

A Chemical Investigation of the Seeds of *Glottidium* *Vesicarium* (Jacq.) Harper*

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Observations on *Glottidium vesicarium* (Jacq.) Harper were begun by the senior author in the fall of 1928. This weed, commonly called the Coffee Bean Weed, is found in the low moist places of Florida in large quantities. Hundreds of acres of it abound, in fact during one summer the larger portion of a four hundred acre tract in Hillsborough County was covered with it. It is common hearsay that Coffee Beans are toxic and that children, chickens and cattle have been poisoned by them. Investigation soon proved that many different beans of various species of plants have been given the name of Coffee Bean by the public. This has led to great confusion.

A description of *Glottidium vesicarium* (Jacq.) Harper as given by Erdman West, Mycologist of the Florida Agricultural Experiment Station, is as follows:

"Plant annual but somewhat woody, 1 to 3 M. tall, sometimes widely branched, often growing in large colonies in damp soil. Leaves, alternate pinnate, with 20 to 52 leaflets; leaflets elliptic, mucronate, 1.5 to 4 cm. long, dark green above, paler beneath. Flowers one to several in axillary peduncles, 2 cm. or more long; corolla, yellow (or dark reddish purple in variety *atrorubrum*), about 1 cm. across; standard reniform, rounded; wings and keel angular; stamens 10, diadelphous, 0 to 1. Calyx 5-lobed, the lobes much shorter than the tube. Pods pendant, usually in pairs, ellipsoid, acuminate at both ends, 5 to 8 cm. long; outer wall dehiscent, inner wall remaining as a white papery envelope. Seeds 2, grey-brown, mottled. Flowers in summer and fall. Pods often remain on the dead stalks all winter. Coastal plain, North Carolina to Florida and Texas. Introduced from the West Indies."

The Texas variety seems to be the same. In the spring of 1931 Dr. S. E. Wolff, Botanist for the Texas Agricultural Experiment Station at Temple, Texas, sent us seeds

which produced plants of the above description. We acknowledge his kindness with thanks.

EXPERIMENTAL

TOXIC PROPERTIES OF THE SEEDS

Rumored cases of children being poisoned by eating the beans could not be substantiated (1). Wolff (2) reported, "I, personally, have cooked the seeds and found them good." Matlack (3) advises that the roasted seeds are non-toxic to guinea pigs. In the fall of 1930 a senior student, C. Kazarian, working in our laboratory found 14 Gm. of the dried ground seed toxic for the cat. The material was mixed with twice its weight of salmon and fed to a cat which had been starved for 43 hours. Within two hours the following symptoms appeared: deep breathing, stupidity, nausea, violent vomiting which ended with yellow and green mucous, several watery stools with griping.

The intraperitoneal injection into rats of 3 cc. of extracts ranging in strength from 10 Gm. per 100 cc. to 100 Gm. per 100 cc., using 15 per cent and 50 per cent ethyl alcohol as menstrua, failed to give any noticeable systemic effects. A rat fed approximately five Gm. of the ground dried seed in twice that quantity of cheese did not show any noticeable ill effects.

The chief work on the toxicity of the seeds has been done by Emmel (4), in 1935, working at the Florida Agricultural Experiment Station. His work was on single comb white leghorn hens. He reported that 150 seeds, when force fed, is the minimum number which will kill a chicken. Investigation by him of the gross pathology, histopathology and blood-picture showed necrotic enteritis and necrosis of the lining of the bulbous portion of the gizzard. The hepatic cells showed fatty degeneration and the remaining parenchymatous organs and all involuntary muscles were in a state of cloudy swelling. There was a destruction of both erythrocytes and leukocytes.

ANALYTICAL

The literature failed to reveal any work of this nature. A thesis by Weedon (5), in 1923, is all that could be found. He reported no alkaloids nor glucosides and so directed his attention largely to the fatty oil. This he obtained by ether extraction in a yield of 6.1 per cent. It had the following properties: specific gravity 0.933 at 35° C.; refractive index 1.480, corrected to 15.0° C.; saponification value 193.0; acid value 3.2; iodine value 132.0. The fatty acids had an iodine value of 138.5. Weedon found the oil to have excellent drying properties and to be a good substitute for linseed oil in paints. He estimated the protein of the seeds to be about 21 per cent.

Fatty Oil.—Our work began by collecting 1 Kg. of the seeds near Gainesville, Florida in the fall of

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1930. Preliminary investigation showed no alkaloids nor glucosides to be present. A saponin was indicated by all the well-known tests. Previous to attempting its isolation the fatty oil was investigated. Eight hundred twenty-nine grams of ground seeds dried at 105° C. yielded 3.39 per cent of fatty oil by petroleum ether extraction. The chemical and physical properties checked well with those found by Weedon. A sample of the oil stored in a well filled bottle in diffused light has become very viscid, indicating polymerization. This, of course, is to be expected from the high iodine value of the oil.

Saponin.—After the above petroleum ether extraction, the seeds were extracted with 80 per cent alcohol. When this was distilled off 8.5 Gm. of a syrupy residue remained. It gave a negative test for saponin. It had an odor of caramel and gave the osazone of dextrose. After standing for several months over concentrated sulfuric acid it partially crystallized. Apparently the saponin had hydrolyzed during extraction and is but another instance illustrating the well known difficulty of isolating these bodies in a pure condition. Repeated efforts, using also methyl alcohol extracts from three Kg. of seeds, failed to produce a pure saponin; therefore, the following efforts were made to get the saponin or product of hydrolysis other than the sugar.

Sapogenin.—Using the procedure of Liang, and Noller (6), the dried alcohol-water extract was dissolved in just sufficient methyl alcohol containing 5 per cent HCl and refluxed continuously for forty-eight hours. The dark brown precipitate which formed was washed with water, dried in air and extracted two hours in a Soxhlet extractor using carbon tetrachloride. The solvent was allowed to evaporate at room temperature, leaving a cream-colored material. Hot methyl alcohol was used to dissolve all the material that it would and normal isopropyl alcohol was used to dissolve the remainder.

The methyl alcohol solution was chilled in an ice-bath and some material separated which on drying in air was a light tan color and glistened. The mother liquor was concentrated and again chilled producing a second crop of material. The quantities were so small that further purification was not attempted, but the material appeared crystalline. Melting points of these two portions were not very sharp, ranging from 48° C. to 54° C.

The normal isopropyl alcohol solution was also chilled and a white crystalline material separated. This was washed with water, dried and the melting point determined as 47° C., uncorrected. Further crystallization from the mother liquor gave crystals whose melting points checked closely. The crystals gave negative tests for sulfur, nitrogen and halogens. Since the quantity of material was so small no further data could be obtained.

It is indicated that an alcoholic-aqueous extract of these seeds yields, upon hydrolysis with hydrochloric acid, one or more sapogenins and it is hoped

that subsequent work with larger quantities will enable their identification.

SUMMARY

The available data on the toxicity of *Glottidium vesicarium* (Jacq.) Harper have been pointed out and the previous work on the seeds, consisting principally of an investigation of the fatty oil, has been reviewed. The data on the fatty oil have been checked and negative tests for alkaloids and glucosides have been obtained. Positive tests for saponin have been obtained but the saponin appears to be hydrolyzed during extraction, and thus far attempts at isolation have failed. One or more hydrolysis products have been obtained by refluxing an alcoholic-aqueous extract with 5 per cent HCl.

REFERENCES

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Crystalline Xanthophyll from Wheat Germ

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While attempting to isolate the tocopherols by new methods, a crystalline xanthophyll was obtained from the nonsaponifiable portion of wheat germ. A small amount of an allophanate of one of the tocopherols was also isolated.

EXPERIMENTAL

Twenty-five pounds of wheat germ were extracted with hot Skelly-solve B in a Barnsdahl continuous extraction apparatus. There was obtained 1200 Gm. of oil which was saponified in the usual manner. The nonsaponifiables were separated from the soap by continuous extraction with

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