Puente Biológico (2019) 9: 1-13

ARTICULO ORIGINAL

Antibacterial activity of Eudesmanolides isolated from *Pluchea carolinensis* (Jacq.) G. Don

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Recibido: 02.12.2018 / Revisado: 13.08.2018 / Aceptado: 8.10.2018.

ABSTRACT

A phytochemical screening of the ethanolic and aqueous extracts of *Pluchea carolinensis* (Jacq.) G. Don leaves and stem revealed the presence of alkaloids, tannins, saponins and terpenes in the leave extracts, meanwhile, tannins, terpenes, steroids and saponins were detected in the ethanolic stem one. To the ethanolic and aqueous extracts, the antimicrobial activity was evaluated, showing the ethanolic dry leave extract the highest inhibition zone against *Acinetobacter* and *Staphylococcus spp*. Two known compounds: 4*α*-hydroxy-3*β*-angeloyloxy-7, 11-dehydroeudesman-3-one (Cuauthemone ester, **1**) and 4*α*-acetoxy-3*β*-angeloyloxy-7, 11-dehydroeudesman-3-one (Cuauthemone diester, **2**) was reported for the first time for *P. carolinensis* and was isolated from the ethanolic dry leave and stem extract. This is the first report of *Pluchea carolinensis* studies in Panamá.

RESUMEN

El análisis fitoquímico de los extractos etanólicos y acuosos de las hojas y tallos de *Pluchea carolinensis* (Jacq.) G. Don revelaron la presencia de alcaloides, taninos, saponinas, glicósidos cardiotónicos y terpenos en los extractos. Para los extractos etanólicos y acuosos, se evaluó la actividad antimicrobiana, mostrando que el extracto etanólico de hojas secas tuvo la zona de inhibición más alta contra *Acinetobacter* y *Staphylococcus spp*. Dos compuestos reportados previamente para otras especies de *Pluchea*, Cuauthemone éster (1) y Cuauthemone diéster (2) se aislaron del extracto etanólico de las partes aéreas de la planta. Este es el primer reporte de estudios de *Pluchea carolinensis* en Panamá.

KEY WORDS; PALABRAS CLAVES

Pluchea carolinensis Jacq, eudesmanolides, antimicrobial activity, Cuauthemone; Pluchea carolinensis Jacq, eudesmanólidos, actividad antimicrobiana, Cuauthemona.

INTRODUCTION

Eudesmanolides are a special class of sesquiterpene lactones, often presented in several genus of the Asteraceae family (Wu, et. al, 2006). *Pluchea carolinensis* Jacq known usually as Salvia de playa (cure for all), is an aromatic herb of 2-3 meters high, from the Asteraceae family, distributed from the Caribbean island, through Mexico to South America. In Panama is widely located from Colón to Chiriquí in low and middlelands. This specie is used in traditional medicine for the treatment of headaches, allergies, fever and muscular pain. Previous reports revealed the isolation of sesquiterpene eudesmane type (Abbas, et. al, 2013), guaianolides, flavonoids and saponins (Aggarwal & Goyal, 2013) from the *Pluchea* genus. The genus comprises 80 species distributed mainly in North and South America, Africa, Asia and Australia (Anderberg, 1994).

EXPERIMENTAL SECTION

PLANT MATERIAL

The aerial parts (leaves and stems) of *Pluchea carolinensis* (Jacq.) G. Don were recollected from Sortova, distrit of Bugaba, Chiriqui Province, Panama, in november 2017 at an altitude of 310 msnm and was identified by Prof. Rafael Rincón. The voucher specimen (N°C-006-04-2017) was deposited in the Autonomous University of Chiriqui Herbarium.

Preparation of *Pluchea carolinensis* crude extracts

Fresh aerial parts (leaves and stem) of *P. carolinensis* were analyzed separately. First, stems (233.1 g, wet weight) and leaves (84.25 g, wet weight) were fine cutted and carried to dryness in the oven at 35° C for three days. After that, both samples (30.0 g and 15.0 g, dry weight, respectively) were extracted with ethanol (95% v/v) and water at room temperature for 7 days, respectively. Subsequently, the extracts were filtrated, concentrated and stored in a freezer at -15° C until further analysis.

The ethanolic dry leaves (2.21 g) and stem (1.68 g) extracts were analyzed by phytochemical screening to determinate its secondary metabolite composition. All the crude extracts were analyzed by infrared spectroscopy in a FT Shimadzu-IRAffinity-1 in solid phase using KBr

tablets. Further purification of the extract (s) with the best chemical profile was realized by column chromatography using Merck silica gel 60, 230-400 mesh. Merck silica gel 60 PF254 plates were used for preparative TLC. Elucidation of the compounds was made by NMR. Spectra were recorded in $CDCl_3$ using the signals of residual non-deuterated solvents as internal reference on a Bruker Avance II 500 MHz spectrometer operating at 500.13 MHz for ^{1}H and 125.13 MHz for ^{13}C. All 2D NMR experiments (COSY, HSQC, DEPT-HSQC, HMBC, NOESY) were performed using standard pulse sequences.

Antimicrobial analysis

The antimicrobial sensibility of all extracts was tested against *Acinetobacter spp* and *Staphylococcus spp*. applying the modified agar diffusion method using Agar-Mueller Hinton (Aquiahuatl et al., 2012). *Staphylococcus spp* was cultivated in Mueller-Hinton medium, meanwhile, *Acinetobacter spp*. strain was put in MacConkey agar, a special medium for gram negative bacteria and preserved until inoculation. The extracts (3 mL) were prepared at concentrations of 10, 20, 40, 60, 80 and 100% and carried to a 1 mL final volume using distilled water as solvent. The modified method of agar wells consists of sowing the inoculum on the surface of the selected agar, followed by the wells with the help of a straw with a diameter of 6 mm. In each well are deposited 10 to 25 μL of the extract to evaluate, considering negative and samples extracts in triplicate. Subsequently, they are incubated for 24 hours at 35 ± 2 ° C. After the incubation period, the inhibition zones are measured.

Inquiry

A total of a hundred habitants with average age of 25 to 65 years old, of the Sortova zone (Bugaba Chiriqui) were selected to obtain information about the traditional uses and administration form of *Pluchea carolinensis*. Every enquire consist in a list of nine question with multiple choice. The data collected was processed and analyzed using Excel program, Microsost Office 365.

RESULTS

The phytochemical analysis of the five extracts studied showed the presence of alkaloids, terpenes, steroids, tannins and phenolic compounds as major metabolites. Also, saponins and carbohydrates were identified.

The dry leaves ethanolic extract was selected for further purification. The extract was passed through a chromatographic column with silica, using cyclohexane: ethyl acetate (1:1) as eluent. Twenty six fractions were collected; fraction 13 was identified as Cuauthemone ester (1) and fraction 16 as Cuauthemone diester (2) by NMR analysis (Figure 1). Compound 1 is a white solid, (9.5 mg) and compound 2 (11.4 mg) is a pale yellow solid. The spectroscopic data of both compounds was compared with the values in literature (Arya & Patni, 2013) and are presented in Table 1 and 2. Further analysis of the ethanolic stem sample showed the same chemistry profile, thus this extract was purified too, using the same methodology, given the above mentioned compounds, and two other non-identified sesquiterpene derivatives.

All the extracts were tested against *gram* negative bacteria (*Acinetobacter* spp.) and *gram* positive (*Staphylococcus* spp.) inhibition *in vitro.* The average values result of all the extract for the antimicrobial analysis are shown in Table 3. The inhibition halo diameter for the ethanolic extracts range from 4 to 12 mm and the aqueous extract no presented inhibition against the bacteria strains. The ethanolic extracts of leaves, dried leaves and stems, presented inhibition halos against the bacterial strains under study at concentrations of 100, 80 and 60% respectively, which indicates a bactericidal response. In the case of the ethanolic extract of stems, an effect was observed at 40% against both microorganisms; the dried leaf extract showed activity at 40% only against *Staphylococcus spp*. No inhibition zone was observed for the negative control.

Finally, the inquiry to 100 persons of the comunnity of Sortova about the traditional uses of *Pluchea carolinensis* (common named Salvia) showed that the plant is mostly used as pain killer (22%) and anti-inflammatory agent (66.7%). Only the 26.7 % of the surveyed used the plant frequently and 44% have used it occasionally. The 90% of the people used the leaves of the plant, via oral administration (infusion). The infusion is prepared using three to four leaves in boiling water for a couple of minutes. **(Figure 1)**

DISCUSSION

From *P. carolinensis,* five extracts were prepared, using fresh and dry leaves and stem, been the dry leave ethanolic extract, the one with the best yield (14.43%). The phytochemical screening allowed to identified alkaloids, phenolic compounds, steroids, terpenes, cardiotonic glycosides and flavonoids as the major secondary metabolites, been the fresh leaves ethanolic extract the one with the most diverse composition. There is no previous report of phytochemical results from *P. carolinensis* aqueous stem and leaves extracts, and we decided to evaluate it since the infusion is one of the most usual forms of administration and use of this plant.

Cowan, 1999 and Gyawali, et. al, 2013 reported the antimicrobial activity of phenolic compounds and flavonoids isolated from *Pluchea* genus. Aggarwal & Goyal, 2013 identified flavonoids, triterpene steroids and sesquiterpenes with eudesmane skeleton as the principal compounds in *Pluchea cass.* This data is in agreement with the results we found. Gunawan et. al, 2015, reported the presence of cardiotonic glycosides in the aqueous leaves extract of *P. indica*, and, they were detected in the ethanolic stem extract of *P. lanceolata* (Arya & Patni, 2013). In this study they were detected in both stem and leaves extracts, been the aqueous extracts the richess one.

It was possible to determine that the major compounds present in the leaves and stem of the species *P. carolinensis* corresponded to sesquiterpenes derivative of modified eudesmane skeleton known as ester of cuauthemone and diester of cuauthemone (see figure 1). These compounds have not been reported for the species under study; however, they have been isolated from some species belonging to the genus Pluchea, such as *Pluchea odorata* (Atanasov et al., 2015; Torres-Valencia et al., 2003).

It can be seen in table 3 that there was no inhibition in the growth of the strains at the evaluated concentrations of the aqueous extracts of *P. carolinensis* and neither inhibition is observed in the negative control. The results obtained are like those exposed by González, Payo and Perera (2006) for *Staphylococcus spp.* The best results were obtained for the ethanolic dry leave and stem extracts, what could be related to the Eudesmanolide compounds. For example, (Talbi, et. al 2015), reported the activity of the eudesmane compounds, taurine, erivanin and herbalbin against

strain of *Escherichia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 9144), *Streptococcus faecalis* (ATCC 10541), *Pseudomonas aeruginosa* (IPP 10536).

The ethanolic and hydro-ethanolic extracts of *P. carolinensis* and *P. odorata* displayed antifungal activity on Candida or Trichophyton spp. ($200 \leq MIC100 \leq 400$ mg/ml), and the hexanic extract of P. *carolinensis* showed a low anti-Pneumocystis activity (MIC100= 100 mg/ml). The identification of some sesquiterpenes (eudesmane and cuauthemone structure), phenolic compounds (flavonols, phenolic acids) and triterpenes has been reported (Ahmed et al., 1998; Arriaga and Borges-Del-Castillo, 1983; Arriaga and Borges-Del Castillo, 1985).

Table 1. NMR spectroscopic data set for compound 1.

Position	^{1}H (ppm)	multiplicity	^{13}C (ppm)	HMBC	COSY
1	1.36	2H, d	14.1	47.2 (w), 59.8, 78.5 (w)	3.09
2	0.96	3H, s	18.6	33.5,36.10, 47.20, 59.97	1.51, 2.24
3	1.6	3H, s	19.4	59.9, 60.2, 169.6	1.34
4	1.27	3H, s	21.1	47.2, 72.2, 78.5	---
5	1.84	3H, s	22.9	23.6, 130.6, 145.4, 202.02	2.04
6	2.04	3H, s	23.6	22.9, 25.5 (w),130.6, 145.5	1.84, 2.24
7	1,92	2H, ddd	23.7	33.5, 36.1,72.2, 78.5	---
8	2.24	2H, (d, dd)	25.5	18.6, 33.5, 36.1, 47.2, 72.2, 130.6, 202.02	2.04, 2.97
	2.97	---	25.5	36.1, 47.2, 130.6, 145.5, 202.02	2.00, 2.24
9	1.56, 1.34	2H (d, d)	33.5	---	1.34, 1.56
10	quat	NC	36.1	---	---
11	2.01	1H, dd	47.2	18.6, 21.1, 25.5, 59.9,72.2	---
12	3.09	1H, q	59.8	14.1, 19.4, 60	1.33
13	2.27	2H	59.9	47.2, 72.2	---
14	quat	---	60.2	---	---
15	quat	---	72.2	---	---
16	4.88	1H, dda	78.5	21.1, 23.6/23.7 (w), 33.5, 47.2, 169.6	1.33, 1.92
17	quat	---	130.6	---	---
18	quat	---	145.5	---	---
19	quat	---	169.6	---	---
20	quat	---	202.02	---	---

Abbreviations. NC: no correlation, s: singlet, d: doublet, dd: double doublet, ddd: doble doble doublet, q: quartet.

Table 2. NMR spectroscopic data set for compound 2.

Position	^{1}H (ppm)	Multiplicity	^{13}C (ppm)	HMBC	COSY
1	1.32	3H, d	13,9	59.6, 60.1	1.55 (w), 3.05
2	1,60	3H, s	18,0	45.1, 73.7, 82.9	
3	1.01	3H, s	19,1	32.9, 35.9, 45.1, 60.1	1.48, 2.27, 2.30
4	1,55	3H, s	19,4	59.7, 60.1, 168.4	1.32 (w)
5	1.99	3H, s	22,2	22.9, 35.9, 169.5	1.32, 1.48, 1.77, 5.89
6	1,86	3H, s	22,9	23.6, 130.2, 145.7, 201.5	2.07, 2.27, 2.90 (w)
7	1,77	1H (tdd)	23,0	32.9	1.32, 1.48, 1.99, 2.00, 5.89
	2,00	1H (dd)	23,0	32.9, 35.9,73.7, 82.9	
8	2,07	3H, s	23,6	22.9, 23.0, 60.4, 130.2, 145.7, 201.5	2.27, 2.90
9	2,24	2H, d	25,8	19.1, 35.9, 45.1, 82.9, 130.2, 201.5	2.30, 2.90
	2,90			19.1, 35.9, 45.1, 82.9, 130.2, 145.7, 201.5	1.86, 2.07, 2.24, 2.27, 2.30
10	1,30	2H (m)	32,9	---	---
	1,48	1 H (td)	32,9	18,0	1.30, 1.32. 1.77, 2.00
11	quat	---	35,9	---	---
12	2.28	1H	45,1	19.1, 25.8, 35.9, 82.9, 130.2	1.01, 2.90
13	3,05	1H, q	59,7	13.9, 19.4, 60.1, 168.4	1.32
14	2.27	2H, m	60,1	---	1.01, 1.86, 2.07, 2.90
15	quat	---	60,4	---	---
16	5,89	1H, dd	73,7	18.0, 23.0, 45.1, 82.9, 168.4	1.77, 1.99
17	quat	---	82,9	---	---
18	quat	---	130,2	---	---
19	quat	---	145,7	---	---
20	quat	---	168,4	---	---
21	quat	---	169,5	---	---
22	quat	---	201,5	---	---

Abreviation. **NC**: no correlation, **s**: singlet, **d**: doublet, **dd**: double doublet, **ddd**: doble doble doublet, **q**: quartet.

Table 3. Average inhibition halos (mm) from the evaluation of crude extracts of *P. carolinensis* (Jacq.) G. Don

[c]	NC	FAL	FAS	NC	FEL	DEL	DES
				Acinetobacter spp			
100%	0	0	0	0	10	12	11
80%	0	0	0	0	8	10	11
60%	0	0	0	0	9	7	5
40%	0	0	0	0	0	0	4
20%	0	0	0	0	0	0	0
10%	0	0	0	0	0	0	0
				Staphylococcus spp.			
100%	0	0	0	0	11	12	10
80%	0	0	0	0	9	7	10
60%	0	0	0	0	8	6	9
40%	0	0	0	0	0	7	5
20%	0	0	0	0	0	0	0
10%	0	0	0	0	0	0	0

Abreviation. **NC**: negative control, **FAL**: fresh aqueous leaves extract, **FAS**: fresh aqueous stem extract, **FEL**: fresh ethanolic leaves extract, **DEL**: dry ethanolic leaves extract, **DES**: dry ethanolic stem extract.

ACKNOWLEDGMENTS

This work was supported by the Centro de Investigacion de Productos Naturales y Biotecnologia (CIPNABIOT) and the Microbiology Laboratory of the Autonomous University of Chiriqui, the Natural Products Laboratory at the University of Buenos Aires, Argentine.

CONCLUSIONS

1. The secondary metabolites identified in the aqueous and ethanol crude extracts of *P. carolinensis* were: alkaloids, carbohydrates, coumarins, terpenes, flavonoids, cardiotonic glycosides, phenolic compounds, saponins and tannins.

2. Only the ethanolic extracts showed biological activity against the pathogenic species analyzed, with the higher inhibition values at concentrations of 80 and 100%.

3. Two eudesmanolides were isolated from the ethanolic dry stem extract of *P. carolinenss* for the first time in Panama.

4. The data collected and information reviewed showed that sesquiterpene compounds should be related to the antibacterial and antiinflamatory activity, but not necessary due to the Cuauthemone metabolites, because exist reports of other sesquiterpene isolated from *Pluchea* species with similar biological activities.

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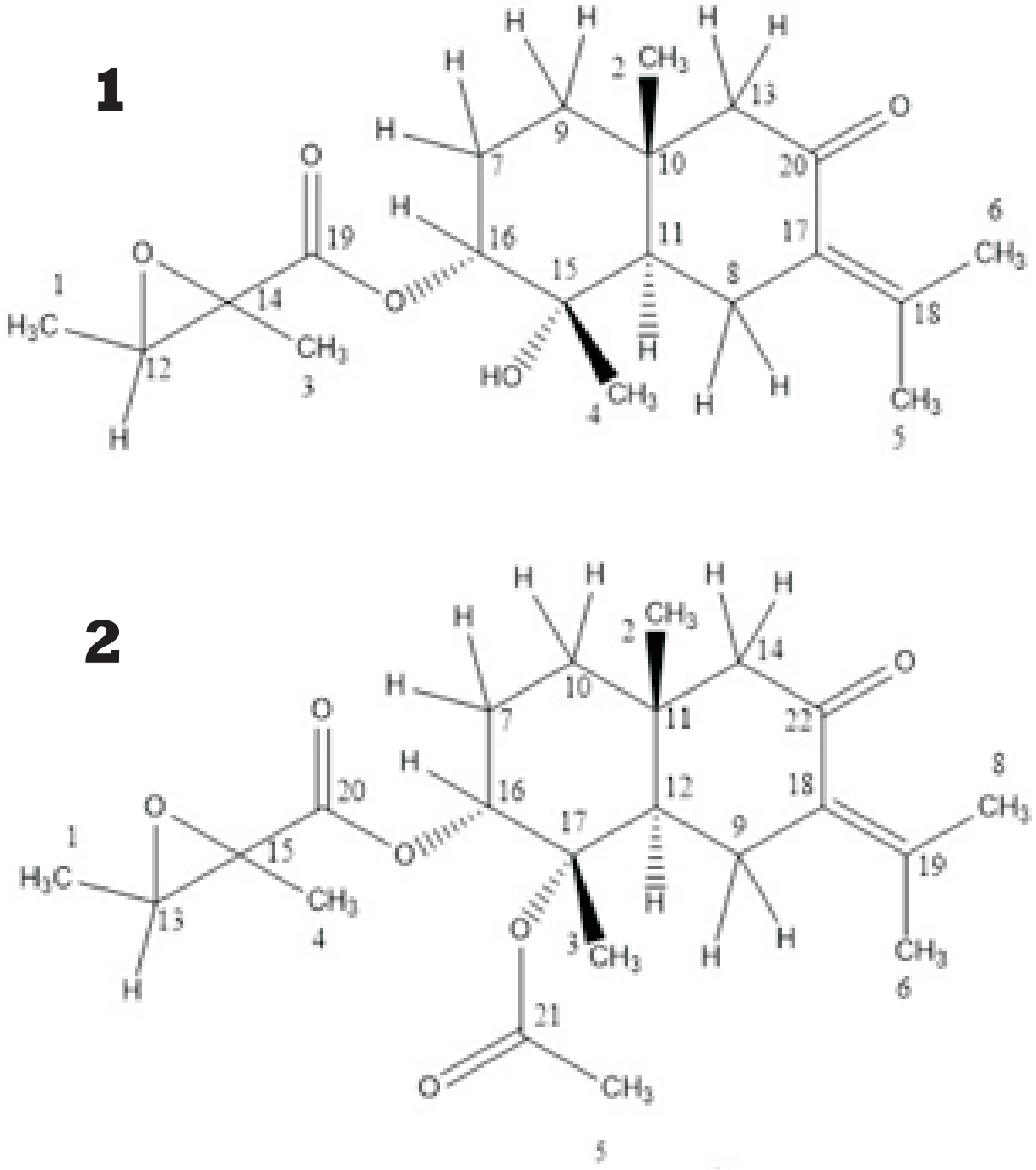

Figure 1. Eudesmanoloides. Cuathermone ester (1) and diester (2) isolated from the ethanolic dry leaves and stem extracts of *Pluchea carolinensis* (Jacq.) *G. Don.*