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Phytochemical investigation and tocolytic activity of the methanolic extract of *Evolvulus linarioides* Meisn. (Convolvulaceae)

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Abstract: *Evolvulus* (Convolvulaceae family) composed of about seventy species widely distributed in Brazil, with some of its species showed a spasmolytic effect in some smooth muscles. *Evolvulus linarioides* Meisn. is a erect sub-shrub with small blue flowers. Previous phytochemical studies have resulted in the identification of chromones and sesquiterpenes. Therefore, the aim of this study was to do phytochemical investigation and evaluate the tocolytic activity of the methanolic extract of *E. linarioides* (EL-MeOH) on rats. After euthanasia, the rat uterus was placed in baths for isolated organ where isotonic contractions were monitored (n = 5). The Ethics Committee on Animal Use of UFPB approved all experiments (Protocol 3559100918). Following the extraction of aerial parts of *E. linarioides* with methanol, the dried extract was successive partitioning in hexane, dichloromethane, ethyl acetate and n-butanol. The n-butanol phase was column chromatographed using Sephadex LH-20 and then further purified by HPLC on C-18 reverse phase (methanol:water, 17:83) affording compound 1 (5 mg). The structure of the compound was elucidated on the basis of spectroscopic analysis, viz. 1D and 2D NMR experiments, chemical study, and comparison with literature data. Thus, compound 1 was characterized as a 5-hydroxy-7-O-(β -D-glucopyranosyl)-2-methylchromone (undulatoside A). In addition, EL MeOH antagonized in a concentration-dependent and equipotent manner both phasic contractions induced by 10^{-2} IU/mL oxytocin ($E_{max} = 100\%$; $IC_{50} = 253.8 \pm 54.6 \mu\text{g/mL}$) and 10^{-5} M carbachol ($E_{max} = 93.6 \pm 2.2\%$; $IC_{50} = 195.5 \pm 47.5 \mu\text{g/mL}$), indicating that the tocolytic effect of EL-MeOH probably involves a common pathway between these agonists. In conclusion, we report for the first time the undulatoside A from Convolvulaceae family, in addition, the species *E. linarioides* presents tocolytic activity on uterus.

Keywords: *Evolvulus linarioides*; chromone; tocolytic activity.

1. Introduction

Evolvulus (Convolvulaceae family) composed of about seventy species widely distributed in Brazil¹. Data showed pharmacological activities for the species of the family Convolvulaceae, such as sedative, anti-inflammatory and spasmolytic in jejunum of rabbit and guinea pig ileum and trachea. Due to this, species of this family have been used to treat disorders such as cough, splenomegaly, anemia, tumors, increased lipid levels, gastric ulcer, obesity, diarrhea, constipation and asthma^{2,3}.

2. Results and Discussion

Comparison of the ¹H and ¹³C NMR data of **1** with those of the known undulatoside A⁷ (Table 1), indicated their similarity. Thus, the structure of **1** was elucidated as 5-hydroxy-7-*O*-(β-*D*-glucopyranosyl)-2-methylchromone, named undulatoside A.

The effect tocolytic was evaluated by comparing the responses before and after the addition of the extract. EL-MeOH antagonized in a concentration-dependent and equipotent manner both phasic contractions (Figure 1) induced by 10⁻² IU/mL oxytocin (E_{max} = 100%; IC₅₀ = 253.8 ± 54.6 μg/mL) and 10⁻⁵ M carbachol (E_{max} = 93.6 ± 2.2%; IC₅₀ = 195.5 ± 47.5 μg/mL), indicating that EL-MeOH showed tocolytic effect and this probably involves a common pathway between these agonists.

Evolvulus linarioides Meisn. is a erect subshrub with small blue flowers⁴. Previous phytochemical studies have resulted in the identification of chromones and sesquiterpenes^{5,6}. Therefore, the aim of this study was to do phytochemical investigation and evaluate the tocolytic activity of the methanolic extract of *E. linarioides* (EL-MeOH) on rats.

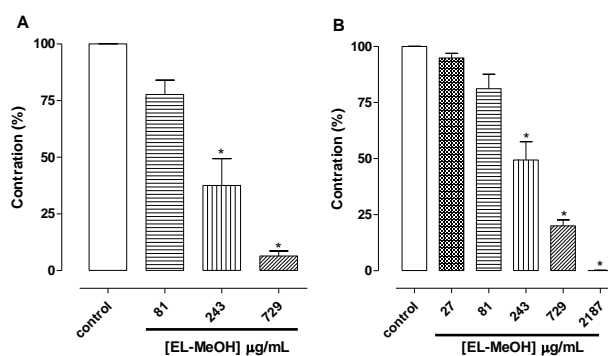
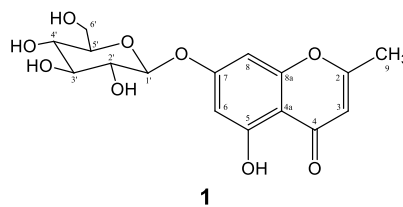


Figure 1. Tocolytic effect of EL-MeOH on phasic contractions induced by 10⁻² IU/mL oxytocin (A) and 10⁻⁵ M CCh (B) on rat uterus. Columns and vertical bars represent the mean and S.E.M., respectively (n = 5). One-way ANOVA followed by the Tukey's post-test. *p < 0.05 (control vs. EL MeOH).



C	Compound 1		Undulatoside A	
	δ _H	δ _C	δ _H	δ _C
2	-	169.9	-	168.4
3	6.11 (s)	109.3	6.12 (d, J = 0.7)	108.3
4	-	184.2	-	182.0
4 ^a	-	106.8	-	105.1
5	-	162.9	-	161.2
6	6.47 (d, J = 2)	101.0	6.47 (d, J = 2.2)	99.5
7	-	164.7	-	162.9
8 ^a	-	159.4	-	157.4
8	6.66 (d, J = 2)	95.9	6.66 (d, J = 2.2)	94.5
9	2.38 (s)	20.3	2.38 (s)	20.0
1'	5.02 (d, J = 7.2)	101.5	5.00-5.04 (m)	99.8
2'	3.45-3.53 (m)	74.6	3.47-3.52 (m)	73.1
3'	3.45-3.53 (m)	78.3	3.47-3.52 (m)	77.1
4'	3.36-3.42 (m)	71.1	3.38-3.42 (m)	69.6
5'	3.45-3.53 (m)	77.8	3.38-3.42 (m)	76.4
6'	3.70 (dd, J = 5.6; 12.4) 3.90 (dd, J = 2; 12.4)	62.3	3.70 (dd, J = 5; 12.1) 3.90 (dd, J = 2.3; 12.1)	60.4

Table 1. Comparison of the ¹H and ¹³C NMR data of **1** (400 e 100 MHz, CD₃OD) with those of the undulatoside A (500 e CD₃OD, 125 MHz e DMSO-d₆)⁷.

3. Materials and Methods

General Experimental Procedures. ^1H and ^{13}C NMR spectra were measured on a BRUKER AVANCE III HD spectrometer. HPLC purification was performed on Shimadzu SCL-10AVP C-18 column (ACE 5 μm 250x21.2 mm).

Plant Material. Aerial parts of *Evolvulus linarioides* was collected from Matureia, Paraíba, Brazil. The plant material was identified by Prof Maria de Fátima Agra, Federal University of Paraíba, and a voucher specimen (AGRA et al. 6970) is kept at the herbarium Lauro Pires Xavier (JPB), Federal University of Paraíba.

Extraction and Isolation. Powdered *E. linarioides* aerial parts (1.5 kg) were extracted with methanol (three times for 72h) at room temperature. The resulting extract was concentrated under reduced pressure using rotavapor at 40 °C, yielded 300 g, which was suspended in distilled water and partitioned with in hexane, dichloromethane, ethyl acetate and *n*-butanol. The *n*-butanol phase (10 g) was

fractionated by column chromatography on Sephadex LH-20 and eluted with methanol, into sixty-eight fractions. The fraction 30-37 (250 mg) were further purified by preparative HPLC on C-18 reverse phase (methanol:water, 17:83) to afford compound **1** (5 mg, $t_{\text{R}} = 65$ min).

All experiments were approved by the Ethics Committee on Animal Use of UFPB (Protocol 3559100918). Wistar female rats were treated with diethylstilbestrol (1 mg/kg, s.c.) 24h prior to experiment to induce estrous hormonal synchronization. Two phasic contractions were obtained with 10^{-2} IU/mL oxytocin or 10^{-5} M carbachol (CCh) with intervals of 15 min among them. The EL-MeOH was pre-incubated individually in at least three concentrations for 15 minutes in different preparations, and the inhibition of the submaximal response to the agonists was evaluated by comparing the responses before (100%, control) and after adding the compounds to the preparation⁸.

4. Conclusions

In conclusion, we report for the first time the undulatoside A from Convolvulaceae family, in addition, the species *E. linarioides* presents tocolytic activity on uterus.

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Conflicts of Interest

The authors declare no conflict of interest.

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