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Original Research Article

## Lipid and volatile composition of borage (*Borago officinalis* L.) leaf

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### ABSTRACT

Lipid and volatile compositions of *Borago officinalis* L. leaf were investigated. The results showed that  $\alpha$ -linolenic acid was the major fatty acid followed by stearidonic acid. Total lipids were composed of neutral and polar lipids. Polar lipids mainly consisted of phosphatidylcholine as the major phospholipid, whereas monogalactosyldiacylglycerol was the major glycolipid. Concerning neutral lipids, they were mainly composed of triacylglycerols. The volatile composition exhibited the presence of many green note compounds such as hexanal, (*E*)-2-hexenal, hexanol, (*Z*)-3-hexenol, (*E*)-2-hexenol and nonanol. Borage leaf appears to be an important source of essential fatty acids, phospholipids, glycolipids and green note volatile compounds for food, cosmetic, pharmaceutical and biomedical applications.

### Abbreviations:

DAG: Diacylglycerol; DGDG: Digalactodiacylglycerol; DMW: Dry matter weight; EPC: Electronic pressure control; FAME: Fatty acid; FFA: Free fatty acids; FID: Flame ionization detector; GC: Gas chromatography; GC-MS: Gas chromatography-mass spectroscopy; GL: Glycolipids; MAG: Monoacylglycerol; MGDG: Monogalactodiacylglycerol; MUFA: Monounsaturated fatty acids; NL: Neutral lipids; PC: Phosphatidylcholine; PE: Phosphatidylethanolamine; PG: Phosphatidylglycerol; PhL: Phospholipids; PL: Polar lipids; PUFA: Polyunsaturated fatty acids; RI: Retention index; SFA: Saturated fatty acids; TAG: Triacylglycerol; TFA: Total fatty acids; TL: Total lipids; TLC: Thin layer chromatography.

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## 1. Introduction

Fatty acids originate from triacylglycerols, phospholipids or glycolipids that are important parts of the cell membranes. Fatty acids are precursors for a large number of volatile compounds of which many are important character-impact aroma compounds responsible for the fresh, green and fruity odor of fruits and vegetables (Berger, 2007). Fatty acid-derived volatile compounds mainly comprise  $C_6$  and  $C_9$  aldehydes, alcohols and esters (Xu et al., 2015). The  $C_6$  compounds are normally the most abundant volatiles in mature borage leaves (Mhamdi et al., 2007a), which are classified as 'Green leaf volatiles' due to their characteristic 'green' and fresh odor (Matsui et al., 2000; Kalua and Boss, 2009). For this reason, these molecules are widely used in food and beverage industries

(Fukushige and Hildebrand, 2005). Green leaf volatiles are quickly generated and released in response to adverse environments owing to membrane breakdown.

Green leaf volatiles are direct products of oxidative cleavage of linoleic acid and  $\alpha$ -linolenic acid through the lipoxygenase-hydroperoxide (Matsui, 2006). Polyunsaturated linoleic acid and  $\alpha$ -linolenic acids, namely essential and unusual fatty acids, are nutritionally vital for human health (Tapiero et al., 2002). Several studies on fatty acids reported that  $\alpha$ -linolenic acid was predominated in leaf lipids of *Borago officinalis* L. (Griffiths et al., 1996; Guil-Guerrero et al., 2001; Mhamdi et al., 2007b; Del Rio-Celestino et al., 2008; Mhamdi et al., 2010; Stähler et al., 2011; Jaffel Hamza et al., 2013; Aidi Wannas et al., 2016). However, so far only few researches have undertaken lipids (Griffiths et al., 1996; Stähler et al., 2011; Aghofack Naguemezi and Tatchago,

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2006) and volatiles (Mhamdi et al., 2007a, 2009, 2010) of borage leaf. To the best of our knowledge, there is no information about lipid classes of Tunisian borage rosette-leaves and only one paper has reported their fatty acid and volatile composition (Mhamdi et al., 2007a).

The main goal of the present investigation was to determine the lipid and volatile composition of borage rosette leaves growing in Tunisia. The findings of this work may be important in order to clarify the biosynthesis relationship between lipids, especially fatty acids, and volatiles as well as to strengthen the functional uses of borage leaf in a wide variety of industrial applications involving food, cosmetics, pharmaceuticals and biomedical disciplines.

## 2. Experimental

### 2.1. Plant material

Mature rosette leaves of *B. officinalis* L. (Fig. 1) were collected at 61 days after cotyledon appearing from spontaneous plants in the region of Bajah at Amdoun area (North Western of Tunisia (Fig. 2) under the geographical coordinates of latitude: 36.81° N; longitude: 9.05° E at an altitude of 448 m.



**Fig. 1.** Representation of mature borage rosette in the sampling area.



**Fig. 2.** Localization of sampling area (North Western of Tunisia in Bajah at Amdoun).

### 2.2. Total lipid extraction

To extract total lipids from *B. officinalis* L., 1.0-g portions of its leaves were extracted using the method of Aidi Wannes et al. (2011). Then, they were placed in boiling water for 5 min and then ground manually

in a china mortar using a mixture of chloroform/methanol/hexane (3:2:1, v/v/v). After washing with this boiling water and decantation during 24 h at +4 °C, the organic phase containing total lipids was recovered and dried under a nitrogen stream. Finally, the residue was dissolved in a known volume of toluene-ethanol (4:1; v/v) and stored at -20 °C for further analyses.

### 2.3. Lipid class fractionation by thin layer chromatography (TLC)

Lipid classes were separated by thin layer chromatography (TLC) using 20 cm×20 cm×0.25 mm silica gel plates (G60, Merck, Darmstadt, Germany). Neutral lipid separation was afforded using the Mangold (1964) method through a developed system composed of petroleum ether-diethyl ether-acetic acid (70:30:0.4; v/v/v). Polar lipids were separated using as mobile phase mixture chloroform-acetone-methanol-acetic acid-water (50:20:10:10:5; v/v/v/v) as described by Lepage (1967). Iodine vapor was used to reveal the presence of all lipid spots and each spot was identified by co-chromatography of pure lipid standards.

### 2.4. Fatty acid transmethylation

Total fatty acids (TFA) of total lipids were transformed into their corresponding methyl esters (Cecchi et al., 1985). Transmethylation was made by the addition of 2 mL of hexane, 0.5 mL of 3.0% sodium methylate, a known amount of heptadecanoic acid methyl ester as the internal standard, 0.2 mL of H<sub>2</sub>SO<sub>4</sub> (1.0 N) and 1.5 mL of sodium chloride (10%). In addition, the hexanic phase containing fatty acid methyl esters (FAME) was recovered and its volume reduced in a stream of nitrogen, prior to analysis.

### 2.5. Volatile compound extraction

Volatile compounds were extracted by hydrodistillation after 90 min using 100 g of fruits. The distillate was extracted with diethyl ether and dried over anhydrous sodium sulphate. All experiments were done in triplicates and results were expressed on the basis of dry matter weight.

### 2.6. Gas chromatographic analysis of fatty acids and volatile compounds

FAME and volatile compounds were analyzed by gas chromatography (GC) using a Hewlett-Packard 6890 apparatus (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and an electronic pressure control (EPC) injector. An HP-Innowax capillary column (polyethylene glycol: 30 m×0.25 mm i.d, 0.25 μm film thickness; Agilent Technologies, Hewlett-Packard, CA, USA) was used; the flow-rate of the carrier gas (N<sub>2</sub>, U) was 1.6 mL/min and

the split ratio was 60:1.

FAME analyses were performed in the split mode under an oven temperature program as follows. Accordingly, the oven temperature was kept constant at 150 °C for 1 min (isothermal mode). Then, it was gradually increased from 150 to 225 °C at a rate of 15 °C/min followed by an extra addition of temperature from 200 to 225 °C at a rate of 2 °C/min. The temperature was finally set isothermal at 225 °C for 2 min. Injector and detector temperatures were held at 250 and 275 °C, respectively. FAMES were identified by comparison of their retention times with those of pure reference standards. Quantitative data were obtained from the electronic integration of the FID peak areas.

Volatile compounds were analyzed by GC, using the same apparatus previously described, under the following temperature programs: oven temps isotherm at 35 °C for 10 min, from 35 to 205 °C at the rate of 3 °C/min, and isotherm at 205 °C over 10 min. Injector and detector temperature were held, respectively, at 250 and 300 °C. Identification of volatile compounds was based on the calculation of their retention indices (RI) relative to (C<sub>8</sub>-C<sub>22</sub>) *n*-alkanes with those of authentic compounds available in our laboratory. Further identification was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library of the GC-MS data system and other published mass spectra (Adams, 2007).

GC-MS analyses of volatile components were carried out on a gas chromatograph HP 5890 (II) coupled to an HP 5972 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) with electron impact ionization (70 eV). An HP-5MS capillary column (30 m×0.25 mm, 0.25 μm film thickness; Agilent Technologies, Hewlett-Packard, CA, USA) was used. The column temperature was programmed to rise from 50 °C to 240 °C at a rate of 5 °C/min. The used carrier gas was helium with a flow-rate of 1.2 mL/min; split ratio was 60:1. Moreover, scan time and mass range were 1 s and 40-300 *m/z*, respectively.

### 3. Results and Discussion

#### 3.1. Lipid composition

The extraction using *n*-hexane from the mature leaf

of borage produced a green oily extract yielding above 5% of dry matter weight (DMW). This result was similar to that reported by Stähler et al. (2011) in the case of borage leaf from New Zealand. As shown in Fig. 3 and Table 1, ten fatty acids were identified which can be divided into three saturated fatty acids (SFA, 19.37% of TFA), three monounsaturated fatty acids (MUFA, 8.61% of TFA) and four polyunsaturated fatty acids (PUFA, 71.97% of TFA). Borage leaf was richer in PUFA mainly due to their higher proportions with 35.74% of TFA for α-linolenic acid, 25.01% of TFA for stearidonic acid, 6.95% of TFA for linoleic acid and 4.27% of TFA for γ-linolenic acid. MUFA were essentially represented by oleic acid with 6.38% of TFA. Concerning hexadecanoic and palmitoleic acids, they belong to the minor fraction of MUFA. SFA were characterized by high levels of palmitic (11.46% of TFA) and arachidic (6.26% of TFA) acids. However, the saturated stearic acid (1.65% of TFA) was weakly represented in borage leaf. Many researchers have reported the fatty acid composition of borage leaf (Griffiths et al., 1996; Guil-Guerrero et al., 2001; Mhamdi et al., 2007b; Del Rio-Celestino et al., 2008; Mhamdi et al., 2010; Stähler et al., 2011; Aidi Wannes et al., 2016) and despite some differences in the obtained results, all these studies confirmed that borage leaf

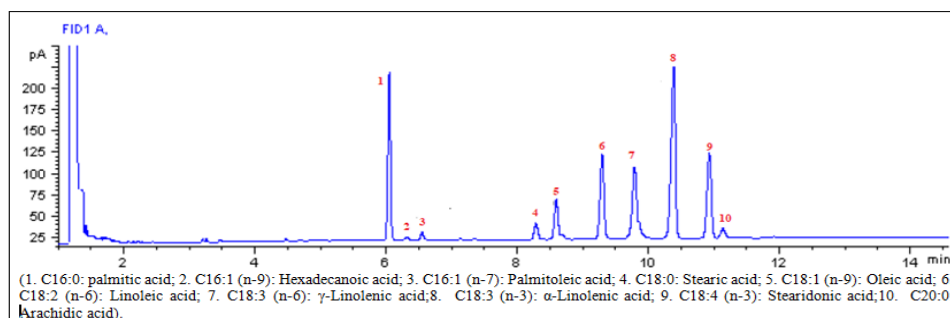
**Table 1**

Fatty acid composition (%) of total lipids from borage rosette leaves.

Fatty acid	Total fatty acids (%)
C16:0	11.46 ± 0.15
C16:1(n-9)	0.54 ± 0.42
C16:1(n-7)	1.69 ± 1.08
C18:0	1.65 ± 0.37
C18:1(n-9)	6.38 ± 0.43
C18: (2n-6)	6.95 ± 0.31
C18: (3n-6)	4.27 ± 0.32
C18: (3n-3)	35.74 ± 0.33
C18:(4n-3)	25.01 ± 0.94
C20:0	6.26 ± 0.11
∑ SFA	19.37 ± 0.21
∑ MUFA	8.61 ± 0.64
∑ PUFA	71.97 ± 0.47
<b>Total</b>	<b>99.95 ± 0.47</b>
<b>Oil yield (%)</b>	<b>4.99 ± 0.44</b>

Values given are the means of three replicates ± standard deviation.

**C16:0**: palmitic acid; **C16:1 (n-9)**: Hexadecanoic acid; **C16:1 (n-7)**: Palmitoleic acid; **C18:0**: Stearic acid; **C18:1 (n-9)**: Oleic acid; **C18:2 (n-6)**: Linoleic acid; **C18:3 (n-6)**: γ-Linolenic acid; **C18:3 (n-3)**: α-Linolenic acid; **C18:4 (n-3)**: Stearidonic acid; **C20:0**: Arachidic acid; **SFA**: Saturated fatty acids; **MUFA**: Monounsaturated fatty acids; **PUFA**: Polyunsaturated fatty acids.



**Fig. 3.** GC chromatographic profile of total fatty acids from borage leaves.

**Table 2**

Lipid composition (%) of borage rosette leaves.

Lipid classes	NL (g/100g of NL)	PL (g/100g of PL)	PhL (g/100g of PhL)	GL (g/100g of GL)
<b>Neutral lipids (NL)</b>				
Monoacylglycerol (MAG)	23.04 ± 0.02	-	-	-
Diacylglycerol (DAG)	25.40 ± 0.04	-	-	-
Triacylglycerol (TAG)	34.60 ± 0.11	-	-	-
Free fatty acids (FFA)	16.94 ± 0.06	-	-	-
<b>Polar lipids (PL)</b>				
Phospholipids (PhL)	-	44.60 ± 0.11	-	-
Glycolipids (GL)	-	55.40 ± 0.15	-	-
<b>Phospholipids (PhL)</b>				
Phosphatidylcholine (PC)	-	-	41.47 ± 0.07	-
Phosphatidylglycerol (PG)	-	-	33.63 ± 0.05	-
Phosphatidylethanolamine (PE)	-	-	24.88 ± 0.04	-
<b>Glycolipids (GL)</b>				
Monogalactosyldiacylglycerol (MGDG)	-	-	-	67.14 ± 0.15
Digalactosyldiacylglycerol (DGDG)	-	-	-	32.67 ± 0.09

Values given are the means of three replicates ± standard deviation.

was a potential source of PUFA especially  $\alpha$ -linolenic and stearidonic acids. PUFA are not synthesized by human body and must be supplied by special diets. It has been well-documented that the UFFA are the most important precursors for active physiological compounds like prostaglandin, thromboxanes and leucotrienes (Barre, 2001). Moreover, a growing literature relating to PUFA illustrated their benefits in alleviating cardiovascular, inflammatory, heart diseases, atherosclerosis, autoimmune disorder, diabetes and other diseases (Finely and Shahidi, 2001). Therefore, the fatty acid composition and the high contents of PUFA made the borage leaf important for a wide range of industrial applications.

Total lipid (TL) content was 46.15 mg/g DMW in borage mature leaf. As can be shown in Table 2, total lipids were composed of neutral (52.76% of TL) and polar (47.24% of TL) components. Neutral lipids (NL) revealed the presence of four fractions namely triacylglycerol (TAG, 34.60% of NL), diacylglycerol (DAG, 25.40% of NL), monoacylglycerol (MAG 23.04% of NL) and free fatty acids (FFA, 16.94% of NL). Polar lipids (PL) were divided in phospholipids (PhL, 44.6% of PL) and glycolipids (GL, 55.40% of PL). PhL referred to PC (41.47% of PhL), PG (33.63% of PhL) and PE (24.88% of PhL). GL fraction, as lipid characteristic of the chloroplast membranes (Andersson and Dörmann, 2009), was also found in borage leaf and it was represented by monogalactodiacylglycerol (MGDG, 67.14% of GL) and digalactodiacylglycerol (DGDG, 32.67% of GL). Similar lipid class components of borage leaf were mentioned by Stähler et al. (2011) but with different proportions. As shown in this work, the total oil of borage leaf respectively contained 53.3% and 46.8% PL and NL, whereas PL fraction included more GL (46.9% of total oil) than PhL (6.3% of total oil) proportions. Apart from the NL, especially TAG natural fats from plant materials and oils contain a number of lipophilic materials of the most diverse chemical make up. Edible plant glycolipids are anticipated to play a key role in human nutrition (Ramadan and Mörsel, 2003). The average daily intake of GL in the human body has been reported to be 90

mg of MGDG and 220 mg of DGDG (Sugawara and Miyazawa, 1999). Therefore, it is important to mention that borage leaf lipid serves as an excellent source of GL in the human diet.

### 3.2. Volatile composition

A clear green volatile oil with a fresh green odor was obtained through the hydrodistillation of borage rosette leaves with a mean yield of 0.06% (w/w). The chemical composition of the volatile fraction from borage rosette leaves was analyzed by GC and the chromatographic profile has been represented in Fig. 4. In the essential oil separated from the leaves of borage rosette, eighteen volatile components were identified representing 98.81% (Table 3). These compounds belong to six chemical classes: aliphatic hydrocarbons constituting 54.38% of the total volatiles with nonadecane as the major compound (42.37%) followed by tetracosane (12.01%). Also, alcohols represented 19.82% of the total volatiles with (*Z*)-3-hexenol as the major compound (10.85%). Aldehydes formed 7.56% with (*E*)-2-hexenal as the main compound (2.02%). Phenols represented only by carvacrol (5.40%) and phenol (1.90%). Ketones formed 6.53% and mainly represented by camphor with a percentage of 5.73%. Finally, monoterpene hydrocarbon classes belong to minor fraction. Most of these volatile compounds have been identified by Mhamdi et al. (2007a) in the essential oil of borage rosette leaves and these authors have also mentioned that nonadecane was the major compound. However, (*E*)-(*E*),2-4 decadienal was found to be the main compound of the essential oils from borage stem (Salem et al., 2014a) and flower (Salem et al., 2014b). In terms of general categories, only nonadecane and tetracosane were non aromatic compounds while the other compounds were mainly characterized by their green note odor like hexanal, (*E*)-2-hexenal, hexanol, (*Z*)-3-hexenol, (*E*)-2-hexenol and nonanol. As reported by Buchhaupt et al. (2012), green note compounds are substances that characterize the aroma of freshly cut grass, cucumber, green apple and foliage. In plants,

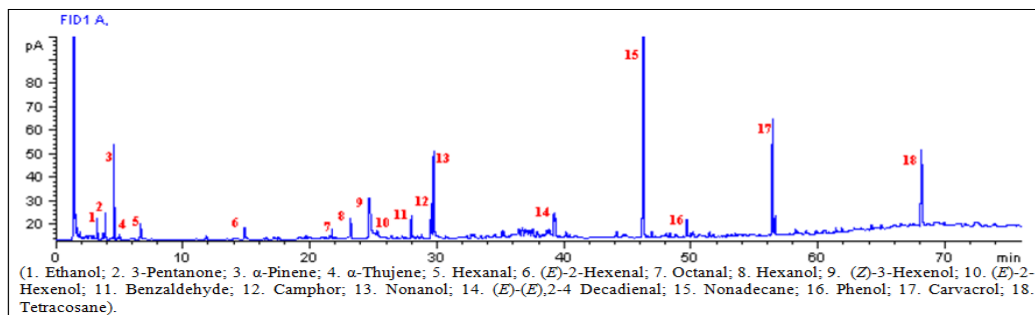
**Table 3**

Volatile composition (%) of the essential oil of borage rosette leaves separated by hydrodistillation and characterized by GC-MS.

Number	Volatile compound	RI <sup>a</sup>	RI <sup>b</sup>	Identification <sup>c</sup>	%
1	Ethanol	1011	668	RI, MS, Co-GC	0.75 ± 0.03
2	3-Pentanone	1024	680	RI, MS, Co-GC	0.80 ± 0.02
3	α-Pinene	1032	939	RI, MS, Co-GC	2.93 ± 0.55
4	α-Thujene	1035	932	RI, MS	0.29 ± 0.03
5	Hexanal	1099	802	RI, MS, Co-GC	0.99 ± 0.01
6	(E)-2-Hexenal	1234	854	RI, MS, Co-GC	2.02 ± 0.21
7	Octanal	1287	1005	RI, MS, Co-GC	1.37 ± 0.12
8	Hexanol	1360	867	RI, MS, Co-GC	3.40 ± 0.33
9	(Z)-3-Hexenol	1370	858	RI, MS, Co-GC	<b>10.85</b> ± 0.99
10	(E)-2-Hexenol	1392	853	RI, MS, Co-GC	1.56 ± 0.17
11	Benzaldehyde	1522	957	RI, MS, Co-GC	1.32 ± 0.15
12	Camphor	1532	1143	RI, MS, Co-GC	<b>5.73</b> ± 0.32
13	Nonanol	1703	1173	RI, MS, Co-GC	3.26 ± 0.21
14	(E)-(E),2-4 Decadienal	1827	1318	RI, MS, Co-GC	1.86 ± 0.30
15	Nonadecane	1900	1900	RI, MS, Co-GC	<b>42.37</b> ± 1.12
16	Phenol	1992	977	RI, MS	1.90 ± 0.03
17	Carvacrol	2239	1292	RI, MS, Co-GC	<b>5.40</b> ± 0.05
18	Tetracosane	2400	2400	RI, MS, Co-GC	<b>12.01</b> ± 0.55
<b>Total</b>					<b>98.81</b>

Values given are the means of three replicates±standard deviation.

RI<sup>a</sup>, RI<sup>b</sup>: Retention indices calculated using respectively a polar column (HP-Innowax) and an apolar column (HP-5); <sup>c</sup>: RI: retention indices relative to C<sub>8</sub>-C<sub>22</sub> n-alkanes on the (HP-Innowax); MS=Mass spectrum; Co-GC=Co-injection with authentic compound; Volatile compound proportions were calculated from the chromatograms obtained on an HP-Innowax column.


**Fig. 4.** GC chromatographic profile of the essential oil volatile compounds from borage rosette leaves.

these compounds are synthesized by conversion of C<sub>18</sub>-polyunsaturated fatty acids, including linoleic and linolenic acids, via the enzymes lipoxygenase and hydroperoxide lyase to short-chained C<sub>6</sub>- and C<sub>9</sub>-aldehydes (Buchhaupt et al., 2012). The first C<sub>6</sub> compound synthesized by the lipoxygenase pathway is (Z)-3-hexenal which is formed after tissue disruption (Matsui et al., 2000) and is then converted to other green note compounds such as (E)-2-hexenal (aldehyde), (Z)-3-hexenol (alcohol) and (Z)-3-hexenyl acetate (ester) (Shiojiri et al., 2006). Due to their aromatic properties, these volatile compounds are widely used in food and beverage industries (Fukushige and Hildebrand, 2005). Some green note compounds, such as hexanal and (E)-2-hexenal, have also some industrial uses in food storage due to their antimicrobial activities (Hubert et al., 2008). Chemical synthesis is the easiest way to produce large amounts of stable green note compounds. However, for food application, consumers have a strong preference for naturally synthesized additives and aromas (Gigot et al., 2010).

#### 4. Concluding remarks

The results of this investigation revealed the importance of exploring the lipid and volatile composition of borage leaf which is a good source of essential fatty acids, particularly α-linolenic acid, for

human nutrition. In addition, these fatty acids, originated from triacylglycerols, phospholipids and glycolipids, are precursors for a large number of borage leaf volatile compounds, namely hexanal, (E)-2-hexenal, hexanol, (Z)-3-hexenol, (E)-2-hexenol and nonanol, which could be further used in food and beverage industry due to their green note.

#### Conflict of interest

The authors declare that there is no conflict of interest.

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