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ORIGINAL ARTICLE

Chemical constituents of the roots of Algerian *Bunium incrassatum* and evaluation of its antimicrobial activity

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Abstract In this study we investigated the chemical composition of the roots of *Bunium incrassatum* growing in Algeria, two coumarins, β-sitosterol, sucrose and oleic acid were isolated from methylene chloride:methanol (1/1) extract of the roots of this species. Furthermore, antimicrobial activity of the crude extract was evaluated using agar diffusion method. The antimicrobial results showed that the crude extract had a great potential antimicrobial activity against all the test microorganisms especially fungal strains.

1. Introduction

Medicinal and aromatic plants have been used for many centuries and still popular in today's alternative therapies. Herbs often represented the original sources of most drugs. *Bunium incrassatum* (Boiss.) Batt. & Trab., a medicinal plant belonging to the Apiaceae family, is widely distributed in the east parts of Algeria and called "*Talghouda*" (Quezel and Santa, 1963).

The genus *Bunium* consists of seven species in Algerian flora, four of which are endemic (Quezel and Santa, 1963). This genus is close to *Carum*. *Bunium* and *Carum* are two of the most important aromatic and medicinal plants, whose seeds and essential oils have been used in food and medicine all over the world for so long (Jassbi et al., 2005).

Bunium incrassatum is an economically important medicinal plant growing in the north of Algeria. The roots of this plant are quite nutritious and usually eaten as potato. There are some preparations in case it is used as an astringent ant diarrhoeal for their virtues, but almost always prefer to consume it directly without saying that properly washed and stripped of the parties.

In the indigenous system of medicines, dried and powdered tubers are regarded as astringent and anti diarrheiques and found to be useful against inflammatory hemorrhoids. In addition, this plant is used for bronchitis and cough treatment.

The chemistry of this species has not been studied before. Previous phytochemical studies on the genus *Bunium* revealed the presence of coumarins (Appendino et al., 1994), sesquiterpenes (Appendino et al., 1991) and especially essential oils

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(monoterppenoids) as frequent metabolites (Salehi et al., 2008). Furthermore, it is well documented that the essential oils and extracts from some *Bunium* spp possess antihistaminic, antibacterial and antifungal effects (Boskabady and Moghaddas, 2004) besides antioxidant activities (Shahsavari et al., 2008).

In this study, we investigated for the first time the constituents of the roots of *B. incrassatum* and their Antimicrobial activity. To the best of our knowledge there are no reports in the literature regarding the chemical constituents or the biological activities of the above mentioned plant.

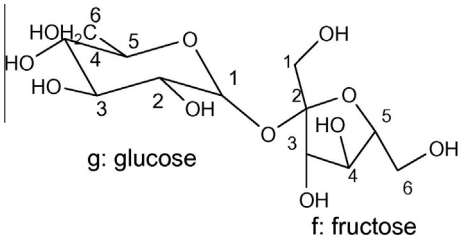

Figure 1 Sucrose.

2. Experimental

2.1. Plant materials

Roots of *B. incrassatum* were collected from Souk Naamane, in the vicinity of Oum El bouaghi (east of Algeria) in May 2007, and the plant was identified by Dr. Amar Zellagui, Department of Biology, University of Oum El bouaghi. A voucher specimen has been deposited in the Herbarium of department of biology, University of Constantine under the code number ZA 103.

All of the clinical stains: *Escherichia coli*, *Staphylococcus aureus, Staphylococcus epidermis, Proteus merabilis, Streptococcus pyogenes, Klebsiella oxytoca, Enterobacter* sp., *Pseudomonas aerogenosa* and *Seratia* sp. were obtained from Bacteriology Laboratory Constantine University Hospital (C.H.U), while the fungi strains Aspergillus flavus, *Penicilium candidum* and *Candida albicans* were isolated in microbiology laboratory, department of biology, Oum El Bouaghi University.

Roots of *B. incrassatum* (800 g) were crushed and extracted with CH_2Cl_2–MeOH (1:1) at room temperature. The extract was concentrated in vacuo to obtain a residue. The residue was fractionated on silica gel CC (3 × 125 cm), eluted with hexane, followed by a gradient of hexane-CH_2Cl_2 up to 100% CH_2Cl_2 and CH_2Cl_2–MeOH.

The extract (1:1) gives a precipitate, which was washed with MeOH to give compound **1**.

The fraction n-hexane-CH_2Cl_2 (25:75) afforded compound **2** also by precipitation.

The fraction n-hexane-CH_2Cl_2 (50:50) afforded **3** and **4** and a precipitate, which was washed with methanol three times to afford compound **5**.

These compounds were identified by using UV, MS, H-^{1}NMR and C-^{13}NMR experiments, and with comparison of their spectroscopic properties with literature data.

2.1.1. Compound *1*: Sucrose

Sucrose, a disaccharide formed from glucose linked to fructose, with the glycoside linkage between the anomeric proton of glucose (α configuration) and the anomeric proton of fructose (β configuration) Fig. 1. It was the major compound of CH_2Cl_2–MeOH (1:1) extract. Its structure was determined by using H-^{1}NMR and C-^{13}NMR experiments.

Compound was obtained as white solid (150 mg); m.p. 186 °C. Literature. 186 °C.

^{1}H-NMR (250 MHz, D_2O) δ: 5.25 (1H, d, J = 3.80 Hz, H-g1) small coupling constant (equatorial–axial coupling), 3.35 (1H, d, J = 3.80, H-g2) because it should be double doublet which broken down into two couplings: a doublet coupling of 9.19 Hz is further split by another doublet coupling of 3.80 Hz which matches the H-g1doublet, 3.58 (1H, t, J = 9.19 Hz, H-g3), 3.27 (1H, t, J = 9.19 Hz, H-g4), the H-g3 and H-g4 are triplets with large coupling constants because both are in axial positions with one neighbour on each side, 4.01 (1H, d, J = 8.44 Hz, H-f3), 3.58 (1H, t, J = 8.36 Hz, H-f4).

A sharp singlet at 3.48 ppm corresponds to the only CH_2 group (H-f1). The signals between 3.60, 3.75 ppm (6 protons) must include the glucose CH_2OH(H-g6), the other fructose CH_2OH (H-f6) and the more complex H-g5 and H-f5 signals.

^{13}C-NMR (62.5 MHz, D_2O) δ: 92.0 (C-g1), 70.9 (C-g2), 72.4 (C-g3), 69.00 (C-g4), 72.2 (C-g5), 59.9 (C-g6), 61.1 (C-f1), 103.5 (C-f2), 76.2 (C-f3), 73.8 (C-f4), 81.2 (C-f5), 62.2 (C-f6).

This results match with Casset et al. (1995) and Jacobsen (2007).

2.1.2. Compound *2*: Oleic acid

Oleic acid contains 18 carbons, having the empirical formula $C_{18}H_{34}O_2$ and involves one double bond, placed symmetrically between the C-9 and C-10 carbon atoms and a carboxylic acid group at one end. Its IUPAC name is Cis-9-octadecenoic acid.

Compound was obtained as colourless oil (10 mg); m.p. 16.5 °C.

m/z: 181.24860 (cal. for $C_{18}H_{33}O_2$, 182.25197).

^{1}H-NMR (250 MHz, $CDCl_3$) δ: 0.98 (3H, t, J = 6.8 Hz, CH3-18), 2.36 (2H, t, J = 7.6 Hz, CH2-2), 5.35 (1H, m, H-9 and H-10).

^{13}C-NMR (62.5 MHz, $CDCl_3$) δ: 179.6 (C-1), 33.9 (C-2), 24.6 (C-3), 29.4 (C-4), 29.2 (C-5), 29.3 (C-6), 29.4 (C-7), 27.2 (C-8), 130.0 (C-9), 129.7 (C-10), 27.1 (C-11), 29.5 (C-12), 29.7 (C-13), 29.6 (C-14 and C-15), 31.9 (C-16), 24.6 (C-17), 14.1 (C-18).

The physical and spectral data showed complete agreement with Ascari et al. (2010).

2.1.3. Compound *3*: Scopoletin

It is obtained as a crystalline solid (8 mg); m.p. 202–204 °C. Literature 203–205 °C.

UV: λ_{max} (MeOH) nm: 252, 297, 344.

m/z: 193.0408 (cal. for $C_{10}H_8O_4$, 192.0422).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.79 (1H, d, J = 9.5 Hz, H-4), 6.89 (1H, s, H_8), 6.82 (1H, s,H_5), 6.24 (1H, d, J = 9.5 Hz, H_3), 3.92 (3H, s, 6-OCH_3).

^{13}C-NMR (100 MHz, $CDCl_3$) δ: 161.84 (C-2), 150.66 (C-7), 144.69 (C-6), 143.69 (C-4), 113.85 (C-3), 111.90 (C-10), 107.85 (C-5), 103.59 (C-8), 56.81 (6-OCH_3).

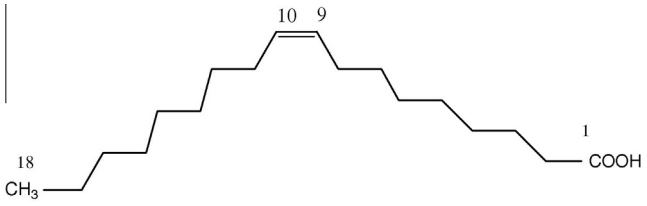

Figure 2 Oleic acid.

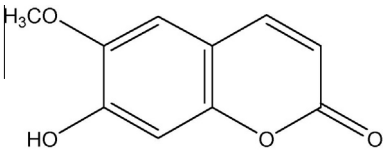

Figure 3 Scopoletin.

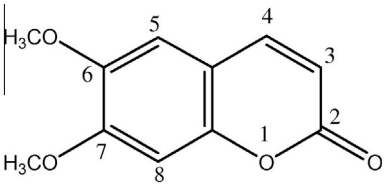

Figure 4 Scoparone.

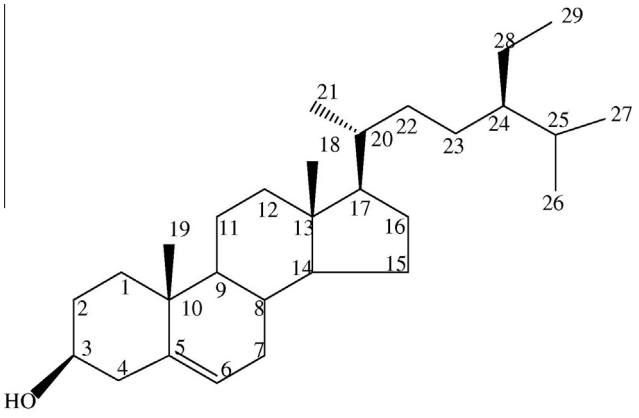

Figure 5 β-Sitosterol.

The physical and spectral data showed complete agreement with Vasconcelos et al. (1998).

*2.1.4. Compound **4**: Scoparone*

This compound obtained as a white solid (4 mg); m.p. 143–145 °C.

UV: (MeOH) λ_{max}: 292, 342 nm.

m/z: 207.12 (cal. for $C_{11}H_{10}O_4$, 206.11).

^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.61 (1H, d, J = 9.4 Hz, H-4), 6.84 (1H, s, H_8), 6.81 (1H, s, H_5), 6.25 (1H, d, J = 9.4 Hz, H_3), 3.94 (3H, s, 6-OCH_3), 3.91 (3H, s, 7-OCH_3).

The physical and spectral data showed complete agreement with Ref. (El-Demrdash and Dawidar, 2009).

*2.1.5. Compound **5**: β-sitosterol*

Compound is isolated as a Colorless powder (75 mg); m.p. 135–137 °C.

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 3.60 (1H, m, H_3), 5.39 (1H, m, H_6), 1.01 (3H, s, H_{18}), 0.68 (3H, s, H_{19}), 0.84 (3H, d, J = 6 Hz, H_{26}), 0.82 (3H, d, J = 6 Hz, H_{27}), 0.85 (3H, m, H_{29}), 0.92 (3H, d, J = 2.9 Hz, H_{21}).

^{13}C-NMR (75 MHz, $CDCl_3$) δ: 37.27 (C-1), 31.64 (C-2), 71.81 (C-3), 42.28 (C-4), 140.75 (C-5), 121.73 (C-6), 31.88 (C-7), 31.91 (C-8), 50.11 (C-9), 36.50 (C-10), 21.10 (C-11), 39.76 (C-12), 42.39 (C-13), 56.75 (C-14), 24.29 (C-15), 28.92 (C-16), 56.75 (C-17), 11.85 (C-18), 19.40 (C-19), 34.97 (C-20), 18.97 (C-21), 33.70 (C-22), 25.41 (C-23), 42.29 (C-24), 28.82 (C-25), 19.40 (C-26), 18.70 (C-27), 21.07 (C-28), 12.25 (C-29) (see Figs. 2–4).

The chemical structure of the β-sitosterol is shown in Fig. 5.

Our result is corresponding with Saxena and Albert (2005).

The Antimicrobial activity tests were carried out on crude extract (CH_2Cl_2/MeOH 1:1) using disk diffusion method (Carbonnelle et al., 1987) against nine human pathogenic bacteria, including Gram positive, Gram-negative bacteria *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Proteus merabilis*, *Streptococcus pyogenes*, *Pseudomonas aerogenosa*, *Klebsiella oxytoca*, *Enterobacter* sp., *and Seratia* sp. and three *fungi*; *Aspergillus flavus*, *Penicilium candidum* and *Candida albicans*.

The bacterial strains were first grown on Muller Hinton medium (MHI) at 37 °C for 24 h prior to seeding on to the nutrient agar but the fungi at 30 °C for 48 h.

A sterile 6-mm-diameter filter disk (Whatman paper no. 3) was placed on the infusion agar seeded with bacteria, and each extract suspended in water was dropped on to each paper disk (40 µl per disk) for all of prepared concentrations (8 mg/ml, 4 mg/ml, 2 mg/ml, 1 mg/ml, 0.5 mg/ml, 0.25 mg/

Table 1 Antimicrobial activity of crude extract (CH_2Cl_2/MeOH: 1/1) at different concentrations on nine strain bacteria.

Strains bacteria	1 mg/ml	2 mg/ml	4 mg/ml	8 mg/ml
E. coli	–	–	–	08.00 ± 1.47
Staphylococcus aureus	06.00 ± 0.00	13.00 ± 1.47	18.50 ± 1.15	20.33 ± 0.86
Staphylococcus epidemidis	–	–	12.66 ± 1.15	14.00 ± 02.00
Proteus mirabilis	–	–	–	07.25 ± 0.57
Streptococcus pyogenes	–	–	07.75 ± 0.95	11.00 ± 0.95
Pseudomonas aerogenosa	–	07.00 ± 01.00	13.00 ± 0.57	16.66 ± 01.15
Klebsiella oxytoca	–	–	08.0 ± 1.47	11.00 ± 01.15
Enterobacter sp.	–	–	07.0 ± 01.00	08.66 ± 01.15
Seratia sp.	–	–	06.0 ± 1.47	09.33 ± 0.57

Table 2 Antifungal activity of crude extract (CH_2Cl_2/MeOH: 1/1) at different concentrations on the three fungi.

Fungies	0.25 mg/ml	0.5 mg/ml	1 mg/ml	2 mg/ml	4 mg/ml	8 mg/ml
Aspergillus flavus	08.00 ± 0.57	12.75 ± 00	19.00 ± 0.57	30.5 ± 0.81	34.00 ± 1.80	35.25 ± 1.47
Penicilium candidum	–	06.00 ± 1.47	07.00 ± 0.86	10.75 ± 0.57	16.66 ± 0.57	23.25 ± 0.57
Candida albicans	–	–	–	–	08.50 ± 0.57	9.33 ± 0.00

ml). The treated Petri disks were 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 disks. Each experiment was carried out in triplicate.

The diffusion test was applied to twelve Gram-positive and Gram-negative microorganisms including three fungi. The results are summarized in Tables 1 and 2 which showed that the crude extract (CH_2Cl_2/MeOH: 1/1) from *Bunium incrassatum* prevented the growth of all the tested microorganisms with an inhibition zone medium diameter increasing proportionally with the concentration. The obtained inhibition varied from 6.00 to 20.33 mm with a highest inhibition zone recorded with *Staphylococcus aureus*. Nevertheless the fungi displayed very high inhibition diameter and varied from 08.00 to 35.25 mm overall with higher concentration of 8 mg/mL. To sum up, the crude extract containing the above compounds exhibited stronger activity against fungi than bacteria strains. The antimicrobial activity of the crude extract of *Bunium incrassatum* (Boiss.) Batt. & Trab. is certainly related to its chemical content such as coumarins.

3. Conclusion

Our study of the Algerian plant *Bunium incrassatum* (Boiss.) Batt. & Trab. led to the isolation and characterization of five compounds followed by the evaluation of antimicrobial activity for the first time.

These results reinforce the previous studies showing that the genus *Bunium* is considered a good source of coumarins. We would like to note here that scopoletin and scoparone were isolated from the first time of this genus.

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