The persistence of action among the aglycones is slight; that is, they are all rapidly eliminated from the circulation. This is particularly true with digitoxigenin in contrast with digitoxin.

Digitoxigenin caused a brief initial stimulation as manifested by convulsions, followed by marked depression of the central nervous system in cats and frogs. Digitoxin has no such action in corresponding doses.

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A STUDY OF THE LEAVES OF IPOMŒA PES-CAPRÆ.*,[1]

BY B. V. CHRISTENSEN[2] AND J. A. REESE.[3]

Ipomœa Pes-Caprœ (*Convolvulaceœ*) is a denizen of nearly every tropical beach (1), the world over. Harshberger (2) regards it as the character plant of the low beaches. It is a perennial plant with a tough woody root as thick as a finger and many feet in length (3). From the enlarged crown of the root grow a number of creeping stems, fleshy and purplish when young, but becoming woody as they mature. The leaves are thick, and the entire plant is mucilaginous. The shape of the leaf is indicated by the name of the plant.

The plant has several scientific names and many colloquial names. Small (4) describes it under *Ipomœa Pes-Caprœ* (L) Sweet. The Index Kewensis (5) gives *Ipomœa Pes-Caprœ*, *Ipomœa biloba*, *Ipomœa maritima*, *Ipomœa bilobata* and *Convolvulus brasiliensis* as synonymous names. Gerth van Wijk (6) lists a number of colloquial names under *Ipomœa biloba*. Bailey (7) considers *Ipomœa Pes-Caprœ*, Roth as synonymous with *Ipomœa maritima*, R. Br. Wehmer (8) gives *Convolvulus brasiliensis* (L) as a synonym for *Ipomœa maritima* R. Br.

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[1] Abstract of Thesis submitted in partial fulfilment of the Degree Master of Science in Pharmacy.

[2] Director, School of Pharmacy, University of Florida.

[3] Graduate Assistant in Pharmacognosy and Pharmacology, University of Florida.

According to Dymock (3) the whole plant, locally named, "Murja Devi," is used in India externally in rheumatism in the form of decoction, and internally in colic. Recently, extracts of the powdered leaves, in the form of an ointment, have been used in several hospitals for the treatment of bed sores. Excellent results are supposed to have resulted from the use of these ointments.

EXPERIMENTAL.

For the present investigation a quantity of leaves of *Ipomœa Pes-Caprœ* was collected at Jacksonville Beach, Cedar Keys and St. Augustine, Florida, in October and November of 1935. After confirming the identity of the species, the leaves were carefully freed from petioles and dried indoors. The material was ground and passed through a number 20 sieve. The ground leaves weighed 12 Kg.

A representative sample was analyzed for moisture and ash.

	Per Cent.				
Sample	A	B	C	D	Average
Total ash	11.46	11.39	11.68	11.60	11.53
Acid-insoluble ash	0.95	0.91	0.98	0.96	0.95
Moisture by oven method (U. S. P. XI)	8.72	8.56	..	..	8.64
Moisture by toluene method (U. S. P. XI)	8.92	8.95	..	..	8.94

By way of preliminary examination several tests were undertaken to determine the general character of the constituents:

1. Test for alkaloid by extracting with warm 1 per cent hydrochloric acid, negative.
2. A portion of the drug was shaken strongly with distilled water containing a little sodium carbonate. No characteristic froth, that might indicate the presence of saponins, was produced.
3. One hundred grams of drug, examined by the "Stas-Otto Method," gave negative tests for alkaloid and glucoside.
4. A quantity of mucilage was precipitated from an infusion made from 200 Gm. of the drug by the addition of an equal volume of alcohol. Upon drying it was converted into an insoluble mass. After removal of the mucilage, the solution readily reduced Fehling's solution. The solution was concentrated on a water-bath and treated with lead acetate and basic lead acetate and the precipitates decomposed with hydrogen sulfide and separately examined, but all tests for glucoside and saponin were negative.
5. Two 50-Gm. samples of the powdered drug were shaken with 500 cc. of petroleum benzin and allowed to stand, with occasional agitation, for eight days. An aliquot portion of the clear supernatant liquid was decanted and allowed to evaporate at room temperature. After drying to constant weight over sulfuric acid, the per cent of extractive was determined. The remainder of the petroleum benzin solution was filtered off from the drug, and the samples, which had been extracted with petroleum benzin, freed from the solvent and extracted successively with ether, alcohol and distilled water in the same manner as with petroleum benzin. The drug was freed each time of the preceding solvent; an aliquot portion of each extraction evaporated on a water-bath.

	Per Cent.		
Sample	A	B	Average
Petroleum benzin (b. p. 30°–40°)	3.76	3.66	3.71
Ether	1.52	1.51	1.52
Alcohol	9.41	9.63	9.52
Distilled water	24.75	23.68	24.22
Total	39.44	38.48	38.97

6. Two 40-Gm. samples of the powdered leaves were extracted in Soxlet extractors with the following liquids. After removal of the solvent the residual extracts were kept over sulfuric acid until of constant weight.

	Per Cent.		
Sample	A	B	Average
Petroleum benzin (b. p. 30°–40°)	3.78	3.80	3.79
Ether	1.98	2.06	2.02
Alcohol	20.00	18.64	19.32
Distilled water	7.20	7.62	7.41
Total	32.96	32.12	32.54

7. Various quantities of the several extracts were administered to small dogs weighing about 4 Kg., at intervals of from two to four days, and in no case was there any noticeable effect attributable to the drug. The maximum quantity of each extract, administered separately in a single dose, was 1 Gm. of the petroleum benzin extract, 1 Gm. of the ether extract, 2 Gm. of the alcoholic extract and 2 Gm. of the aqueous extract.

ANTISEPTIC DETERMINATION OF OINTMENTS.

Since this investigation was begun because of the use of ointments made from the powdered drug and extracts of the drug, it was decided to determine the antiseptic value of ointments made from extracts of the drug. A quantity (1 Kg.) of the powdered leaves was extracted successively in a large modified Soxhlet apparatus with petroleum benzin (b. p. 30°–40°), ether, alcohol and distilled water. After removal of the solvents, the extracts were kept in a desiccator over calcium chloride until used in the preparation of the various ointments. All the ointments used in the tests were made using U. S. P. Petrolatum as the base. The method used for testing was the agar-plate method of the U. S. Department of Agriculture (9) and *Staphylococcus aureus* was used as the test organism.

Petroleum Benzin Extract.—Ointments representing, in terms of petroleum benzin extractive, 10, 15, 20, 25, 50, 100 and 200 per cent of the strength of the drug showed no inhibitory (antiseptic) action. These tests, as all the succeeding ones, were run with two strains of *Staphylococcus aureus.*

Alcoholic Extract.—Ointments representing, 10, 15, 25 and 50 per cent of the strength of the drug showed no inhibitory action.

Ether Extract.—Ointments representing 10, 25, 50 and 100 per cent of the drug had no inhibitory action.

Aqueous Extract.—Ointments representing 12, 25 and 50 per cent of the strength of the drug were prepared. The organisms grew adjacent to and in some cases under the test ointment.

Petrolatum Extract.—Since an ointment had been used made by heating the leaves with petrolatum and straining, two ointments were prepared by this method. Ten grams of the powdered leaves were heated for one hour on a boiling water-bath with 90 Gm. of petrolatum, and strained. This ointment showed no antiseptic action. An ointment was made in the same manner using 20 Gm. of the powdered leaves and 80 Gm. of petroleum. This ointment, likewise, showed no inhibitory action.

LARGE SCALE EXTRACTION.

For the purpose of a more complete examination a quantity (9 Kg.) of the powdered leaves was extracted by continuous percolation with petroleum benzin (b. p. 30°–40°), and after removal of this solvent, extracted by continuous percolation with hot alcohol. The petroleum extract, after removal of the solvent, weighed 330 Gm. The alcoholic extract, after removal of the solvent, weighed 1325 Gm.

Examination of the Petroleum Benzin Extract.—One hundred and fifty grams of this extract were dissolved in a large volume of ether and shaken successively with aqueous ammonium carbonate, aqueous potassium carbonate and finally with a 10 per cent solution of potassium hydroxide. The alkaline extracts were decomposed with diluted sulfuric acid and the liberated substances removed with ether. The ammonium carbonate and potassium carbonate extractives consisted of traces of resinous material. The potassium hydroxide extractive, however, consisted of several Gm. of a black, oily liquid. After repeated attempts to obtain a crystalline material, a white flocculent material precipitated from hot ethyl acetate on cooling. This melted at 86°–88° C.

When boiled with acetic anhydride for one hour under a reflux condenser, it separated on cooling the solution; the melting point was not changed.

Examination of the Alcoholic Extract.—The alcoholic extract was mixed with water and steam passed into the mixture until the volatile products were removed. The distillate, which contained oily drops floating on the surface, was extracted with ether. The ethereal solution was dried with anhydrous sodium sulfate and the solvent removed, when a small amount (0.25 Gm.) of a pale yellow, volatile oil was obtained. This possessed a persistent odor and gave the color reactions of furfural.

After the steam distillation there remained in the distillation flask a dark-colored liquid (A) and a quantity of soft, dark, resinous mass (B). The resinous mass was repeatedly washed with hot water until the washings were colorless and the latter were added to the main portion of the aqueous liquid.

Examination of the Aqueous Liquid (A).—The aqueous liquid which had been separated from the resinous mass amounted to about 4 liters, and was acid to litmus. It was concentrated at a low temperature and repeatedly extracted with ether. The combined ethereal shakings were successively shaken with 5 per cent ammonium carbonate, 5 per cent potassium carbonate and 10 per cent potassium hydroxide solutions. The three alkaline extracts were decomposed with diluted sulfuric acid and the liberated material extracted with ether. Attempts to obtain crystalline substances failed.

The ethereal liquid, which had been extracted with the alkaline solutions, was allowed to evaporate, when a very small quantity of a pale yellow, crystalline substance was obtained. This substance was not very soluble in ether and alcohol. After washing with ether and drying it melted at 118° C. The quantity of this substance was too small to permit further investigation.

The original aqueous liquid was then repeatedly extracted with chloroform, and the combined chloroform extracts shaken with aqueous potassium hydroxide. The potassium hydroxide solution was acidified with diluted sulfuric acid and extracted with chloroform. Upon evaporation of the chloroform a light, brown residue was obtained.

The original aqueous liquid, which had been extracted with ether and chloroform, had a reddish brown color and a salty taste. It gave a green color with ferric chloride and reduced Fehling's solution. A portion of the aqueous liquid was treated with basic lead acetate and an unsuccessful attempt was made to prepare an osazone from the filtrate. Steam was then passed into the remainder of the aqueous liquid (A) in order to expel the last traces of chloroform. The liquid was treated with an excess of lead acetate, and to the filtrate from the lead precipitate a slight excess of basic lead acetate was added. The filtrate resulting from the lead precipitates, and also the lead acetate and basic lead acetate precipitates after being suspended in water were decomposed with hydrogen sulfide. The final filtrate remaining after treatment with basic lead acetate and also the solutions resulting from the decomposition of the lead precipitates were likewise examined for an active principle by shaking them separately with chloroform, and by treatment with potassium hydroxide, acidifying and extracting with ether. As the result of this treatment only bitter residues, red coloring matter and a bright yellow powder, which could not be crystallized, were obtained.

Examination of the Resinous Mass (B).—This resin, which had been separated from the aqueous liquid (A) as previously described, was mixed with purified white sand, dried and extracted in a modified Soxhlet apparatus successively with petroleum benzin (b. p. 30°–40°), ether and chloroform.

Petroleum Benzin Extract of the Resin.—This extract was a dark green, semi-solid and weighed 135 Gm. This was refluxed for four hours with saturated alcoholic potassium hydroxide and the alkaline liquid diluted with water. The alcohol was removed in a current of steam and the unsaponifiable material extracted with ether. The ethereal solution was washed with water, dried with anhydrous sodium sulfate and the solvent removed; a reddish yellow residue was obtained. This residue was dissolved in hot alcohol. On cooling an amorphous substance separated which gave the color reactions of the phytosterols.

The alkaline liquid, which had been extracted with ether, was acidified with sulfuric acid. The liberated material was collected by filtration, washed with water and as much water as possible removed from the mass by suction. The mass was transferred to a large flask and re-

fluxed with ether for several hours. The ethereal solution was filtered and the ether removed, when several Gm. of a mixture of fatty acids were obtained. They have not been identified.

Ether Extract of the Resin.—The ether extract of the resinous mass (B), after removal of the solvent and drying in a desiccator over sulfuric acid under reduced pressure, was a dark green, soft, resinous mass weighing 18 Gm. This was dissolved in hot alcohol, boiled with decolorizing charcoal and filtered. A black solution was obtained. The liquid was again boiled with decolorizing charcoal and a black solution was again obtained. The alcohol was removed; the resinous residue obtained dissolved in ether and extracted successively with ammonium carbonate, potassium carbonate and potassium hydroxide solutions. These alkaline solutions extracted only resinous material.

Chloroform Extract of the Resin.—This extract was a dark brown, resinous mass weighing 6 Gm. from which no pure substance could be obtained.

SUMMARY AND CONCLUSIONS.

1. The most important constituents of the leaves of *Ipomœa Pes-Caprœ* are: mucilage, a volatile oil, a complex resin, fat, a phytosterol, bitter substances and red coloring matter.

2. Neither the leaves nor extracts of the leaves appear to have any noticeable pharmacological activity.

3. Ointments prepared from the leaves and extracts of the leaves have no antiseptic action.

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A COMPARATIVE STUDY OF THE COLORIMETRIC, VITAMETER AND BIOLOGICAL TESTS FOR VITAMIN A.*

BY A. BLACK, R. D. GREENE, H. L. SASSAMAN AND C. SABO.[1]

Although the biological method of testing vitamin A is still considered by many to be the only absolutely reliable one, certain chemical tests and physical measurements have been very extensively used in recent years. Rosenheim and Drummond (1) reported in 1925 that arsenic trichloride gave a blue color with fish oils which was proportional to the vitamin A content. A little later Carr and Price (2) developed the antimony trichloride test. This latter procedure has been modified and improved by many and especially by Wokes and Willimott (3) and Norris and Danielson (4).

In 1928 Morton and Heilbron (5) reported that the absorption of light of 3285 Ängström units by fish liver oils and "A" concentrates ran closely parallel

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[1] Biological Laboratories, E. R. Squibb & Sons, New Brunswick, N. J.