

Short Communication

Petunia violacea: hallucinogen or not?

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A report in an Ecuadorian anthropological monograph that *Petunia violacea* was used as a hallucinogen by some native South American people under the name *Shanin* (Alvear, 1971) stimulated interest in the ethnobotanical literature (Schultes, 1975). This was particularly interesting because the species is a member of the alkaloid-rich Solanaceae family. No reports of its containing alkaloids have been published to date (Raffauf, 1970). We have unsuccessfully attempted to isolate an alkaloid from this plant grown in the greenhouse, and recently interviewed the author of the original report. This communication will summarize the results of the laboratory work and the interview.

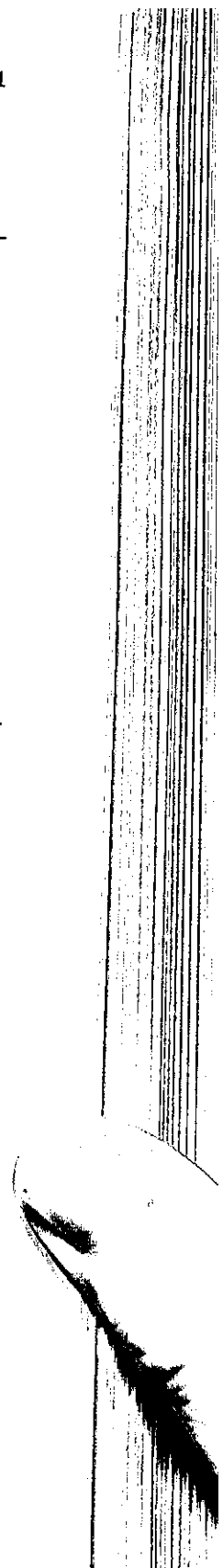
Experimental

Plants

The seeds of *Petunia violacea* were purchased from J. L. Hudson, Seedsman, P. O. Box 1058, Redwood City, California. The plants were cultivated in a greenhouse in non-sterile soil under natural lighting conditions (no growth lights were used). The seeds were broadcast in 30 × 100 cm flats and transplanted to 10 cm diameter pots at about six weeks. The plants were fertilized twice monthly with Peterson's 20:20:20 agricultural grade fertilizer. The greenhouse was fumigated once with nicotine sulfate for white flies. The plants took about a week and a half to germinate and appear, a month and a half to reach 3 cm (transplanting size) and roughly two and a half months to flower.

Methods

Two alkaloid extraction and separation methods were employed to determine whether or not *Petunia violacea* contained any alkaloids.



Extraction and separation I

The first procedure involved homogenizing fresh plants with enough 95% ethanol to solvate the homogenate in a commercial size Waring Blendor. This homogenate was heated over steam for about half an hour and then filtered with a Buchner funnel using Whatman No. 3 paper. The filtrate was acidified with an equal volume of 0.2 M HCl and partitioned against hexane. The procedure was repeated five times in a separatory funnel with roughly one third the total ethanol-acid (aqueous) volume of fresh hexane each time. The aqueous layer was then made basic by adding sufficient 2 M NaOH to neutralize the 0.1 M HCl and bring the aqueous phase to 0.1 M OH⁻. This was partitioned against chloroform five times with one third the volume of basic layer. The chloroform extractions were condensed and the chloroform was evaporated with a Buchi rotovapor evaporator under reduced pressure. Enough 95% ethanol was added to visibly solvate the residue. Spinach coated with known quantities of quinidine was extracted using this method and good yields were obtained.

Extraction and separation II

The second procedure was a slightly modified extraction method described by Farnsworth and Euler (1962). A Soxhlet extractor with cellulose thimbles was used in lieu of the reflux apparatus and the sample was extracted until clear rather than for a specified amount of time. A known weight of dried plant material was moistened with 8 M NH₄OH and dried on a steam-bath. This was then extracted in the Soxhlet with seven times its weight in milliliters of chloroform. The extract was evaporated to dryness and solvated in 95% ethanol. This was called fraction 1. Fraction 2 was obtained by extracting the air-dried chloroform-exhausted marc with a volume of ethanol containing 0.5% HCl (w/v) equal to the volume of chloroform. This was reduced to dryness and resuspended in ethanol. Good yields were obtained with the spinach control.

Alkaloid detection

All alkaloid detection was done using the modified Dragendorff's reagent suggested by Rifauff (1962). Small volumes of sample were quantitatively spotted with calibrated micropipets onto Eastman 13181 silica gel thin-layer chromatograms. These plates were developed with the solvent system methanol-NH₄OH (99:1) (Jackson and Moss, 1969). The chromatograms were then sprayed with the modified Dragendorff's reagent. Quantitative spotting allowed calculation of the plant weight corresponding to each spot (see Tables 1 and 2). All chromatograms were developed with an atropine standard to insure that the detection system was working properly.

Results and discussion

The first part of the analysis was an attempt to discover if and when *Petunia violacea* reached a peak alkaloid production. Groups of about

TABLE 1

Extraction of whole plants at various stages of development using extraction and separation method I

Developmental stage (days)	Total wet weight (g)	Volume of extract (ml)	Volume spotted (μ l)	Weight ^a represented (mg)	Reaction with Dragendorff's reagent
Seeds	—	—	—	—	negative
44 d	30	5	10	60	negative
49 d	—	175	15	—	negative
59 d	—	175	20	—	negative
66 d	361	215	20	34	negative
72 d ^b	156	815	30	5	negative
80 d ^c	209	775	30	6	negative

^aWeight of wet plant mass represented by volume spotted.

^bFifty per cent flowered.

^cOne hundred per cent flowered.

TABLE 2

Extraction of flowers, stems and roots using extraction and separation method II

Plant part	Dry weight extracted (g)	Fraction 1 ^a		Fraction 2 ^b	
		Weight ^c represented (mg)	Reaction with Dragendorff's reagent	Weight ^c represented (mg)	Reaction with Dragendorff's reagent
Roots	5	30	negative	10	negative
Stems	2	30	negative	10	negative
Flowers	4.3	30	negative	10	negative

Fraction 1 was the first extraction done with chloroform.

Fraction 2 was the second extraction done with acidified ethanol.

Dry weight of the *Petunia* extracted represented by spot.

Twenty plants were harvested at various stages of development. These plants were then homogenized in enough ethanol to solvate the marc and extracted using extraction method I. Table 1 summarizes these results.

The second part of the analysis was an extraction of the flowers, stems and roots separately using extraction method II. This was performed on frozen plants which had flowered. These results are outlined in Table 2.

We were unable to detect alkaloids with the Dragendorff's reagent at any stage in the plant's development or in any portion of the mature plant grown in the greenhouse. However, not all hallucinogens are alkaloids, not all alkaloids will react with Dragendorff's reagent, nor would all alkaloids have been extracted with the extraction methods employed. No absolute conclusion can be reached on the basis of these data; but if there is a hallucinogen in *Petunia violacea*, it is either non-alkaloidal or an atypical alkaloid,

or even an alkaloid produced under very specific conditions not reproduced in this study.

An interesting sidelight to this issue is that, in a survey of 256 species of plants collected in Iowa, *Petunia hybrida* was shown to be the most powerful inhibitor of cholinesterase (Orgel, 1963), an enzyme responsible for the breakdown of the neurotransmitter acetylcholine found in the central nervous system. Normally inhibition of this enzyme does not result in hallucinations, but it has profound physiological consequences.

In an effort to follow up the original report (Alvear, 1971) the author, Obispo Alvear, was interviewed in his home in Ibarra, Ecuador. The meeting took place on July 30, 1979. During the course of our discussion, he said that he had found the information about *Shanin*, *Petunia violacea*, in a book on Argentine folklore, but he could not remember the title or author of the book.

Conclusion

In summary, the interest in *Petunia* as a possible source of hallucinogen appears to be unwarranted. Preliminary investigations show the plant to contain no alkaloids detectable by Dragendorff's reagent, and the original reference which stimulated the interest could not be substantiated.

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