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Alkaloids from the seed, calyx, and corolla of *Erythrina americana* Miller and *Erythrina coralloides* A.DC.

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Citation: Sánchez-Herrera, S. G., Soto-Hernández, R. M., Garín-Aguilar, M. E., Vibrans-Lidermann, H., García-Mateos, M. del R., & Martínez-Vázquez, M. (2021). Alkaloids from the seed, calyx, and corolla of *Erythrina americana* Miller and *Erythrina coralloides* A.DC. *Agro Productividad*. https://doi.org/10.32854/agrop.v14i12.2025

Editor in Chief: Dr. Jorge Cadena Iñiguez

Received: May 4, 2021.
Accepted: December 12, 2021.
Published on-line: December 30, 2021.

Agro Productividad, 14(12). December. 2021. pp: 153-157.

ABSTRACT

Objective: To identify the main alkaloids present in the seeds, calyx, and corolla of *E. americana* and *E. coralloides* (Fabaceae) using HPLC-MS.

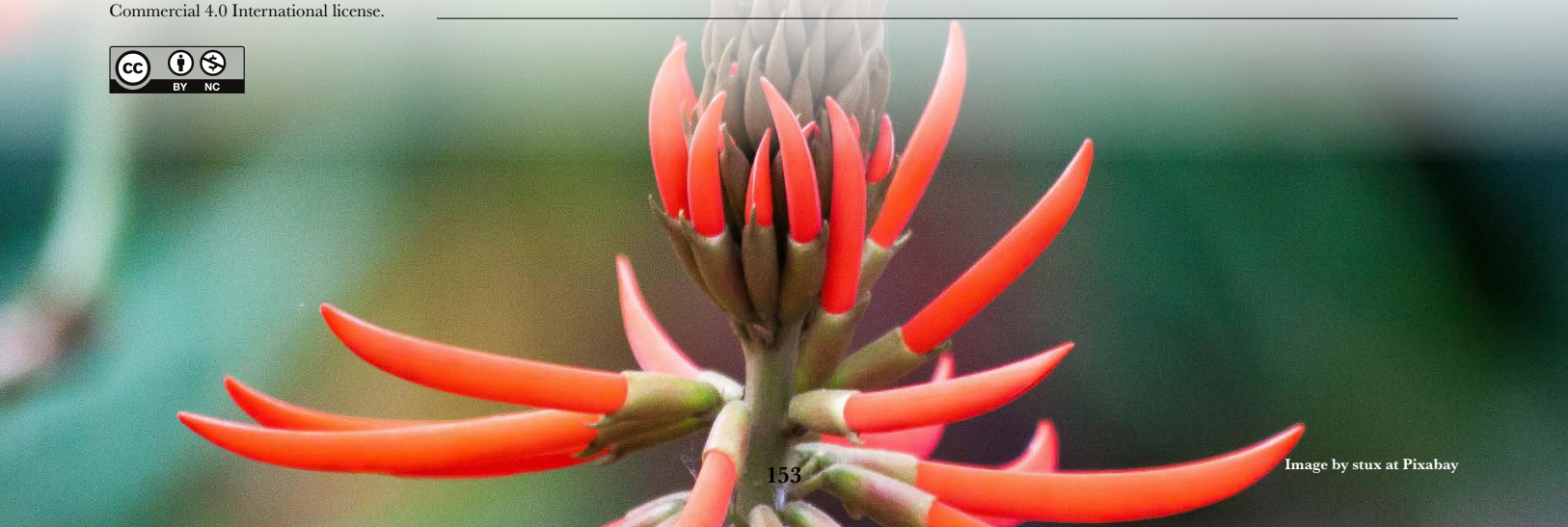
Design/methodology/approach: The seeds, calyx, and corolla of both species were separated; the crude alkaloid extracts were prepared according to the method described by Games *et al.* (1974). The crude alkaloid extracts were analyzed using a high-performance liquid chromatograph interfaced to a quadrupole ion trap mass spectrometer.

Results: The lactonic alkaloids -α-erythroidine and β-erythroidine were the main alkaloids found in *E. americana*, whereas the presence of erythrinine and 8-oxo erythraline is typical of *E. coralloides*. These compounds can be used to distinguish both species.

Limitations on study/implications: The use of the HPLC-MS technique allowed the detection of a large numbers of alkaloidal structures in different parts of the plants; however, this technique is not available in any laboratory.

Findings/conclusions: A large number of erythrinane-like alkaloids were found in seed, calyx, and corolla. The use of seeds and flowers in the preparation of diverse traditional dishes can cause soothing and sedating effects in consumers, as a result of the presence of lactonic alkaloids. The HPLC-MS method allowed the detection of alkaloidal structures in flowers and seeds that had not been identified in previous studies about this species.

Keywords: Alkaloids; chemotaxonomy; liquid chromatography-mass spectrometry.



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INTRODUCTION

The search for new active ingredients of natural origin based on the traditional use of certain plants is a well-documented fact. The genus *Erythrina* (Fabaceae) is one of the many Mexican plants used in traditional medicine. Several species of this genus are distributed throughout the world and a large number of these have been found in Mexico. In recent years the genus *Erythrina* was the subject of various researches throughout the world. Different aspects of the presence of flavonoids (Kumar *et al.*, 2013; Tanaka *et al.*, 2002; Chukwujekwu *et al.*, 2011), lectins (Turton *et al.*, 2004), or alkaloids (Garín-Aguilar *et al.*, 2005) have been studied. In particular, the following aspects of alkaloids have been the subject of study: structural analysis, biosynthesis (Maier *et al.*, 1999), pharmacology (Dantas *et al.*, 2004, Setti-Perdigão *et al.*, 2013, Saidu *et al.*, 2000), or chemotaxonomy (Yoshida *et al.*, 2009).

Previous studies have reported the presence of various alkaloids, but especially those that have an effect on the peripheral nervous system. These alkaloids include erysodine, an alkaloid isolated from the seeds of the genus *Erythrina*, which is an antagonist of neuronal nicotinic receptors $\alpha 4\beta 2$. This alkaloid was used to determine the role that these receptors play in memory consolidation (Garín-Aguilar *et al.*, 2009).

Two species of *Erythrin*, *E. americana* and *E. coralloides*, were studied to determine the alkaloid profile of their respective seeds and flowers (calyx and corolla), and therefore to compare their chemotaxonomic identity (Krukoff and Barneby, 1973).

MATERIALS AND METHODS

Seeds and flowers of *E. coralloides* and *E. americana* were collected in Texcoco and in Colorines, respectively; both towns are located in the State of Mexico. Specimens of each of the species were deposited in the CHAPA herbarium (Postgrado en Botánica, Colegio de Posgraduados). The authenticity of the specimens was certified by Mr. Joaquin Becerra Zabala from the CHAPA herbarium.

The flowers of both species were separated into calyx and corolla, and dehydrated in a Felisa FE-291 drying oven.

Crude extracts were prepared according to the method described by Games *et al.* (1974) for the extraction of alkaloids. Each plant material was extracted with hexane in a Soxhlet equipment for 48 hours. Vacuum evaporation was applied to the solvent and the residue was washed with 2% H_2SO_4; afterwards, the pH of the acidic phase was adjusted to 8 using $NaHCO_3$. Finally, extractions were carried out with CH_2Cl_2 (3x100), obtaining the hexane fraction of "free" alkaloids. The plant material was once more extracted with CH_3OH in the Soxhlet, the solvent was vacuum evaporated, and the residue was washed with 2% H_2SO_4. The acidic solution was extracted with CH_2Cl_2 (3x100) to remove traces of fat, and later adjusted to a pH of 8 with $NaHCO_3$, obtaining the methanolic fraction of "free" alkaloids.

The aqueous phase was acidified to a pH of 2 with HCl and refluxed at 60 °C for 3 hours to hydrolyze the esterified alkaloids. Then, the solution was adjusted to a pH of 8 and extracted with CH_2Cl_2 (3x100) to obtain the fraction of "released" alkaloids.

The alkaloid extracts were analyzed using the liquid chromatography-mass spectrometry (HPLC-MS) technique. For this purpose, the crude extract was analyzed using a Waters 600 high-performance liquid chromatograph, connected with a Finnigan LCQ mass spectrometer, using an atmospheric pressure chemical ionization (APCI) source. The alkaloids were separated by means of a 250 mm × 4.6 mm Superco Discovery C-18 (5um) column, at a 1 ml/min speed, using a linear mobile-phase with a programmed gradient of three solvents: A (0.1% ammonium acetate), B (Methanol), and C (acetonitrile) as follows: t=0 min, 75% A, 20% B, 5% C; t=10 min, 50% A, 45% B, 5% C; t=15 min, 50% A, 45% B, 5% C. The APCI source was vaporized at 450 °C; the nitrogen gas pressure was 80 and 20 psi respectively; and the capillary temperature was 150 °C. The alkaloids were confirmed and identified by means of mass spectrometry (MS), comparing the spectra with the authentic samples (standards) and with spectral libraries.

RESULTS AND DISCUSSION

Table 1 shows the crude extract amounts of *E. americana* and *E. coralloides*, expressed as mg of crude alkaloids/100 g of dry weight of each plant material. In the case of *E. americana*, the highest amount of crude extract was found in the seeds of the fraction of free alkaloids in methanol, while for *E. coralloides*, the highest fraction was found in the calyx of the fraction of free alkaloids in methanol.

The tissue analysis shows a high concentration of free alkaloids in CH_3OH and released alkaloids for both species; the concentration was lower for the fraction of free alkaloids in hexane. The hexane fraction was included because other studies (Hargreaves *et al.*, 1974; Sotelo *et al.*, 1993) have shown that it contains significant amounts of alkaloids. Some authors report that alkaloids represent 0.05 to 0.1% of the total plant of some species and that these substances were located in seeds, roots, bark, leaves, and flowers (Sotelo *et al.*, 1993; Dyke and Quessy, 1981); however, the concentration and type of alkaloids present in calyx and corolla have not been mentioned in any reports.

Table 2 shows that the erysodine (1), erythrinine (5), α-erythroidine (8), and β-erythroidine (9) alkaloids are distributed in both species; such alkaloids are characteristic of the species of the genus *Erythrina* found in the American continent. The erysovine (2) and erysopine (3) alkaloids were found only in the seeds of *E. coralloides*.

The erythraline (4), erythrinine (5), crystamidine (6), α-erythroidine (8), β-erythroidine (9), and 8-oxo-α-erythroidine (10) alkaloids were found in the *E. americana* calyx. The erythrinine (5), α-erythroidine (8), β-erythroidine (9), and 8-oxo-α-erythroidine (10) alkaloids were found in the corolla.

Table 1. *E. americana* and *E. coralloides* crude alkaloid fractions (mg/100 g dry tissue).

Specie	***E. americana* (mg)**			***E. coralloides* (mg)**		
Fraction	Calyx	Corolla	Seeds	Calyx	Corolla	Seeds
Free alkaloids in hexane	26	25	28	45	3.2	24.6
Free alkaloids in methanol	167.9	88	662	520	201	489
Liberatedalkaloid fraction	388	73	95	324	103.4	74

Table 2. Content and distribution of total alkaloids in *E. americana* and *E. coralloides*.

Specie	***E. americana***			***E. coralloides***		
Alkaloids	Calyx	Corolla	Seeds	Calyx	Corolla	Seeds
1.- Erysodine		*	*	*	*	*
2.- Erysovine						*
3.- Erysopine						*
4.- Erytraline	*		*	*	*	
5.- Erytrinine	*	*	*	*	*	*
6.- Cristamidine	*		*	*	*	*
7.- 8-oxo-erytraline			*	*	*	*
8.- α-erythroidine	*	*	*	*	*	
9.- β-erythroidine	*	*	*	*	*	
10.- 8-oxo-α-erythroidine	*	*	*	*		
11.- MW 289			*			
12.- MW 465					*	

The same alkaloids were found in *E. coralloides* calyx and corolla, with the exception of 8-oxo-α-erythroidine (10). The 8-oxo-erythraline (7) alkaloid was detected in the seeds of *E. americana* and in all the tissues of *E. coralloides*.

The structures found by means of this technique were diene (1-7) and lactonic (8-10) alkaloids. According to the spectra produced by HPLC-MS, an alkaloid with a PM of 289 (11) was observed in the seeds of *E. americana*, while an alkaloid with a PM of 465 (12) was found in the corolla of *E. coralloides*; these alkaloids had not been previously reported in these species. The HPLC-MS combination has the advantage of quickly and accurately determining the presence of alkaloids in a sample from a few milligrams. Likewise, the use of HPLC-MS technique allows the detection of a large number of alkaloidal structures in the calyx and corolla of the genus *Erythrina*. The use of their flowers in the preparation of traditional dishes benefits from this fact.

CONCLUSIONS

A large number of erythrinane alkaloids were found in seeds, calyx, and corolla. The use of seeds and flowers in the preparation of diverse traditional dishes can cause soothing and sedating effects in consumers, as a result of the presence of lactonic alkaloids. The HPLC-MS technique allowed the detection of alkaloids in the flowers and seeds of these species that had not been previously identified in other studies.

ACKNOWLEDGEMENTS

The authors would like to thank CONACyT for the grant awarded during this research.

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