

Research Article



Selective and High Yield Isolation of Pure Wogonin from Aerial Parts of *Scutellaria havanensis* Jacq.

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ABSTRACT

Pure Wogonin (5,7-dihydroxy-8-methoxyflavone) as well as part of different extracts from *Scutellaria* (Lamiaceae) genus plants, has demonstrated to possess anti-inflammatory, anti-cancer, anxiolytic, neuroprotective, anti-atherosclerotic, anti-arthritis, anti-tumor, anti-allergic, anticoagulant, antioxidant and antiviral properties. Because of the growing interest in this substance, many methods for its isolation and high purity have been developed and reported in the literature. However, such methods are laborious, inefficient and expensive. In the present investigation, two new fast, selective and simple methods for isolating pure wogonin with high yields from *Scutellaria havanensis* were developed. The methods involve fresh aerial parts extraction with ethyl ether by sonication (5 min) and electrical shaker (2h), filtration and solvent evaporation without using any chromatographic techniques. Quantities of ca 0.8 to 1.2 g of wogonin (>95 % purity) were obtained from 50 g of plant material, yielding 8 to 12 % of recoveries (dry weight). The structure of wogonin was confirmed by PMR and ¹³C-NMR, UV, FT-IR and GC-MS. This is the first report on the wogonin isolation and characterization from this endemic Cuban plant by simple extraction and direct crystallization from the extract.

Keywords: *Scutellaria havanensis*, wogonin, Lamiaceae, NMR, GC-MS, ultrasound.

INTRODUCTION

Scutellaria (Lamiaceae) genus is widely distributed throughout the world and it is represented by about 300 species.¹⁻⁵ Several pharmacologic researches have confirmed that total extracts or flavones of this genus, such as wogonin (5,7-dihydroxy-8-methoxy flavone) and baicalin (5,6,7-trihydroxyflavone) possess anti-inflammatory,⁶⁻¹⁰ anxiolytic,¹¹⁻¹⁴ neuroprotective,^{9,15-17} anticonvulsant,¹⁸⁻¹⁹ antithrombotic,^{20,21} antioxidant,^{22,23} anticancer,²⁴ antitumor,²⁵ anti-arthritis,²⁶ antiviral, anti-allergic, antispasmodic and antimicrobial properties.^{5,27,28}

Considering wogonin's pharmacological relevance and the fact that it occurs together with other flavonoids in the different parts of *Scutellaria* species,²⁹⁻³³ several trials have been carried out for its isolation and production, even at large scale. Among them, increasing the growth rate and flavonoid content of *Scutellaria* species with CO₂ enrichment in a controlled environment,³⁴ cultivation of *Scutellaria* cells,^{16,35-37} synthesis methods,³⁸ biotechnology production,^{4,39,40} classical column chromatography methods, analytical assay,^{31,33,41} and preparative high-performance liquid chromatography,²² high-speed countercurrent chromatography⁴² and a low-pressure preparative chromatography (LPPC) after endogenous baicalinase catalyzed hydrolysis.⁴³ However, these methods have disadvantages of a long extraction time, low yield of wogonin, high solvent consumption, multiple steps for the isolation, high cost of the procedures and very laborious ones.

Scutellaria havanensis Jacq. (Havana skullcaps) is an endemic plant native in Havana, Cuba.⁴⁴ Until present days, a qualitative phytochemical screening of the ethereal, methanol and aqueous extracts⁴⁵ as well as the study of the volatile constituents⁴⁶ from aerial parts of this plant have been performed. In this work, flavonoids, alkaloids, coumarins, triterpenoids and steroids, alkaloids, free amine groups, sugars, quinones, resins, saponins and bitter principles were detected, besides β-caryophyllene (75.6%), α-humulene (11.6%), caryophyllene oxide (2.6%) among other volatile compounds in minor proportions were identified. A total absence of anthracenic and cyanogenic glycosides was also demonstrated. Continuing the investigation of Cuban Lamiaceae, in the present paper we report two new specific and single methods for obtaining pure wogonin with high yield from aerial fresh parts of *S. havanensis*.

MATERIALS AND METHODS

Plant material

Aerial parts (leaves and stems) of *S. havanensis* were collected in October 2011 in the National Botanic Garden, Havana, Cuba. Identification of the plant material was carried out by PhD. Angela Leyva (voucher 087485-HAJB).

Wogonin extraction and analysis

Fresh aerial parts (leaves and stems) of *S. havanensis* (50 g) were extracted with diethyl ether (100 mL) for 2 h in an electrical shaker (ZRM, CNIC, Cuba) at room temperature and for 15 min in an ultrasonic bath (35 KHz, Bioblok Scientific, Germany). After filtering the extracts, the solvents were evaporated at room temperature to yield



only yellow needles in high percent. Methods were applied three times. The purity of the yellow needles was evaluated by GC-MS and chemical structure elucidate with all spectroscopic techniques available.

Equipment

Melting point (m.p.) was determined on a Reichert-Thermovar apparatus (Germany). UV- spectrum was recorded on an UltrospecPlus Pharmacia, LKB (Switzerland) from 200 to 800 nm. IR spectrum was recorded on an IFS-48 Bruker spectrophotometer (Germany), from 400 to 4000 cm^{-1} . PMR and ^{13}C NMR measurements were obtained on Bruker AC- 250 F (Germany) at 250 and 62.9 MHz, respectively. Chemical shifts (δ) reported as parts-per-million and referenced to the tetramethylsilane (TMS) internal standard.

The mass spectrum was obtained from an Agilent 6890N gas chromatograph coupled to 5975B mass spectrometer (USA). An HP-5 Ms column (30m x 0.25mm, 0.25 μm film thickness) was used with helium as carrier gas (1 mL/min). The GC oven temperature was kept at 60 $^{\circ}\text{C}$ for 2 min and programmed to 200 $^{\circ}\text{C}$ at a rate of 20 $^{\circ}\text{C}/\text{min}$, then from 200 $^{\circ}\text{C}$ to 300 $^{\circ}\text{C}$ at a rate of 8 $^{\circ}\text{C}/\text{min}$ and kept constant at 300 $^{\circ}\text{C}$ for 5 min. The injection and source temperature were 320 $^{\circ}\text{C}$ and 250 $^{\circ}\text{C}$, respectively. MS interface temperature was 250 $^{\circ}\text{C}$. EI/MS spectrum was taken at 70 eV. Mass range was from m/z 35 to 800. Peak identification was achieved by computer matching mass spectra against commercial libraries (NIST 2011 GC/MS), as well as MS literature data.^{10,47,48}

RESULTS AND DISCUSSION

Because of the current interest in Wogonin (Fig 1) as well as the low proportions at which this flavone has been detected and isolated so far, (0,01 - 0,12 %) either in the different parts of the *Scutellaria* plants (*S. baicalensis*, *S. laterifolia*, *S. barbata*) among others, or in their commercial products,^{22, 29-33,48} in the present investigation were developed two new, simple and rapid procedures for selective isolation of pure wogonin from the fresh aerial parts of *Scutellaria havanensis* without using any chromatographic or other isolation techniques.

As it was previously described, after a single extraction, using fresh plant material, with diethyl ether, filtration and solvent elimination, yellow needles were obtained, with high percent recovery of the flavonoid based on dry plant material (Table 1). Higher recoveries were reached too, when ultrasonic bath was employed for extraction, using fresh plant material, while reducing significantly the extraction time. It was also noted that both methods were reproducible, batch to batch, and high yield of wogonin were obtained in comparison to other reported methods developed for the same purpose. By the contrary, those methods previously mentioned, are time-consuming and employ multiple chromatographic or purification steps yielding lower total recoveries (0.002% to 1.4%) of this flavonoid.^{22, 29-33}

Harbone,⁴⁹ reported that this type of flavonoid occurs in the outside of the leaves of the plant so when previous methylation is used, it is possible the isolation using solvents of middle polarity. According to this, wogonin has been detected in ethyl acetate in *S. schachristanica*⁴⁷ and dichloromethane in *S. baicalensis*.¹² In addition, empirical evidence and practical work, have both indicated that fresh herb extraction is likely to be more efficacious than the dried herb,^{33,50} because the instability of the latter and the presence of fewer flavonoids content. This report supports the results of the extraction methods used in the present investigation.

Table 1: Methods for obtaining wogonin from *S. havanensis* (n=3)

Method	Mass (g)	Mean recoveries (%) \pm SD
Electrical shaker	0.8	8.0 \pm 0.50
Ultrasonic bath	1.2	12.0 \pm 0.45

The obtained yellow needles were characterized by the spectral data (GC-MS, UV; IR, PMR and ^{13}C NMR) as well as a comparison with the literature reports^{10,48,51} allowing to identify the product as 5,7-dihydroxy-8-methoxyflavone or wogonin with over 95% of purity directly from the extracts (Fig 1). Thus, the GC-MS analysis indicated the presence of a single major component; its retention time was 20.98 min (Fig. 2a). The molecular formula was established as $\text{C}_{16}\text{H}_{12}\text{O}_5$ based on EIMS (Fig. 2b) that showed the presence of molecular ion (M^+) at m/z 284 Dalton (54.2 %), other important ions were detected at m/z 269 $[\text{M}-\text{Me}]^+$ (base peak, 100%), 283 $[\text{M}+1]^+$ (10%), 139 $[\text{A1}-\text{Me}-\text{CO}]^+$ (38.7%), 241 $[\text{M}^+-43:\text{Me}-\text{CO}]$ (26.7%), 69 (12.3), 103 (7), 254 $[\text{M}-\text{OCH}_2]^+$ (8%), 209 $[\text{M}-\text{Ph}]^+$ (6.4%), and 167 $[\text{A1}-\text{Me}]^+$ (6.9%). Fragmentation pattern was carried out according to Mabry and Markham.⁵² The 8-methoxy position was also established because of the fact that 6-methoxylated aglycones (flavones) still exhibit intensive fragments at both $[\text{M}-\text{CH}_3]^+$ (32-56%) and $[\text{M}-\text{H}_2\text{O}]^+$ (42-29.5%), whereas in case of 8-methoxy derivatives $[\text{M}-\text{CH}_3]^+$ ions have higher intensities (90-100%) than $[\text{M}]^+$ while $[\text{M}-\text{H}_2\text{O}]^+$ fragments show only very small intensities or are not present at all.⁵³

Other spectroscopic data and physical constants which confirm that the isolated compound was 5,7-dihydroxy-8-methoxyflavone or wogonin (Fig.1) are:

Yellow needles: mp 203–204 $^{\circ}\text{C}$.^{48,51}

UV spectrum (MeOH, λ_{max} , nm): 245, 276, 316.⁴⁷

IR spectrum (ν , cm^{-1}): 3460-3230 (OH), 2925 (OCH₃), 1661 (C=O), 1615 and 1580 (C=C).⁴⁷

PMR spectrum (250 MHz, DMSO- d_6 , δ , ppm, J/Hz, 0 = HMDS): 3.88 (3H, s, OCH₃), 6.32 (1H, s, H-3), 6.86 (1H, s, H-6), 7.60 (3H, m, H-2',4',6'), 8.01 (2H, d, H-3',5'), 12.39 (1H, s, 5-OH).^{10,47,48,54}



^{13}C NMR spectrum (62.9 MHz, $\text{DMSO}-d_6$, δ): 162.3 (C-2), 104.4 (C-3), 181.1 (C-4), 148.8 (C-5), 98.5 (C-6), 156.6 (C-7), 127.2 (C-8), 155.5 (C-9), 103.1 (C-10), 130.2 (C-1'), 125.4 (C-2'), 128.4 (C-3'), 131.1 (C-4'), 128.4 (C-5'), 125.4 (C-6'), 60.2 (OCH_3).^{10,37,48,54}

Data of the ^{13}C NMR is also coincident with the information on 5,7-dihydroxyflavonoids reported by Breitmaier and Voelter.⁵⁵ To summarize, the NMR data obtained is consistent with the chemical shifts of wogonin (Fig 3).

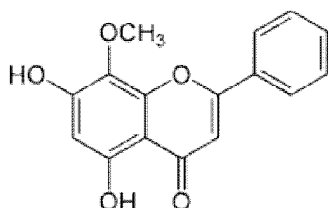


Figure 1: Structure of wogonin (5,7-dihydroxy-8-methoxyflavone)

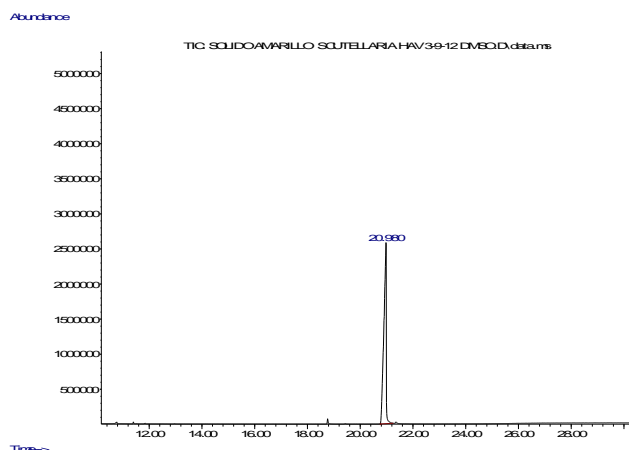


Figure 2a: GCMS chromatogram of wogonin extracted from *S. havanensis* aerial parts.

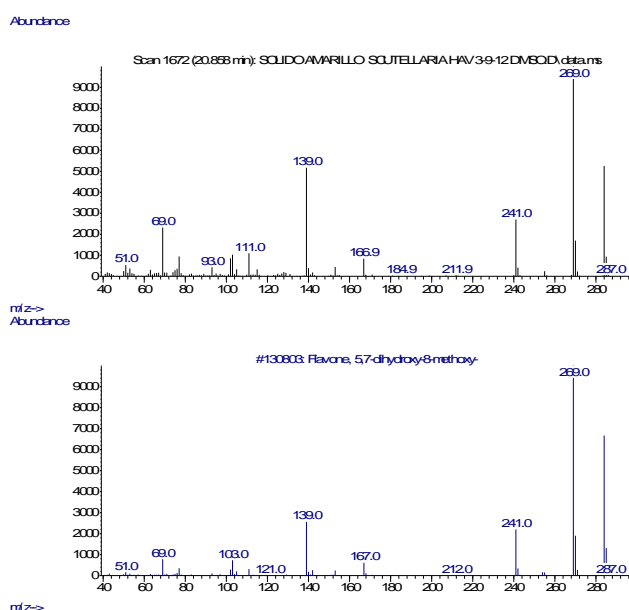


Fig. 2b: Mass spectrum of wogonin from *S. havanensis* (upper) compared with the mass spectrum of NIST 2011 library (lower).

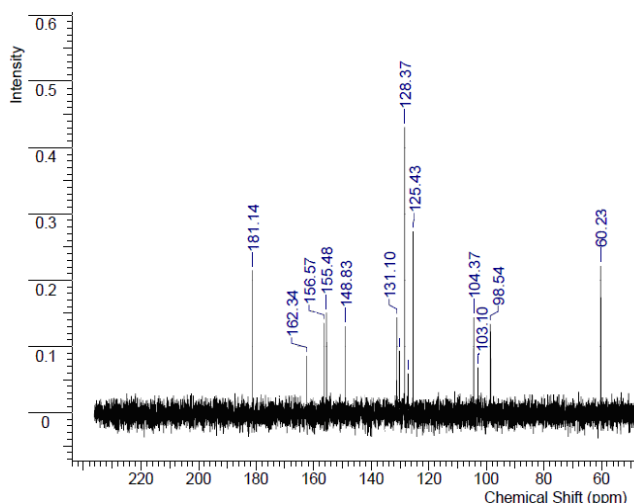


Figure 3: ^{13}C -NMR spectrum of wogonin extracted from *S. havanensis* aerial parts.

CONCLUSION

Two fast, selective and ship methods for the isolation of pure wogonin with high yields, directly from the extracts of fresh plant material of *Scutellaria havanensis* were developed. The results of the present investigation indicated that the developed procedures are efficient methods for obtaining pure wogonin without using any chromatographic techniques directly from extracts. This compound is reported for the first time from the aerial parts of this specie that grow in Cuba. These results are a contribution to the chemical composition of this endemic Cuban plant and could support future research on its possible pharmacological effects.

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