SOME OBSERVATIONS ON THE EFFECT OF DIFFERENT DRYING METHODS ON THE GLYCOSIDE CONTENT OF THE LEAVES OF *DIGITALIS LANATA*

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There is little information available on the effect of different drying methods on the differential glycoside content of the leaves of *Digitalis lanata*. This has been due largely to the absence of adequate methods for differential analysis, most methods being dependent on a biological assay of total cardiac activity. For the determination of the component cardiac glycosides, a method using paper chromatography and fluorescence photography has been devised[1], and was used in this investigation of the genuine glycosides, digilanids A, B and C.

Method

A batch of leaves of *D. lanata* was collected in the early morning. Drying of the leaves was commenced on the same day, using the following methods:—(1) Low temperature vacuum drying; (2) sun drying; (3) hot air oven at 100° C.; (4) hot air oven at 70° C.; and (5) hot air oven at 50° C.

In low temperature vacuum drying the leaves were maintained at 5° to 10° C. under vacuum (0·01 mm. mercury) and took about 18 hours to dry. Drying was considered to be complete when the leaves had become brittle. The sun-dried leaf was exposed on a steel mesh in the open for 7 days, being left out overnight and subject to some light rain on the 5th day. Mild to warm weather prevailed, with a shade temperature of 70° to 75° F. Oven drying at 70° and 100° C. was completed within 3 hours and that at 50° C. within 7 hours. Approximately 2·3 g. of each sample of dried leaf, previously broken up in a mortar, were placed in 30 ml. of solvent, chloroform and methanol 1 : 1, for extraction. A moisture determination was carried out on each sample, using the Karl Fischer method.

Extraction

The leaf was shaken for 3 hours with the 30 ml. of chloroform-methanol solvent, followed by filtration on a Buchner funnel. This was repeated twice, shaking the residue with 10 ml. of solvent for 1 hour. The combined filtrates were evaporated to small bulk under reduced pressure, at a temperature not exceeding 30° C., and then adsorbed by diatomaceous earth. Chlorophyll and other impurities were next removed by Soxhlet extraction of the powder with light petroleum (low-boiling) in which the cardiac glycosides are insoluble. The glycosides were redissolved from the diatomaceous earth with chloroform-methanol, followed by filtration and evaporation to very small bulk under reduced pressure. The extract was finally adjusted to 10 ml. in 80 per cent. ethanol.

Chromatography

A small volume of the extract, usually 0·01 ml., was run on a chromatogram. The starting line was about 1 inch from the lower edge. The solvent used was benzene, ethyl acetate and water, 16:84:50, the aqueous layer being the stationary phase. To the organic layer, the mobile phase, 1 to 2 per cent. of ethanol was added. This strength increases the R_F value sufficiently to bring the digilanid C completely out of the spot.

Two tanks were used during the investigation, a glass one 6 × 8 × 14 inches high, and a wooden one with glass windows, 11 × 11 × 17 inches high. The wooden tank, being larger, was more difficult to equilibrate and required a much greater volume of solvent for saturation. Complete saturation of the atmosphere in the tank with both phases is important for a clear separation of the digilanids. This was achieved by hanging strips of filter paper down the sides of the tank and dipping into both solvents. Whatman No. 1 paper was used throughout, placing 5 spots on a 6-inch wide paper and 7 spots on an 8-inch wide paper. The system was allowed to equilibrate overnight and then run usually for about $3\frac{1}{2}$ to 4 hours at 22° to 24° C.

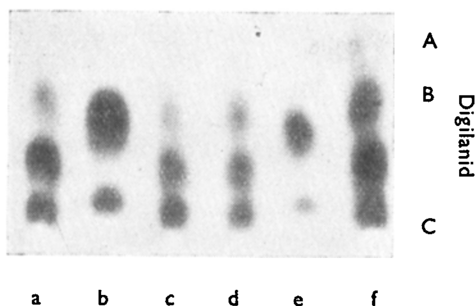

Fig. 1. The analysis of 2 plant samples by visual comparison from the photographic record.

a. 0·01 ml. of extract from sun-dried plant.
b. 5·0 μg. of digilanid B, 10·0 μg. of digilanid C } standards.
c. 0·005 ml. of extract from sun-dried plant.
d. 0·005 ml. of extract from plant dried at 50° C.
e. 2·5 μg. of digilanid B, 5·0 μg. of digilanid C } standards.
f. 0·01 ml. of extract from plant dried at 50° C.

After development, in which the ascending method was used, the chromatogram was allowed to dry in the air for at least 1 hour, and then sprayed with a mixture of chloroform and trichloracetic acid 2·5: 1. Approximately 15 to 20 g. of spray was used for each chromatogram, best results being obtained for digilanids B and C if the spray was freshly prepared. The chromatogram was immediately heated in a hot air oven for 10 minutes at 110° C. The digilanid B and C treated in this way yield a bright blue fluorescence in ultra-violet light. Digilanid A, however, appears as a yellow spot. It was observed that variations in temperature during heating appeared to lessen the intensity of the fluorescence, although the temperature itself was not critical. Heating for 5 to 10 minutes at temperatures ranging from 105° to 115° C. has been found satisfactory.

Known amounts of standard solutions of digilanids A, B and C are run on each chromatogram. The estimation of each glycoside in the plant sample is obtained by comparing the size and intensity of the unknown spot with that of the appropriate standard. Comparisons are made

directly from the chromatogram, and also by examination of photographs of the chromatograms made under ultra-violet light using the method described in the paper of Silberman and Thorp.[1]

The intensity of the fluorescence of digilanid B is much greater than that of an equivalent amount of digilanid C. Usually two concentrations of digilanids B and C were run on each chromatogram, 2·5 and 5 μg of digilanid B, and 5 and 10 μg of digilanid C. A preliminary investigation of each plant sample is necessary to ascertain the extent to which the extract must be diluted to contain glycosides within these limits. Above these limits the fluorescence is too strong to allow accurate comparison, and below the fluorescence is too weak. It was thought that traces of plant pigment might potentiate the fluorescence of the plant glycosides. This possibility was eliminated by adding known amounts of digilanids B and C to spots of plant extracts whose digilanid content had been previously estimated. The fluorescence produced compared well with that of the standard digilanids run concurrently.

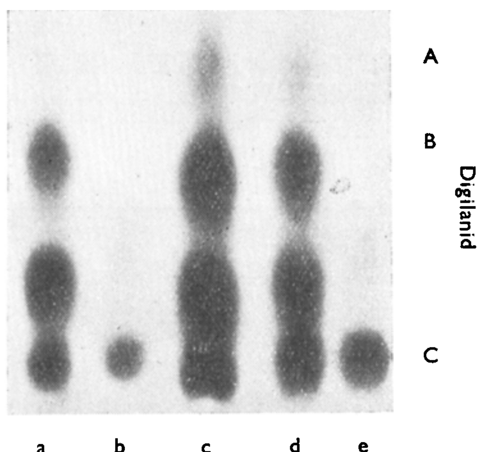

FIG. 2. Photographic record of a chromatogram showing the estimation of digilanid C in a plant sample.

a. 0·005 ml. of extract from plant dried at 100° C.
b. 5·0 μg. of digilanid C.
c. Plant sample corresponding to 0·01 ml. of the extract from the plant which was oven-dried at 100° C.
d. 0·005 ml. of the above extract and 5·0 μg. of digilanid C added.
e. 10·0 μg. of digilanid C.

Digilanid A could not be estimated quantitatively. A blue fluorescence is given by a substance in the plant extract which travels at the same rate at the yellow-fluorescing digilanid A standard. The plant extract and standards were run on a system of horizontal chromatography using benzene, ethyl acetate and *n*-butanol 12 : 78 : 5 on formamide-impregnated paper. This plant substance again travelled at the same rate as the digilanid A standard and therefore we have assumed that this plant substance is digilanid A, the blue colour perhaps being due to traces of plant pigment present.

In each plant extract a considerable amount of an unknown substance was found which appeared on the chromatogram between digilanids B and C. Digoxin, gitoxin and digitoxin travel to the solvent front in the ascending system, and as no other pure samples of digitalis glycosides are obtainable we were not able to investigate this unknown substance.

RESULTS

The results are summarised in Tables I, II and III.

TABLE I

No.	Extract	Moisture per cent.	Digilanid B		Digilanid C	
			μg. per 0·01 ml.	Percentage of anhydrous leaf	μg. per 0·01 ml.	Percentage of anhydrous leaf
1	Low temperature vacuum dried ..	6·00	6	0·27	24	1·065
2	Sun-dried	8·00	2 to 3	0·12	23 to 24	1·165
3	Oven at 100° C. ..	6·08	3	0·13	13 to 14	0·624
4	Oven at 70° C. ..	3·65	3	0·13	20 to 21	0·903
5	Oven at 50° C. ..	5·48	3 to 4	0·16	17 to 18	0·802

This table shows the number of mg. of digilanids B and C in 10 ml. (μg. in 0·01 ml.) of the extract, corresponding to approximately 2·3 g. of dried leaf. The digilanids B and C have been calculated as percentages of the anhydrous leaf. The apparent inconsistencies in the number of μg. per 0·01 ml. and the percentage of digilanids calculated on anhydrous leaf are due chiefly to the differences in weight of leaf taken for each extract.

TABLE II

Extract	Mean	Limits of error P = 0·95	Error per cent.
1	1·065	1·091–1·039	± 2·4
2	1·165	1·211–1·119	± 4
3	0'624	0.660–0·588	± 6
4	0'903	0·940–0·866	± 4
5	0'802	0·847–0·757	± 6

Table II shows the statistical analysis of the results obtained for digilanid C. The percentage error shown indicates only that error due to observation or variations in chromatographic technique; it does not include error due to sampling or extraction procedure.

TABLE III

No.	Extract	Digilanid A
1	Low temperature vacuum dried	10 x
2	Sun-dried	2·5 x
3	Oven at 100° C.	less than 1·25 x
4	Oven at 70° C.	5 x
5	Oven at 50° C.	6 x

Table III shows the digilanid A content of the plant extracts compared with the vacuum-dried extract. The arbitrary figure of 10 x was taken for the freeze-dried extract, as comparison with the digilanid A standard was not possible.

The figures shown in Table I represent the mean value for each extract, estimated independently by 3 observers from a series of chromatograms. Each extract was run approximately 12 times, excluding the preliminary investigation.

A statistical analysis of the results obtained for digilanid C was made and is shown in Table II. It indicates only the errors due to observation and variations in spraying and heating techniques in duplicate chromatograms.

As the fluorescence of the plant digilanid A could not be compared with that of the digilanid A standard, the plant extracts were compared with the vacuum-dried extract, and the results are shown in Table III.

DISCUSSION

The method of low temperature vacuum drying preserved the digilanids better than any other method. Of the three glycosides, digilanid A was the least stable, being almost completely decomposed by oven-drying at 100° C., and decomposed to a considerable extent by sun-drying. Oven-drying at 50° and 70° C. also resulted in some decomposition. Digilanid B was also considerably decomposed by all methods of drying, other than

low temperature vacuum drying. Oven-drying at 50° C. resulted in slightly less decomposition than oven-drying at 70° and 100° C. Sun-drying resulted in slightly more decomposition than any of the other methods. Digilanid C was apparently unaffected by sun-drying, and a subsequent experiment on a fresh batch of leaf confirmed this result. The digilanid C content in the sample dried at 50° C. appeared to be lower than in that dried at 70° C. Whilst the sample heated at 70° C. took 3 hours to dry, that heated at 50° C. took 7 hours, and the exposure to the lower temperature for a longer period may account for the decrease in digilanid C content. It has been assumed in these estimations that the standard digilanids are pure. However, on careful examination of the chromatograms, traces of impurities have been observed in the digilanid C standard. Therefore whilst the results are still comparative, the potency of the digilanid C in these extracts may be slightly overestimated.

Summary

1. A batch of leaves of *Digitalis lanata* was dried by 5 different methods.

2. The leaf samples were extracted and the digilanid B and C content estimated by paper chromatography and fluorescence photography. A comparative estimate indicating the decomposition of digilanid A was also made.

3. The digilanids were decomposed least in leaves which were dried at a low temperature *in vacuo*, although sun-drying was equally effective for the preservation of digilanid C.

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Reference

1. Silberman and Thorp, *J. Pharm. Pharmacol.*, 1953, 7, 438.