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Furanocoumarin content in *Bituminaria bituminosa* varieties and *Cullen* species

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Abstract. *Bituminaria bituminosa* and several species from the *Cullen* genus are being evaluated in Spain and Australia as forage species for Mediterranean environments. In these environments, summer drought is the main limiting factor for the utilization of commercial perennial legumes such as *Medicago sativa*. Species from *Bituminaria* and *Cullen* genera are known to produce furanocoumarins (psoralen and angelicin) among other secondary compounds. Furanocoumarins derive from the phenylpropane, and their pharmacological properties are well known. In this study we are presenting the psoralen and angelicin content in two varieties of *Bituminaria bituminosa* and different species of the genus *Cullen*. All species of the genus *Cullen* showed the lowest mean concentration values of psoralen (948 ppm) and angelicin (1155 ppm). In *Bituminaria bituminosa* var *albomarginata* the mean content of psoralen and angelicin was 2796 and 3055 ppm respectively, while the concentrations of these furanocoumarins were highest in *B. bituminosa* var. *bituminosa*, with 2853 and 7302 ppm for psoralen and angelicin.

Keywords. *Bituminaria* – *Cullen* – Angelicin – Psoralen – Grazing.

Résumé. *Bituminaria bituminosa* et certaines espèces du genre *Cullen* sont en cours d'évaluation en Espagne et en Australie, et sont sélectionnées pour être utilisées comme espèces pour le pâturage en milieux méditerranéens. La sécheresse, en particulier estivale, est un facteur limitant pour d'autres légumineuses pérennes déjà connues comme *Medicago sativa*. Il a été décrit que ces genres accumulent des furanocoumarines comme le psoralène et l'angélicine, entre autres composés provenant du métabolisme secondaire des plantes. Les furanocoumarines sont un groupe de composés dérivés du métabolisme des phénylpropanes, dont les propriétés pharmacologiques sont largement connues. Dans cette communication sont présentés les résultats obtenus concernant la teneur en psoralène et en angélicine de différentes variétés et clones de *Bituminaria* et d'espèces de *Cullen*. Les effets de ces composés secondaires sur le métabolisme et la santé animale sont discutés.

Mots-clés. *Bituminaria* – *Cullen* – Angélicine – Psoralène – Pâturage.

I – Introduction

The genus *Bituminaria* is a perennial legume belonging to the Fabaceae family. In the Canary Islands (Spain) the species presents a large diversity with three botanical varieties: *bituminosa*, *albomarginata* and *crassiuscula* (Méndez *et al.*, 1990; Muñoz *et al.*, 2000). Several species from the genus *Cullen* (*C. australasicum*, *C. discolor*, *C. pallidum* and *C. patens*) are perennial, highly productive, persistent and native to south-central Australia (Bennett *et al.*, 2006).

Furanocoumarins are a subgroup of phenolic compounds included in the coumarins group. Biosynthetically, they are mainly originated from shikimic acid pathway, with the intermediated

of cinnamic acid. In the case of *Bituminaria bituminosa*, the presence of two furanocoumarins has been reported by different authors as psoralen (linear furanocoumarins) and angelicin (angular furanocoumarins) (Innocenti *et al.*, 1991, 1997; Méndez *et al.*, 2001; Tava *et al.*, 2007). However, little information exists concerning the presence of these secondary compounds in the genus *Cullen*.

This work quantifies by HPLC the psoralen and angelicin content in two varieties of *Bituminaria bituminosa* and different species of the genus *Cullen*. The objective is to identify plants with low furanocoumarin (FC) content to be selected for grazing animals, avoiding possible photosensitisation effects reported in other plant species.

II – Material and methods

1. Plant material

Bituminaria bituminosa, var. *bituminosa*, accession Calnegre (OMV F19) and var. *albomarginata*, accession Famara (PA-L10) were cultivated in IMIDA (Murcia-Spain). The plants of Famara accessions were established in 1998 and were an F2 from seeds of an F1 from original seeds sent by Pilar Méndez. The *Cullen* species (*C. australasicum*, *C. cinereum*, *C. tenax*, *C. pallidum* and *C. patens*) were cultivated at The University of Western Australia, Perth, Australia.

2. Extraction and analysis of furanocoumarins (FCs)

Leaves of the different plant materials described above were dried at 50°C to constant weight immediately after collecting. The leaves were ground and shaken with methanol-water (1:1, v/v). Each extract was hydrolyzed with 1N HCl acid previously to quantification, and the solutions were filtered through a 0.45 µm nylon membrane before analysis. For the HPLC analysis, a Jasco liquid Chromatograph was used and an analytical column LiChroCARTR C₁₈ (Agilent, USA), with an average particle size of 5 µm (250 x 4 mm i.d.) at 30°C. For the separation of FCs, a mixture of acetonitrile/water was used as solvent.

III – Results and discussion

The psoralen, angelicin and total FC content of *Bituminaria bituminosa* var. *albomarginata* accession Famara are shown in Table 1. The psoralen concentration in leaves of the plants analyzed varied between 2204 and 3416 ppm, with a mean value of 2796 ppm. For angelicin, the concentrations ranged from 2530 to 3918 ppm, with a mean value of 3055 ppm. Angelicin represented 52% of FCs in var. *albomarginata*, which contains a mean total FC of 5864 ppm.

The psoralen and angelicin content of *Bituminaria bituminosa* var. *bituminosa*, accession Calnegre are presented in Table 2. The concentrations of FCs in all the analyzed plants of var. *bituminosa* were higher than the obtained with var. *albomarginata*. The psoralen content varied between 2283 and 3920 ppm, with a mean value of 2853 ppm. The angelicin content varied between 5437 and 10,571 ppm, with a mean value of 7302 ppm. This variety presents the highest concentration of FCs (angelicin + psoralen) with a mean value of 10,155 ppm.

The psoralen and angelicin content of different species of *Cullen* are shown in Table 3. All species of *Cullen* analyzed showed the lowest concentration of psoralen and angelicin. The psoralen content varied between 426 and 1384 ppm, with a mean value of 948 ppm. The angelicin content varied between 839 and 1640 ppm, with a mean value of 1155 ppm. The total mean FC content of *Cullen* species was 2103 ppm.

Table 1. Psoralen and angelicin content in leaves of *Bituminaria bituminosa* var *albomarginata*, accession Famara

Plant	Psoralen (ppm)	Angelicin (ppm)	Total furanocoumarin (ppm)
1	2769 ± 125	2610 ± 132	5379 ± 148
2	3416 ± 165	2567 ± 187	5983 ± 203
3	2907 ± 152	3918 ± 201	6825 ± 204
4	2685 ± 95	2530 ± 164	5215 ± 149
5	2204 ± 75	2652 ± 81	4856 ± 90
Mean value	2796 ± 122	3055 ± 153	5864 ± 159

Table 2. Psoralen and angelicin content in leaves of *Bituminaria bituminosa* var *bituminosa*, accession Calnegre

Plant	Psoralen (ppm)	Angelicin (ppm)	Total furanocoumarin(ppm)
1	2552 ± 136	6796 ± 232	9348 ± 212
2	2558 ± 155	6404 ± 287	8962 ± 255
3	3920 ± 162	10571 ± 261	14491 ± 244
4	2283 ± 99	5437 ± 164	7720 ± 151
5	2953 ± 175	7300 ± 181	10253 ± 205
Mean value	2853 ± 145	7302 ± 225	10155 ± 213

Table 3. Psoralen and angelicin content in leaves of different species of *Cullen*

Specie	Psoralen (ppm)	Angelicin (ppm)	Total furanocoumarin (ppm)
<i>C. australasicum</i>	1191 ± 257	899 ± 331	2090 ± 339
<i>C. cinereum</i>	426 ± 120	1249 ± 187	1675 ± 177
<i>C. tenax</i>	1263 ± 428	839 ± 261	2102 ± 397
<i>C. pallidum</i>	478 ± 159	1640 ± 89	2118 ± 143
<i>C. patens</i>	1384 ± 403	1146 ± 187	2530 ± 340
Mean value	948 ± 273	1155 ± 211	2103 ± 279

The FC content obtained for the two botanical varieties of *Bituminaria* are similar to the ones published by Innocenti *et al.* (1997) using a chemical hydrolysis method of extraction. The psoralen and angelicin content varied from 260 ppm and 940 ppm for an accession from Padova to 3700 ppm and 2300 ppm for an accession from Consenza respectively.

IV – Conclusions

(i) All the studied species of *Cullen* and *Bituminaria* are able to produce psoralen and angelicin in their leaves.

(ii) There is a large variability in the content for these furanocoumarins in all species, being the *Cullen* species the ones with the least amount.

(iii) The high concentrations of furanocoumarins present in *Bituminaria bituminosa* var. *bituminosa*, accession Calnegre, makes it an ideal source for the extraction of these compounds for pharmaceutical purposes.

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