# Constituents and Biological Activities from *Muehlenbeckia hastulata*

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*Muehlenbeckia hastulata*, Chemical Constituents, Biological Activities

*Muehlenbeckia hastulata* (J. E.Sm.) Johnst. used mainly for its abortive properties was found active for oxytoxic and analgesic activities in a biological activity screening (oxytoxic, analgesic, antimicrobial, cytotoxic and xanthine oxidase assay). Epicatechin, emodin-8-glycoside and rutin were isolated from the aerial part and the root of the plant.

## Introduction

Polygonaceae are known as ornamentals and as crop plants (Heywood, 1978). *Muehlenbeckia* is one of them and well known in Chile. Extracts of this plant have been traditionally used as diuretic, hypotensive, antihemorragic, sedative and for treating rheumatism; besides it roots shows an abortive effect in human and animal species due to its uterine motility (Muñoz *et al.*, 1981; Houghton and Manby, 1985; Zin and Weiss, 1980).

Previous chemical and biological studies on this species, have shown hypotensive effects (Navarrete *et al.*, 1998) and the presence of emodine, crysophanic acid and anthraquinone glycosides (Montes and Wilkomirsky, 1985).

As part of a research program on endemic species used in Chilean folk medicine, we report another study on chemical composition and pharmacological activities of the aerial part and the root of *Muehlenbeckia hastulata.*

## Materials and Methods

### *General experimental procedures*

Solvents used for NMR were $CDCl_3$ and DMSO-$d_6$. The measurements of the NMR spectra were carried out on a Bruker AMX-300 [$^1$H NMR (300 MHz) $^{13}$C NMR (75 MHz)] spectrometer. Column chromatography (CC) was carried out using silica gel 60 G (Merck 7734). TLC were performed on silica gel GF 254 (Merck 5554) with EtOAc/MeOH (95:5 v/v), spots were detected by UV (254,366 nm), Liebermann Burchard test, KOH 5% and $AlCl_3$ 5% reagents.

Separation of the diverse compounds were performed on Chromatotron 8924 (Harrison Research, Palo Alto, California, 7924T.) on silica gel 60 PF 254 (1 mm) and eluted with EtOAc/MeOH (96:4 v/v).

### *Plant material*

The aerial part and the root of *Muehlenbeckia hastulata* (J. E. Sm.) Johnst., were collected in Cuesta Zapata, 55 km north of Santiago and identified by the botanist Sebastian Teillier. A voucher specimen is kept at the Herbario de Escuela de Química y Farmacia (SQF 21033).

### *Extraction and isolation*

Ground dried aerial part (4.5 kg) was extracted with EtOH at room temperature (3 × 3 l) for four days. The extract was concentrated to dryness (534.5 g) and the residue was successively extracted with hexane (2 × 0.5 l) (53.6 g) and then with $CH_2Cl_2$ (2 × 0.5 l) (42.55 g).

The *n*-hexane extract (20.0 g) was applied with CC on silica gel 60 with *n*-hexane/EtOAc gradient (0, 5, 10, 20, 50, 100% EtOAc) yielding seven fractions of increasing polarity. Fraction 3 (5 g) was applied to a column chromatography on silica

  

gel with $CH_2Cl_2$/EtOAc gradient (0, 50, 100%) and then EtOAc/MeOH 0, 50, 100%) yielding crude **1** and **3** Additional purification by chromatotron with EtOAc/MeOH (1%) yielded **1** (27.7 mg, 0.0006%).

Fraction 4 and 5 (11.2 g) were purified by CC with $CH_2Cl_2$/MeOH gradient (0, 30, 50, 100% MeOH) yielding crude **2–3**. Chromatotron analysis with EtOAc/MeOH (10%) yielded **2** (20 mg, 0.0004%). Purification by column chromatography on silica gel with $CH_2Cl_2$/EtOH gradient (0, 20, 60, 100%) and then by chromatotron with EtOAc/MeOH (30%) yielded **3** (12 mg, 0.0003%) The compounds isolated were identified by $^1$H NMR and $^{13}$C NMR spectroscopy and compared with bibliographic data (compound **1**: Breitmaier, 1993, Harborne and Mabry, 1982; compound **2**: Coskun *et al.*, 1990 and compound **3**: Mabry *et al.*, 1970).

Ground dried root (1.1 kg) was extracted with hexane (3 × 2 l) ( 95 g) at room temperature. The hexane extract was dried and dissolved in ethanol (3 × 2 l) (51 g). Both ethanol and indissoluble hexane extract were used for the evaluation of the oxytoxic activity.

*Oxytoxic assays*

Uteri from young virgin guinea pig (210–250 g) were used. A piece of the uterus was set up in the organ bath of 14 ml of capacity containing Tyrode's physiological solution (Laurence *et al.*, 1964) aerated with a mixture of oxygen (95%) and carbon dioxide (5%), maintained at 37 °C. Increasing aliquots of standard solution of 0.025 I. U./ml of oxytoxin (Syntocinon ®,Laboratorio Sandoz, Santiago, Chile) were added to the organ bath and contractions changes were registered on a kymograph. The organ bath was rinsed three times with Tyrode and the tissue allowed to rest for 3 min. before suspended aqueous aliquots of dried hexane extracts (15 mg/100 ml) were added.

*Analgesic assays*

The analgesic activity of the hexane extract (HE) was evaluated in groups of 8 mice and 16 control subjects, using a intraperitoneal injection of 0.5 ml of acetic acid 0.6%. The analgesic effects were calculated by comparing the number of abdominal writhes of the treated and the control group, which only received the vehicle (Davies *et al.*, 1997). The number of abdominal writhes of each mouse was counted for 30 min, beginning 5 min after acetic acid administration.

The following equation was used to calculate the mean dolour percentage:

$$\% \text{ D} = [\text{C sample/Control}] \times 100$$

where D is dolour, C sample is the mean writhes reached in sample-treated animals and C control (41.6 ± 3.79) is the mean writhes reached in control animals which received only the vehicle. The analgesic effect (An) was calculated according to the following equation:

$$\% \text{ An} = 100 - \% \text{ D}$$

In analgesic assays, the extracts were orally administered by means of an intragastric catheter, suspended in saline arabic gum 1 h before the acetic acid, at doses of 600 mg/kg. The drug induced changes were statistically estimated using the Wilcoxon test for independent data (Hollander and Wolfe, 1973). The effects were significant for $p \leq 0.05$. The SEM ($SD/\sqrt{nn}$) values were calculated for the mean writhes.

Sodium naproxen (SN), obtained from Laboratorios Saval, Santiago, Chile, was used as a reference drug and was suspended in the same vehicle.

*Antimicrobial assays*

The antimicrobial activity of the extracts were determined against *Escherichia coli* (ATCC 8739), *Klebsiella pneumoniae* (isolated from a patient), *Salmonella aviatum (ATCC 2228), Salmonella aeruginosa* (ATCC 14207), *Staphylococcus aureus* (ATCC 6538P), *Micrococcus flavus* (ATCC 10290) *and Bacillus* subtilis (ATCC 6633).

The extracts were dissolved in DMSO. Dilutions of 100 and 200 μg/ml were added to a fixed volume of Plate Count Agar (PCA). They were then superficially inoculated with a single line of an overnight culture of the different microorganisms and incubated at 37 °C for 24 h. Results were recorded as growth or growth inhibition at each extract concentration. (Erazo *et al.*, 1997)

*Cytotoxicity studies*

A screening procedure was used to assess the cytotoxicity activity of the global ethanol extract against the following cell lines: P-388 (lymphoid

neoplasm from DBA/2 mouse, ATCC CCL-46), A-549 (human lung carcinoma, ATCC CCL-185) and HT-29 (human colon carcinoma, ATCC HTB-38). Cells were seeded into 16 mm wells (multidishes) (NUNC 42001) at concentrations of $1 \times 104$ (P-388), $2 \times 104$ (A-549) (HT-29) cells/well, respectively, in 1 ml aliquots of MEM (minimum essential medium) 10 FCS containing the compound to be evaluated at the concentrations tested. In each case, a set of control cells was incubated in the absence of sample and counted daily to ensure the exponential growth of cells. After four days at 37 °C, under a 10% $CO_2$, 98% humid atmosphere, P-388 cells were observed by inverted microscopy and the degree of inhibition was determined by comparison with the control, whereas A-549 and HT-29 were stained with crystal violet before examination (San Feliciano *et al.*, 1993). As reference drug adriamicin was used.

*Xanthine oxidase activity*

Both xanthine and xanthine oxidase (XO) from cow's milk were purchased from Sigma Co. and the standard inhibitor allopurinol was obtained from Laboratorios Saval, Chile. The global methanol extract was evaluated at 50 μg/ml and if it showed an inhibition value > 50% it was further tested for $IC_{50}$ determination (Noro *et al.*, 1983; Schmeda-Hirschmann *et al.*, 1992). The inhibition of XO activity using xanthine as the substrate was spectrophotometrically measured in relation to the amount of uric acid, which was determined at 290 nm using a UNICAM spectrophotometer. The assayed mixture consisted of 1.0 ml of test solution, 2.9 ml of phosphate buffer ($Na_2HPO_4$/$KH_2PO_4$; pH = 7.5) and 0.1 ml of enzyme solution. After preincubation of the mixture at 25 °C for 15 min, the reaction was initiated by adding 2.0 ml of substrate solution. This assayed mixture was incubated at 25 °C for 30 min. The reaction was stopped by adding 1.0 ml of 1 N HCl, the absorbance was measured. The percent inhibition of xanthine oxidase activity (% I) was calculated as:

$$\% \text{ I} = (\text{A-B}) - (\text{C-D})/(\text{A-B}) \times 100$$

where A is the activity of XO without test material (total uric acid); B, the blank of A without XO; C, the enzyme activity with test material (residual uric acid); and D, the blank of C without the enzyme.

The IC50 determination of allopurinol was 0.035 μg/ml (0.267 μM). For xanthine oxidase activity, the drug-induced changes were statistically estimated using the Wilcoxon test for independent data (Hollander and Wolfe, 1973). Effects were significant for $p \leq 0.05$.

## Results and Discussion

Three compounds were isolated and characterized from Muehlenbeckia hastulata: Epicatechin (**1**), emodin-8-glycoside (**2**) and rutin (**3**).

The oxytoxic studies showed that the root extract was more active than leaf extract. The ethanol dissolution of the dried hexanoic root extract present 62% of oxytoxin activity and its effect is reverted by phenoterol (0.54 μg/ml) and papaverin (23.2 μg/ml) (Table I).

Table I. Oxitoxic activity measured by contraction amplitude of guinea pig uterine smooth muscle of *Muehlembeckia hastulata* hexane extract compared with oxitoxin (μg × ml/organ bath).

| Hexane extract [μg × ml] | Contraction amplitude [cm] | Oxitoxin [μg × ml] | Contraction amplitude [cm] |
|---|---|---|---|
| 54 | 2.0 | 8.9 | 1.00 |
| 107 | 3.10 | 17.9 | 3.20 |
| 268 | 5.60 | 26.8 | 4.20 |
| 536 | 7.20 | 35.7 | 5.60 |
| 804 | 7.80 | 44.6 | 6.50 |
| 1018 | 8.80 | 53.5 | 7.40 |
| | | 62.5 | 8.10 |
| | | 71.4 | 8.60 |
| | | 80.4 | 8.80 |

1 IU of oxitoxin = 0.5 mg of oxitoxin.
The relation of activities was obtained by the slope relation of linear plot of contraction amplitude vs. log concentration for the hexane extract and oxitoxin, yielding a 62% of oxitoxin activity.

The hexane extract showed analgesic activity (42%), similar to sodium naproxen at doses of 12.5 mg/kg of 70% (Delporte *et al.*, 2002) (Table II).

Hexane, dichloromethane and ethanol extracts exhibited no antimicrobial activities against all tested microorganisms.

Cytotoxical studies carried out with the ethanol extract showed to be non- cytotoxic, tested on P-388 murine leukaemia, A-549 human lung carcinoma and HT-29 colon carcinoma with an $IC_{50}$ major 20 μg/ml.

Table II. Analgesic activity (An) of hexane extract (EH) and sodium naproxen (SN).

| S | Dose [mg/kg] | % An ± SEM |
|---|---|---|
| EH | 600 | 42.0* ± 5.6 |
| SN | 12.5 | 70.0* ± 4.3 |

* $p \leq 0.05$.
S Sample; sodium naproxeno (d-2-(6-methoxy-2-naphthyl) propionic acid sodium salt).

*Muehlenbeckia hastulata* showed a weak inhibitor toward xanthine oxidase (XO) at 50 $\mu$g crude extract/ml (Schmeda-Hirschmann *et al.*, 1992) and isolated epicatechin was inactive.

In conclusion, this study demonstrates that *Muehlenbeckia hastulata* possesses significant oxytoxic and analgesic activities. These effects would explain the traditional use of this plant to induce uterine contractions in domestic animals and for treating rheumatism by the traditional medicine of Mapuche people.

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