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Phytochemical study of Algerian *Bupleurum atlanticum* Murb (Apiaceae)

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ABSTRACT

Phytochemical investigation of chloroform soluble parts of the aqueous-EtOH extract of the aerial parts of Bupleurum atlanticum Murb*, led to isolation of three compounds: scoparone* ***1,*** *isoscopoletin* ***2*** *and chinensin* ***3*** *by the use of different chromatographic techniques. Structure elucidation of these compounds was achieved by UV, IR, EI-MS and NMR spectroscopy: 1H, 13C and 2D NMR.*

Key words: *Bupleurum atlanticum,* Apiaceae, coumarins, lignans.

INTRODUCTION

Bupleurum is a genus of family Umbelliferae (Apiaceae), comprising about 200 species and primarily located in the Northern Hemisphere, Eurasia, and North Africa. It is represented by only14 species in north Africa [1].

Under the name of *Chaihu* (*Saiko* in Japanese and *Shiho* in Korean), the roots of several species from the genus have been frequently used in the prescriptions of oriental traditional medicine, for the treatment of common cold with fever, influenza, inflammation, hepatitis, malaria, and also menopausal syndrome in China for 2000 years[2].

Many species from the genus have been studied chemically, resulting in the isolation of approximately 120 derivatives of saikosaponins, more than 50 lignans, as well as a number of coumarins, flavonoids, polyacetylenes, polysaccharides, sterols, phenylpropanoids, and organic acids[3].

Its richness in secondary metabolites explains its traditional use and rationalize its various therapeutic activities scientifically proven such as cytotoxicity, anti-inflammatory, antiproliferative and hepatoprotective activities antiulcer, antibacterial and antifungal effects [4].

As part of a systematic examination and valorization of the Algerian species, we have investigated *B, atlanticum,* which is endemic to Algeria and Morocco [5] and has not been previously investigated. This work concerns the phytochemical study of the chloroform soluble part of the aqueous ethanol extract of the leaves and flowers.

In this paper, we report the isolation and the structure elucidation of three known secondary metabolites **1-3** (Figure 1).To the best of our knowledge, this is the first report on the isolation of these compounds from *B.atlanticum* species.

MATERIALS AND METHODS

Plant material

Bupleurum atlanticum was collected during the flowering phase in May, in southern East of Algeria, and was authenticated by Dr. A. Hamchi (gerent in Belezma national park). A voucher specimen has been deposited at the Herbarium of Belezma Park.

Extraction and Isolation

Air-dried leaves and flowers (500 g) of *Bupleurum atlanticum* were macerated at room temperature with EtOH–H_2O (70:30, v/v) for 24 h, three times. After filtration, the filtrate was concentrated and dissolved in H_2O (200 ml) under magnetic agitation. The resulting solution was filtered and successively extracted with hexane, $CHCl_3$, EtOAc and *n*-butanol. The organic phases were dried with Na_2SO_4 filtered and concentrated *in vacuo* at room temperature to obtain the following extracts: hexane (4.5g), chloroform (9.00 g), EtOAc (1.56g) and *n*-butanol (25 g).

A part of the chloroform extract (5 g) was fractionated by CC on silica gel using petroleum ether, dichloromethane, and methanol with increasing polarity to yield 16 fractions (F1–F16) obtained by combining the eluates on the basis of TLC analysis. Fraction 8,9 and 12 are rechromatographed separately on silica gel flash column using hexane/ EtOAc and then the purification was achieved on preparative plates of silica gel to yield the three compounds.

Structural identification

Nuclear Magnetic Resonance: NMR spectra (^{1}H-NMR, ^{13}CNMR, COSY, HSQC and HMBC) for all the isolated compounds were recorded in deuterated chloroform (99.8% $CDCl_3$), on a Bruker 400 MHz NMR spectrometer. Mass spectrometry EI (Electronic Impact) on a Jeol JMS 600 mass spectrometer. IR spectra were registered on KBr pellets on a Shimadzu FT/IR-460 spectrophotometer and UV were registered in Methanol on a Shimadzu Mini 1240 spectrophotometer.

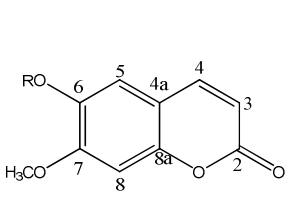
Compound (1): $C_{11}H_{10}O_4$, mp 145^0 C; UV (λmax, MeOH, nm): 230, 250, 290 and 345. IR(KBr, cm^{-1}): 1734(C=O), 1633 (C=C), 1513 et 1449 (C=C aromatics).
^{1}H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 3,92 (3H, s OCH_3 -7, 3,94 (3H, s = OCH_3 -6), 6,28 (1H d, J = 9,3 H-3). 6,84 (1H s, H-8), 6,85 (1H s, H-5). 7,62 (1H d, J = 9,3 , H-4). ^{13}C RMN : 161,3 (C-2), 113,5 (C-3), 143,2 (C-4), 111,4 (C-4a), 107,9 (C-5), 146,3 (C-6), 152,8 (C-7), 100,0 (C-8), 150,0 (C-8a), 56,3 (2 OCH_3)

Characterized as 6,7-dimethyl coumarin (*scoparone*) [6].

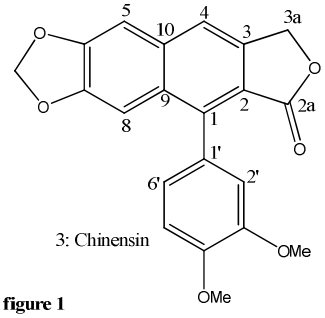
Compound (2): $C_{10}H_8O_4$, Yellow needles mp 184°C; UV (λmax, MeOH, nm): 230, 255, 295 and 350. IR (KBr, cm^{-1}): 1712 (C=O), 3346 (OH), 1617 (C=C), 1514 et 1565 (C=C aromatics); ^{1}H NMR (400 MHz, $CDCl_3$δ, ppm, J/Hz): 3,95 (3H, s, OCH_3). 5,54 (1H, s, OH). 6,26 (1H, d, J = 9,4, H-3). 6,81 (1H, s, H-8). 6,94 (1H s, H-5). 7,57 (1H, d, J = 9,4, H-4). ^{13}C RMN : 161,4 (C-2), 113,9 (C-3), 143,3 (C-4), 112,2 (C-4a), 111,0 (C-5), 142,6 (C-6), 150,0 (C-7), 99,3 (C-8), 149,2 (C-8a), 56,4 (OCH_3).
EI-MS (rel. int): m/z 192,0 (M$^+$, 100%) 164 (43,4%), 149 (70%), 121(16,6%).
Characterized as 7-methoxy 6-hydroxy coumarin (*isoscopoletin*) [7].

Compound (3): $C_{21}H_{16}O_6$, mp 224°C; UV (λmax, MeOH, nm): 220, 255, 310 and 350. IR (KBr, cm^{-1}): 1762 (C=O), 1615 et 1467 (C=C aromatic), 934 (methylenedioxy); ^{1}H NMR (400 MHz, $CDCl_3$,δ, ppm, J/Hz): 3,85 (3H, s,= OCH_3- 3'). 3,95 (3H s, OCH_3- 4'). 5,35 (2H, s, H3a, H3a'). 6,09 (1H, s , CH_2O_2). 7,69 (1H, s, H-4). 7,20 (1H, s, H-5). 7,12 (1H, s, H-8). 6,86 (1H, d, J = 1,9, H-2'). 6,91 (1H dd, J1 = 8,2 and J2 = 1,9 Hz, H-6'). 7,03 (1H, d, J = 8,2, H-5'). ^{13}C RMN : 140,4 (C-1), 118,7 (C-2), 169,7 (C-2a), 67,8 (C-3a), 139,8 (C-3), 118,9 (C-4), 103,6 (C-5), 149,9/148,9 (C-6), 148,9/ 149,9 (C-7), 103,7 (C-8), 130,5 (C-9), 134,6 (C-10), 127,1 (C-1'), 113,3 (C-2'), 148,5 (C-3'), 148,6 (C-4'), 110,7 (C-5'), 122,4 (C-6'), 55,9 (OCH_3 -3'), 55,8 (OCH_3 -4'), 101,7 (OCH_2O).
EI-MS (rel. int): m/z 349 (M$^+$, 100%), 321(2,7%), 176 (19,5 %), 163 (37%).

Characterized as (5-(3,4-Dimethoxyphenyl)furo[3',4':6,7]naphtho[2,3-d]-1,3-dioxol-6(8H)-one;6,7-(Epoxy methanoxy)-9-(3,4-dimethoxyphenyl)-1,3-dihydronaphtho[2,3-c]furan-1-one (*Chinensin*). [8].



1: R = CH_3 scoparone
2: R = H isoscopoletin



3: Chinensin

figure 1

CONCLUSION

Bupleurum genus plants are used as herbal medicines for the treatment of various diseases.

The present study carried out for the first time on Algerian *Bupleurum atlanticum* species, resulted in isolation and identification of coumarins and lignans. These results were in accordance with those reported for *Bupleurum* species.

These chemical compounds, which are known for their strong antioxidant activity, are likely to be responsible for medicinal significance of the genus. Therefore, this plant should be valued and subject to more detailed further deeper studies.

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