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Quantification of 5-methylcoumarin-4-glucoside and 11,12-dihydroxy-5-methylcoumestan in Six Peruvian Species of the Genus *Mutisia* (Asteraceae)

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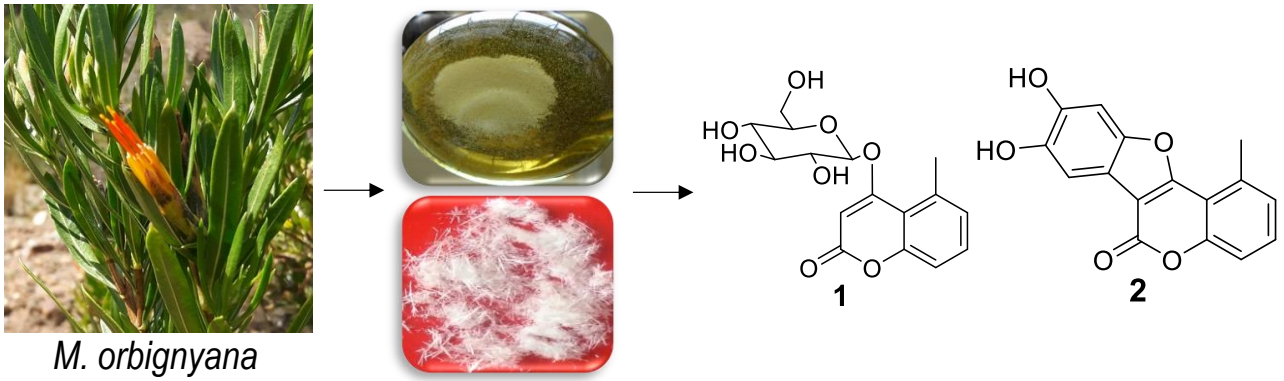
HIGHLIGHTS

- A 5-methylcoumarin and a coumestan were isolated from *M. orbignyana*.
- An HPLC method was established for the quantification of both compounds.
- The concentrations of both compounds were assessed in six samples of *Mutisia*.
- *Mutisia orbignyana* displayed the highest contents of both isolated compounds.

Abstract: *Mutisia* L. f. (Mutisiinae, Mutisieae, Mutisioideae, Asteraceae) is a South American genus that contains several medicinal species. *Mutisia acuminata* Ruiz & Pav. and *M. orbignyana* Wedd. are known sources of the 12-dihydroxy-5-methylcoumestan and 5-methylcoumarin-4-glucoside respectively. The 12-dihydroxy-5-methylcoumestan exhibited notable antihepatotoxic activity in a previous study. In the present work, 5-methylcoumarin-4-glucoside and 12-dihydroxy-5-methylcoumestan were isolated from a Peruvian collection of *M. orbignyana*. Additionally, the relative concentrations of those two isolated compounds in six Peruvian species of *Mutisia* (*M. acuminata*, *M. cochabambensis* Hieron., *M. lanata* Ruiz & Pav., *M. orbignyana*, *M. venusta* S.F. Blake and *M. wurdackii* Cabrera) were determined by an HPLC method. The highest concentrations of both constituents were observed in the sample of *M. orbignyana*.

Keywords: *Mutisia*; Asteraceae; 5-methylcoumarin; coumestan.

GRAPHICAL ABSTRACT



INTRODUCTION

Among the main antihepatotoxic agents of natural origin silymarin stands out for its great commercial impact and wedelolactone due to its high dissemination because it comes from *Eclipta alba* (*Asteraceae*), a common weed that occurs in the lowlands of India subcontinent [1]. In 1988, Daily and coauthors [2] reported the presence of 11,12-dihydroxy-5-methylcoumestane among other substances in the methanolic extract of *Mutisia acuminata* (Mutisieae, Mutisioideae, Asteraceae). Furthermore, in 2009, Flores and coauthors [3], established the presence of the 5-methylcoumarin-4-glucoside (**1**) and 11,12-dihydroxy-5-methylcoumestane (**2**) in *M. orbignyana* (Figure 1). The chemical structure of compound **2** is related to wedelolactone and a remarkable antihepatotoxic effect has also been demonstrated for it [2]. Regarding compound **1**, a high concentration of it has recently been reported in *Vernonia glaberrima* (Vernoniaeae, Cichoroideae, Asteraceae) [4] showing cytotoxic activity. The presence of 5-methylcoumarins in member of *Mutisioideae* subfamily has also been extensively reviewed by Vestena and coauthors 2022 [5], and members of *Mutisia* genus stands out as potential sources of 5-methylcoumarins with potential antihepatotoxic activity. Therefore, in the present work, both 5-methylcoumarin-4-glucoside (**1**) and 11,12-dihydroxy-5-methylcoumestane (**2**) were purified from a Peruvian assession of *M. orbignyana.* Additionally, the relative concentrations of both substances in six Peruvian species of *Mutisia* genus (*M. acuminata*; *M. cochabambensis*; *M. lanata*; *M. orbignyana*, *M. venusta* and *M. wurdackii*) were determined by an HPLC method as part of our program for the search of new source of 5-methylcoumarins.

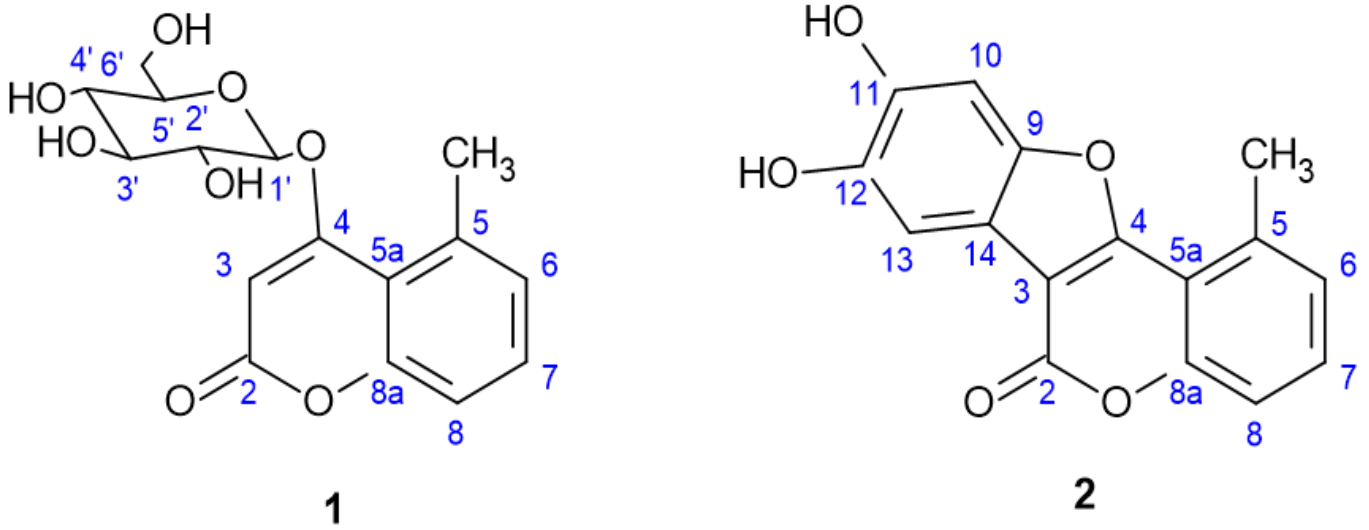


Figure 1. Structures of 5-methylcoumarin-4-glucoside (**1**) and 11,12-dihydroxy-5-methylcoumestan (**2**)

MATERIAL AND METHODS

Plant material

Aerial parts (leaves) of *M. acuminata* Wedd.*, M. cochabambensis* Hieron., *M. lanata* Ruiz & Pav. and *M. venusta* S.F. Blake were collected in Cusco region, Perú. *Mutisia orbignyana* was collected in Moquegua region, Perú whereas *M. wurdackii* Cabrera was collected in Amazonas region, Perú. The plants were translated to the phytochemistry laboratory (Chemistry Department, UNSAAC), and dried at room temperature.

Isolation of 5-methylcoumarin-4-glucoside (1) and 11,12-dihydroxy-5-methylcoumestan (2)

50 g of dried *M. orbignyana* were exhausted with 96% ethanol. The dried ethanolic extract was suspended in hot water (85 ºC) and vacuum filtered to remove waxes and chlorophylls. The aqueous extract was partitioned with ethyl acetate. The ethyl acetate extract was evaporated and vacuum liquid chromatographed with a gradient of hexane and ethyl acetate, the fraction of hexane:ethyl acetate (7:3) yielded by ethanol recrystallization 15.4 mg of **2** (0.034%) and the ethyl acetate fraction yielded by ethanol recrystallization 27 mg of **1** (0.054%). The structures of the isolated compounds were established based on NMR spectroscopy, high-resolution MS and comparison with literature data as 5-methylcoumarin-4-β-glucoside (**1**) and 11,12-dihydroxy-5-methylcoumestan (**2**) [2,3].

5-Methylcoumarin-4-β-glucoside (**1**): ^{1}HNMR (DMSO-d_6, 400MHz) δ 5.98 (s, 1H, H-3), δ 7.16 (d, 1H, H-6), δ 7.51 (t, 1H, H-7), δ 7.25 (d, 1H, H-8), δ 2.71 (s, 3H, C_5-CH_3), δ 5.20 (d, 1H, H-1'), δ 3.41 (t, 1H, H-2'), δ 3.39 (t, 1H, H-3'), δ 3.20 (t, 1H, H-4'), δ 3.49 (m, 1H, H-5'), δ 3.72 (t, 1H, H-6'), δ 4.63 (m, 1H, -OH-6'), δ 5.11, 5.20, 5.49 (3d, 3H, -OH- 2',3',4'). ^{13}CNMR (DMSO-d6, 100 MHz) δ 161.7 (C-2), δ 93.4 (C-3), δ 167.0 (C-4), δ 114.2 (C-5a), δ 137.5 (C-5), δ 128.2 (C-6), δ 132.4 (C-7), δ 115.3 (C-8), δ 154.8 (C-8a), δ 23.6 (CH_3), δ 100.3 (C-1'), δ 73.5 (C-2'), δ 77.0 (C-3'), δ 70.0 (C-4'), δ 77.8 (C-5'), δ 61.0 (C-6'). LC-HRESIMS negative mode *m/z* 383.0984 [M - H]$^-$ (calcd for $C_{16}H_{18}O_8$+CH_2O_2, 383.0978).

11,12-Dihydroxy-5-methylcoumestan (**2**): ^{1}HNMR (DMSO-d_6, 400MHz) δ 9.60 (br, 2H, 11,12-OH), δ 7.53 (t, 1H, H-7), δ 7.40 (d, 1H, H-6) δ 7.30 (s, 1H, H-10), δ 7.30 (d, 1H, H-8), δ 7.23 (s, 1H, H-13), δ 2.85 (s, 3H, C_5-CH_3). ^{13}CNMR (DMSO-d6, 100 MHz) δ 158.8 (C-2), δ 104.7 (C-3), δ 157.5 (C-4), δ 113.4 (C-5), δ 111.8 (C-5a), δ 134.1 (C-6), δ 130.5 (C-7), δ 126.7 (C-8), δ 153.0 (C-8a), δ 105.5 (C-9), δ 114.7 (C-10), δ 149.4 (C-11), δ 146.4 (C-12), δ 98.8 (C-13), δ 144.7 (C-14), δ 20.8 (-CH_3). LC-HRESIMS negative mode *m/z* 281.0445 [M - H]$^-$ (calcd for $C_{16}H_{10}O_5$, 383. 281.0450).

Quantification of 5-methylcoumarin-4-glucoside (1) and 11,12-dihydroxy-5-methylcoumestan (2)

For quantification purposes, 500 mg of each dried plant material was macerated for 24 hours with 20 mL of 70% ethanol, this operation was repeated 3 times and the filtrates were made up to 60mL. Five mL of each extract were evaporated at 40°C, weighed and redissolved in one mL of methanol and filtrated by 0.22µm PTFE membrane for conditioning in chromatographic vials. The chromatographic method was developed in a Thermo Scientific Ultimate 3000 UHPLC chromatograph with automatic injection and DAD detector. A Zorbax Eclipse Plus C_{18} Column (1.8µm particle size, 4.6 × 100 mm) was used. Column temperature was set at 35°C. Mixtures of 0,1% formic acid in water (A) and acetonitrile (B) were used as mobile phase with the following gradient program (minutes, %B): 0,2; 5,30; 10,80; 12,80; 15,100; 17,100; 19,2; 20,2. DAD: 200-400 nm: 280, 345, 254, 330 nm. The total run time was 20 minutes and a flow rate of 0.4 mL/min. The relative concentrations of both compounds were quantified using calibration curves prepared with standard solutions of **1** and **2** dissolved in methanol with six data points. The calibrations curves were obtained by potting the peak area signals as a function of the concentration. The equation curves were obtained (Y=64.55X-2.71 for **1**, Y=115.84X-1.43 for **2**) and linearity was evaluated by least-squares regression analysis (r^2 = 0.9996 for **1**, and r^2 = 0,9995 for **2**). For quantification of **1** the chromatograms at 280 nm were used, whereas **2** was quantified at 345nm.

RESULTS AND DISCUSSION

In the Figure 2, typical UHPLC chromatograms of *M. orbignyana* are displayed. Compound **1** eluted at a retention time of 8.20 min whereas compounds **2** eluted at 12.09 min. In addition to the isolated constituent additional chromatographic peaks were observed, highlighting the occurrence of two peaks with retention times of 6.99 (peak **a**) and 8.89 (peak **b**) which displayed the characteristic UV spectra of the phenylpropanoids. Although phenylpropanoid are ubiquitous constituent in members of Asteraceae family,

there is no previous report on the occurrence of phenylpropanoids in *M. orbignyana*. The phenylpropanoids 3-*O*-caffeoylquinic acid and 5-*O*-caffeoylquinic acid were previously describe in *M. friesiana* [6].

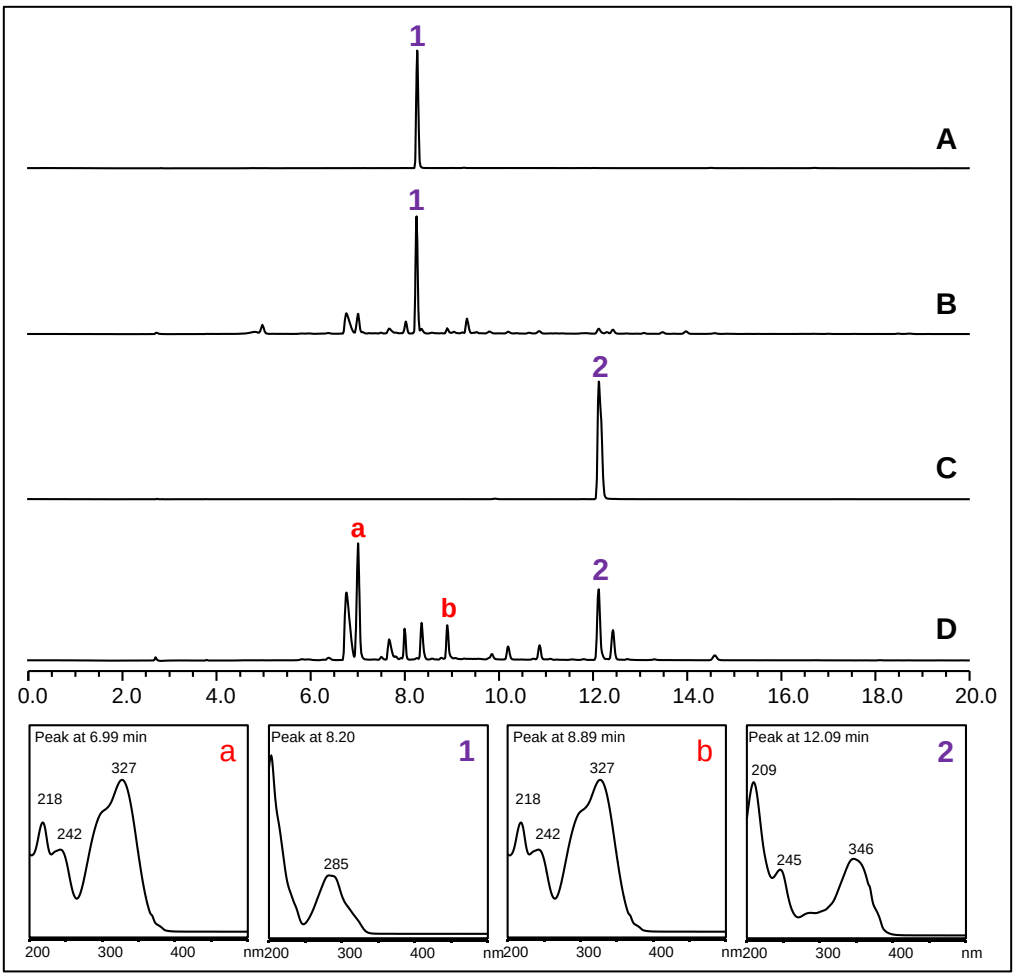


Figure 2. Typical chromatograms of standard compounds and *Mutisia orbignyana*. (A) Chromatogram of a standard solution of 5-methylcoumarin-4-β-glucoside (**1**) monitored at 280nm. (B) Chromatogram of the ethanolic extract of *M. orbignyana* monitored at 280nm. (C) Chromatogram of a standard solution of 11,12-dihydroxy-5-methylcoumestan (**2**) monitored at 345 nm. (D) Chromatogram of the ethanolic extract of *M. orbignyana* monitored at 345nm. In the bottom, four selected UV spectra of unidentified phenylpropanoid derivatives (**a** and **b**) and the standard compounds **1** and **2**.

The identity of those phenylpropanoids in *M. orbignyana* as well as their occurrence in other member of *Mutisia* genus need to be elucidated in further investigations. The Table 1 displays the content of **1** and **2** in six Peruvian species from *Mutisia*. *Mutisia orbignyana* is the plant with the highest content of both substances while in *M. cochabambensis* neither of those compounds were present. According to Cabrera [7], who divided the genus *Mutisia* into six sections based on morphological aspects, *M. orbignyana* belongs to the *Isantha* section, the most basal. *Mutisia orbignyana* has a particularly high concentration of **1** comparable to the African plant *Vernonia glaberrima* (*Cichoroideae*). On the other hand, to our knowledge there is no other genus apart from *Mutisia* that displays the presence of **2**.

Table 1. Content of 5-methylcoumarin-4-glucoside (**1**) and 11,12-dihydroxy-5-methylcoumestan (**2**) in six *Mutisia*[1].

Species	**1**	**2**
M. acuminata	3.6	0.78
M. cochabambensis	-	-
M. lanata	0.45	-
M. orbignyana	47.6	6.83
M. venusta	0.61	-
M. wurdackii	0.31	0.34

[1] mg of compound/g of dry plant. Concentrations were obtained with the following calibration curves: Y=64.55X-2.71 for **1**, Y=115.84X-1.43 for **2**.

CONCLUSION

Mutisia acuminata and especially *M. orbignyana* are candidate species to extract both 5-methylcoumarin-4-glucoside (**1**) and 11,12-dihydroxy-5-methylcoumestan (**2**). The other Peruvian *Mutisia* have none or small amounts of these compounds. However, further analysis of samples from different geographical origin and collection times are required.

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Conflicts of Interest: The authors declare no conflict of interest.

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