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# PROFILING OF PHENOLIC AND FLAVONOID COMPOUNDS OF IN *VITRO BORAGO OFFICINALIS* L. PLANTLETS: A PROMISING PLANT IN THERAPEUTIC NUTRITION

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**ABSTRACT**

*Borago officinalis*, sometimes known as borage, a medicinal and ornamental plant that grows throughout the Mediterranean basin, Western Asia, and parts of North Africa, South America, and Continental Europe. As a result of the importance of this plant, the aim of this study is to propagate it using tissue culture technique and identify the most bioactive compounds (phenolic and flavonoid). Shoot tip explants were grown on MS-medium without growth regulators or with 0.5 and 1.0 mg/l of the following growth regulators: *kinnetin (kin), 6-(,-dimethylallylamino) purine (2iP),* and *thidiazuron (TDZ).* After 30 days, shoot length, number of auxiliary buds, and observed rooting were recorded. The produced plantlets were subjected to extraction with 80% methanol, followed by HPLC profiling of phenolic and flavonoid compounds. The result revealed that, basal MS-medium without cytokinins resulted in higher quality shoots with root development than the other used media. The formation of small flowers was also observed on the plantlets that formed on the basal MS-medium after three subcultures. Rosmarinic acid showed the most frequent and prevalent compound, recorded 166.64 µg/g DW, followed by apigenin (55.63 µg/g DW), and caffeic (27.25 µg/g DW). *In vitro Borago officinalis* can be utilized as a source of phenolics and flavonoids, especially rosmarinic acid.

**Keywords**: *Borago officinalis*, *in vitro* culture, phenolics and flavonoids

## INTRODUCTION

Borage (*Borago officinalis* L.) is a Boraginaceae herbaceous plant native to North Africa that has spread to several Mediterranean countries. This plant is utilized in Algeria not only for making beverages and salads, but also for a variety of therapeutic applications and landscape coordination **(Mhamdi *et al.*, 2009)**. Borage leaves contain the following compounds: alkaloids, sopinin, sopindian, fatty acids including α-Linolenic acid, ALA (55%) and gamma-Linolenic acid GLA (4%); silicic acid (22%), and organic acids (acetic, lactic, and malic acid). The leaves of borage contain almost 30% mucilage that can be hydrolyzed to glucose, galactose, arabinose, and alantoein **(Murkovic *et al.*, 2002; Herrmann *et al.*, 2002)**. Furthermore, few studies on its antioxidant capabilities have been undertaken. According to **Conforti *et al.* (2008)**, borage leaf extract shows excellent antioxidant activity. Other investigations have discovered phenolic acids, antioxidants, which enable the scavenging of free radicals in ethanolic extracts of the seeds **(Mhamdi *et al.*, 2010)**. Phenolic compounds like rosmarinic acid, synergic acid, and synaptic acid are the principal chemicals that have antioxidant action **(Mhamdi *et al.*, 2009; Sánchez‐Escalante *et al.*, 2003)**. Borage raw leaves are used as an anticonvulsant, bronchodilator, and vasodilator **(Huang *et al.*, 1995)**. It also has anti-depressant properties, furthermore used to treat thrombosis, inflammation, and cancer **(Horrobin 1992; Campra-Madrid & Guil-Guerrero, 2002)**. GLA is used to treat local eczema, heart disease, cyclical mastalgia, diabetes, arthritis, and multiple sclerosis as a food supplement and medicine prescription **(Campra-Madrid & Guil-Guerrero, 2002)**. Besides, some chronic diseases such as cancer, burn, diabetes, hyperlipidemia, and amnesia have all been proven to benefit from medicinal herbs with natural antioxidants. These substances can prevent or treat the negative effects of other compounds **(Shirzad *et al.*, 2011; Ansari *et al.*, 2013; Khosravi-Boroujeni *et al.*, 2012; Baradaran *et al.*, 2012)**. Borage flour contains a high concentration of phenol compounds after oil extraction and can be used in place of antioxidants in oils and meat products to prevent fat oxidation **(Mhamdi *et al.*, 2009)**. Because of its potential to absorb reactive oxygen species and DPPH radicals, borage extract could be employed as an alternative medicine to treat disorders caused by free radicals that can harm tissues **(Ciriano *et al.*, 2009)**. As a result of increased interest in the quality of fatty acids, as well as their amount in human diet in terms of health and influence on improving atherosclerosis, the demand for borage has increased **(Meyer *et al.*, 1999)**. Based on the high importance of this promising plant, it was thought to provide it wildly spread by tissue culture technique. Large-scale plant tissue culture approaches have been shown to be an appealing a substitute for conventional plantations, offering a Plentiful quantities of secondary metabolites regardless of the availability of plants and a more reliable product in quantity and quality **(Sajc *et al.*, 2000)**. To meet pharmaceutical industry demand while conserving natural resources,

researchers focused their efforts in the previous decade on optimizing culture conditions for maximizing the obtained yield of specified secondary metabolites through the use of many artificially generated procedures **(Isah *et al*., 2018)**. A whole plant can be regenerated from an organ, tissue, or plant cell using plant tissue culture procedures, but it must be performed on an appropriate culture medium and in a controlled condition. Under these conditions, the obtained plantlets are true to type and have the same properties as the mother plant **(Hassanein & Soltan, 2000)**.

The current study's goal is to propagate *Borago officinalis* L. *in vitro* and identify the phenolics and flavonoids profile compound as the most significant and bioactive one in the resulting plants.

## MATERIALS AND METHODS

### Preparation of explants

The Faculty of Pharmacy, Cairo University, provided seeds of *Borago officinalis* L. from its Medicinal and Aromatic Plants Farm. After rinsing the seeds in sterile distilled water, they were submerged in 70% ethanol for two to three minutes. After that, the seeds were sterilized for 20 minutes in a 20% commercial Clorox (5% NaOCl) solution with 0.5% Tween 20. This was followed by three sterile distilled water rinses. The seeds were cultivated in aseptic conditions on MS-medium **(Murashige & Skoog, 1962)**, which included 3% (w/v) sucrose, and then they were solidified using 0.2% (w/v) Gelzan (Gelrite). pH 5.8 was achieved in the culture medium. The cultures underwent a 16-hour photoperiod consisting of fluorescent light tubes with 45 µmol cool white light and 8 hours of darkness, all cultures incubated at 26±2°C.

### *In vitro* propagation of *Borago officinalis* L.

Shoot tip explants obtained from 30 days-old seedlings were cultured on MS-medium either without growth regulators or with 0.5 and 1.0 mg/l of kinetin (kin), 6-(γ,γ-dimethylallylamino) purine (2iP), and thidiazuron (TDZ) as growth regulators. The cultures were maintained under 16 hours of fluorescent light, 45 µmol cool white light tubes, and 8 hours of darkness in a culture room maintained at 26±2°C. Shoot length, the quantity of auxiliary buds, and the observed rooting were measured thirty days later.

### HPLC of phenolic compounds

### Preparation of extracts

Harvested micropropagated plantlets on the best medium were lyophilized using a Labconco freeze dryer. The drying was performed at −50°C, 0.1 mbar, and 48 h. The dried plant materials were macerated in 80% methanol for 12 h, then filtered and concentrated on a rotary evaporator under vacuum. The extracts were then kept at -20°C until use.

### Instrument and chromatographic conditions

Agilent Technologies 1100 series liquid chromatograph, which was outfitted with a diode-array detector and an auto sampler, was used to perform the HPLC analysis. An Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) analytical column with a C18 guard column was used (Phenomenex, Torrance, CA). Acetonitrile (solvent A) and 2% acetic

acid in water (v/v) (solvent B) made up the mobile phase. The flow rate was maintained at 0.8 ml/min for the duration of the 60-minute run. 50 µl was the injection volume. The peaks were monitored simultaneously at 280 and 320 nm for the benzoic acid and cinnamic acid derivatives, respectively as well as 360 nm for flavonoids, then identified by congruent retention times and UV spectra and compared with those of the standards.

**Statistical analysis**

Twenty-four explants were used per each treatment. Means and standard errors (SE) were obtained from analysis for each treatment by the use of computer program Microsoft Excel 2010. Data were presented as means ± SE.

## RESULTS AND DISCUSSIONS

### *In vitro* propagation of *Borago officinalis* L.

*In vitro* propagation of medicinal plants has become critical to delivering high-quality stock plants for conservation and pharmaceutical purposes **(Nilanthi & Yang, 2014)**. Basal MS-medium and MS-medium supplemented with different cytokinins (kinetin, 6-dimethylallylaminopurine, and thidiazuron) were utilized in this experiment to study their effect on the growth of the resulting *Borago officinalis* L. plantlets. The results in Table 1 demonstrated that, the shoots were initiated in all media with varying percentage responses, with 0.5 mg/l TDZ recording the highest value at 83.3%, followed by basal MS-medium recording 68.8%. The use of kinetin led to modest response. However, with media containing 2iP, the % response recorded the lowest value. While rooting formation occurred only on the basal MS-medium with maximum shoot length (4.2 cm) and highest number of auxiliary buds (3.0), in contrast to the media containing cytokinins, no rooting formation has occurred. Also, all media containing cytokinins recorded lower shoot length and number of auxiliary buds compared to the basal MS-medium; the minimum values were recorded by using 1.0 mg/l 2iP (1.3, 1.7 cm, respectively), and this medium recorded a minimum shoot formation of 12.5%. The high quality of resultant shoots with root formation was also noticed by using basal MS-medium without cytokinins compared to other used media (Fig. 1). The formation of small flowers was also observed on the plantlets that formed on the basal MS-medium after three subcultures. Rooted small flowered plantlets on MS-medium are presented in Fig. 2. There has been little research on *Borago officinalis* L. *in vitro* propagation, including an attempt to grow doubled haploid (DH) plantlets from anther culture. They discovered that, the addition of 200 mg/l colchicine over 4 days, and a 5-hour pretreatment of anthers with 0.2% n-butanol produced the best results **(Hoveida *et al*., 2017)**. Plant tissue culture is the most promising solution for medicinal plants that have low yields and are susceptible to biotic or abiotic stress. It can also be employed for conservation, propagation, induction of polyploidy or aneuploidy, plant engineering, and bioreactor applications.

Many endangered and endemic medicinal plants have undergone *in vitro* multiplication such as *Bacopa monnieri* **(Mathur *et al*., 2003)**, *Paedaria foetida* **(Srivastava & Srivastava, 2004)** and *Picrorhiza kuroa* **(Sood & Chauhan, 2009)**. High multiplication was obtained in various plant species by using shoot meristems, including *Citrullus colocynthis* **(Meena & Patni, 2007)**, *Zephyranthes bulbous* **(Gayathri & Ramagopal, 2007)**, *Glossocardia bosvallea* **(Geetha & Gopal, 2007)**, *Cannabis sativa* **(Monthony *et al*., 2021)**, and *Caralluma retrospeciens* **(Shawky & Mussa, 2020)**.

**HPLC of phenolic compounds**

Antioxidant study of various plant sources was prompted by the growing interest in natural substitutes for synthetic antioxidants. In the current study, 80% methanol has been used to extract the most bioactive phenols and flavonoids from *Borago officinalis* plantlets. Table 2 shows the 14 phenolic and flavonoid compounds that were found. The most common and abundant compound was rosmarinic acid, which was recorded at 166.64 µg/g DW. Apigenin came in second with 55.63 µg/g DW, while caffeic acid was found at 27.25 µg/g DW. Vanillic acid had the lowest concentration (0.305 g/g DW), whereas the remainder of the components, including gallic, protocatechuic, p-hydroxybenzoic, gentisic, cateachin, chlorogenic, syringic, ferulic, cinnamic, and kaempferol, had intermediate values ranging from 1 to 12 µg/g DW. Ethanolic and aqueous extracts of *Borago officinalis* leaves were previously assessed for phenolics and falvonoids content. Also various methods were used to assess the antioxidant capabilities of these extracts. They reported that the ethanolic extract had higher phenolics and flavonoids content as well as better antioxidant potential compared to the aqueous extract. LC MS/MS analysis of *Borago officinalis* extract of leaves revealed the existence of flavonoids and polyphenol (oleuropein), which were identified in borage for the first time **(Zemmouri *et al*., 2019)**.

Methanolic extract of Borago seeds subjected to identification of phenolics and flavonoids, caffeic acid, phydroxyphenyl, and p-hydroxybenzoic acid were the main components **(Zadernowskia *et al*., 2002)**. Indeed, ferulic acid, cinnamic acid, syringic acid, sinapic acid, and coumaric acid were found in the methanolic leaf extract of borago **(Mhamdi *et al*., 2010)**. Which is consistent with the presence of these phenolic acid derivates in our extract. In our work as well, the rosmarinic compound was present in greater quantities than the other compounds, implying that this plant may be used to generate rosmarinic with high efficiency.

Because of their diverse benefits for human health, research investigations concentrating on flavonoids and other phenolic compounds from medicinal plant species have risen significantly in recent decades. The majority of recent evaluations concentrated on a single element of flavonoids or phenolics' influence on human health **(Wang *et al*., 2016; Okpuzor *et al*., 2009; Oki *et al*., 2002)**. Borago is an edible plant that grows throughout the parts of North Africa, South America, and Continental Europe. It has traditionally been

exploited as a valuable culinary resource in several regions of Spain, Italy, France, and Germany **(Hoveida *et al.,* 2017)**. Therefore, its cultivation in Egypt can be expanded, whether in the field or using tissue culture technique.

## CONCUSIONS

Plant tissue culture is a well-known method for creating a large number of genetically identical plantlets. In our work, using basal MS-medium without cytokinins produced shoots with root development that were of higher quality than those produced with other media. The methanolic extract of *Borago officinalis* revealed the existence of 14 phenolic and flavonoid compounds. Rosmarinic acid was the most prevalent and plentiful component. Its known that rosmarinic acid has antioxidant activity and in addition to the fact that the plant of borage contains high amount of α-Linolenic acid and gamma- Linolenic acid. Therefore, the plant is considered a promising source for treating many diseases.

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**Table (1): Effect of different growth regulators on shoot and root formation of *Borago officinalis* L.**

| Growth regulators | Concentration (mg/l) | Response (%) | Shoot length | Number of auxiliary buds | Rooting |
|---|---|---|---|---|---|
| Basal | | 68.8 | 4.2 ± 0.2 | 3.0 ± 0.5 | + |
| Kin | 0.5 | 60.0 | 2.7 ± 0.7 | 2.7 ± 0.7 | - |
| | 1.0 | 50.0 | 2.3 ± 0.3 | 2.0 ± 0.0 | - |
| 2iP | 0.5 | 44.4 | 2.5 ± 0.3 | 1.7 ± 0.3 | - |
| | 1.0 | 12.5 | 1.3 ± 0.2 | 1.7 ± 0.3 | - |
| TDZ | 0.5 | 83.3 | 2.5 ± 0.3 | 2.0 ± 0.0 | - |
| | 1.0 | 27.3 | 2.2 ± 0.2 | 2.3 ± 0.3 | - |

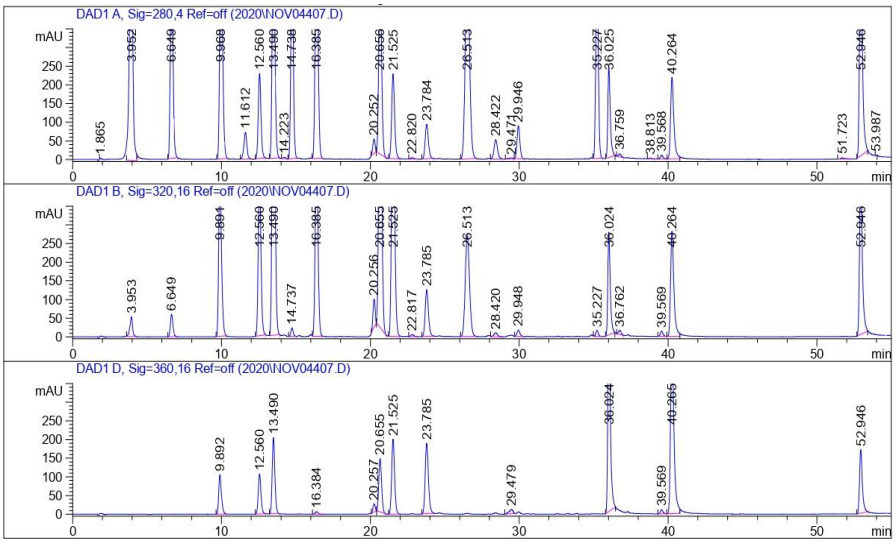

**Fig. (1): Shoots formation from shoot tip explant after one month on MS-medium containing 0.5 mg/lkin (A), 0.5 mg/l2iP (B), and 0.5 mg/lTDZ (C), on basal MS-medium (D).**

**Fig. (2): Rooted and flowered plantlets on MS-medium without any growth regulators.**

**Table (2): Profile of phenolic and flavonoid compounds in the methanolic extract of *Borago officinalis* L. plantlets.**

| No. | Compound | Retention time (min) | Molecular formula | Concentration (µg/g DW) |
|---|---|---|---|---|
| 1. | Gallic | 3.9 | C7H6O5 | 2.77 |
| 2. | Protocatechuic | 7.7 | C7H6O4 | 1.63 |
| 3. | *p*-hydroxybenzoic | 12 | C7H6O3 | 6.77 |
| 4. | Gentisic | 12.1 | *C7H6O4* | 2.70 |
| 5. | Cateachin | 15.2 | C15H14O6 | 3.00 |
| 6. | Chlorogenic | 16.5 | C16H18O9 | 1.14 |
| 7. | Caffeic | 17.2 | C9H8O4 | 27.25 |
| 8. | Syringic | 19.3 | C9H10O5 | 5.86 |
| 9. | Vanillic | 21.2 | *C8H8O4* | 0.305 |
| 10. | Ferulic | 28.9 | C16H12O5 | 7.24 |
| 11. | Rosmarinic | 39.2 | *C18H16O8* | 166.64 |
| 12. | Cinnamic | 46.9 | *C9H8O2* | 3.56 |
| 13. | Apigenin | 54.2 | *C15H10O5* | 55.63 |
| 14. | Kaempferol | 54.9 | C15H10O6 | 11.32 |

A




B

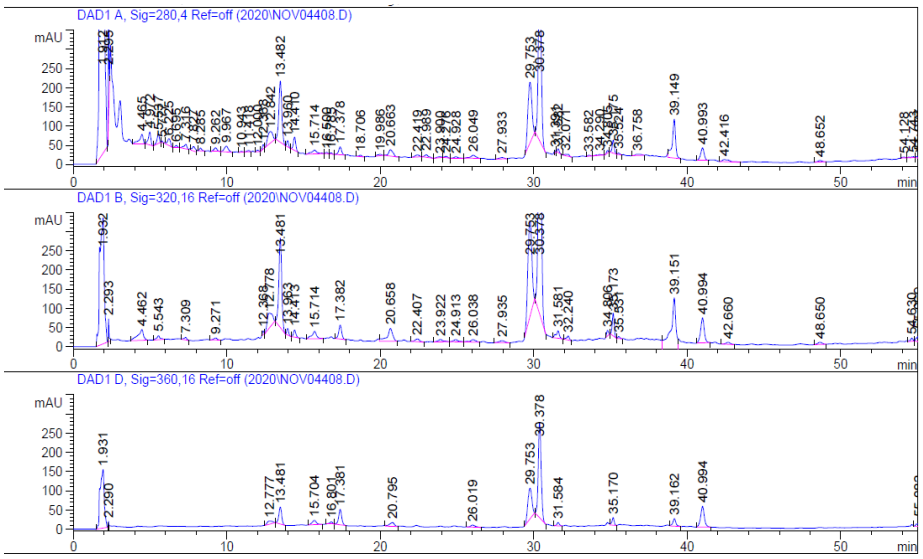



**Fig. (3): HPLC chromatogram of phenolic and flavonoids standard compounds (A) and methanolic extract (B) showing the peaks obtained for the mixture containing 14 compounds at: $\lambda$=280 nm (gallic acid, protocatechuic, *p*-hydroxybenzoic , cateachin, syringic, vanillic, and cinnamic); $\lambda$=320 nm (gentisic, chlorogenic, caffeic, ferulic, cinnamic, and apigenin); and $\lambda$=360 nm (kaempferol).**

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**الملخص العربي**

## المركبات الفينولية والفلافونيدية في نباتات البوراج الناتجة معمليا: نبات واعد في التغذية العلاجية

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[2]قسم نباتات الزينة والأشجار الخشبية – المركز القومي للبحوث – الدقي 12622 – مصر

بوراغو أوفيشياليس، المعروف أحيانًا باسم لسان الثور، هو نبات طبي وزخرفي ينمو في جميع أنحاء حوض البحر الأبيض المتوسط، وغرب آسيا، وأجزاء من شمال أفريقيا، وأمريكا الجنوبية، وأوروبا القارية. ونظرا لأهمية هذا النبات فإن الهدف من هذه الدراسة هو إكثاره بتقنية زراعة الأنسجة وتحديد المركبات الحيوية الأكثر نشاطا (فينولات وفلافونيدات). تمت زراعة براعم النباتات الناتجة من البذور على بيئة MS بدون منظمات نمو أو باستخدام 0.5 و1.0 ملغم/لتر من منظمات النمو التالية: *kinnetin (kin), 6-(,-dimethylallylamino) purine (2iP), and thidiazuron (TDZ).*

وبعد 30 يومًا، تم تسجيل طول اللأفرع وعدد البراعم المساعدة والتجذير الملحوظ. تم إخضاع النباتات الناتجة للاستخلاص باستخدام 80% من الميثانول، متبوعًا بتحليل HPLC للمركبات الفينولية والفلافونيدية. أظهرت النتائج أن بيئة ال MS التي لا تحتوي على السيتوكينينات أدى إلى الحصول على براعم ذات جودة أعلى مع تطور الجذر مقارنة بالبيئات الأخرى المستخدمة. كما لوحظ تكوين أزهار صغيرة على النباتات التي تكونت على بيئة ال MS بعد ثلاث مرات نقل. أظهرت نتائج التحليل الHPLC للمركبات الفعالة أن حمض الروزمارينيك ه المركب الأكثر شيوعًا وانتشارًا من بين 14 مركب فينولي، حيث سجل 166.64 ميكروجرام/جرام وزن جاف، يليه الأبيجينين (55.63 ميكروجرام/جرام وزن جاف)، والكافيين (27.25 ميكروجرام/جرام وزن جاف). ملخص الدراسة أنه يمكن زراعة هذا النبات في المختبر و استخدامه كمصدر للفينولات والفلافونيدات، وخاصة حمض الروزمارينيك.