferric hydroxide to remove tannins and gums, and then concentrated under reduced pressure until 1 Gm. of the extract was equivalent to 30 Gm. of dried leaves.

The potency and stability of the extract, after storage under various conditions for five months, were determined by comparison with U. S. P. XI Reference Tincture of Digitalis (1 cc. = 1 U. S. P. Digitalis Unit) by the 18-hr. frog method. At the time of the first assay, which was seven months after the extract was manufactured, two samples

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[1] This preparation was furnished by Dr. C. W. Sondern of George A. Breon and Company, Kansas City, Mo.

of the extract and two of a 10% solution of the extract in distilled water were set aside, one of each being stored in a refrigerator and the other at room temperature, all protected from light.

EXPERIMENTAL

Male frogs (*Rana pipiens* Schreber), weighing from 16 to 30 Gm., were stored in running water about 1 cm. deep at 10° C. for one week prior to use. Twenty-four hours before injection, the frogs were transferred to the assay tank, held exactly at 20° C. Each frog was wiped gently with a towel, the urine expressed and the animal weighed to the nearest one-half gram. Injections were made into the pectoral lymph sac through the floor of the mouth. Observations were made at 18 (±1/2) hrs. after injection, a frog being considered dead when pain reflexes on pinching the toes were absent and subsequent examination showed that the heart was stopped.

The concentrations of the solutions injected were such that for all doses each frog received 0.02 cc. per Gm. of body weight. In addition, the alcohol content of the dilutions of the Reference Tincture of Digitalis was adjusted, by evaporation or addition of alcohol, to contain about 23% of alcohol by volume. Except for the first and last doses of convallaria extract in Table I, the doses are in equal logarithmic increments.

TABLE I.—FIRST ASSAY: COMPARISON OF CONVALLARIA EXTRACT AND U. S. P. XI REFERENCE TINCTURE OF DIGITALIS

Reference Tincture		Convallaria Extract	
Dose, Cc./Kg.	Mortality[a]	Dose, Mg./Kg.	Mortality[a]
1.78	0/15	17.8	0/15
2.24	4/15	22.4	1/15
2.82	6/15	25.1	5/15
3.55	13/15	28.2	8/15
4.47	15/15	31.6	12/15
5.62	15/15	35.5	14/15
...	...	44.7	15/15

LD_{50}: 2.80 ± 0.13 cc./Kg. 27.7 ± 0.7 mg./Kg.

0.1 Gm. of convallaria extract is equivalent to 10.1 ± 0.5 U. S. P. Digitalis Units.

[a] Mortality of controls, 4/66.

The first assay (Table I) was conducted in December, 1940, and the second (Table II) in May, 1941. The second assay was conducted in two sections on successive days. Using the logarithms of the doses, log LD_{50} (average lethal dose) was calculated by Kärber's method (7). The standard deviation of the logarithm of the LD_{50} was derived by the formula $\sqrt{\Sigma(pq/n)d^2}$, in which p = mortality as a fraction, $q = 1 - p$, n = number of animals and d = difference between logarithms of the successive doses (8). The standard error of the ratios of the LD_{50}'s of the standard to the extracts (standard error of the potency of the extracts) was calculated by the formula $\sqrt{S_1^2 + S_2^2}$, in which S_1 and S_2 are the

TABLE II.—SECOND ASSAY: COMPARISON OF U. S. P. XI REFERENCE TINCTURE OF DIGITALIS AND SAMPLES OF EXTRACT AND OF A 10% SOLUTION STORED FIVE MONTHS AT ROOM TEMPERATURE AND IN A REFRIGERATOR

PART 1

Reference Tincture		Convallaria Extract		
Dose, Cc./Kg.	Mortality[a]	Dose, Mg./Kg.	Mortality[a] Refrigerated	Mortality[a] At Room Temperature
2.37	0/12	23.7	0/12	0/12
2.82	0/12	28.2	0/12	0/12
3.35	3/12	33.5	2/12	2/12
3.98	5/12	39.8	5/12	7/12
4.73	5/12	47.3	8/12	8/12
5.62	10/12	56.2	11/12	12/12
6.68	12/12	66.8	12/12	12/12
LD_{50}:	4.41 ± 0.20 cc./Kg.		42.2 ± 1.8 mg./Kg.	40.4 ± 1.6 mg./Kg
Equivalent of 0.1 Gm. of convallaria extract in U. S. P. Digitalis Units:			10.4 ± 0.7	10.9 ± 0.7

PART 2

Reference Tincture		10% Solution of Convallaria Extract		
Dose, Cc./Kg.	Mortality[b]	Dose, Mg./Kg.	Mortality[b] Refrigerated	Mortality[b] At Room Temperature
...	...	23.7	1/12	1/12
2.82	0/12	28.2	2/12	0/12
3.35	3/12	33.5	0/12	1/12
3.98	4/12	39.8	4/12	4/12
4.73	9/12	47.3	7/12	11/12
5.62	9/12	56.2	9/12	10/12
6.68	11/12	66.8	12/12	12/12
LD_{50}:	4.34 ± 0.21 cc./Kg.		44.0 ± 2.1 mg./Kg.	41.6 ± 1.6 mg./Kg.
Equivalent of 0.1 Gm. of convallaria extract in U. S. P. Digitalis Units:			9.9 ± 0.7	10.4 ± 0.6

[a] Mortality of controls, 0/21.
[b] Mortality of controls, 1/25.

respective standard deviations of the LD_{50}'s expressed in per cent (8).

Our results show that the potency in all cases was well within the limits of the standard deviations; hence there has been no deterioration of the extract. A preliminary experiment, conducted in July, 1940, two months after the manufacture of the extract and five months before the first assay given above, showed that 0.1 Gm. of the extract was equivalent to approximately nine U. S. P. XI Digitalis Units. The absence of change, even when diluted 1 to 10 with water, is in line with the finding by Straub (6) that aqueous solutions of convallaria extract in sealed ampuls retained their full strength for four years.

SUMMARY

The potency of a concentrated, purified, aqueous extract of the leaves of *Convallaria majalis* was determined by comparison with digitalis on frogs. The extract and a 10% aqueous dilution thereof were found to be stable at room temperature during the course of five months.

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A Chemical Study of Oklahoma Plants

V. *Ephedra Nevadensis* Watson*

By Jess L. Beasley, Jr.,† and Loyd E. Harris‡

Fig. 1.—Habit photograph of *Ephedra Nevadensis*.[1]

Fig. 2.—*E. Nevadensis* showing staminate cones.[1]

Six species of Ephedra have been reported in North America, where they are found growing in the desert or semi-desert regions of Mexico, Colorado, Texas and Oklahoma (1). *Ephedra Nevadensis* was described by Watson (2) in 1879. Cross (3) reported in 1937 that collections of Ephedra were first made in Oklahoma, southwest of Hollis, in Harmon County, January 28, 1932.

Nagai (4) isolated the alkaloid ephedrine from *Ephedra vulgaris* in 1887. The use of this alkaloid in medicine has made it a drug of great importance during the past two decades. The chief sources of ephedrine are *Ephedra sinica* Stapf, *Ephedra equisetina* Bunge and other species of Ephedra (5)

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[1] By courtesy of Dr. G. L. Cross, Botany Department, University of Oklahoma.