The Value of Palisade Ratios in the Differentiation of Official Belladonna, Digitalis, Hyoscyamus and Stramonium Leaves

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INTRODUCTION

Zörnig and Weiss (1) in their anatomical study of the leaves of certain composite plants found that the average number of palisade cells beneath an upper epidermal cell was of diagnostic value. They also stated that the ratio of the number of palisade cells included within the area of any epidermal cell remained almost constant. Wallis and Dewar (2) later made a thorough study of the comparative anatomy of leaves belonging to several species of *Barosma*, and they also concluded "that variation in the palisade ratio is independent of the position of the leaf." Furthermore, they were the first to use the term "palisade ratio," stating in their paper that "the total number of palisade cells beneath four upper epidermal cells. . . . when divided by four, gave the recorded figure for the 'palisade ratio,' this being the term which we have adopted to express the average number of palisade cells beneath an upper epidermal cell." Wallis and Dewar also found that the palisade ratio was of value in the differentiation of certain other species of *Barosma* from the official *Barosma betulina* and that it was a useful diagnostic character. Markwell and Cross (3) utilized this diagnostic character as a means of detecting an adulterant of rubbed spearmint leaves.

Later, Dewar (4, 5, 6), working with various species of digitalis, showed that "there is no constant increase or decrease of the ratio in going from the base to the apex of the leaf." Wallis and Forsdike (7) confirmed this constancy of the palisade ratio within a species by their investigation on the leaves of *Atropa belladonna, Scopolia carniolica* and *Solanum nigrum;* and, in addition, they concluded that the palisade ratio does not change with the age of the leaf, the habitat or from year to year within any definite species.

Since the palisade ratio appears to be a fairly constant character and of diagnostic value, the authors thought that it may prove to be of important pharmacognostical value in the microscopical differentiation of the leaves of *Atropa belladonna* Linné, *Digitalis purpurea* Linné, *Hyoscyamus niger* Linné and *Datura stramonium* Linné by students.

EXPERIMENTAL

The experimental work consisted of three parts, (*a*) a study of 2–3 mm. squares cut from the apex: center and base of leaves obtained in 1939; (*b*) an examination of powdered (No. 60) four-year-old leaves obtained from stock; and (*c*) an attempt to identify each leaf, or leaves, in unknowns consisting

Table I.—Palisade Ratios of the Leaf Segments of the Four Species

Species	Segment of Leaf	No. of Leaves Examined	No. of Determinations	Average Palisade Ratio	Range of Palisade Ratio
Hyoscyamus niger	Center	2	14	4.6	3.25–5.0
	Base	2	14	5.4	4.25–7.0
	Apex	4	18	5.1	4.25–7.0
			46	(Av.) 5.0	3.25–7.0
Atropa belladonna	Center	3	18	5.9	4.0 –8.7
	Base	2	15	6.9	5.0 –8.5
	Apex	2	15	6.5	4.5 –8.2
			48	(Av.) 6.4	4.0 –8.7
Datura stramonium	Center	2	17	5.3	3.75–7.5
	Base	4	21	5.1	2.75–6.7
	Apex	2	16	5.0	3.5 –8.5
			54	(Av.) 5.1	2.75–8.5
Digitalis purpurea	Center	3	22	4.3	3.5 –5.2
	Base	2	19	3.9	2.0 –6.2
	Apex	2	17	4.0	3.0 –5.0
			58	(Av.) 4.1	2.0 –6.2

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of either an unadulterated powder or of mixtures of two or more species.

The clearing solution which was used throughout this work consisted of 50 Gm. of chloral hydrate dissolved in 20 cc. of distilled water. The 2–3 mm. squares and the powders were cleared by boiling in a test-tube on a water-bath for 15 minutes, the material being completely covered by the clearing solution. The material was then mounted in the clearing solution and examined under a magnification of 450×. In each determination the number of palisade cells under any four adjacent epidermal cells, when divided by four, represented the palisade ratio; and in no case was any palisade cell included where more than one-half of its area lay outside of the epidermal cells.

The results obtained from the study of the leaf segments of the four species are shown in Table I.

Table II.—Palisade Ratios of the Four Species of Powdered Leaves

Species	No. of Determinations	Average Palisade Ratio	Range of Palisade Ratio
Hyoscyamus niger	15	4.7	4.0 –5.2
Atropa belladonna	17	6.5	5.7 –9.5
Datura stramonium	18	5.0	4.0 –7.0
Digitalis purpurea	14	3.8	2.75–4.7

Table III.—Palisade Ratios of the Unknown Powdered Species

Unknown	Specimen Examined	No. of Determinations per Specimen	Average Palisade Ratio	Range of Palisade Ratio
No. 1 (digitalis)	*a*	6	3.75	3.25–40
	b	7	4.75	4.25–5.2
	c	6	4.75	4.0 –5.25
	d	5	5.75	5.5 –6.0
	e	7	4.30	4.0 –5.0
	f	7	3.80	3.5 –4.25
	g	8	4.75	4.0 –5.75
No. 2 (digitalis and hyoscyamus)	*a*	7	3.75	3.0 –4.5
	b	5	4.30	4.0 –4.75
	c	4	3.74	3.25–4.0
	d	5	4.25	3.75–4.5
	e	5	3.50	2.5 –3.75
	f	5	4.25	3.75–5.0
	g	5	4.3	4.0 –4.75
No. 3 (digitalis, hyoscyamus, stramonium and belladonna)	*a*	5	7.0	5.75–8.25
	b	5	4.5	4.0 –5.0
	c	5	3.6	3.25–4.5
	d	6	5.75	4.5 –7.0
	e	5	3.6	2.5 –4.25
	f	5	7.25	6.25–8.25
	g	5	6.5	5.25–7.75
	h	5	4.25	3.5 –5.5
	i	5	4.2	3.5 –5.0
	j	5	4.8	4.0 –5.25
	k	5	5.4	4.25–6.0

From a study of Table I, it is readily seen that the average palisade ratio of any leaf does not differ sufficiently from those of the other species to be of value in differentiating all of the four species studied. However, it appears to be possible to distinguish belladonna leaves from the remaining three species because of their somewhat higher palisade ratio. This has been successfully carried out by the authors.

The results of the study of the powdered leaves in Table II show that the average palisade ratios of the powdered belladonna, digitalis, hyoscyamus and stramonium leaves are of little practical value in the differentiation of the four powders—with the possible exception of belladonna leaves.

In order to confirm the results obtained in Table II, unknowns shown in Table III were studied.

From the results in Table III, it is shown that the palisade ratios cannot be utilized to distinguish between digitalis, hyoscyamus and stramonium leaves. However, as seen in Unknown No. 3, it is possible to differentiate the belladonna leaf as it has a higher average palisade ratio (6.5–7.25) when compared with the remaining species.

CONCLUSIONS

1. A study has been made of the palisade ratios of the official leaves of belladonna, digitalis, hyoscyamus and stramonium.

2. The palisade ratios are of practically no value in the differentiation of the official hyoscyamus, digitalis and stramonium leaves.

3. It is possible to use the palisade ratio of the official belladonna leaves as a means of differentiating them from hyoscyamus, digitalis and stramonium leaves.

REFERENCES

(1) Zörnig and Weiss, *Arch. Pharm. Berl.*, 263 (1925), 462.

(2) Wallis and Dewar, *Quart. J. Pharm. Pharmacol.*, 6 (1933), 347.

(3) Markwell and Cross, *Analyst*, 60 (1935), 784.

(4) Dewar, *Quart. J. Pharm. Pharmacol.*, 6 (1933), 448.

(5) Dewar, *Ibid.*, 7 (1934), 15.

(6) Dewar, *Ibid.*, 7 (1934), 342.

(7) Wallis and Forsdike, *Ibid.*, 11 (1938), 700–708.

The Netherlands has honored the following scientists by portraying them on postage stamps: Antonj van Leeuwenhoek, the first microbe hunter; Hermann Boerhaave, founder of the modern clinic; Frans Cornelis Donders, physiologist and ophthalmologist.