VEINLET TERMINATION NUMBER—SOME FURTHER OBSERVATIONS

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IN a previous communication[1] the present authors introduced the term veinlet termination number and described how it may be used to differentiate commonly used medicinal leaves in the whole or coarsely powdered condition. The present paper is an extension of the work to further species.

EXPERIMENTAL

Whole leaves of *Atropa belladonna* L. and *Digitalis purpurea* L. were examined by the method previously described. The ranges, means and standard deviations of veinlet termination number, vein islets per 1 sq. mm. and vein islets per 4 sq. mm. appear in Table I. The ranges, means and standard deviations of veinlet termination number for the coarse powders appear in Table II. The means for veinlet termination number obtained on coarse powders again show close agreement with those obtained on whole leaves. Thus there appears to be sufficient evidence to justify dispensing with the more laborious process of making determinations on whole leaves and for determining the veinlet termination number of any species on coarse powders only.

Accordingly, veinlet termination number was determined on 3 different samples of coarse powder of each of the species investigated in this and the previous paper, and also on *Atropa acuminata* Royle ex Lindley, *Hyoscyamus niger* L., and *Datura stramonium* L. The number of counts and the area of the field of view was varied in some instances to see if this

TABLE I

WHOLE LEAVES—RANGES AND MEANS OF CHARACTERS

Character	Species	Range	Mean	Standard deviation
Veinlet termination number.	*A. belladonna*	2—4·49—12·03—19	8·26	3·77
	D. purpurea	0—1·48— 5·18— 9	3·33	1·85
Vein islets, 1 sq. mm.	*A. belladonna*	3—5·13—15·73—29	10·43	5·30
	D. purpurea	1—2·75— 5·61— 9	4·18	1·43
Vein islets— (4 sq. mm.) 1 sq. mm.*	*A. belladonna*	3—3·89—13·92—22	8·90	5·02
	D. purpurea	1—2·06— 4·13— 6	3·09	1·04

* Vein islets determined on 4 sq. mm. and expressed as vein islets per 1 sq. mm.

TABLE II

COARSE POWDERS—RANGES AND MEANS OF VEINLET TERMINATION NUMBER

Species	Range	Mean	Standard deviation
A. belladonna ..	2—4·18—11·83—13	8·32	3·51
D. purpurea ..	0—0·64— 4·32— 6	2·48	1·84

affected the means. Sample No. 1 was used as a standard in each species, 50 counts being made and the range of the mean of 20 counts being calculated. Samples 2 and 3 were then estimated using 20 counts to ascertain that their mean fell within the calculated range. These results are shown in Table III.

TABLE III

VEINLET TERMINATION NUMBER—COARSE POWDERS, RANGES AND MEANS FOR 20 COUNTS

Species	Sample	Number of counts	Area of field sq. cm.	Range for 20 counts, P = 0·01	Mean	Standard deviation
E. truxillense	1	50	0·6943	23·1—32·3	27·68	8·02
	2	20	0·6943		27·14	
	3	20	0·6943		27·21	
E. coca ..	1	50	0·6943	16·8—21·0	18·87	3·64
	2	20	0·6943		20·09	
	3	20	0·6943		21·96	
C. acutifolia ..	1	50	0·6943	32·7—40·2	36·46	6·58
	2	20	0·1521		36·16	
	3	20	0·1521		33·52	
C. angustifolia	1	50	0·6943	25·9—32·8	29·35	5·94
	2	20	0·1925		29·53	
	3	20	0·1925		31·69	
A. belladonna	1	50	0·6943	6·3—10·3	8·32	3·51
	2	20	0·6943		7·56	
	3	20	0·6943		9·94	
A. acuminata ..	1	50	0·6943	1·4—3·5	2·48	1·84
	2	20	0·6943		2·16	
	3	20	0·6943		2·45	
D. purpurea ..	1	50	0·6943	2·5—4·2	3·37	1·47
	2	20	0·6943		3·53	
	3	20	0·6943		2·95	
H. niger ..	1	50	0·6943	12·4—19·0	15·7	5·73
	2	20	0·6943		14·19	
	3	20	0·6943		13·83	
D. stramonium	1	50	0·6943	12·7—20·1	16·36	6·42
	2	20	0·6943		17·07	
	3	20	0·6943		18·72	

TABLE IV

VEINLET TERMINATION NUMBER AND VEIN ISLETS PER 1 SQ. MM.—CORRELATION COEFFICIENTS

Species	Correlation coefficient	Standard error of correlation coefficient	Range of correlation coefficient	Correlation coefficient P = 0·01; N = 100
E. truxillense ..	0·2473	0·0939	0·1534—0·3412	
E. coca	0·2407	0·0942	0·1465—0·3349	
B. betulina ..	0·1844	0·0660	0·1184—0·2504	
B. crenulata ..	0·0668	0·0996	−0·0328—0·1664	
C. acutifolia ..	0·4376	0·0811	0·3565—0·5187	0·2540
C. angustifolia ..	0·1384	0·0808	0·0576—0·2192	
A. belladonna ..	0·7227	0·0478	0·6749—0·7705	
D. purpurea ..	0·3509	0·0877	0·2632—0·4386	

Correlation coefficients were calculated for all the species of whole leaves so far examined, showing the relationship between the number of veinlet terminations and the number of vein islets determined in the same area of 1 sq. mm., and between veinlet termination number and leaf area. These appear in Tables IV and V.

TABLE V

VEINLET TERMINATION NUMBER AND LEAF AREA—CORRELATION COEFFICIENTS

Species	Correlation coefficient	Standard error of correlation coefficient	Range of correlation coefficient	Correlation coefficient P = 0·01; N = 100
E. truxillense ..	−0·5134	0·1764	−0·6898 to −0·3370	0·2540
E. coca	−0·1471	0·2344	−0·3815 to +0·0873	
B. betulina ..	−0·2295	0·1134	−0·3429 to −0·1161	
B. crenulata ..	−0·0274	0·2378	−0·2652 to +0·2104	
C. acutifolia ..	−0·7823	0·0930	−0·8753 to −0·6893	
C. angustifolia ..	−0·2771	0·2212	−0·4983 to −0·0559	
D. purpurea ..	−0·0629	0·2386	−0·3015 to +0·1757	

DISCUSSION

Comparison of the means obtained on coarse powders (Table III) with those obtained on whole leaves (Table I; also Table III of the previous paper) show them to be practically identical. Similarly the means of 20 counts in samples 2 and 3 of each species (Table III) show close agreement, falling within the calculated range, the results being unaffected by different areas of fields of view. This further supports the claim that the character may be accurately determined on coarse powders of leaves, and shows the comparatively uniform distribution of veinlet termination number throughout the leaf surface.

The ranges of the means of 20 counts overlap in certain cases, i.e., *A. acuminata* and *D. purpurea*; *H. niger* and *D. stramonium*; *E. truxillense* and *C. acutifolia*, but the respective leaves in each of these pairs of species may readily be identified by other characters such as trichomes. Taken in conjunction with such other characters, veinlet termination number remains useful in these instances. Leaves, either whole or in coarse powder, of all the co-generic species examined in this and the previous paper may be differentiated by their veinlet termination number, with the exception of *B. betulina* and *B. crenulata.*

In the light of further evidence, the ranges of the correlation coefficients in Table IV show a tendency towards correlation in a positive direction between veinlet terminations and vein islets per 1 sq. mm. This indicates, generally, that as the number of veinlet terminations increases so also does the number of vein islets, or, in another form, that as the number of veinlet terminations increases the vein islet areas decrease. This phenomenon is rather surprising since, from a physiological point of view, it would be expected that as the vein islet areas increased the veinlet terminations would also increase. The degree of correlation varies somewhat however, and, with the exception of *A. belladonna* and *C. acutifolia*, is not of a high order.

The ranges of the correlation coefficients in Table V show a general tendency towards correlation in a negative direction between veinlet termination number and leaf area. In four species, *E. coca*, *B. betulina*, *B. crenulata* and *D. purpurea*, the coefficient is not significant, hence correlation within each of these species may be discounted. Only in the cases of *E. truxillense*, *C. acutifolia* and *C. angustifolia* does any significant correlation appear to exist. This indicates that, in these cases, the larger

the area of the leaf the smaller the veinlet termination number. In commercial samples, however, leaves of varying area are likely to be present in random amounts, hence the veinlet termination number of any particular sample is not likely to be affected to any great extent by leaf area.

The implications of the variations in these relationships are not clear, and an investigation on a much larger number of species would be necessary to obtain sufficient evidence for further examination and discussion. However, as the whole leaves used in this work were selected so as to represent as wide a range of size as possible, and as the means of each of the 3 different samples of coarse powder used in each species agree very closely with each other and with the mean for the whole leaves, for practical purposes these relationships appear to exert no influence on the value of veinlet termination number as a diagnostic character.

Veinlet termination number appears to possess certain marked advantages over other methods. No special apparatus is required, all counts being made using a microscope the area of the field of view of which is known. This area itself forms the reference area within which counts are made, and no squared grid in the eyepiece nor the use of a camera lucida is required as is the case for vein islet determinations. Palisade ratio, stomatal number and stomatal index necessitate the counting of fairly large numbers of comparatively small cells which lie adjacent to each other, and their determination is therefore somewhat laborious and fatiguing. In contrast, the determination of veinlet termination number entails the counting of comparatively small numbers of discrete characters which are fairly widely separated and easily observable. With a little practice the counting of 20 areas, as conducted above on coarse powders, may be completed in 15 minutes. Furthermore, it is immaterial which surface of the leaf lies uppermost.

The use of veinlet termination number, either alone or in conjunction with other histological characters, differentiates all of the species so far examined with the exception of *B. betulina* and *B. crenulata*. In the latter instance, vein islet number was found by Levin[2] to be unsuitable, and it is possible that in any genus which contains a large number of species such characters may prove of little value. In all genera so far examined which contain only a small number of species, these characters are of value. The veinlet terminations appear to be more uniformly distributed throughout the leaf surface than the vein islets, hence the former character would appear to be more suitable for the differentiation of leaves.

Although restricted in this work to commonly used medicinal leaves, veinlet termination number should be equally suitable to application to the wider botanical field.

SUMMARY

1. Veinlet termination numbers for further species of commonly used medicinal leaves, both whole and coarsely ground, are presented.

2. The relationship between veinlet termination number and the number of vein islets per 1 sq. mm. of leaf surface, and between veinlet termination number and leaf area are discussed.

3. The practical advantages of veinlet termination number are set out.
4. The ranges of the means of 20 counts (P = 0·01) for coarse powders of the following species are:—

A. belladonna ..	.. 6·30–10·34
A. acuminata ..	.. 1·42– 3·54
D. purpurea ..	.. 2·52– 4·22
H. niger ..	.. 12·40–19·00
D. stramonium ..	.. 12·66–20·06

References

1. Hall and Melville, *J. Pharm. Pharmacol.*, 1951, **3**, 934.
2. Levin, *Quart. J. Pharm. Pharmacol.*, 1929, **2**, 17.