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Short communication

Gamma-linolenic acid from fourteen boraginaceae species

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Abstract

The seed oil of 14 Boraginaceae species was surveyed in a search for new sources of γ-linolenic acid (GLA, 18:3ω6). GLA content on total fatty acids ranges from the absence in *Cordia* and *Halgania* species to 23.9% in *Lithospermum latifolium*. The percentage of GLA on total seed weight reaches 3.9% in *L. latifolium* and *Pulmonaria officinalis*. The relatively high GLA percentage for *Echium russicum* suggest a distant taxonomic relationship when compared to other continental *Echium*.
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1. Introduction

The fatty acid γ-linolenic acid (GLA, 18:3ω6) appears in the seeds of some plant species besides the more common α-linolenic acid (ALA, 18:3ω3). New sources of GLA are being sought because of its claimed beneficial effect on a variety of human diseases, as well as its importance as a dietary and a cosmetic component (Gunstone, 1992; Horrobin, 1992; Ugnarius, 1996; Reddy et al., 1998; Das, 1998). Traditional plant sources of GLA are *Oenothera biennis* (evening primrose) (Pina et al., 1984), *Borago officinalis* (borage) (Gunstone, 1992) and *Ribes nigrum* (black currant) (Lercker et al., 1988). GLA is also found in the seed oil of all Boraginaceae species and its content on total saponifiable oil has been used as a taxonomic marker (Velasco and Goffman, 1999; Guil-Guerrero et al., 2000, 2001a,b).

The finding of new species producing GLA is a desirable option, taking into account its high economic value, both as a medicinal oil or as a pure compound used for research or medicinal applications. In addition, GLA species could become new crops adapted to marginal lands that are not suitable for traditional agriculture, with the additional beneficial ecological effects on the ecology of many degraded habitats.

This paper reports on the fatty acid composition of 14 species from the Boraginaceae, most of them unreported to date. On the other hand, the taxonomical significance of the fatty acid profiles for these species is discussed.

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2. Material and methods

2.1. Materials

Some seeds were collected at maturity from their natural habitats: seeds of *Echium lusitanicum, E. vulgare* and *Myosotis arvensis* were gathered in June 2001 in Salamanca (Spain). The remaining seeds were purchased from B&T World Seeds®, Olonzac (France).

The whole seed was used in the analyses, except in the case of *Cordia dichotoma*, in which the seed coat was discarded.

Just prior to analysis, seeds were freeze-dried and ground to powder with a mortar, then they were immediately analysed.

2.2. Oil extraction and transesterification

Rapid simultaneous oil extraction and transesterification were made according to the method of Lepage and Roy (1984). About 10 mg of seed were placed in test tubes containing 1 ml of the methylation mixture (methanol/acetyl chloride, 20:1 v/v) and 0.5 ml hexane and heated at 100 °C for 10 min. After cooling to room temperature, 1 ml of distilled water was added and the upper hexanic layer was taken for gas chromatography (GLC analysis). Duplicates were used for each sample and mean values are reported in the tables (variation among the duplicate samples was routinely $< 5\%$).

2.3. Gas-liquid chromatography (GLC)

Mixed fatty acid methyl esters (FAME) were analysed in a Hewlett-Packard HP5890 series II GC provided with FID and HP3394 integrator. A capillary column of fused silica of high polarity (Supelco SP2330; length: 30 m; internal diameter: 0.25 mm; thickness of the film: 0.2 μm) was used. The flow rate of the carrier gas (N_2) was 0.75 l/min. Split ratio in the injector was 100:1. Injector temperature was 240 °C and the detector temperature was 260 °C. The oven starting temperature was 205 °C and was increased at a rate of 6 °C/min to 240 °C. Injection volume was 5 μl and a blank was run every ten analyses. Peaks were identified by comparison with known methyl ester standards ('Rapeseed oil mix' and 'PUFAS-1' from Sigma). Oil contents in samples were determined using methyl heptadecanoate (17:0) as internal standard.

3. Results and discussion

Seed oil content and fatty acid composition from 14 Boraginaceae species here analysed are given in Table 1. Most of data about fatty acids of these species have not been reported, while the fatty acid profiles of *Buglossoides purpureocaeruleum* (= *Lithospermum purpureocaeruleum*; Kleiman et al., 1964; Miller *et al.*, 1968), *E. vulgare* (Miller et al., 1968; Tétényi, 1974; Velasco and Goffman, 1999), *M. arvensis* (Tétényi, 1974), *Nonea pulla* (Miller et al., 1968) and *Symphytum uplandicum* (Tétényi, 1974) have been previously reported, showing data in agreement with our results.

Seed oil content ranges from 11.8% in *L. incisum* to 50.2% in *C. dichotoma*. An oil content of 4% (dry basis) was previously reported in *C. dichotoma* (oilseed chemicals database at http://www.ncaur.usda.gov/nc/ncdb), in great contrast to our reported 50.2%. This discrepancy might be attributed to the inclusion or not of the thick coat of this seed in the analysed material or in the calculation of the whole seed weight. This may also explain striking differences in the lipid content among other *Cordia* species found in the same database.

The percentages of GLA on total fatty acids ranges from an absence in *Cordia* and *Halgania* species to 23.9% in *L. latifolium*. The GLA content on total seed weight reaches a maximum of 3.9% for *L. latifolium*. The GLA percentage exhibited by *L. latifolium* seed oil was within the range of *B. officinalis* (21–23%), which is the best crop source of GLA until now (Gunstone, 1992). However, before considering this species as a new source of GLA oil for human consumption, the absence of toxic components in the oil, such as pyrrolizidine alkaloids, should be checked. These compounds have been reported to be present in the seeds of related Boraginaceae species (Krenn et al.,

Table 1
Fatty acid composition of 14 plant seeds from the Fam. Boraginaceae Juss[a]

	Saponifiable oil[b]	% GLA total seed	Fatty acids[c]									
			16:0	18:0	18:1ω9	18:1ω7	18:2ω6 (LA)	18:3ω6 (GLA)	18:3ω3 (ALA)	18:4ω3 (SDA)	20:0	20:1ω9
Subfam. *Cordioideae* Link (Fam. Cordiaceae R. Br)												
Cordia dichotoma G. Forst.	50.2	–	16.5	7.9	37.1	0.4	32.9	–	–	–	1.7	0.4
Subfam. Ehretioideae (Mart. ex Lindl.) Arn (Fam. Ehretiaceae Lindley)												
Halgania andromedifolia Gaudich.	12.6	–	9.1	3.0	22.8	1.0	62.9	–	–	–	–	–
Halgania argyrophyla Gaudich.	12.2	–	15.4	5.9	31.7	–	44.8	–	–	–	–	–
Subfam. Heliotropioideae (Schrad.) Arn.												
Heliotropium paniculatum L.	19.6	–	10.1	5.4	13.4	0.7	66.1	0.2	–	–	–	–
Subfam. Boraginoideae Arn.												
Tribe *Lithospermeae* (DC.) Guerke												
Buglossoides purpureocaeruleum (L.) I.M. Johnst	12.6	2.3	8.23	4.5	10.3	–	18.7	18.5	33.7	9.2	–	1.8
Echium lusitanicum L.	20.6	2.2	6.7	2.5	16.4	–	16.3	10.9	33.3	12.3	–	1.0
Echium vulgare L.	28.7	3.1	6.5	3.7	9.4	–	16.9	10.9	39.3	13.3	–	–
Echium russicum J.F. Gmel.	18.6	2.9	5.7	2.4	14.4	–	22.6	15.8	26.6	10.6	–	1.3
Lithospermum incisum Lehm.	11.8	0.7	10.0	3.0	14.0	–	19.0	5.5	34.0	11.0	–	1.5
Lithospermum latifolium Michaux	16.3	3.9	7.2	3.0	12.9	1.5	18.5	23.9	27.4	8.2	–	2.9
Tribe Myosotideae												
Myosotis arvensis (L.) Hill	41.5	1.7	8.8	3.3	26.5	0.5	24.4	4.1	14.8	7.0	0.7	4.2
Tribe Boragineae												
Nonea pulla (L.) DC.	16.8	2.1	8.7	2.9	25.0	–	24.8	12.6	13.2	3.0	–	4.2
Pulmonaria officinalis L.	31.0	3.6	8.0	3.5	27.5	0.5	32.0	11.6	8.8	2.5	–	3.3
Symphytum uplandicum Nym.	22.7	3.6	8.8	4.1	22.0	–	42.5	15.9	1.1	–	–	3.2

[a] Fatty acid percentages based on saponifiable oil.
[b] (%) Based on total seed, determined by GLC.
[c] Other fatty acids of undetermined structure account for 100% of the fatty acids.

1994). Nevertheless, pyrrolizidine alkaloids are not lipophillic and therefore, would not be expected to be present in any substantial quantity in the oil.

Another good source of GLA found in this study is *B. purpureocaeruleum*, which belong to the Tribe Lithospermeae, as well as the species mentioned before.

GLA amounts in *E. vulgare* (10.9%) and *E. lusitanicum* (10.9%) were very similar to other Continental *Echium* species (Guil-Guerrero et al., 2000). Conversely, the relatively high GLA percentage in *E. russicum* (15.8%) suggests a distant taxonomic relationship with the above mentioned *Echium*. This is in good agreement with the genetic data of Böhle et al. (1996), in which *E. russicum* appears separated from the cluster made up by the rest of continental *Echium* species. The GLA level in *E. russicum* seeds might indicate a closer relationship of this plant to the *Echium* ancestor that colonized the Macaronesian area, since high GLA contents are also found in all Macaronesian *Echium* species (Guil-Guerrero et al., 2000, 2001a). Nevertheless, this hypothesis requires further investigation.

Among the 14 Boraginaceae species here analysed, only three lack GLA in their seed oil. These species belongs to subfamilies Cordioideae Link (*C. dichotoma*) and Ehretioideae (Mart. ex Lindl.) Arn (*Halgania andromedifolia* and *H. argyrophyla*). The absence of GLA in species from these subfamilies agree with previous reports of Kleiman et al. (1964) and Miller et al. (1968) on related taxa. There is increasing evidence that GLA content constitutes a good taxonomical marker in Boraginaceae. In this context, the absence of GLA in *Cordia* and *Halgania* species support their proposed inclusion in separate families (see Table 1).

4. Conclusion

GLA content in the species tested in this work indicate that it appears in significant amounts in some species, especially among those of the genus Lithospermeae. Moreover, data from our screening reinforces the notion that Boraginaceae should be considered as a main target when looking for new sources potentially rich in GLA.

Among the species surveyed in this work, *L. latifolium* (American Gromwell) seems to be the best option to attempt an alternative crop to the current producers of GLA oils.

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