Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 4, No. 3, p. 151-155, 2014
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Evaluation of oil content and composition seed oil of Iranian borage *Echium amoenum* subjected in leshkan accession in North of Iran

Esmail Babakhanzadeh Sajirani[1*], Javad Hadian[2], VahidAbdossi[1], Kambiz Larijani[3]

[1]*Department of Horticulture Science, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[2]*Department of Agriculture, Medicinal Plants and Drug Research Institute, Shahid Beheshti University, G. C., Evin, Tehran, 1483963113, Iran*

[3]*Department of Chemistry, Science and Research Branch, Islamic Azad University, Tehran, Iran*

Article published on March 06, 2014

Key words: linolenic acid, α-Linolenic, oleic acid, oil components, Iranian borage.

Abstract

Iranian Gole Gav Zaban *(Echium amoenum* fich & mey), a perennial endemic Iranian medicinal plant, belonged to Boraginaceae family, naturally grown in mountainous regions of North of Iran. The violet dry petals have been used as tonic, tranquillizer, diaphoretic, cough suppressant and a remedy for sore throat in folk Iranian medicine. This study is the first report about the analysis of seeds oil of *Echium amoenum* in leshkan accession. The results showed that the oil content of seeds was 36.4% and eleven fatty acids were identified and quantified by gas chromatography (GC). The major fatty acidsr espectively were α-Linolenic acid (35.69%), Linoleic acid (20.68%) and Oleic acid (17.08%).

***Corresponding Author:** Esmail Babakhanzadeh Sajirani ✉ esmailbabakhanzade@yahoo.com

Introduction

Echium amoenum (Iranian Gole GavZaban) is a perennial endemic Iranian medicinal plant, belonged to Boraginaceae family, naturally grown in mountainous regions of North of Iran. Four species of this genus are found in Iran, which *E. amoneum* is the only species in cultivation and consumption placed (mozafarian, 1996). The violet dry petals have been used as tonic, tranquillizer, diaphoretic, cough suppressant and a remedy for sore throat in folk Iranian medicine (Mehrabani *et al.,* 2005). Phytochemical investigation on this plant represents several chemical compounds, including anthocyanins (13%), flavonoid aglycones (15.%), saponins, unsaturated terpenoids, sterols and essential oils (0.05%) (Heidari *et al*., 2006; Shafaghi *et al*., 2002).Also, many fatty acids have been reported in seeds oil of *Echium amoenum* that showsa remarkable amount of linoleic acid (40-75%) (Mojab *et al*.,2008; Daneshfar *et al*., 2013). Linolenic acid as omega-3 is an essential fatty acid that cannot be synthesized in the body and must be provided by diet(Salunkhe and Kadam, 1998). Fatty acid patterns in the seeds as biochemical tools were also proved to explain the phylogenetic relations in most studies (Hohn and Meinschein 1976; Aitzetmüller *et al*., 1999). The seeds of *Boraginaceae* were reported to contain very high level of gamma linolenic acid (GLA) having nutritious and biomedical importance in addition to taxonomical utility (Velasco and Goffman 1999; Guil-Guerrero *et al*., 2001a; 2003). This study is the first report to evaluate the compositions of seed oil in Iranian borage *Echium amoenum* subjected in leshkan accession, Guilan, in North of Iran. Considering the limited information related to Iranian borage oil. The aim of this study was to investigate content and composition seed oil of of fatty acids and 1000 seed weight of Iranian borage in this accession.

Materials and methods

Plant materials

Leshkan accession seeds were collected from the leshkan,Eshkevari region, Guilan,Iran (36°40'N, 50°20'E; 1772 m above sea level) in September 2013. The soil characteristics are shown in Table 1.

Table 1. Soil properties.

Sand (%)	Silt (%)	Clay (%)	K (Av.) (p.p.m)	P (Av.) (p.p.m)	O.C (%)	T.N.V (%)	EC (dS/m)	pH
36	58	6	428	68.6	5.3	2.06	1137	6.73

T.N.V = Total Neutralizing Value; Av = available; O.C= Organic carbon.

Table 2. Fatty acids content and composition seed oil of Echium amoenum subjected in leshkan accession.

Common Name	Structural Formula	Lipid Numbers	Concentration (%)
Palmitic acid	$CH_3(CH_2)_{14}COOH$	C16:0	7.120
Palmitoleic	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	C16:1	0.083
Margaric acid	$CH_3(CH_2)_{15}COOH$	C17:0	0.136
Stearic acid	$CH_3(CH_2)_{16}COOH$	C18:0	5.162
Oleic acid	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	C18:1(n-6)	17.087
Linoleic acid	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$	C18:2(n-6)	20.684
Nonadecylic	$CH_3(CH_2)_{17}COOH$	C19:0	6.097
α-Linolenic*acid*	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$	C18:3(n-)ω3	35.694
Arachidic acid	$CH_3(CH_2)_{18}COOH$	C20:0	6.944
Gondoic acid	$C_{20}H_{38}O_2$	C20:1	0.712
Arachidonic acid	$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_3COOH$	C20:4(n-6)	0.282

Oil Exteraction

After cleaning and grinding the seeds, The 10 grams of seeds were weighed and oil extraction was carried out by Soxhlet for 6 to 7 hours. And the hexane was used as solvent. To separate the solvent from the oil the sample was placed in a rotary evaporator. After removal of solvent, oil percentage was calculated.

Fatty acid profiles

The derivatizaion of the lipid fraction of *Echium amuenum* was carried out according to Metcalfe *et al.* (1996). A lipid fraction sample (0.05 g) was placed in a test tube, a 3 ml mixture of boron tri floride methanol was added and then the test tube and its contents were heated in a water bath at 70 °C for 20 minutes. To recover the fatty acid methyl esters, the derivatized mixture was washed into a separatory funnel with 15 ml of hexane and 20 ml of distilled water. The organic and aqueous phases were then separated. The organic phase containing the fatty esters was dried and the solvent was evaporated at 50 °C. The fatty acid profiles were then determined by GC. Finally, for Dewatering of fatty acids in the sample, one ml of the supernatant phase was separated and combined with 0. 5 g Sodium sulfate (as an absorbent moisture) and centrifuged by 2500 rpm for two to five minutes to mix, then supernatant phase was injected into the GC. The GC analysis was performed on the obtained oil using a Unicam 4600. The chromatographic column for the analysis was a BPX70 capillary column (30 m × 0.25 mm i.d., film thickness 0.22 μm). The helium gas was used at 1ml/min as carrier gas. The temperature of injector and detector respectively were 250°C and 300°C.

Percentage of oil extraction

To determine the percentage of oil extraction was used method of Uquiche *et al.*, 2008.

Results and Discussion

Oil percentage and 1000 seed weight

The result of this study showed that the oil content of seeds was 36.4% and 1000 seed weight was 10.13 gr.

Composition of seed oil

The results of study showed that the leshkan accession of Iranian borage seed have containing eleven fatty acids (Table 2). α-Linolenic *acid (35.69%)*, linolenic acid (20%) and oleic acid (17.08%) were as the major fatty acids in the oil and have been identified in over 75% of the oil fatty acids. In other hand, Four fatty acids (palmitic acid (13.9%), linoleic acid (75.7%), Arachidic acid (6.2%) and oleic acid (4.1%)) by Mojab *et al*, (2008) and Seven fatty acids (linoleic acid (40.2%), linoleic (18.58%), Arachidic acid (10.6%),palmitic acid (6.64%), gamalinolenic (6.64%) Stearic acid (3.62%)) by Daneshfar *et al*, (2013) reported in seed oil of *Echium amoenum*. More than 75% of the fatty acids in Iranian borage are unsaturated fatty acids. Due to the high percentage of unsaturated fatty acids, especially α - linoleic acid, Iranian borage oil is valuable in terms of nutrition. Unsaturated fatty acids are involved in the cell membrane, gene expression, prostaglandin biosynthesis, nervous system and improve the immune system (Yehuda, 2001). Reduce the amount of palmitic acid in this accession can be due to the high altitude and cold of this area Considering that the amount of palmitic acid in cold areas is reduced (Jose *et al*., 1990). Fatty acid profiles were studied at various levels of ecotypes may be the result of enzymatic activity in ecotypes is due to geographical conditions. Fatty acid Biosynthesis pathway are shown in fig 1. the Another approach can be deduced, the contents and composition of the fatty acids in the seed oil of this plant, can be useful for assessing taxonomic relationships between interspecies and interaspecies. Unsaturated fatty acid content of oil seeds in cold areas is higher than in temperate zones (Jose *et al*., 1990). The high amounts of oleic acid in our study can be attributed to the increasing altitude and decreasing temperature.

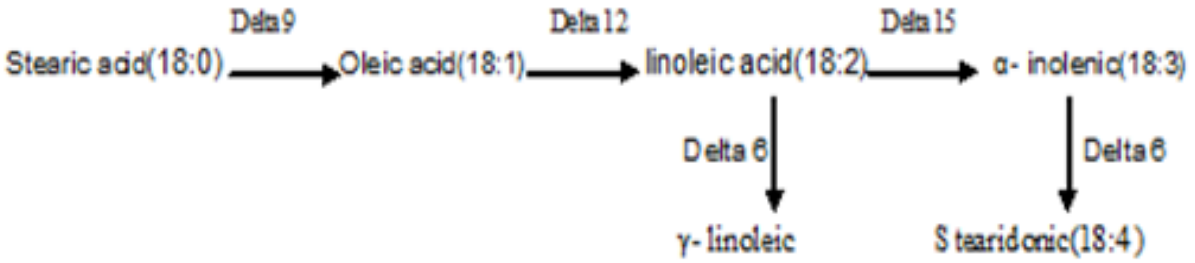

Fig 1. Biosynthesis pathway of fatty acids (Guil-Guerrero *et al.,* 2001).

More than 75% of the fatty acids are unsaturated fatty acids, borage Iran.

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