Secondary Metabolites from *Pterocaulon alopecuroides* and their Antiproliferative Activities

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**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-methylene-dioxy-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 cells showing GI50 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.

**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*.1 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 1H and 100 MHz in 13C NMR respectively. The chemical shift (δ) values are given in ppm and coupling constants (J) are given in Hz. CDCl₃ or (CD₃)₂CO were used as solvents. Column chromatography was performed on a Biotage Isola 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 F₂₅₄ 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 5% Pb (OAc), 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, EtOAc and AcOEt (3 × 500 mL each). The EtOAc 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...
 RESULTS AND DISCUSSION
The Et₂O 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), 10.8 mg of 5,6-methylenedioxy-7-(2,3-dihydroxy-3-methylbutoxy)coumarin (2), 48.0 mg of Dihydromarkenpor (3), 125.4 mg of 5,7,4′-trihydroxy-6-(α,α-dimethylallyl)dihydroflavonol (4), and 11.1 mg of 5,4′-dihydroxy-7-(γ,γ-dimethylallyloxy)dihydroflavonol (5) (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; 1H NMR (400 MHz, CDCl₃) 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-4´), 1.28 (3H, s, H-5´). 13C NMR (100 MHz, CDCl₃) 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 GI₅₀. 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 GI₅₀ 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 GI₅₀ 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; Et₂O: Ethyl Ether; AcOEt: Ethyl Acetate; PTLC: Preparative Thin Layer Chromatography.

REFERENCES
1. Cabrera AL, Ragonese AM. Revisión del género *Pterocaulon* (Compositae). Darwiniana. 1978;21(2-4):185.
2. Liogier AH. La flora de la Española VIII. 1st ed. San Pedro de Macoris: Universidad Central del Este. 1996.
3. Castillo QA, Triana J, Eiroa JL, Padrón JM, Plata GB, Abel-Santos EV, Báez LA, et al. Flavonoids from *Eupatorium Illitum* and Their Antiproliferative Activities. Phcog J. 2015;7(10):178-81.
4. Monks A, Scudiero D, Skehan P, Shoemaker R, Paull K, Vistica D, Hose C, et al. Feasibility of High Flux Anticancer Drugs Screen Using a Diverse Panel of Cultured Human Tumor Cell Lines. J Natl Cancer Inst. 1991;83(11):757-66.
5. Stadler M, Padrón JM, González-Cardenete MA. Antiproliferative Activity and Effect on GABA Receptors of Calitrisic Acid Derivatives. Planta Med Int Open. 2017;4:e69-92.
6. Vilegas W, Boralle N, Cabrera A, Bernardi AC, Pozetti GL, Arantes SF. Coumarins and A Flavonoid from *Pterocaulon alopecuroides*. Phytochemistry. 1995;38(4):1017-9.
7. Magalhaes AF, Magalhaes EG, Leitao FHF, Frighetto RTS, Barros SMG. Coumarins from *Pterocaulon balansae* and *P. lanatum*. Phytochemistry. 1995;38(4):1017-9.
8. Alarcón R, Picciaroni A, Peñaloza L, Uriburu ML, Boerno A, Sosa V. Phenolic compounds from *Pterocaulon alopecuroides*. Biochem Syst Ecol. 2010;38(5):1059-64.
9. Alarcón R, Carrozo FR, Ocampo S, Lucatti A, Flores GL, Tonn C, et al. Flavonoids from *Pterocaulon alopecuroides* with Antibacterial Activity. Planta Med. 2008;74(12):1483-7.
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*.

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Cite this article: Castillo QA, Padrón JM, Emiliano A. Secondary Metabolites from *Pterocaulon alopecuroides* and their Anti-proliferative Activities. Pharmacog J. 2019;11(3):493-5.