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## Phytochemical and biological study of Aerial parts extracts of *Blackstonia grandiflora* (Viv.) Maire

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### ABSTRACT

*Blackstonia grandiflora* (Viv.) Maire is a medicinal plant used in Algerian traditional medicine as a natural remedy to treat fever. Two compounds were isolated from aerial parts of *Blackstonia grandiflora*, halenaquinone (1) and gentiopicroside (2). The structures of the isolated compounds were determined by CPG-MS, 1D- and 2D-NMR techniques. The dichloromethane, ethyl acetate and *n*-butanol (CH<sub>2</sub>Cl<sub>2</sub>, EtOAc and *n*-BuOH respectively) extracts were evaluated for *in vitro* antibacterial activity and to determine the zone inhibition of extracts on some bacterial strains, the antibacterial activity were tested against two gram negative (*Pseudomonas aeruginosa*, *Escherichia coli*) and two gram positive (*Staphylococcus aureus*, *Bacillus sp*) bacterial strains using diffusion method. The results indicated a good inhibition of EtOAc extract on the growth of gram negative bacteria. Furthermore, CH<sub>2</sub>Cl<sub>2</sub> extract showed a significant inhibitory effect against gram positive bacteria.

**Keywords:** xanthenes; secoiridoids glycosides; *Blackstonia grandiflora*, Anti bacterial activity

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### INTRODUCTION

The Gentianaceae, or Gentian family, is distributed worldwide with approximately 100 genera and about 1,800 species that include monocarpic and perennial herbs, shrubs, trees, and lianes, with terrestrial and epiphytic representatives. The plants are diverse in habit, the majority being herbaceous. *Gentiana* (360 species), *Gentianella* (250 species), and *Swertia* (135 species) are the three largest genera [1].

The etymology of the name Gentian comes from Gentius, the second-century BC king of Illyria who has discovered the antipyretic properties of Gentians [2]. The Gentianaceae have been widely used in traditional medicine and also as constituents in bitters and similar concoctions [3, 4]. They contain many species with interesting pharmacological proprieties because of their secondary metabolites. They have also been identified as chemotaxonomic markers; among the natural compounds of this family there are iridoids, xanthenes such as mangiferine, and C-glucoflavonoids [5, 6].

The *Blackstonia* genus belongs to the family Gentianaceae; the genus comprises four species: *Blackstonia acuminata* (Koch & Ziz) Domin, *Blackstonia grandiflora* (Viv.) Maire, *Blackstonia imperfoliata* Samp and *Blackstonia perfoliata* Huds; they are distributed in Mediterranean area and western to central European. The species of *Blackstonia* grow in at least temporarily humid patches in open places [7]. The genus has not been deeply analyzed from a pharmacological and phytochemical point of view; reports about their chemical composition are

very few. The biologically active components: flavonoid glycosides [8, 9], xanthenes and secoiridoid glycosides [10, 11] were isolated from entire plants of *B. perfoliata*.

*Blackstonia grandiflora* (Viv.) Maire is annual plant, 20-40cm high, stems single, erect, simple or ramified. In Algeria, this plant is growing in the forests, damp areas and in the high plateaus. It is widespread in northern Algeria [12]. The aerial parts of *B. grandiflora* used in Algeria as a traditional remedy for fever and it is also known as a tonic [13]. However, no phytochemical or biological studies were carried out in this regard. In previous report, we demonstrated that the aerial parts of *Blackstonia grandiflora* extracts have a potent antiglycation activity against advanced glycation endproducts (AGEs) formation [14]

The present study is a report of phytochemical investigation and antibacterial activity of the aerial parts extracts of *Blackstonia grandiflora* yielded four compounds were isolated and identified, halenaquinone **1** and gentiopicroside **2**, it is worth mentioning that this is the first phytochemical and biological studies carried out on *Blackstonia grandiflora* (Viv.) Maire.

## MATERIALS AND METHODS

### Plant material

Aerial parts of *B. grandiflora* (Gentianaceae) were collected during the flowering phase in may 2014 from El-kala, in the extreme north-east of the Algeria. Dr Gerard De Belair (Department of biology, Annaba University, Algeria) ascertained botanical identity of the plant and voucher specimen was deposited in the Herbarium of our laboratory.

### Extraction, isolation and identification:

The aerial parts were dried and pulverized to a coarse powder. The powdered plant material (500 g) was extracted three times with 70 % (V/V) aqueous methanol at room temperature overnight. The extracts were combined and concentrated under reduced pressure on a rotary evaporator and dissolved in distilled water (500 ml). The resulting solution was extracted successively with (3 × 300 ml) dichloromethane, ethyl acetate (3 × 300 ml) and *n*-butanol (3 × 300 ml). The organic phases were dried with Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated in vacuum at room temperature to obtain dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>), ethyl acetate (EtOAc), *n*-butanol (BuOH) and the remaining hydromethanolic (MeOH) extracts

The CH<sub>2</sub>Cl<sub>2</sub> extract (0,86 g) was subjected to silica gel column chromatography using a gradient elution of cyclohexane-CH<sub>2</sub>Cl<sub>2</sub>-EtOAc to give sixteen fractions, **A-P** of 50 ml each. A solid crystals (2,7 mg) in fraction **F** were investigated by gas chromatography-mass spectroscopy (CPG-MS) method to give a peak was determinate Halenaquinone **1**.

The EtOAc extract (2,144 g) was chromatographed on a column of silica gel and eluted with a gradient elution of cyclohexane-CH<sub>2</sub>Cl<sub>2</sub>-EtOAc with increasing polarity to give twelve fractions, **A-K** of 100 ml each. Fraction **I** (128,8 mg) was applied to a silica gel column using CHCl<sub>3</sub>: MeOH: acetone (15:2:1) mixtures to give five fractions (**I-1** → **I-5**). Fraction **I-3** (36,2 mg) was purified by Sephadex LH-20 using MeOH to yield Gentiopicroside **2** (32,6 mg).

### Antibacterial assay

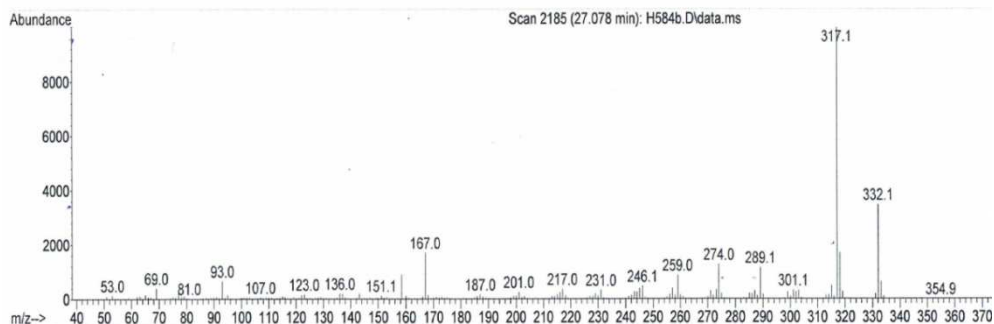
#### Biological material

All of the bacteria, standard strains [*Escherichia coli* ATCC 25922 (*E. coli*), *Staphylococcus aureus* ATCC 29213 (*S. aureus*), *Bacillus sp.*] were obtained from Laboratory of Mycology, Biotechnology and Microbial Activity (*LaMyBAM*), University Constantine 1, Algeria. Whereas, *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*) was obtained from Bacteriology Laboratory Constantine University Hospital (C.H.U).

#### Biological test

The extracts (CH<sub>2</sub>Cl<sub>2</sub>, EtOAc, *n*-BuOH) were used to investigate the antibacterial activity of *B. grandiflora* using the disk diffusion method [15]. The bacterial strains were first cultivated on Mueller-Hinton agar at 37 °C for 24 h prior to seeding on to the nutrient agar.

A sterile 6 mm diameter filter disk (Whatman No. 3 filter paper) was placed on the infusion agar seed with bacteria, and each extract suspended in ethanol 60% was dropped on to each paper disk (40  $\mu$ L per disk) for all the concentrations (2.00 mg/ml, 1.00 mg/ml, 0.50 mg/ml, 0.25 mg/ml). The treated Petri dish was kept at 4 °C for 1 h, and incubated at 37 °C for 24 h. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the disk. Each experiment was carried out in triplicate and the results were expressed as mean $\pm$ SD. The antibacterial activity of the plant extracts is depicted in **Table 1**.

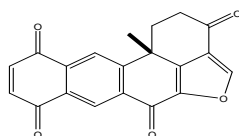


## RESULTS AND DISCUSSION

### Structures elucidation

The  $\text{CH}_2\text{Cl}_2$  extract of the aerial parts of *Blackstonia grandiflora* was separated by silica gel column chromatography (CC) to yield a known compound including pentacyclic quinone (Helenaquinone **1**).

**Compound 1:** Helenaquinone **1**; yellow – whitish crystals. The GC-MS analysis revealed the presence of two volatile compounds from the EtOAc fraction of *Blackstonia grandiflora*. The major constituent was detected by CPG/MS as Helenaquinone **1** (RT: 27.07 min) (**figure 1** and **2**). Identification of compound based on comparison of CPG retention time and mass spectra data described in the library Wiley 275 (1995). The formula of compound **1** was determined as  $\text{C}_{20}\text{H}_{12}\text{O}_5$  by CPG-MS showing a molecular ion peak at  $m/z$  332 (34.04 %). The formation of a strong ion at  $m/e$  317 (base peak) [M-15, 95.75 %] due to loss of a  $\text{CH}_3$  group and at  $m/e$  274 [M-15-44] lead to loss of  $\text{CH}_3$  and  $\text{CO}_2$  groups respectively. Another fragment at  $m/e$  289.1 [M-43] was obtained by loss of  $\text{CH}_3\text{CO}$  and the presence of the fragment at  $m/e$  301.1 [M-31] shows the loss of  $\text{CH}_3\text{O}$  group. These peaks were compared to those reported in the literature [16].



**Figure 1:** GC-MS chromatogram and mass spectra of (12bs)-12b-methyl-1H-tetrapheno[5,4-bc]furan-3,6,8,11(2h,12h)-tetrone (Helenaquinone **1**)

Phytochemical investigation of the EtOAc extract of *Blackstonia grandiflora* led to isolation of a secoiridoid glycoside, named Gentiopicroside **2**.

**Compound 2:** (3S,4R)-4-ethenyl-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-4,6-dihydro-3H-pyrano[3,4-c]pyran-8-one (Gentiopicroside **2**, **figure 2**); Amorphous,  $\text{C}_{16}\text{H}_{20}\text{O}_9$ , **MW:** 356, **EIM S:**  $m/z$  357 [M+H]<sup>+</sup>, <sup>1</sup>H-NMR (MeOD, 600 MHz,  $\delta$  in ppm) 7.35 (1H, s, H-1), 5.65 (1H, ddd,  $J = 16.24, 10.19, 6.89$  Hz, H-11), 5.56 (1H, d,  $J = 3.58$  Hz, H-3), 5.51 (1H, m, H-6), 5.13 (1H, d,  $J = 17.52$  Hz, H-10a), 5.10 (1H, d,  $J = 9.63$ , H-10b), 4.95 (1H, m, H-7a), 4.91 (1H, m, H-7b), 4.55 (1H, d,  $J = 7.91$  Hz, H-1'), 3.8 (1H, dd,  $J = 12.27, 1.32$  Hz, H-6'a), 3.55 (1H, dd,  $J = 11.98, 5.58$  Hz, H-6'b), 3.28 (1H, t,  $J = 8.3$  Hz, H-3'), 3.24 (1H, t, H-4), 3.21 (1H, m, H-5'), 3.17 (1H, t,  $J = 8.87$  Hz, H-4'), 3.076 (1H, dd,  $J = 10.2, 8.4$  Hz, H-2'); <sup>13</sup>C-NMR (MeOD, 600 MHz,  $\delta$  in ppm) 166.34 (C-9), 150.66 (C-1), 135.01 (C-11), 127.06 (C-5), 118.56 (C-12), 117.22 (C-6), 104.95 (C-10), 100.20 (C-1'), 98.52 (C-3), 78.42 (C-3'), 77.97 (C-5'), 74.54 (C-2'), 71.52 (C-4'), 70.94 (C-7), 62.77 (C-6'), 46.61 (C-4). Gentiopicroside was further supported structurally by 2D NMR studies (H-H COSY, HSQC and HMBC). <sup>13</sup>C and <sup>1</sup>H-NMR data are in agreement with the literature [17].

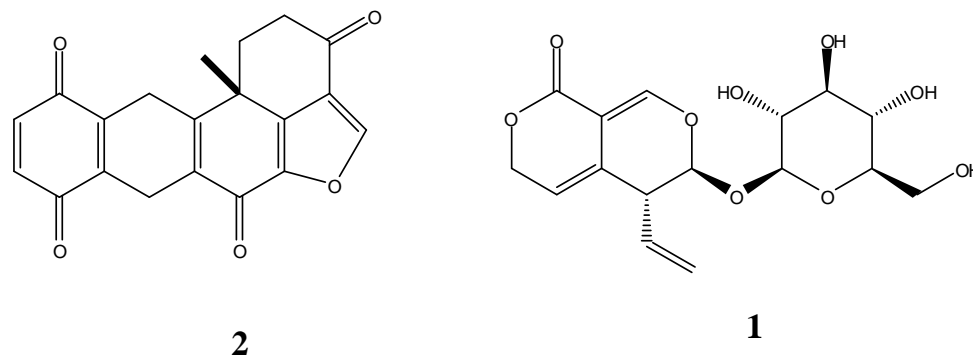


Figure 2: Chemical structures of compounds 1 and 2 isolated from *Blackstonia grandiflora*

### Antibacterial assay

In-vitro antibacterial effectiveness of the extracts of *B. grandiflora* against the four strains [*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Bacillus sp.*, *Pseudomonas aeruginosa* ATCC 27853] was evaluated via the determination of the surrounding inhibition zones; the inhibition zones diameters measured in different samples were shown in **Table 1**.

Table1: Antibacterial properties of CH<sub>2</sub>Cl<sub>2</sub>, EtOAc and n-ButOH extracts of *Blackstonia grandiflora* at four different concentrations

Strains tested	Extracts	Antimicrobial Activity *				Control	
		2 mg/ml	1mg /ml	0,5mg/ml	0,25/ml		
Gram -	<i>E. coli</i>	CH <sub>2</sub> Cl <sub>2</sub>	12,50±0,50	12,66±0,76	12,5±0,50	12,16±0,28	-
		EtOAc	12,41±0,52	12,00±0,00	11,83±0,76	11,08±1,12	-
		n-ButOH	12,16±1,25	12,33±0,57	12,16±1,2	11,33±1,15	-
	<i>P. aeruginosa</i>	CH <sub>2</sub> Cl <sub>2</sub>	-	-	-	-	-
		EtOAc	13,00±0,50	12,16±0,28	11,83±0,76	11,50±0,50	-
		n-ButOH	-	-	-	-	-
Gram+	<i>Bacillus sp</i>	CH <sub>2</sub> Cl <sub>2</sub>	14,16±0,28	13,16±1,25	11,5±1,32	10,16±1,04	-
		EtOAc	11,33±1,52	11,16±1,25	11,66±0,76	09,50±0,50	-
		n-ButOH	10,86±1,60	10,33±1,15	08,50±0,50	08,50±0,50	-
	<i>S. aureus</i>	CH <sub>2</sub> Cl <sub>2</sub>	10,5±1,00	11,00±1,50	-	-	-
		EtOAc	11,00±1,00	10,50±0,50	10,5±0,50	-	-
		n-ButOH	12,5±0,50	11,66±0,75	10,83±0,76	10,00±1,73	-

\*measured by the diameter of zone of inhibition in mm ± SD, the control: Ethanol 60%, (-): no detected activity

The CH<sub>2</sub>Cl<sub>2</sub> extract demonstrated the strong inhibitory activity against three resistant strains tested (*E.coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Bacillus sp*) especially gram positive bacteria with inhibition diameter of 14,16 mm for *Bacillus sp* and gram negative bacteria with inhibition diameter of 12, 50 mm for *E.coli* ATCC 25922; the both at high concentration of 2 mg/ml. While the CH<sub>2</sub>Cl<sub>2</sub> extract of *B. grandiflora* showed no-inhibitory activity against *Pseudomonas aeruginosa* ATCC 27853 (gram negative).

The EtOAc extract inhibited the growth of four resistant strains tested. At a concentration 2 mg/ml of EtOAc extract has a significant effect against *E.coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Bacillus sp*, with inhibition zones ranging from 11 to 12,41 mm in diameter. In contrast, the extract showed a strong inhibitory action against *Pseudomonas aeruginosa* ATCC 27853 (Gram negative) with inhibition diameter of 13 mm.

The n-BuOH extract showed an inhibition zone varied from 10,5 mm to 12,5 mm with higher concentration of 2 mg/ml against three resistant strains tested (*E.coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Bacillus*), whereas the extract showed no activity against *Pseudomonas aeruginosa* ATCC 27853.

Based on the above results, we have highlighted that the EtOAc extract of *B. grandiflora* showed the most pronounced antibacterial activities against the four bacterial strains especially against gram negative strains. On the other hand, CH<sub>2</sub>Cl<sub>2</sub> and n-ButOH extracts showed no inhibitory activity against *Pseudomonas aeruginosa* ATCC 27853 (Gram negative).

The activity of CH<sub>2</sub>Cl<sub>2</sub> extract from *B. grandiflora* against gram positive strains (*Staphylococcus aureus* ATCC 29213 and *Bacillus sp*) may be attributed to polyphenol content as reported by some authors [18]. Among the phenolic compounds with known antibacterial activity xanthenes [19]. Xanthenes are endowed with a broad spectrum of biological activities; they have a good antibacterial activity especially against *Staphylococcus aureus*

that is resistant to antibiotic methicillin, and responsible for many infections such as nosocomial infections [20, 21]. Recently, several studies demonstrated that the hydroxyl xanthenes exhibited a strong antibacterial activity against gram positive strains [22-24]. The antibacterial potential of hydroxyl xanthenes correlated significantly with their ability to generate reactive oxygen species such as singlet oxygen, with induced photoinactivation of bacteria [25]. Moreover, Gram negative bacteria are inherently resistant to inactivation by these compounds due to the barrier properties of the outer membrane that prevents the necessary localization [22].

Secoiridoid glycosides are present in various traditional medicine preparations and are reported to have variety pharmacological proprieties including antibacterial activity [26-28]. According to Kumarasamy (2003), two secoiridoid glycosides isolated from *Centaureum erythraea* Rafn have shown the inhibition growth gram negative strains (*Bacillus cereus*, *Bacillus subtilis*, *Citrobacter freundii* and *Escherichia coli*) [29]. Whereas, secoiridoid glycosides isolated from *Centaureum pulchellum* extracts demonstrated a strong antibacterial activity [30].

In the light of our results, the EtOAc extract has significant antibacterial activity especially against gram negative strains: *Pseudomonas aeruginosa* and *E.coli* with inhibition diameters of 13 mm and 12.50 mm respectively at high concentration 2 mg/ml. Antibacterial potential of the extract probably related to the presence of gentiopicroside 2 isolated as major compound from aerial part extract of *Blackstonia grandiflora*. gentiopicroside 2 is principal component found in many species of Gentianaceae family such as *Gentiana Rigescens* Franch.; *Gentiana lutea* L and *Gentiana scabra* Bunge [31-33]. According to several studies demonstrated that gentiopicroside 2 showed high antibacterial efficiency [29, 34, 35]. These authors found that the compound inhibit the growth of 12 of 17 pathogenic bacterial species tested. The gentiopicroside 2 was showed most active against *Serratia mercerscens* (gram negative) with MIC value estimated at  $6.3 \times 10^{-3}$  mg/ml. The effect due to radical-scavenging activity when compared with the activity of quercetin, which served as a control [29]

## CONCLUSION

In the present study, the phytochemical evaluation of aerial parts of *Blackstonia grandiflora* extracts lead to isolation of halenaquinone (1) and gentiopicroside (2). The EtOAc extract showed the most potent antibacterial effect especially against gram negative bacterial species tested. On the other hand, the CH<sub>2</sub>Cl<sub>2</sub> extract exhibited a good inhibition on the growth of gram positive bacteria. The mechanism of anti bacterial action of secondary metabolites of *Blackstonia grandiflora* (Viv.) Maire involves their ability in photoinactivation of bacteria.

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