A PRELIMINARY STUDY

of

THE EXTRACT OF ECHINACEAE ANGUSTIFOLIA

by

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INTRODUCTION

In the latter part of August, 1926, a quantity of the products of Echinaceae Angustifolia was received from Lloyd Brothers of Cincinnati, accompanied by a letter and descriptive matter from John Uri Lloyd. In his letter Professor Lloyd says:

"By this days' express or parcel post, will go to you the product of fifty pounds, first quality, recent Echinaceae. This is the total product, excepting a small amount of a crude green oil that on standing overlies the extract, of which oil, eight fluid ounces, was sent to Philadelphia. This, however, is a fragment; the remainder can be used, if you desire in your calculation."

"Echinaceae now heads the list of drugs used in Lloyd Brothers laboratory. It is steadily increasing in sales and is persistently being knocked by the Chicago managers of the American Medical Association. Much does it remind me of the history of Cinchona!"

The descriptive material sent by Professor Lloyd is as follows:

Description of Echinaceae Products

forwarded to

Professor Edward Kremers

by John Uri Lloyd.

"Fifty pounds recent Echinaceae root were ground and percolated with condensed official alcohol in my concentration.* The percolate was distilled until the alcohol was exhausted, the extract being then reduced to an extractive consistency. Specimen A.

"After standing 48 hours, sixteen ounces of a green oil came to the top. Part of this oil (eight ounces) was sent to Dr. Cohen, of Philadelphia, for clinical and physiological investigation. The remainder is forwarded as Specimen B."

"The alcohol-exhausted drug was then exhausted with water. This sweet extract is marked Specimen C."

"Remarks. The extract, A, on standing, has liberated further supplies of the crude oil.

This oil, B, contains, among other constituents, a very acrid substance, and also the volatile oil previously used by you."

"This sweet substance, C, was by me considered a 'sugar', or 'glucose', possibly a mixture. Miss Stewart sent a good sized lot to a friend, Mr. Luther Carpenter, then Chemist (now Superintendent), of one of the largest beet-sugar factories in Bay City, Michigan, for examination. To my surprise he reported,

'I found no trace of sugar in the molasses submitted to me; but a great mass of impurities of all kinds. In my opinion,

* "The apparatus referred to is the Lloyd extraction and concentration apparatus."

the plant cannot be utilized at all in the sugar line.' From report on the Sweet Principles of Echinaceae Angustifolia, by Mr. Luther Carpenter, Bay City, Michigan."

"In my opinion, however, that 'great mass of impurities' may prove to be of exceptional interest. Especially do I wonder what that 'sweet principle' can be. In this, I naturally think of glycyrrhiza or its relatives. You may find it a fundamental."

The material referred to in the foregoing description consisted of two glass jars of extractive matter, (Specimens A and C), and an eight ounce bottle of a heavy green oil, (Specimen B).

Specimen A consisted of approximately 1500 grams of a dark brown, oily or tarry extract with a characteristic strong, penetrating and persisting odor suggesting fatty acids.

Specimen B consisted of 189 grams of dark green viscid oil having the same penetrating, persisting odor as the alcoholic extract from which it had separated. Upon standing, another small portion of similar oil separated from Specimen A, making, in all, about 200 grams of this product.

The jar containing the aqueous extract (Specimen C) had leaked considerably in transit. There remained, however, a quantity of a dark brown, molasses-like material with a sweet taste, about equivalent in amount to the alcoholic extract making up Specimen A.

EXPERIMENTAL PART

The Alcoholic Extract.

Work was begun upon the alcoholic extract by carrying out preliminary tests in order to determine the best way to separate the fixed and volatile oils contained therein. It was decided to first distill with steam and afterwards to extract the fixed oil with petroleum ether.

The entire alcoholic extract was accordingly transferred to a five liter flask and distilled with steam as long as oil separated from the aqueous distillate. This was a long and tedious process. 150 cc. of volatile oil was obtained. This oil was light greenish yellow in color. It had a characteristic disagreeable and lasting odor. Its specific gravity of 0.789 at 23° C.; 0. 791 at 20° C., and its other physical properties indicate that this oil is undoubtedly identical with that studied by Bischoff and obtained from the same source. Its higher boiling point may be accounted for by assuming the presence of small quantities of non-volatile material carried over mechanically during the long period of distillation. No further work was done upon the volatile oil.

The large volume of aqueous distillate obtained in the above distillation was cohobated three times. The volatile oil obtained in this way was added to that previously obtained. The final aqueous distillate was acid in reaction. No tests for definite chemical compounds were obtained.

The non-volatile residue in the flask was evaporated to dryness and extracted with petroleum ether. During the process of evaporation, a large portion of this residue accidently burned and was lost. Upon the evaporation of the petroleum ether, about 50 cc. of fixed oil resulted. This, upon boiling with alcoholic potassium hydroxide gave the following saponification values:

Sample No. I.

Wt. of oil	1.6998	gm.
N/2 alcoholic KOH	23.3	cc.
N/2 HCL	20.92	cc.
Blank		
N/2 alcoholic KOH	28.3	cc.
N/2 HCL	28.8	cc.
Result		
Saponification Value	129.98	

Sample No. II.

Wt. of oil	1.4832	gm.
N/2 alcoholic KOH	28.3	cc.
N/2 HCL	22.16	cc.
Blank		
N/2 alcoholic KOH	28.3	cc.
N/2 HCL	28.8	cc.
Result		
Saponification Value	125.55	

Sample No. III.

Wt. of oil	2.2794	gm.
N/2 alcoholic KOH	28.3	cc.
N/2 HCL	19.8	cc.

Blank

N/2 alcoholic KOH	28.3	cc.
N/2 HCL	28.8	cc.

Result

Saponification Value	110.79

Sample No. IV.

Wt. of oil	1.7552	gm.
N/2 alcoholic KOH	28.3	cc.
N/2 HCL	21.48	cc.

Blank

N/2 alcoholic KOH	28.3	cc.
N/2 HCL	28.8	cc.

Result

Saponification Value	116.90

The residue from the petroleum ether extract consisted of a large quantity of dark brown semisolid material, without the former characteristic odor. It resembled in appearance the aqueous extract, but it was not further examined.

Specimen B. The dark colored heavy oil which had separated from the alcoholic extract in the Lloyd Brothers Laboratories in Cincinnati was next examined.

The small portion of oil which separated from the alcoholic extract after it was sent to us was added to Specimen B and the whole distilled with steam so long as volatile oil was obtained. By this process 48 cc. of volatile oil, to all appearances exactly like that distilled from the alcoholic extract, was obtained, and having practically the same boiling points and specific gravities.

The non-volatile portion in the distilling flask was shaken with petroleum ether, in an attempt to separate the fixed oil. An exceedingly permanent emulsion resulted. After standing six weeks, sufficient material separated to warrant the determination of saponification values after evaporation of the petroleum ether solvent. The results of these saponifications follow:

Sample No. I.

Wt. of oil	1.4858	gm.
N/2 alcoholic KOH	28.3	cc.
N/2 HCL	26.1	cc.
Blank		
N/2 alcoholic KOH	28.3	cc.
N/2 HCL	28.8	cc.
Result		
Saponification Value	49.27	

Sample No. II.

Wt. of oil	1.5534	gm.
N/2 alcoholic KOH	28.3	cc.
N/2 HCL	26.21	cc.

Blank

N/2 alcoholic KOH	28.3	cc.
N/2 HCL	28.8	cc.

Result

Saponification Value	48.87

It will be observed that the saponification value of this oil is much lower than that shaken out from the alcoholic extract. Whether this discrepancy is due to incomplete removal of the petroleum ether solvent, to the occlusion of water, or to some other factor does not become apparent. The slow separation of the emulsion obviates further work upon this material at present.

Specimen C, the aqueous extract, was molasses-like in appearance and had a sweet taste. Samples of this material treated with phenyl hydrazine in the usual way gave an abundant yield of osazone. Examined under the microscope, these osazone crystals appeared to be identical with those obtained from a sample of pure dextrose. Recrystallization from alcohol did not change their crystalline form.

An aqueous solution of the extract heated with Fehling's solution gave a copious precipitate of cuprous oxide.

To determine the total quantity of reducing sugars in the aqueous extract, several samples of the extract were accurately weighed and allowed to stand with dilute hydrochloric acid for several hours. They were then neutralized carefully and boiled with an excess of Fehling's solution. The resulting cuprous oxide was cooled in Gooch crucibles, dried and weighed with the following results com-

puted as dextrose:

Sample No. I.

Wt. of extract	0.5 gm.
Wt. of residue	0.29 gm.
Dextrose	133.0 milligrams.
Dextrose	26.6 per cent

Sample No. II.

Wt. of extract	0.5 gm.
Wt. of residue	0.31 gm.
Dextrose	138.4 milligrams.
Dextrose	27.68 per cent.

SUMMARY

The products from 50 lbs of fresh Echinaceae, received from Lloyd Brothers Laboratories and consisting of Specimen A, about 1500 grams of alcoholic extract; Specimen B, 189 grams of oil which separated from Specimen A; and Specimen C, about 1500 grams of aqueous extract, were investigated with the following results:

Specimen A yielded about 10 grams of oil like Specimen B. Distilled with steam, 150 cc., about 10%, of volatile oil, undoubtedly identical with that examined by Bischoff in 1924 was obtained. Extracted with petroleum ether, 50 cc., approximately 3 1/3%, of a fixed oil with a characteristic and persisting odor and a saponification value of about 120 resulted. The residue resembled Specimen C.

Specimen B, distilled with steam, gave 48 cc., nearly 25% of volatile oil. The remainder, shaken with petroleum ether formed a very stable emulsion. A small quantity of oil separated had a saponification value of approximately 50. This oil was probably mixed with petroleum ether, or water.

Specimen C was sweet and resembled molasses. It gave an osazone which appeared to be identical with dextraosazone. It reduced Fehling's solution. Total reducing sugars, determined by means of Fehling's solution and computed as dextrose equalled approximately 27%.

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Echinaceae Angustifolia

Proc. A. Ph. A., 41, p. 479.

Echinaceae Angustifolia contains 4½ per cent of oleoresin, nearly ½ of which is a straw-colored volatile oil, and 1/6 colorless fixed oil, the remainder being resin.

Lloyd, J. U. 1897

Echinaceae

Pharm. Rev., 15, p. 206; (Proc. A. Ph. A., 46, p. 868.)

Quotations from an address by Prof. Lloyd giving the history of the discovery of echinaceae, also its introduction to the medical profession, together with the characteristics of the drug.

Sayre, L. E. 1898

Echinaceae Root

Drugg. Circ., 42, p. 124; (Proc. A. Ph. A., p. 869.)

Statement by Prof. Sayre giving a short history of the investigation of the drug, a complete description of the two species together with a quotation from Prof. Lloyd and an analysis of the drug.

Sayre, L. E. 1904

Echinaceae Angustifolia

Drugg. Circ., 48, p. 5; (Proc. A. Ph. A., p. 737.)

Article by Prof. Sayre in which he relates the demand for the plant in the past ten years, possibility of its preservation or cultivation and description of the plant.

Moses, John 1910

Echinaceae and a Spurious Root That Appeared in the Fall of 1909

Am. Jour. Phar., 82, p. 224; (Proc. A. Ph. A., 58, p. 191.)

Article by Moses giving the description of Echinaceae and a spurious root which exhibits similar characteristics.

Kraemer, Henry 1911
Sollenberger, Maud

The Pharmacognosy of Echinaceae

Am. Jour. Pharm., 83, p. 315; (Proc. A. Ph. A., 59, p. 204.)

An article by Kraemer and Sollenberger giving botanical synonyms, description of the plant, description of the rhizome and root, microscopical structure and intercellular substance.

Beringer, George M. 1911

Fluid Extract of Echinaceae

Am. Jour. Pharm., 83. p. 324.

An article by Beringer in which he lauds the use of Echinaceae and gives various strengths of menstra for the fluid extract with the view of its introduction into the National Formulary.

---------- 1913

Echinaceae

Jour. A. Med. A., 60, p. 69; (Y. B. A. Ph. A., 2, p. 114.)

"Echinaceae was considered by the Council on Pharmacy and Chemistry, and was rejected on the ground of insufficient evidence for its therepentic efficiency. So far as can be learned, no reliable evidence for the claims made for the drug has been presented since the report was made by the Council."

---------- 1913

Echinaceae

Jour. A. Med. A., 61, p. 2089; (Y. B. A. Ph. A., 2, p. 114.)

Statement giving the claimed therepentic uses of the drug with a decision not to recognize it in New and Nonofficial Remedies.

---------- 1914

Echinaceae

Am. Jour. Pharm., 86, p. 450; (Y. B. A. Ph. A., 3, p. 220.)

The results of a chemical analysis of the roots of Brauneria Angustifolia and Brauneria Purpurea.

---------- 1915

Echinaceae

Jour. A. Med. A., 64, p. 71; (Y. B. A. Ph. A., 4, p. 131.)

Worthlessness of it and its preparations, echtisia, echthol and echitone.

---------- 1918

Echinaceae

Eclect. Med. J.; (Pract. Drug., 1918, p. 22; Y. B. A. Ph. A., 7, p. 216.)

"Echinaceae, first an aboriginal medicine, barely escaped wide exploitation as a "quack medicine" at the hands of an illiterate but shrewd herb doctor, and was finally introduced into current medicine by Prof. John King."

Giltner, L. T. 1920
Couch, J. F.

An Experimental Study of Echinaceae Therapy

Jour. Agric. Research, 20, p. 63; (Am. Jour. Pharm., 93, p. 227.)

Abstract by Giltner and Couch giving the results of treatment of disease in animals with echinaceae and their conclusion of its usefulness.

Beal, J. H. 1921

* Echinaceae

Am. Jour. Pharm., 93, p. 229; (Y. B. A. Ph. A., 10, p. 235.)

J. H. Beal critizes the paper of Couch and Giltner opining that their experiments were too few in number to be conclusive.

* Comment on the Paper by Couch and Giltner on "An Experimental Study of Echinaceae Therapy."

Giltner, L. T. 1921
Couch, J. F.

Echinaceae--A Reply to Dr. Beal

Am. Jour. Pharm., 93, p. 324; (Y. B. A. Ph. A., 10, p. 236.)

Giltner and Couch reply to Dr. Beal giving further details as to their experiments and stating that they see no reason to withdraw any of the conclusions stated in the former paper.

---------- 1921

Echinaceae

Jour. A. Med. A., 76 (Jan. 1, 1921), 39; (Y. B. A. Med. A., 10, p. 235; Am. Jour. Pharm., 10, p. 330.)

Statement that Fluid-extract of Echinaceae should not be in the National Formulary. "Animal experiments made by Couch and Giltner of the U. S. Bureau of Animal Industry, to determine whether the drug possessed the properties ascribed to it, gave negative results in every instance."

Bischoff, F. 1924

Oil of Echinaceae Angustifolia

Jr. A. Ph. A., 13, p. 898.

The volatile oil from Echinaceae Angustifolia contains a hydrocarbon, $C_{15}H_{28}$, with two double bonds. It is apparently not a straight chain hydrocarbon.

APPROVED Nellie Wakeman

June 11, 1927