

CHEMICAL EXAMINATION OF THE SEEDS
OF PRUNUS VIRGINIANA

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PREFACE.

Of the five subfamilies of the rose family, viz. the

- (1) Spiraeoideae
- (2) Pomoideae
- (3) Rosoideae
- (4) Prunoideae
- (5) Chrysobalanoideae

about all yield economically more or less important products.¹ Thus the first yields soap bark; the second the quince, apple and pear; the third the strawberry, raspberry, black berry, kusso, the roses and their valuable oils; the fourth the almond, peach, apricot, prune, plum, and the cherries. It is but natural that these products which have added to man's comfort for a thousand years and more, should have been made the subject of chemical research. But this investigation has not been restricted to the more important economic products. The Ulmaria has made important contributions to plant chemistry and the mountain ash berry has been the subject of numerous investigations. Even many of these genera and species which have not been otherwise examined have been tested for cyanogenetic glucosides, i.e. for their capacity to yield hydrogen cyanide.

Inasmuch as the bark of the wild cherry is used for medicinal purposes on the American continent, and

1 Wehmer, Die Pflanzenstoffe.

has been official in eight editions of the U. S. Pharmacipoeia, the bark of Prunus virginiana L. (P. serotina Ehrb.) has been examined repeatedly. The leaves have been tested for the hydrogen cyanide which they yield upon distillation, and the seeds have been extracted for fatty oil. It seemed desirable, therefore, to learn something more about the chemistry of the fruit which grows so abundantly in different sections of this country. With this object in view, the following examination was undertaken.

Review of the chemistry of Prunus Virginiana.

The earliest chemical statement on record is that by Lemberger of 1871, who states that the variation in the color of the infusion of wild cherry bark is due to the presence of tannin in greater or lesser extent.

In 1875 Williams attempted to isolate the bitter principle from the bark, but did not succeed.

In 1887 Power and Weimar report on the following constituents: amygdalin, emulsin, bitter principle, fluorescent principle, but none of these were isolated in pure or crystalline form.

No more successful were Rother's investigations into the nature of the fluorescent principle reported on the same year.

In 1889, Hawkins examined into the hydrogen cyanide content of commercial wild cherry bark and found that it varied considerably.

In 1890 Schimmel & Co. distilled from the bark an oil which very much resembles that of bitter almonds.

As the result of a microscopic examination of the bark, Bastin in 1895 reported on the observation of starch and of crystals of calcium oxalate.

In 1895 and 1896, the hydrogen cyanide content of the bark was further investigated by Stevens and Judy on the one hand and by Dohme and Engelhardt on the other. Stevens concludes that the thicker bark has the higher hydrogen cyanide content, whereas Dohme arrives at the conclusion that the young, green bark, contains more than the thick brown bark. The latter also describe (1896) in detail the laboratory method of assay.

In 1897 Grace E. Cooley looked into the demand made by the U.S. P. that wild cherry bark should be collected in fall. She found the starch content to be at its maximum in fall, the tannin content (color test) in spring. Tests for the more important hydrogen cyanide and emulsin gave negative results.

A. B. Stevens, resuming his work in 1899, arrives at the conclusion that wild cherry bark deteriorates (as to its hydrogen cyanide glucoside content) with age and that only fresh bark should be used in the manufacture of galenical preparations.

A year later A. W. Stevens reports that the inner layer of the bark contains practically all of the glucoside.

It thus becomes apparent that our knowledge of the

chemistry of this species is practically restricted to the dried bark, the drug of the U. S. Pharmacopoeia and the shops; that even of this no direct constituents have been isolated in a pure state; and that our principle knowledge is restricted to the volatile oil, products of hydrolysis of the glucoside, which the bark unquestionably contains.

Collection of the fruit and
preparation of the material.

The choke cherry used for the experimental part of this thesis was collected in August, 1912, near Webster, S. D. Being collected by hand, only the ripe or nearly ripe fruits were taken. These were placed in small bags and allowed to stand for a day to insure a thorough ripening. The berries were then separated from the leaves and twigs and thoroughly washed, dried and weighed. Fifteen pounds of the well ripened cherries were thus obtained.

Small portions of the cherries were thrown into hot water, which process softened them thoroughly, without, however, rupturing the skins. They were then thoroughly macerated by squeezing with the fingers, after which they were extracted for one half hour with boiling water.

The aqueous extract was separated from the pits and skins by straining and expressing through muslin bags, after which it was evaporated to a thick soft extract and this weighed.

The pits and skins were allowed to dry by exposure to sunlight after which they were separated by hand and weighed separately.

The results thus obtained from the 15 lbs of cherries (6804 grams) are herewith tabulated.

Evaporated extract	1048	grams	(15.3 p.c.)
Pits	1531	"	(22.5 p.c.)
Skins	369	"	(5.4 p.c.)
Loss due to evaporation of water	3857	"	(56.6 p.c.)

Examination of the Seeds.

In order to ascertain the ratio of seeds to pits, ten grams of the pits were carefully cracked and the seeds removed and weighed. The pits were found to consist of 27.6 p.c. of seeds.

One hundred (100) grams of the pits were now taken and ground to a No. 70 powder in a drug mill. Ninety grams of this powder were placed in a Soxhlet extraction apparatus and extracted with ether until exhausted of its oily content. The ethereal extract was then set aside in a tared open dish and allowed to evaporate spontaneously until the weight was constant. The weight of oil thus obtained was 13.5 grams corresponding to 15 p. c. of the pits or 3.37 p. c. of the seeds. This yield is much more in harmony with the yield of oil of other rosaceous seeds than the yield of 5 p. c. obtained by Betz according to the reports of Czapek.¹

The oil obtained was of a viscid consistency with a slight benzyaldehyde odor and had a specific gravity of 0.916 at 20°.

The saponification number (Koettstorfer number) was determined by heating a weighed quantity of the

1 (Biochemie, vol. 1. p. 119).

fixed oil with a definite alcoholic potassium hydroxide solution and titrating back the excess of alkali with standard hydrochloric acid solution. The results are herewith tabulated.

	I		II	
Amt of oil used	2.0046 gm.		0.5566 gm.	
Amt of $\frac{N}{2}$ taken for saponification	25.0	cc.	25.0	cc.
Amt of HCL(factor 1.69) used for back titration	12.3	cc.	14.2	cc.
Amt of $\frac{N}{2}$ KOH consumed in saponification	2.6	cc.	0.7	cc.
Saponification value	35.59		35.05	

A small portion of the pits were carefully cracked and exactly ten grams of seeds were separated therefrom. The seeds were ground finely in a mortar, transferred to a Soxhlet extraction apparatus, extracted with ether until exhausted. Upon evaporation of the ethereal extract 4.45 grams of oil were obtained, corresponding to 44.5 p.c. of seeds, or 2.7 p.c. of the entire fresh fruit.

The iodine absorption number of the oil from the pits was determined by allowing a weighed quantity of oil to stand in contact with an alcoholic solution of iodine and mercuric chloride for a definite length

of time. (U. S. Pharmacopoeia, 8th revision, p. 527).

The excess of iodine was titrated by means of standard sodium thiosulphate solution. The following tabulation shows the results obtained.

Amount of oil used	0.3098 gm.
Amount of Iodine T. S. used.....	12.5 cc.
Amount of Mercuric Chloride T. S. used.....	12.5 cc.
Amount of Sodium Thiosulphate(factor 1.15) used.....	17.6 cc.
Amount of Sodium Thiosulphate (factor 1.15) used in blank.....	40.7 cc.
Difference(= equivalent of amount of iodine absorbed.....	23.1 cc.
Iodine number	107.9

Examination of Skins.

One hundred grams of the dried skins were ground to a fine powder (No. 80) in a drug mill and then placed in a Soxhlet extraction apparatus and extracted with heptane. The resulting liquid (i. e. the heptane containing extractive matter) was of a yellowish-green color.

Bibliography.

Bastin, E. S. 1895.

Some further observations on cherry barks.

Am. Journ. Pharm., 67, p. 595. (Proc. A. Ph. A., 44, p. 623.)

The author submits microscopic drawings of the bark of Prunus demissa Walters and agrees with Sargent that this western tree is but a "form", not even a distinct "variety" of the choke cherry, Prunus Virginiana. He also comments on the bitter taste, starch, and crystals of calcium oxalate, but does not otherwise refer to chemical constituents.

Cooley, G. E. 1897.

An investigation of the official Prunus Virginiana, to distinguish it from barks collected at other seasons.

Am. Journ. Ph., 69, p. 414. (Proc. A. Ph. A., 46, p. 845.)

The U. S. P. calls for the bark collected in fall. Inasmuch as the bark collected at other seasons cannot be distinguished histologically, chemical methods of distinction were tried. The starch content was found to be at its maximum in fall, the tannin content (color test) in spring. Tests for hydrocyanic acid and emulsin gave negative results.

(Bibl. -2)

Dohme, A. R. L., and H. Engelhardt.

1896.

Further experiments with wild cherry bark.

Pharm. Review, 14, p. 13. (Jahresb. d. Pharm., 31, p. 193;

The authors correct an error occurring in a previous publication on the hydrocyanic acid content and describe their laboratory method for assaying the bark.

Dohme, A. R. L., and H. Engelhardt.

1895.

Untersuchungen der Cortex Pruni Virginianae.

Pharm. Rundsch., 13, p. 260. (Jahresb. d. Pharm., 30, p. 154.

The authors found that, in conformity with the common assumption, and contrary to the statement of A. B. Stevens, the young green bark contained considerably more hydrocyanic acid than the thick brown bark.

(Bibl. -3)

Hawkins, L. W.

1889.

Wild Cherry Bark and its Preparations.

Pharm. Journ., 49, p. 355. (Jahresb. d. Pharmacie, 24, p. 10.

The author made an investigation of the hydrocyanic acid content of commercial wild cherry bark and found that its content from various sources varied considerably.

Lemberger, J. L.

1871.

On Wild Cherry Bark.

Proc. A. Ph. A., 19, p. 503. (Year-Book Ph., 9, p. 46;

The author found that the variation of the color of infusion of wild cherry bark is due to the existence of tannin in greater or less quantity.

Power, R. and Weimar, H.

1887.

On the Constituents of Wild Cherry Bark.

Pharm. Rundsch., 5, p. 203. (Jahresb. d. Pharm., 22, p. 24.

The authors report on the following substances:-

Amygdalin

Bitter principle

Emulsin

Fluorescent principle.

(Bibl. -4)

Rother, R.

1887.

The Fluorescent Principle of Wild Cherry Bark.

Am. Journ. Pharm., 59, p. 286. (Pharm. Ztg.,
p. 484.

The author reports on the results of his investigations into the nature of the fluorescent principle.

Schimmel & Co.

1890.

Oel der Rinde von Prunus Virginiana.

Bericht von Schimmel & Co., April, p. 48. (Jahresb.
d. Pharm., 25, p. 35.)

An oil, which very much resembles the oil of bitter almonds, was obtained from the bark.

Stevens, A. W.

1900.

Wild Cherry Bark and its Preparations.

Am. Journ. Pharm., 72, p. 300. (Jahresb. d.
Pharm., 35, p. 36;

The author finds that the inner layer of the bark contains practically all of the glucoside.

(Bibl. -5)

Stevens, A. B.

1899.

Wild Cherry Bark.

Pharm. Review, 17, p. 445. (Jahresb. d. Pharm., 35, p. 36.

As a result of numerous assays the author finds that wild cherry bark deteriorates with age and arrives at the following conclusions:

1.) The bark is best preserved in air tight "retainers."

2.) That only the fresh bark should be used in the manufacture of galenical preparations.

Stevens, A. B. and J. N. Judy.

1895.

The relative hydrocyanic acid content of the thick and thin bark of *Prunus Virginiana*.

Proc. A. Ph. A., 43, p. 226. (Pharm. Rundsh., 13, p. 204; Jahresb. d. Pharm., 30. p. 154;

The authors conclude that the thick bark has the higher hydrocyanic acid content.

Williams, J. L.

1875.

Bitter principle of wild cherry bark.

Am. Journ. Pharm., 47, p. 53. (Proc. A. Ph. A., 23, p. 209;

The author reports an attempt to isolate the bitter principle.

Approved by Edward Kremer

Prof. of Pharm. Chemistry

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