

subjects, but that quantity produced little or no cathartic effect with guinea pigs; and, though 50 mg. of aloe-emodin was quite active for guinea pigs, Tutin and Clewer (12) reported that this material was only slightly active with human subjects in 100-mg. dosage. It is of some interest to note that although the guinea pig serves as a satisfactory test animal, the dosage per unit body weight, compared to the human dosage, is about 250:1.

The marked supplementary or synergistic effect observed when a mixture of three products was fed is of special interest because it provides evidence to account for the high activity of the crude extracts in comparison with the relatively low activity of any of the isolated or highly purified fractions contained in the conventional types of extracts.

#### SUMMARY

Aloe-emodin and chrysophanic acid have been isolated from cascara sagrada extract, subsequent to dialysis, acid hydrolysis and treatment with ferric chloride. Syringic acid and emodin were also isolated a number of times, confirming earlier identifications.

The cathartic activity of weighed single dosages of three of the substituted anthraquinones that have been isolated from cascara sagrada extract was assayed by the technique previously described, using guinea pigs as test animals. Known, weighed mixtures of the three ingredients were also assayed and found to be markedly more active than similar quantities of any one of the compounds given alone. A mixture of 10 mg. each of aloe-emodin and emodin plus 5 mg. of chrysophanic acid was distinctly more

active than 25 mg. of any one of the three compounds when tested separately.

Chrysophanic acid anthrone, aloin and chrysarobin were practically inactive in 25-mg. doses when assayed individually. The supplementary or synergistic effect observed for mixtures of the compounds appears to provide an explanation of the marked activity of crude extracts compared to the relatively poor activity of any of the purified fractions thus far studied.

#### REFERENCES

- (1) Schwabe, P., *Arch. Pharm.*, 226 (1888), 569; *C.*, 59 (1888), 1283; *Proc. A. Ph. A.*, 37 (1889), 491.
- (2) Jowett, H. A. D., *Proc. A. Ph. A.*, 52 (1905), 288.
- (3) Sipple, H. L., King, C. G., and Beal, G. D., *Jour. A. Ph. A.*, 23 (1934), 205.
- (4) Green, M. W., King, C. G., and Beal, G. D., *Ibid.*, 25 (1936), 107; 27 (1938), 95.
- (5) Daels, M. F., *Bull. acad. roy. méd. Belg.*, 30 (1920), 129.
- (6) Beal, G. D., and Tumminckatti, M. C., *Jour. A. Ph. A.*, 14 (1925), 865; 15 (1926), 847.
- (7) Eder, R., and Widmer, C., *Helv. Chim. Acta*, 6 (1923), 966.
- (8) Jacobson, R. A., and Adams, Roger, *J. Am. Chem. Soc.*, 46 (1924), 1312.
- (9) Gunton, J. A., and Beal, G. D., *Jour. A. Ph. A.*, 11 (1922), 681.
- (10) Cahn, R. S., and Simonsen, J. L., *J. Chem. Soc.* (1932), p. 2573.
- (11) Gardner, J. H., et al., *J. Am. Chem. Soc.*, 57 (1935), 1074; 58 (1936), 757; 58 (1936), 597.
- (12) Tutin, F., and Clewer, H. W. B., *J. Chem. Soc.*, 99 (1918), 946.

## A Study of Wild and Cultivated Stramonium in Puerto Rico\*

By Carl H. Johnson† and Esteban Nunez-Melendez‡

Since stramonium grows profusely in Puerto Rico and since the leaves are widely used in the island for the relief of asthma and

\* This paper is based on a thesis presented to the Graduate Council of the University of Florida in partial fulfillment of the requirements for the degree of Master of Science in Pharmacy.

Presented to the Scientific Section of the A. Ph. A., Detroit meeting, 1941.

† Assistant Professor of Pharmacognosy and Pharmacology, School of Pharmacy, University of Florida, Gainesville, Fla.

‡ Instructor in Pharmacy, College of Pharmacy, University of Puerto Rico.

other ailments, a study of the content of the active medicinal principles of Puerto Rican stramonium was deemed desirable. A comparison of the wild and cultivated forms was made, together with the effects of fertilizer.

The three species of the genus *Datura* in Puerto Rico according to Britton (1) are *Datura Stramonium* L., *Datura Metel* L. and *Datura fastuosa* L. Their morphology has been studied by de Grosourdy (2), Stahl

(3), Britton (1) and other investigators. Only the first species, *Datura Stramonium*, was used in this investigation.

The common name used in Puerto Rico, *belladonna del pobre*, signifies that the poorer classes cannot afford to buy preparations of the imported belladonna, but instead use the wild stramonium that grows so abundantly. For the relief of asthma the sun-dried leaves are made into cigarettes; when mixed with lard as an ointment they are used as an antispasmodic and for healing sores.

In 1936 stramonium seeds were obtained from the United States Department of Agriculture and were planted in the Medicinal Plant Garden of the College of Pharmacy of the University of Puerto Rico, with the purpose of comparing the plants of the wild and cultivated stramonium.

It is generally known that stramonium is abundant on the island, but the wild drug is frequently of inferior quality and below the strength of the United States Pharmacopoeia (4). Among the factors that might affect the plant in the production of its active constituents are fertilization, soil, temperature, selection and other conditions. A study of the literature on stramonium reveals that there are many conflicting opinions regarding the various factors which affect the alkaloidal content of this plant.

That stramonium grown in different countries varies in the percentage of active constituents has been shown by many investigators. It has been demonstrated that leaves from South Africa contain over 0.5% of alkaloids (5), whereas leaves from China contain less than 0.2% (6).

## EXPERIMENTAL

### FIELD WORK

In view of the objectives of the present investigation, it was believed advisable to make an outline for the work on fertilization and on the cultivation of the seeds. Therefore the work was arranged as follows:

1. Study of the wild plants.
2. Study of cultivated plants from seeds obtained from wild plants.
  - (a) Without using fertilizer.
  - (b) Using a commercial fertilizer.
3. Study of cultivated plants from seeds obtained from the Department of Agriculture.

- (a) Without using fertilizer.
- (b) Using a commercial fertilizer.

From the wild plants the seeds were collected in September.

The seeds were planted in early spring in large seed beds about 3 ft. wide and 5 ft. long. The beds were carefully prepared by thoroughly mixing equal parts of rich garden soil, well-rotted manure and clean sand. After leveling off the surface the seeds were planted in drills about 1 in. deep and 4 in. apart. The seeds were barely covered with soil. Watering was done with a very fine spray to maintain the proper moisture in the soil, especially during dry weather.

Germination began quickly, the plants appearing above the ground in one week. The percentage of germination was high and plants of a uniform size were produced. The seedlings were allowed to grow in the beds until they attained a height of about six inches when they were strong enough to withstand transplanting. The seedlings were planted about 20 in. apart in the row with two rows about 3 ft. apart in each plot. It was observed that the seedlings developed rapidly into quite strong and healthy plants.

For the preparation of the plots, a new ground plow was used after first clearing off the brush. The plowing was repeated three times at one-week intervals. Because of good weather conditions the soil was well pulverized; in addition all the weeds were destroyed and the soil was aerated. Practically no clods remained and the soil was in good condition for putting out the plants. The field was divided into plots about 40 ft. long and 5 ft. wide, separated by furrows 1 ft. wide and deep. Six plots were established for each group of plants.

The plants grew very well and proper care was exercised to keep the soil free from weeds at all times. The soil was cultivated with a hoe about twice a month, particularly after each hard rain. In hot, dry weather the cultivation was shallow to conserve the natural moisture of the soil. Water was used when necessary.

The commercial fertilizer used contained 4% nitrogen, 8% phosphorus and 4% potash.

The leaves were harvested at the beginning of the month of July. At this time the plants were in full bloom and appeared to be at the height of their development. There was no appreciable difference in the size of the leaves of plants grown under different conditions nor did the plants themselves give any indication of the soil variations.

### LABORATORY WORK

The harvested leaves were air dried at a temperature of about 40° C. by spreading them out in a thin layer in an attic. As the weather was dry and warm, no artificial heat was necessary. After about one week's time, the leaves were ground in a hand drug mill until all of the drug could pass through a 60-mesh sieve. After the material was sifted, it was placed in a tightly stoppered, amber-colored con-

tainer which was kept away from the light in a cool, dry place.

In addition to the assay for alkaloidal content of the plant, other determinations were carried out which might give some valuable information as to the influence of fertilization and climatic factors. Therefore, the following assays were carried out on each sample of the drug cultivated under the different conditions set forth:

1. Total alkaloidal content.
2. Total ash and acid-insoluble ash.
3. Moisture by the oven and toluene methods.
4. Total ether-soluble extract.
5. Volatile and non-volatile ether-soluble extracts.
6. Crude fiber.

1. The extraction of the total alkaloids was carried out by means of a Soxhlet extractor followed by shaking out with immiscible solvents to obtain the purified alkaloids as directed in the U. S. P. XI. They were determined volumetrically by treating with an excess of 0.02 *N* sulfuric acid and back titrating with 0.02 *N* sodium hydroxide solution. About 10 Gm. of the finely powdered drug was used as a sample each time. The assays were carried out on the leaves obtained from two crops, 1939 and 1940.

The alkaloidal content is indicated in Table I, which summarizes the results of the assays.

TABLE I.—ALKALOIDAL CONTENT

Sources of Leaves	Total Alkaloids, Per Cent by Weight	
	1939 Crop	1940 Crop
Wild plants	0.28	0.29
Cultivated plants, without fertilizer, from seeds of wild plants	0.32	0.36
Cultivated plants, with fertilizer, from seeds of wild plants	0.34	0.38
Cultivated plants, without fertilizer, from seeds obtained from the U. S. D. A.	0.14	0.19
Cultivated plants, with fertilizer, from seeds obtained from the U. S. D. A.	0.28	0.25

2. The total ash and the acid-insoluble ash were determined by the U. S. P. methods. The results are tabulated in Table II.

TABLE II.—TOTAL ASH AND ACID-INSOLUBLE ASH

Source of Leaves	Total Ash, Per Cent		Acid-Insoluble Ash, Per Cent	
	1939 Crop	1940 Crop	1939 Crop	1940 Crop
Wild plants	16.86	16.85	0.79	0.56
Cultivated plants, without fertilizer, from seeds of wild plants	10.11	9.41	0.65	0.43
Cultivated plants, with fertilizer, from seeds of wild plants	11.52	10.28	0.11	0.19
Cultivated plants, without fertilizer, from seeds obtained from the U. S. D. A.	14.63	10.24	1.09	0.27
Cultivated plants, with fertilizer, from seeds obtained from the U. S. D. A.	15.08	13.23	0.54	0.31

3. The moisture was determined on a 10-Gm. sample by the oven method of the U. S. P. and on a 20-Gm. sample by the toluene method. The results are given in Table III.

TABLE III.—MOISTURE CONTENT

Source of Leaves	Moisture Content, Per Cent			
	Oven Method 1939 Crop	Oven Method 1940 Crop	Toluene Method 1939 Crop	Toluene Method 1940 Crop
Wild plants	10.48	7.71	10.20	9.25
Cultivated plants, without fertilizer, from seeds of wild plants	13.86	10.71	12.40	10.10
Cultivated plants, with fertilizer, from seeds of wild plants	13.20	10.64	10.80	9.25
Cultivated plants, without fertilizer, from seeds obtained from the U. S. D. A.	13.47	10.69	11.20	10.50
Cultivated plants, with fertilizer, from seeds obtained from the U. S. D. A.	12.45	10.49	10.80	9.50

4. The total ether-soluble extract was determined by the U. S. P. method; the results are given in Table IV.

TABLE IV.—TOTAL ETHER-SOLUBLE EXTRACT

Source of Leaves (1939 Crop Only)	Yield, Per Cent
Wild plants	4.37
Cultivated plants, without fertilizer, from seeds of wild plants	4.76
Cultivated plants, with fertilizer, from seeds of wild plants	4.80
Cultivated plants, without fertilizer, from seeds obtained from the U. S. D. A.	4.82
Cultivated plants, with fertilizer, from seeds obtained from the U. S. D. A.	7.23

5. The volatile and non-volatile ether extracts were determined by heating the total ether-soluble extracts at 110° C.; the results are given in Table V.

TABLE V.—VOLATILE AND NON-VOLATILE ETHER-SOLUBLE EXTRACTS

Source of Leaves (1939 Crop Only)	Ether-Soluble Extracts, Per Cent	
	Volatile	Non- Volatile
Wild plants	0.22	4.15
Cultivated plants, without fertilizer, from seeds of wild plants	1.32	3.53
Cultivated plants, with fertilizer, from seeds of wild plants	0.21	4.59
Cultivated plants without fertilizer, from seeds obtained from the U. S. D. A.	0.22	4.60
Cultivated plants, with fertilizer, from seeds obtained from the U. S. D. A.	1.32	5.99

6. The U. S. P. method for crude fiber was carried out with the results shown in Table VI.

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.

## REFERENCES

- (1) Britton, N. L., "Scientific Survey of Porto Rico and the Virgin Islands," New York Academy of Science, New York, N. Y., 1923-30, Vol. VI, p. 174.
- (2) De Grosourdy, D. R., "El Medico Botanico Criollo," Libreria de Francisco Brachet, Paris, 1864, Vol. II, p. 175.
- (3) Stahl, A., "Flora de Puerto Rico," Federal Emergency Relief Administration, San Juan, Second edition, 1936, Vol. III, p. 218.
- (4) "The Pharmacopœia of the United States of America," Mack Printing Co., Easton, Pa., Eleventh revision, 1936, p. 360.
- (5) *Bull. Imp. Inst.*, 14 (1916), 27; through *Am. J. Pharm.*, 88 (1916), 422.
- (6) Tsao, P. N., and Chen, S. Y., *J. Chinese Chem. Soc.*, 3 (1935), 372; through *Chimie & industrie*, 36 (1936), 966.

## 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<sup>1</sup> 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

\* From the Department of Physiology and Pharmacology, College of Pharmacy, University of Nebraska, Lincoln, Neb.

† George A. Breon Fellow in Pharmacology, 1940.

<sup>1</sup> This preparation was furnished by Dr. C. W. Sondern of George A. Breon and Company, Kansas City, Mo.