Pharmacogn J. 2019; 11(3): 493-495
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

Secondary Metabolites from *Pterocaulon alopecuroides* and their Antiproliferative Activities

Quírico A Castillo[1,*], José M. Padrón[2], Anastacio Emiliano[3]

ABSTRACT

Objective: To isolate secondary metabolites from the aerial parts of *Pterocaulon alopecuroides*, elucidate their structures and evaluate their antiproliferative activities on selected human cancer cell lines. **Materials and Methods:** The ethanolic extract of *P. alopecuroides* afforded five compounds, which were characterized using spectroscopic techniques and by comparison with data from the literature. Antiproliferative activities of all isolates were evaluated. **Results:** The compounds 7-(2,3-dihydroxy-3-methylbutoxy)-6-methoxycoumarin (**1**), 5,6-methylenedioxy-7-(2,3-dihydroxy-3-methylbutoxy) coumarin (**2**), Dihydrokaempferol (**3**), 5,7,4´-trihydroxy-6-(α,α-dimethylallyl)dihydroflavonol (**4**) and 5,4´-dihydroxy-7-(γ,γ-dimethylallyloxy)dihydroflavonol (**5**) were isolated. The antiproliferative activity of all compounds was evaluated in a panel of six human solid tumor cell lines showing GI_{50} values for the most active compounds in the low micromolar range. **Conclusion:** Compound **2** is reported for first time from *P. alopecuroides*. Isolated coumarins show no antiproliferative activity, whilst among flavonoids compound **5** showed the best antiproliferative activity.

Key words: *Pterocaulon alopecuroides*, Coumarins, Flavonoids, Antiproliferative activities, 5,4´-dihydroxy-7-(γ,γ-dimethylallyloxy)dihydroflavonol.

Quírico A Castillo[1,*], José M. Padrón[2], Anastacio Emiliano[3]

[1]Escuela de Química and Instituto de Química, Universidad Autónoma de Santo Domingo (UASD), Facultad de Ciencias, Ciudad Universitaria, Santo Domingo, D.N., DOMINICAN REPUBLIC.
[2]BioLab, Instituto Universitario de Bio-Orgánica "Antonio González" (IUBO-AG), Centro de Investigaciones Biomédicas de Canarias (CIBICAN), Universidad de La Laguna, 38206, La Laguna, SPAIN.
[3]Universidad Ana G. Méndez at Gurabo, PO Box 3030, Gurabo, PR 00778, PUERTO RICO.

Correspondence

Dr. Quírico A. Castillo

Escuela de Química and Instituto de Química, Universidad Autónoma de Santo Domingo (UASD), Facultad de Ciencias, Ciudad Universitaria, Santo Domingo, D.N., DOMINICAN REPUBLIC.

Phone no : +1 809 535 8273

E-mail: qcastillo55@uasd.edu.do

History

- Submission Date: 05-11-2018;
- Review completed: 03-01-2019;
- Accepted Date: 21-01-2019

DOI : 10.5530/pj.2019.11.78

Article Available online
http://www.phcogj.com/v11/i3

INTRODUCTION

The genus *Pterocaulon* (Asteraceae) has 18 species, of which twelve are American and six are Australian.[1] Two of them are present in the island of Hispaniola: *P. alopecuroides* and *P. virgatum*.[2]

Continuing our interest in studying the flora present in Hispaniola, the phytochemical study of the species *Pterocaulon alopecuroides* (Lam.) DC. was carried out, which led to the isolation and structure elucidation of compounds **1**-**5**. To the best of our knowledge, this is the first time that compound **2** is reported to be isolated from *P. alopecuroides*.

MATERIALS AND METHODS

General

NMR spectra were recorded using a Bruker Ascend Aeon spectrometer with cryoprobe operating at 400 MHz in ^{1}H and 100 MHz in ^{13}C NMR respectively. The chemical shift (δ) values are given in ppm and coupling constants (*J*) are given in Hz. $CDCl_3$ or $(CD_3)_2CO$ were used as solvents. Column chromatography was performed on a Biotage Isolera One flash purification system (Biotage, Charlotte, North Carolina, USA) using SNAP ULTRA silica gel cartridges. Analytical and preparative TLC were developed on silica gel 60 F_{254} plates (Merck KGaA, Darmstadt, Germany).

Plant Material

Aerial parts of *P. alopecuroides* were collected on October 2015 at Cordillera Central, Municipio Rancho Arriba, San José de Ocoa province, Dominican Republic. The plant material was identified by Teodoro Clase, botanist at Jardín Botánico Nacional "Dr. Rafael Ma. Moscoso", Santo Domingo, Dominican Republic, where a voucher specimen (JBSD 126571) has been deposited.

Extraction and Isolation

Aerial parts of *P. alopecuroides* were air-dried and ground to a fine powder. The ground material (105 g) was extracted with 95% EtOH using a Soxhlet apparatus. The resulting crude extract (18.5 g) was dissolved in 95% EtOH (250 mL) and treated with a 5% Pb $(OAc)_2$ solution (250 mL) to precipitate chlorophyll. After 24 h, the mixture was filtered, concentrated *in vacuo* to remove most of the EtOH and extracted successively with hexanes, Et_2O and AcOEt (3 × 500 mL each). The Et_2O residue (2 g) was subjected column chromatography, eluting with mixtures of hexanes-acetone with increasing polarity to afford 40 fractions. Repeated column chromatography, followed by PTLC afforded compounds **1**-**5**.

Antiproliferative assays

The cell lines used in this study were A549 and SW1573 (lung), HBL-100 and T-47D (breast), HeLa (cervix) and WiDr (colon) and were a kind gift of

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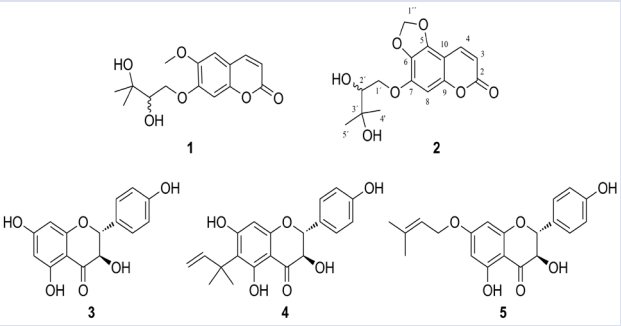

Figure 1: Compounds 1-5 isolated from the aerial parts of *Pterocaulon alopecuroides*.

Table 1: Antiproliferative Activity (GI_{50}) of compounds 1-5 against Human Solid Tumor Cells[a].

Compound	A549	HBL-100	SW1573	HeLa	T-47D	WiDr
1	> 100	> 100	> 100	> 100	> 100	> 100
2	> 100	> 100	> 100	> 100	> 100	> 100
3	31±6.0	55±8.2	27±4.2	32±5.6	31±2.6	35±6.2
4	33±3.7	> 100	40±7.3	82±20	20±7.6	18±4.1
5	16±0.9	20±0.6	17±0.3	16±1.6	17±1.0	19±2.2

*a*Values are given in μM and are means of two to three experiments.

Prof. Godefridus J. Peters (VUMC, The Netherlands). Compounds **1**-**5** were evaluated for antiproliferative activity using the protocol of the National Cancer Institute (NCI) of the USA with minor modifications.[3-5] Cells were seeded onto 96-well plates at cell densities of 2,500 (A549, HBL-100, HeLa and SW1573) or 5,000 (T-47D and WiDr) cells per well, depending on their doubling times. The results, expressed as GI_{50} (50% growth inhibition) values after 48 h of drug exposure, were calculated according to NCI formulas.

RESULTS AND DISCUSSION

The Et_2O residue (2.0 g) of the ethanolic extract from *P. alopecuroides*, afforded, after different chromatographic procedures, 11.8 mg of 7-(2,3-dihydroxy-3-methylbutoxy)-6-methoxycoumarin **(1)**[6], 10.8 mg of 5,6-methylenedioxy-7-(2,3-dihydroxy-3-methylbutoxy)coumarin **(2)**[7], 48.0 mg of Dihydrokaempferol **(3)**[8], 125.4 mg of 5,7,4´-trihydroxy-6-(α,α-dimethylallyl)dihydroflavonol **(4)**[9], and 11.1 mg of 5,4´-dihydroxy-7-(γ,γ-dimethylallyloxy)dihydroflavonol **(5)**[9] (Figure 1). Their chemical structures were elucidated using mainly 1D and 2D NMR and by comparison with data reported in literature. Below is shown found spectral data for compound **2**.

5,6-methylenedioxy-7-(2,3-dihydroxy-3-methylbutoxy) coumarin (2)

White solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.97 (1H, d, *J* = 9.7, H-4), 6.58 (1H, s, H-8), 6.24 (1H, d, *J* = 9.7, H-3), 6.04 (2H, s, H-1´´), 4.51 (1H, dd, *J* = 10.3, 2.9, H-1´a), 4.38 (1H, dd, *J* = 10.3, 8.0, H-1´b), 3.83 (1H, m, H-2´), 1.33 (3H, s, H_3-4´), 1.28 (3H, s, H_3-5´). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 161.2 (C-2), 152.5 (C-7), 151.5 (C-9), 138.6 (C-4), 136.7 (C-5), 132.3 (C-6), 112.1 (C-3), 106.9 (C-10), 102.1 (C-1´´), 93.1 (C-8), 76.4 (C-2´), 73.7 (C-1´), 71.6 (C-3´), 26.7 (C-4´), 24.8 (C-5´). Assignments were confirmed by HSQC-DEPT and HMBC experiments.

Antiproliferative activity

All isolates were evaluated for their antiproliferative activity against the human solid tumor cell lines A549, HBL-100, HeLa, SW1573, T-47D and WiDr. The bioactivity of compounds **1**-**5** on the mentioned cell lines was expressed as GI_{50}. The results (Table 1) shows that coumarins **1**-**2** are inactive whilst flavonoids **3**-**5** display growth inhibition. The most active compound of the series is flavonoid **5**, which show GI_{50} values in the range 16-20 μM.

CONCLUSION

In summary, we have reported the isolation of five secondary metabolites from the aerial parts of *Pterocaulon alopecuroides*. Compound **2** is reported for first time as isolated from this species. The study of the antiproliferative activity against selected human solid tumor cell lines showed that the isolated coumarins (**1**, **2**) were not active, while the isolated flavonoids (**3**-**5**) were more active, being compound **5** the most active of all the ones tested with GI_{50} values ranging from 16 to 20 μM.

ACKNOWLEDGEMENT

This project has been partially supported by the program FONDOCYT of the Ministerio de Educación Superior, Ciencia y Tecnología (MESCYT), Dominican Republic (Grant 2014-1D4-02). QAC thanks Dr. Ernesto Abel-Santos (University of Las Vegas, Nevada, USA) for his help in this project.

CONFLICT OF INTEREST

the authors declare no conflict of interest.

ABBREVIATIONS

EtOH: Ethyl Alcohol; **Pb (OAc)$_2$:** Lead Acetate; **Et$_2$O:** Ethyl Ether; **AcOEt:** Ethyl Acetate; **PTLC:** Preparative Thin Layer Chromatography.

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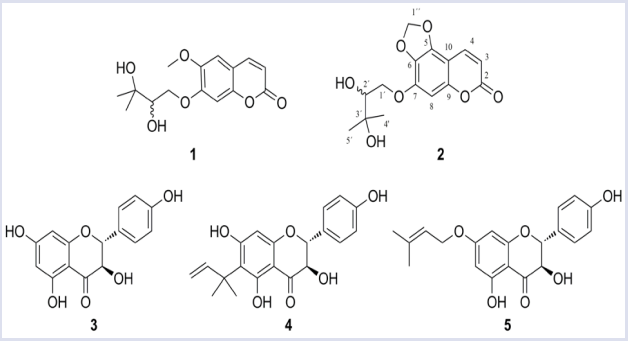
GRAPHICAL ABSTRACT



SUMMARY

- Phytochemical investigation of the ethanolic extract of the aerial parts of *Pterocaulon alopecuroides* (Asteraceae) afforded the compounds 7-(2,3-dihydroxy-3-methylbutoxy)-6-methoxycoumarin (1), 5,6-methylenedioxy-7-(2,3-dihydroxy-3-methylbutoxy) coumarin (2), Dihydrokaempferol (3), 5,7,4´-trihydroxy-6-(α,α-dimethylallyl)dihydroflavonol (4) and 5,4´-dihydroxy-7-(γ,γ-dimethylallyloxy)dihydroflavonol (5). All isolates were evaluated for their antiproliferative activities on a panel of six human tumor cell lines. Compound 2 is reported for first time from *P. alopecuroides*.

ABOUT AUTHORS

Quírico A. Castillo: Professor of Organic Chemistry. Universidad Autónoma de Santo Domingo, Dominican Republic.

Anastacio Emiliano: Professor of Organic Chemistry. Universidad Ana G. Méndez, Gurabo, Puerto Rico.

José M. Padrón: Associate Professor in Organic Chemistry of Universidad de La Laguna - Tenerife, Canary Islands, Spain.

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