Polyphenols contents, heavy metals analysis and in vitro antibacterial activity of extracts from *Cladanthus arabicus* and *Bubonium imbricatum* of Moroccan Origin

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**ABSTRACT**

Aim of this study was to evaluate polyphenols and major and trace elements of *Cladanthus arabicus* and *Bubonium imbricatum*, along with their in vitro antibacterial activity against six multidrug resistant *Enterobacteriaceae* (*Escherichia coli* S33/16, *E. coli* S34/16, *Proteus mirabilis* S32/16, *Klebsiella pneumoniae* S12/16, *Enterobacter cloacae* S5/16, and *Salmonella* sp S12/14). UV spectrophotometry, ultra-high-performance liquid chromatography coupled to mass spectrometry and inductively coupled plasma mass spectrometry were used to evaluate total polyphenol content, quali-quantitative profile of single polyphenols and inorganic elements of the extract. The antibacterial activity was investigated by standard methods. Twelve polyphenols were identified in both plants and these were more concentrated in *B. imbricatum* than *C. arabicus* extracts. High levels of minerals, essential trace elements and tolerable levels of heavy metals (Cd, As and Pb) were found. Furthermore, the extracts showed also a strong in vitro antibacterial activity, particularly versus *E. coli* S33/16 (MIC, 0.125 mg ml⁻¹).

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1. Introduction

Nowadays the development of health products from natural sources, that have demonstrated to exert beneficial effects for human and animal health, is a widely explored field (Tropea et al. 2013; Alesci et al. 2015; Certo et al., 2017; Costa et al., 2017; Gervasi et al. 2018). The benefic effects of plants may come from their essential oils (Aghraz et al. 2018; Bennameur et al. 2018; Pluchtová et al. 2018), as well as from several bioactive compounds, such as and polyphenols whose presence represent a distinctive character of several plant species belonging to Compositae (Caprioli et al. 2017; Venditti et al. 2015).

Consequently, the chemical characterization of medicinal plants becomes necessary for studying the potential presence of bioactive compounds and for assessing their toxicity related to potential environmental pollution (Pejin et al 2012; Naccari et al. 2015; Cicero et al. 2017; Graci et al. 2017; Cammilleri et al. 2018).

Cladanthus arabicus (L.) Cass. belonging to the genus Cladanthus (family Compositae) is a medicinal plant commonly used for its anti-icteric properties, antifeedant activity and as an ornamental plant.

Bubonium imbricatum (Cav.) DC., syn. Asteriscus imbricatus (Cav.), a species considered a member of the genus Asteriscus (family Compositae), is an endemic plant whose presence is restricted to certain areas.

Essential oils from these two plants from Morocco have been already characterized for the chemical composition, together with various bioactivities (Aghraz et al. 2016; Aghraz et al. 2017). Therefore, the aim of this study was to elucidate the chemical composition and the antibacterial activity of the aerial parts, yet not investigated, of Moroccan B. imbricatum and C. arabicus. To this purpose, the total phenol contents, along with the determination of single polyphenols, and the major and trace element profiles were determined. Additionally, the in vitro antibacterial activity against multidrug resistant Enterobacteriaceae was evaluated. In this age, in fact, multidrug resistance patterns bacteria are difficult to treat and may even be untreatable with conventional antibiotics, for these reason the search for new antimicrobial agents is essential for preventing the post antibiotic era (Gervasi et al. 2014a, 2014b).

2. Results and discussion

2.1. Total phenolic content and single polyphenols

Aerial parts of B. imbricatum were characterized by a higher total phenolic content (33.74 µg gallic acid equivalent (GAE) mg\(^{-1}\)), thus reflecting the presence of single polyphenols at higher concentrations, when compared to C. arabicus (14.89 µg GAE mg\(^{-1}\)) (Table 1).

Indeed, concerning the analysis of single polyphenols, both plants showed a similar qualitative polyphenol profile characterized by phenolic acids, such as protocatechuic acid, p-hydroxybenzoic acid, vanillic acid and p-coumaric acid, and flavonoids, such as apigenin, vanillin, apigenin-7-O-glucoside, diosmetin, and luteolin. Nonetheless, most of these compounds were found at significantly higher levels in the B. imbricatum extract (\(p < 0.05\), Table 1). Overall, aerial parts from B. imbricatum had caffeic and
ferulic acids (7.50 and 7.35 mg kg$^{-1}$) as the most abundant phenolic compounds (Table 1). Conversely, in the *C. arabicus* extract, caffeic and protocatechuic acids were found at the highest concentrations (4.88 and 4.72 mg kg$^{-1}$, respectively) (Table 1).

### 2.2. Inorganic elements in the aerial parts of *C. arabicus* and *B. imbricatum*

The amounts of major and trace elements, including heavy metals, in the aerial parts of *C. arabicus* and *B. imbricatum* were determined using ICP-MS, one of the most sensitive analytical techniques for the rapid and reliable determination of inorganic elements (Albergamo et al. 2017; Mottese et al. 2018; Bua et al. 2016; Di Bella et al. 2015). Almost all the investigated elements were found at significantly higher levels in *B. imbricatum* than *C. arabicus* ($p < 0.05$). However, Na, Mg, K, and Ca were found at the highest concentrations, comprised between 6000 and 65,000 mg kg$^{-1}$ (Table 2). Conversely, the element characterized by the lowest amounts was Cu, being 19.95 mg kg$^{-1}$ and 12.04 mg kg$^{-1}$ in *B. imbricatum* and *C. arabicus* respectively ($p < 0.05$). The significantly elevated concentrations of such minerals in both samples may reflect the high mineralization degree of the soils where the plants grow, and also the age of the plants (Namwiba 2007). As reordered for major and essential trace elements, also heavy metals were demonstrated to be generally more concentrated in *B. imbricatum* than *C. arabicus* ($p < 0.05$). Specifically, Cr and Pb were the metals found at the highest levels (1931 and 515 μg kg$^{-1}$, $p < 0.05$) in both plants, followed by Cd (103 and 1024 μg kg$^{-1}$, $p < 0.05$). However, As, Pb and Cd did not exceeded the maximum permissible levels, fixed respectively at 1.0, 10 and 0.5 mg kg$^{-1}$, for consumed medicinal herbs (WHO 1998; European pharmacopeia 2008). These results confirm the safety of both plants in terms of toxic heavy metals.

### Table 1. Formula, retention time, theoretical, measured mass and concentrations (mg kg$^{-1}$, dw) of the main polyphenol compounds identified in the extracts from *C. arabicus* and *B. imbricatum* by UHPLC-MS and total phenolic contents (μg GAE mg$^{-1}$, dw) revealed in the extracts from *C. arabicus* and *B. imbricatum* by UV spectrophotometry.

| Phenolic compounds | Formula | Theoretical mass [M-H] | Measured mass [M-H] | mm | min. | Concentrations mg kg$^{-1}$ (ppm) |
|--------------------|---------|------------------------|---------------------|----|------|-----------------------------------|
| 4-hydroxybenzoic acid | C$_7$H$_6$O$_3$ | 137.024 | 137.023 | −0.021 | 13.51 | 1.51 ± 0.28 | 2.60 ± 0.35 |
| Tyrosol | C$_6$H$_10$O$_2$ | 137.060 | 137.059 | −0.146 | 3.23 | — | 0.11 ± 0.007 |
| Vanillin | C$_8$H$_6$O$_4$ | 151.040 | 151.038 | −0.371 | 17.43 | 1.46 ± 0.35 | 2.16 ± 0.44 |
| Protocatechuic acid | C$_7$H$_6$O$_4$ | 153.019 | 153.017 | −0.515 | 10.32 | 4.71 ± 0.35 | 4.42 ± 0.78 |
| p-Coumaric acid | C$_8$H$_6$O$_3$ | 163.040 | 163.038 | −0.301 | 18.51 | 0.54 ± 0.06 | 0.75 ± 0.07 |
| Vanillic acid* | C$_8$H$_8$O$_4$ | 167.034 | 167.033 | 0.105 | 14.40 | 1.46 ± 0.35 | 2.16 ± 0.44 |
| Gallic acid | C$_7$H$_6$O$_3$ | 169.014 | 169.013 | 0.020 | 7.48 | 0.60 ± 0.08 | — |
| Caffeic acid* | C$_9$H$_8$O$_4$ | 179.034 | 179.034 | 0.805 | 16.24 | 4.88 ± 0.42 | 7.49 ± 0.96 |
| Ferulic acid* | C$_{10}$H$_{10}$O$_4$ | 193.050 | 193.050 | 0.635 | 19.06 | 1.77 ± 0.19 | 7.35 ± 1.13 |
| Apigenin-7-glucoside | C$_{21}$H$_{20}$O$_{10}$ | 431.09872 | 431.09854 | 1.112 | 20.87 | 0.58 ± 0.04 | 0.92 ± 0.06 |
| Apigenin* | C$_{15}$H$_{10}$O$_5$ | 269.045 | 269.045 | −0.150 | 23.86 | 0.10 ± 0.001 | 2.47 ± 0.41 |
| Luteolin* | C$_{15}$H$_{10}$O$_6$ | 285.040 | 285.040 | 1.206 | 23.00 | 0.24 ± 0.005 | 0.72 ± 0.003 |
| Diosmetin* | C$_{16}$H$_{12}$O$_6$ | 299.056 | 299.056 | 0.985 | 24.04 | 0.14 ± 0.002 | 0.75 ± 0.004 |
| Total phenolic content (μg GAE mg$^{-1}$) | | | | | | 14.89 ± 2.17 | 33.74 ± 4.28 |

*Compounds found at statistically different concentrations ($p < 0.05$).
Table 2. a) Major, essential trace element and b) heavy metals composition of *B. imbricatum* (B.I.) and *C. arabicus* (C.A.) revealed by ICP-MS.

|       | Na$^{(23)}$ | Mg$^{(24)}$ | K$^{(39)}$ | Ca$^{(44)}$ | Mn$^{(55)}$ | Fe$^{(56)}$ | Cu$^{(63)}$ | Zn$^{(66)}$ | Se$^{(82)}$ |
|-------|-------------|-------------|------------|-------------|-------------|-------------|-------------|-------------|-------------|
| B. I. | 64,208 ± 598 | 9250 ± 285  | 26,695 ± 379 | 57541 ± 791 | 67.43 ± 11.31 | 1489 ± 57   | 19.95 ± 2.35 | 65.38 ± 20.12 | 381 ± 25    |
| C. A. | 30,491 ± 832 | 6954 ± 193  | 39,387 ± 428 | 21,554 ± 682 | 112 ± 15.26  | 492 ± 38    | 12.04 ± 1.09 | 49.79 ± 13.56 | 116 ± 32    |

b) | Cr$^{(52)}$ | Co$^{(59)}$ | Ni$^{(60)}$ | As$^{(75)}$ | Cd$^{(111)}$ | Pb$^{(208)}$ |
|-------|-----------|-----------|-------------|------------|-------------|-------------|
| B. I. | 1931 ± 235 | 379 ± 153 | 540 ± 175   | 507 ± 187  | 103 ± 35    | 1182 ± 239  |
| C. A. | 515 ± 123  | 144 ± 67  | 362 ± 123   | 319 ± 144  | 1024 ± 78   | 1312 ± 110  |

*Elements found at statistically different concentrations ($p \leq 0.05$). Levels of major and trace essential elements are expressed as mg kg$^{-1}$, dw; whereas contents of Se and heavy metals as µg kg$^{-1}$, dw.*
2.3. Antibacterial activity of B. imbricatum and C. arabicus extracts

The results for antibacterial activity of the two extracts, evaluated with disc diffusion assay and Minimal Inhibitory Concentration (MIC) are reported in Table 3. The antibacterial activity of extracts stopped the growth of most of the tested MDR Enterobacteriaceae by forming significant inhibition zones (inhibition zone ≥ 10 mm).

C. arabicus extract showed the highest inhibition against Escherichia coli (S34/16), whereas Proteus mirabilis found to be the most sensitive to the extract from B. imbricatum. The inhibition zone was respectively 13.5 ± 0.17 mm and 14 ± 0.22 mm. From literature research, substances forming inhibition diameters in the range between 9 and 13, are considered moderately active, instead if these are larger than 14 mm are considered very active (Mothana and Lindequist 2005).

Aligianis et al. (2001) classified the antibacterial activity of plants extracts on their minimal inhibitory concentrations indicating a strong activity when the MIC is up to 0.5 mg ml⁻¹, moderate activity with a MIC between 0.6 and 1.5 mg ml⁻¹ or weak activity if the MIC is above 1.6 mg ml⁻¹. The MIC results showed that both extracts exhibited significant antibacterial activities against all the tested Enterobacteriaceae, being between 0.25 mg ml⁻¹ and 0.125 mg ml⁻¹. E. coli was the most sensitive bacteria to B. imbricatum extract and E. coli, Klebsiella pneumoniae and P. mirabilis to C. arabicus extract. The activity of plants extracts could be related to the effect of bioactive compounds (phenolic acids and flavonoids) (Rubio-Moraga et al. 2013), and this is in good agreement with previous studies in which flavonoids and phenolic acids have been associated with high antibacterial activities (Hossain et al. 2014). Moreover, caffeic acid, that has been revealed as the main compound in B. imbricatum extract, was shown to possess a wide range of biological properties, particularly antimicrobial activity (Matejczyk et al. 2018). Despite the few amounts revealed in the extracts, other polyphenols could be responsible for the antibacterial activity of the aerial parts from such plants, such as apigenin, vanillin, p-hydroxybenzoic and protocatechuic acids (Stojković et al. 2013).

3. Experimental

See Supplementary materials for chemicals and standards, plant materials, total polyphenol content, single polyphenol analysis, elemental analysis, evaluation of antibacterial activity of plant extracts and statistical analysis.
4. Conclusion

The aerial parts of *B. imbricatum* and *C. arabicus* were thoroughly analyzed for total polyphenol contents, single polyphenols, major and trace elements, including heavy metals, and antibacterial activity. Both plants were characterized by relevant amounts of single polyphenols, significant levels of major and trace essential metals, and demonstrated a good safety in terms of toxic heavy metals. Additionally, *in vitro* antibacterial activity against a collection of MDR *Enterobacteriaceae*, that may be explained by the presence of certain polyphenols, was reported. Obtained results should encourage further *in vivo* studies for the development of phytoterapeutic products.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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