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Time-Dependent Accumulation of Phenolic Compounds in Linum Album Kotschy Cell Culture Exposed to Methyl Jasmonate

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Abstract

Linum album can produce many valuable phenolic compounds, especially lignans, potent anticancer agents. In the present study, we attempted to determine the inducing effect of Methyl Jasmonate (MeJa) (50µM) as an abiotic elicitor on the production of phenolics such as phenolic acids, flavonoids, and lignans in cell culture of L. album over a period of time. The content of cinnamic acid, p-coumaric acid, and ferulic acid increased at 72 h in response to elicitation. However, the level of caffeic acid increased at 48 h of elicitation, 1.53 times higher than the untreated sample. According to the results, the content of flavonoids, including catechin, quercetin, myricetin, and daidzein, also significantly increased during the last hours of the treatment with 50 µM of MeJA in L. album cells. The maximum levels of quercetin and myricetin were observed at 48 h of elicitation, while catechin and daidzein contents peaked at 72 h, 2-fold and 2.42-fold over the control, respectively. Significant time-dependent changes also occurred in Lariciresinol (LARI) and 6-Methoxypodophyllotoxin (6MPTOX) contents under MeJa treatment, while there was no change in Secoisolariciresinol (SECO) content in treated cells compared to the control. The level of 6MPTOX reached 4 times the control sample after 72 h of elicitation. In conclusion, the induction of phenolic contents in response to MeJA in a time-dependent manner can suggest that treatment time adjustment is essential to increase and obtain specific phenolic compounds in the cell culture of L. album at a shake-flask scale.

Keywords: Cell culture, Flavonoids, Lignans, Linum album, Phenolic compounds.

1 | Introduction

To date, various bioactive agents are derived from plants as the main source of natural biocompounds, which can be used in the pharmaceutical, nutritional, and cosmetic industries [1], [2]. Among phytochemicals, phenolic compounds form a major group synthesized by the phenylpropanoid pathway, including phenolic

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acids, flavonoids, lignans, and lignins [3]. These substances play a pivotal role in preserving plant biological machinery, especially crucial macromolecules, including carbohydrates, proteins, lipids, DNAs, and RNAs, versus the harmful impacts of oxidative processes [4], [5]. On the other hand, phenolic substances have long been used for human health due to their antioxidant potential to prevent many chronic and degenerative disorders as protective dietary ingredients [6], [7]. Many in vitro and in vivo studies on polyphenols exhibit their anticancer, anti-inflammatory, antifungal, antiviral, and antibacterial properties [5], [8], [9]. Therefore, the biosynthesis of these compounds in various plants for their food and medicinal applications has been studied largely in recent years [10], [11].

Linum album Kotschy ex Boiss, a member of the Linaceae family, is a medicinal herb belonging to the endemic plant species of Iran [12]. This plant can produce many phenolic compounds, especially lignans [13], [14]. Podophyllotoxin (PTOX), 6-Methoxy Podophyllotoxin (6MPTOX), Secoisolariciresinol (SECO), and Lariciresinol (LARI) are the main lignans of this plant, which have antiviral and antineoplastic effects [15]. Based on the previous studies, it is evident that in vitro culture is an effective protocol to facilitate the production of large quantities of plants to isolate valuable metabolites while preventing the exploitation of wild plants [16]. In this case, in vitro studies have suggested that different elicitors can induce the accumulation of phenolic compounds such as lignans, lignin, phenolic acids, and flavonoids in L. album [17–19]. MeJa, as a potent elicitor, is a methylated derivative of jasmonic acid, which can act as a plant defense regulator involved in the induction of secondary metabolite production [20]. In our previous study, we represented the effect of MeJa on the production of lignans and the accumulation of amino acids and soluble sugars at only one-time point [21]. In the present work, we investigated the time-dependent production of phenolic compounds in *L.* album cell culture under MeJA elicitation by determining changes in phenolic acids, flavonoids, and lignans profiles.

2 | Materials and Methods

2.1 | Cell Culture and Elicitation

L. album seeds were obtained from wild plants in the Sohanak region (35°480'N, 51°32'E, and altitude of 1900 m) placed in Tehran province, Iran. Dr. Shahrokh Kazempour-Osaloo, a professor of plant systematics, identified this plant. Seeds were surface-sterilized and cultivated in a sterile MS medium jar [22] under dark conditions. After seed germination, seedlings as a source of explants were grown in a growth chamber at 16 h photoperiod under 45µmol m^{-2} s^{-2} of irradiance level, 21/17 °C (Day/night), and 50% humidity. A callus line was established from leaf explant grown on solidified MS medium supplemented with 2 mg L^{-1} of NAA and 0.4 mg L^{-1} Kin. After callogenesis, suspension culture was provided by crushing friable callus in liquid MS medium supplemented with NAA (2 mg L^{-1}) and Kin (0.4 mg L^{-1}). The samples were placed on a rotary shaker at 110 rpm and 25±2 °C under continuous darkness. Every 14 days, 2 g of cells were subcultured into the fresh media in a 100 mL flask. MeJA with a final concentration of 50µM was added to the cells after 7 days of culture [22]. The samples were harvested at 24, 48, and 72 h after elicitation.

2.2 | Quantification of Phenolic Acids

The samples were extracted based on [23] procedure for determining phenolic acids. Briefly, frozen cells (0.2 g) were ground in methanol with a mortar and pestle. After that, the extracts were centrifuged, and supernatants were collected. After evaporation of the solvent, the residual was resuspended in acetonitrile (4 mL), and the solution was extracted three times with 3 mL of n-hexane. Determination of phenolic acids was performed by HPLC system (Agilent Technologies 1260 infinity, USA) using a C-18 column (Perfectsil Target ODS-3 (5µm), 250 × 4.6 mm; MZ Analysentechnik, Mainz, Germany). A photodiode array (278-300 nm) was set on HPLC to detect UV spectral data. The mobile phase was aqueous acetic acid 2% (A) and methanol (B) [24].

2.3 | Determination of Flavonoids

To measure flavonoids, the cells (0.5g) were homogenized in 1.5 mL of methanol (40%, v/v) containing 0.5% acetic acid. All samples were shaken for 4 h and centrifuged at 13,000 rpm for 12 min. The detection of individual flavonoids was performed based on an HPLC system equipped with a C-18 column (Perfectsil Target ODS-3 (5µm), 250×4.6 mm; MZ Analysentechnik, Mainz, Germany). The UV spectra were 254, 280, 300, and 350 nm. Phosphoric acid (0.5%) in deionized water (Solvent A) and acetonitrile (solvent B) were utilized as the mobile phase in the gradient program [25]. Identification of flavonoids was achieved according to the retention time and peak area of their authentic standards.

2.4 | Analysis of Lignans

Lignans contents were determined by Agilent 1260 HPLC chromatography with a Diode Array Detector (DAD) based on [26] protocol. A total of 200 mg of dried cells were used to extract lignans, as described by Yousefzadi et al. [27], with a few modifications. Finally, HPLC-grade methanol (0.5mL) was mixed with the residues, and after centrifuging, 20µL of the solution was injected into the HPLC system. A C18-ODS3 (5µm, 250×4.6 mm) column was used as the stationary phase. The elution solvent was composed of acetonitrile and water with a standard gradient system, as described by [27].

2.5 | Statistical Analysis

The experiment was accomplished following a completely randomized design. All measurements were performed in triplicate, representing data as the mean ± standard error. To analyze data, SPSS software version 25 was used. Differences between groups were calculated using an Analysis of Variance (ANOVA) test followed by Duncan's multiple range test (α=0.05).

3 | Results and Discussion

Today, the increasing demand for bioactive products has made cultured plant cells, tissues, and organs widely used as an important alternative source for producing and accumulating many valuable phytochemicals [28], [29]. In the last decade, L. album has also been introduced into different in vitro cultures, and their capacity for biosynthesis of desired phenolic compounds such as phenolic acids, flavonoids, and lignans has been evaluated in response to different biotic and abiotic elicitors [30], [18], [31–33]. In our previous work, the effect of several concentrations of MeJa elicitor was investigated on the accumulation of phenolic compounds, especially lignans, in L. album cell suspension [21]. Here, we detailed the accumulation of phenolics over a time when L. album cells were treated with the optimal concentration of MeJa (50µM).

The results showed that the contents of some phenolic acids, including cinnamic, p-coumaric, ferulic, and caffeic acids, changed in the treated cells. According to the results, cinnamic acid content altered in L. album cells during MeJa treatment, with its peak achieved after 72 h (*Fig. 1a*). On the other hand, changes in p-coumaric acid were found in the treated cells, which was up to 1.13-fold in comparison to the control after 72 h of exposure (*Fig. 1b*). Also, caffeic acid content increased at 48h of MeJa treatment, which was 1.53 times more than the control sample (*Fig. 1c*). As shown in *Fig. 1d*, the results indicated a decrease in ferulic acid content after 24h of MeJa treatment, while its content increased after 72h. Phenolic acids are a group of phenolic compounds with antimicrobial and antioxidant properties and play key roles in plant tolerance to cope with adverse conditions and decrease the toxic effects of Reactive Oxygen Species (ROS) [34]. Among the phenolic acids, cinnamic acid can also serve as a substrate to form other complex phenolic compounds with ubiquitous distribution in fruits and vegetables [35]. Furthermore, phenolic acids are commonly utilized as flavor and aroma compounds in the food, beverage, and perfumery industries [36]. Our observations were quite similar to the results reported by [18], where they considered higher concentrations of cinnamic, p-coumaric, ferulic, and caffeic acids in hairy roots of L. album exposed to chitosan in a time-dependent manner.

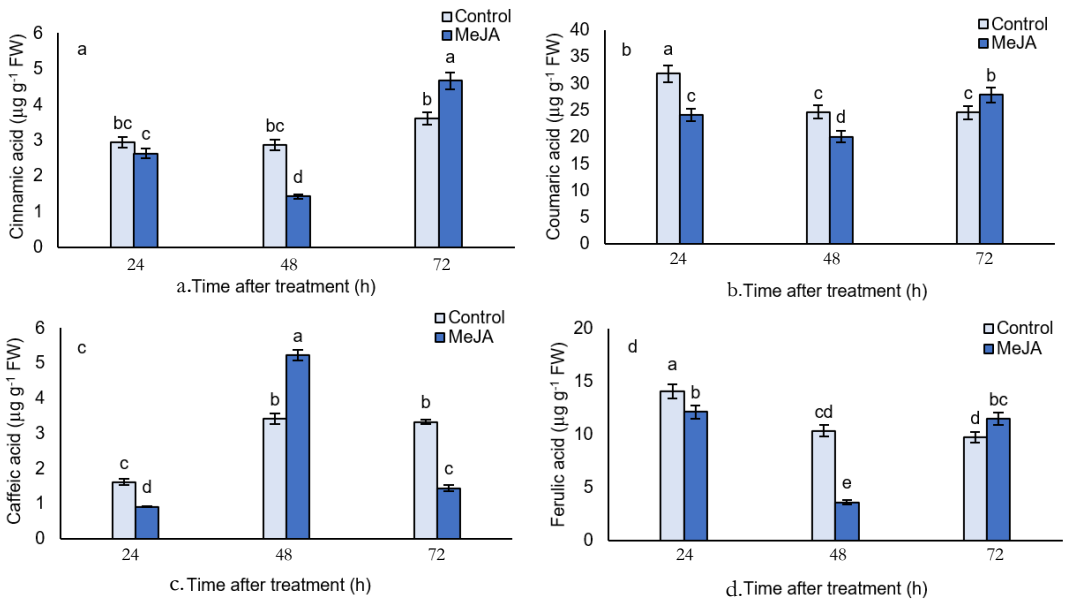
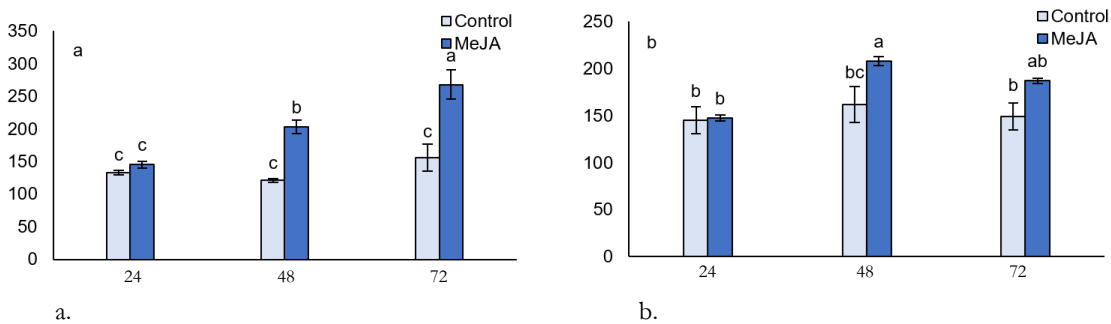


Fig. 1. Effect of MeJa elicitation on the contents of cinnamic acid; a. coumaric acid, b. caffeic acid, c. and Ferulic acid, d. in L. album cell culture.

Data are means±SD. Significant differences (P≤ 0.05) are indicated by different letters.

The effect of MeJa elicitation on the contents of various flavonoids was tested by measuring the levels of individual flavonoids catechin, quercetin, myricetin, and daidzein, where HPLC chromatogram of the treated samples showed the significant changes in their contents (*Fig. 2*). According to the results, the content of catechin enhanced during MeJa treatment, which its peak achieved at 72h (267.8µg g^{-1} FW) (*Fig. 2a*). Quercetin content remained unaffected at 24 h of MeJa treatment compared to the control. However, its content showed a significant enhancement after 48h and 72h as a time-dependent response, where its content was up to approximately 1.26 and 1.28-fold, respectively (*Fig. 2b*). A significant increase was also observed for myricetin at 48h of treatment, which was 1.21 times higher than that of the control sample (*Fig. 2c*). Likewise, daidzein showed a linear increment in content during the treatment period, with a peak of 2.42 times that of the control sample after 72h of elicitation (*Fig. 2d*). It has been determined that flavonoids are abundant polyphenols that are involved in various plant life processes such as regulating cell growth, attracting pollinators, and protecting versus adverse conditions [37].

Likewise, these molecules can act as signaling agents, UV filters, and ROS scavengers, and thus, they can modulate the plant defense system against different stresses like drought, heat, and freezing [38]. In humans, flavonoids exhibit a broad spectrum of health benefits, which are dependent on their natural structures and bioactive functions, including anti-inflammatory, anticancer, anti-aging, cardio-protective, neuroprotective, immunomodulatory, antidiabetic, antibacterial, antiparasitic, and antiviral properties [39]. The hydroxyl group in flavonoids is well-known as a functional group for donating electrons through resonance, which deactivates free radicals and ameliorates the antioxidant system [40].



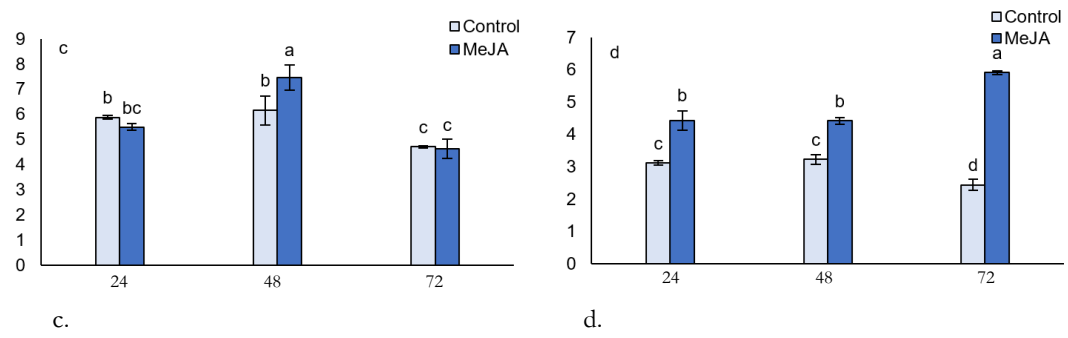


Fig. 2. Effect of MeJa elicitation on the contents of catechin (time after treatment), a. catechin, b. quercetin, c. Myricetin, d. daidzein.

Data are means±SD. Significant differences (p≤0.05) are indicated by different letters.

On the other hand, we quantified 3 main lignans of 10-day-old cultured cells of L. album following MeJa elicitation at different time points. As shown in *Fig. 3a*, SECO content did not change significantly during MeJa elicitation. Moreover, LARI content significantly increased during MeJA elicitation over a period of 24 h in comparison with the untreated sample. However, its content was decreased at 48h, which was approximately 0.77-fold lower than the control (*Fig. 3b*). The content of 6MPTOX increased after MeJa elicitation so that a 4-fold increase was observed after 72h (*Fig. 3c*). Previous studies have been shown that lignans, as a subgroup of monolignols derived from phenylpropanoid route, are associated with various defense mechanisms in L. album that lead to the modulation of the effect of different biotic and abiotic elicitors [17], [18]. Clinician reports have shown that lignans and their glycosidic derivatives (e.g., etoposide, etopophos, and teniposide) have functional roles in treating several types of cancers as antineoplastic drugs [41], [42].

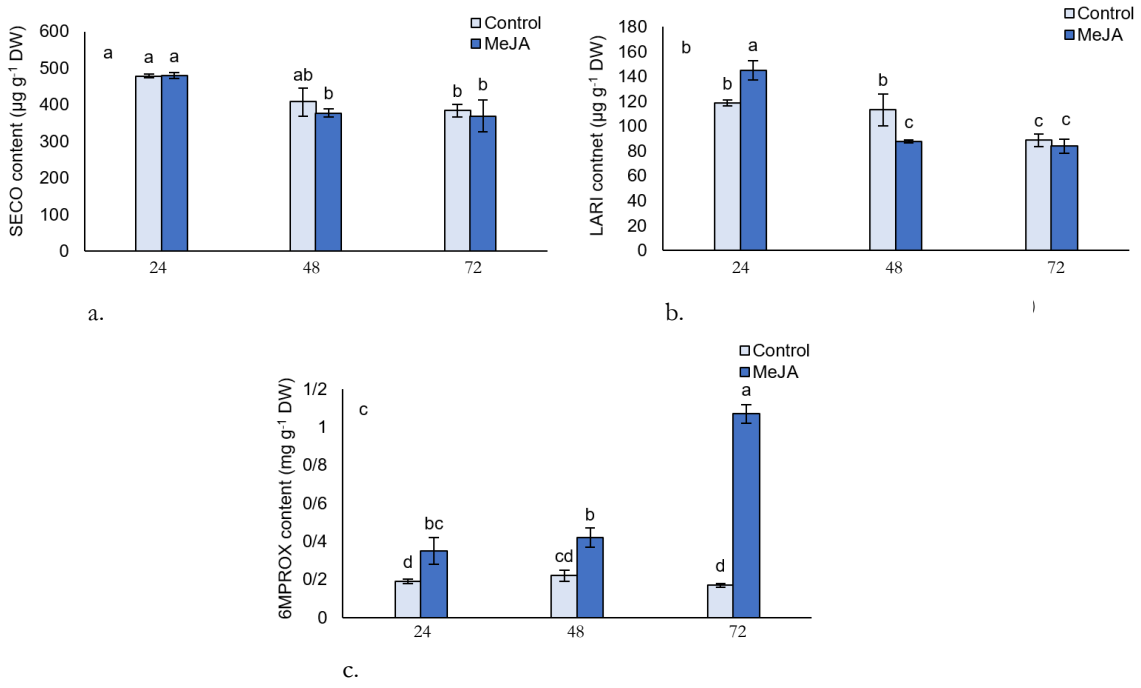


Fig. 3. Effect of MeJA elicitation on the contents of SECO (Time after treatment (h)); a. LARI, b. and 6MPTOX, c. in L. album cell culture. Data are means±SD. Significant differences (P≤0.05) are indicated by different letters.

Fig. 4a shows a positive correlation between phenolic acids, flavonoids, and lignans was found. Cluster analysis of phenolic compounds revealed 4 distinct clusters: 1) myricetin to caffeic acid, 2) 6MPTOX to catechin, 3) ferulic acid to cinnamic acid, and 4) LARI to SECO. Principal Component Analysis (PCA) of the HPLC data

also revealed correlation patterns between the accumulation of phenolic compounds and MeJa elicitation (*Fig. 4b*). According to the data mining by PCA, the components were significantly separated into the treated and untreated cells during a time-point of elicitation. PCA score frame (PC1 and PC2) explained the differences between the cells treated with MeJa and the control samples. MeJa at 72h showed a clear separation by the first component (PC1~62.4%), while different time points were dedicated by the second component (PC2~27.2). In summary, this study indicated that MeJA can induce phenolic acids, flavonoids, and lignans accumulation in L. album cell culture in a time-dependent manner.

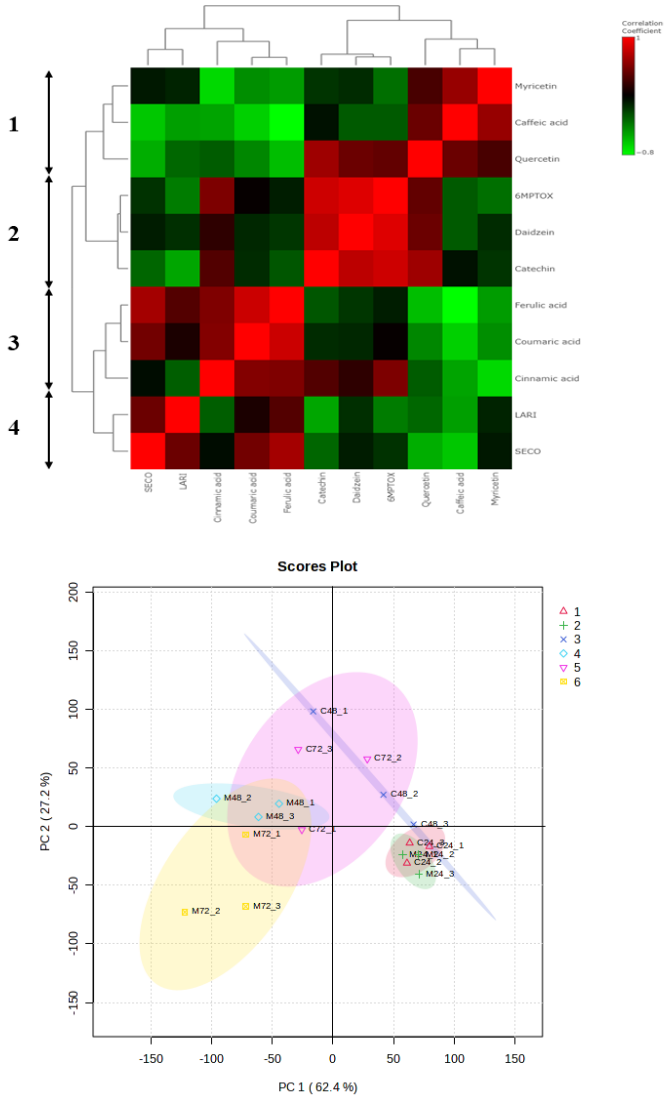


Fig. 4. An HCA map was employed to cluster various metabolites according to the pearson correlations coefficient.

Positive and negative correlations are described by red and blue color, respectively; a. Score plots of the PCA conducted on the data obtained from samples treated with MeJa and their control at the 24, 48, and 72h time courses (b).

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Author Contributions

All authors contributed to this research paper. The investigation, Methodology, Data curation, Resources, Formal analysis, Software, Performing the experiments, Analyzing the data and writing the manuscript, M.S., Design and Supervise the study, Project administration, Supervision, Methodology, Data curation, Manuscript review, and editing, M.SH., Advise the study, Manuscript review, and editing, E.S. All authors have read and agreed to the published version of the manuscript.

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Data Availability

The data supporting the findings of this study are available from the corresponding authors upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Yahya, N. A., Attan, N., & Wahab, R. A. (2018). An overview of cosmeceutically relevant plant extracts and strategies for extraction of plant-based bioactive compounds. *Food and bioproducts processing*, *112*, 69–85. DOI: 10.1016/j.fbp.2018.09.002

[2] Nguyen, T. L., Ora, A., Häkkinen, S. T., Ritala, A., Räisänen, R., Kallioinen-Mänttäri, M., & Melin, K. (2023). Innovative extraction technologies of bioactive compounds from plant by-products for textile colorants and antimicrobial agents. *Biomass conversion and biorefinery*, *14*(20), 24973–25002. DOI: 10.1007/s13399-023-04726-4

[3] Vogt, T. (2010). Phenylpropanoid biosynthesis. *Molecular plant*, *3*(1), 2–20. DOI: 10.1093/mp/ssp106

[4] Ghasemzadeh, A., & Ghasemzadeh, N. (2011). Flavonoids and phenolic acids: role and biochemical activity in plants and human. *Journal of medicinal plant research*, *5*(31), 6697–6703. DOI: 10.5897/JMPR11.1404

[5] Khalid, M., Saeed-ur-Rahman, Bilal, M., & HUANG, D. feng. (2019). Role of flavonoids in plant interactions with the environment and against human pathogens - a review. *Journal of integrative agriculture*, *18*(1), 211–230. DOI: 10.1016/S2095-3119(19)62555-4

[6] Tungmunnithum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. *Medicines*, *5*(3), 93. DOI: 10.3390/medicines5030093

[7] Mutha, R. E., Tatiya, A. U., & Surana, S. J. (2021). Flavonoids as natural phenolic compounds and their role in therapeutics: an overview. *Future journal of pharmaceutical sciences*, *7*(1), 25. DOI: 10.1186/s43094-020-00161-8

[8] Ahmad, A., Kaleem, M., Ahmed, Z., & Shafiq, H. (2015). Therapeutic potential of flavonoids and their mechanism of action against microbial and viral infections - a review. *Food research international*, *77*, 221–235. DOI: 10.1016/j.foodres.2015.06.021

[9] Nemitz, M. C., Argenta, D. F., Koester, L. S., Bassani, V. L., von Poser, G. L., & Teixeira, H. F. (2016). The international scenario of patents concerning isoflavones. *Trends in food science and technology*, *49*, 85–95. DOI: 10.1016/j.tifs.2016.01.008

[10] Costa, P., Gonçalves, S., Valentão, P., Andrade, P. B., Coelho, N., & Romano, A. (2012). Thymus lotocephalus wild plants and in vitro cultures produce different profiles of phenolic compounds with antioxidant activity. *Food chemistry*, *135*(3), 1253–1260. DOI: 10.1016/j.foodchem.2012.05.072

[11] Cheynier, V. (2012). Phenolic compounds: from plants to foods. *Phytochemistry reviews*, *11*(2–3), 153–177. DOI: 10.1007/s11101-012-9242-8

[12] Suzuki, S., & Umezawa, T. (2007). Biosynthesis of lignans and norlignans. *Journal of wood science*, *53*(4), 273–284. DOI: 10.1007/s10086-007-0892-x

[13] Fuss, E. (2003). Lignans in plant cell and organ cultures: an overview. *Phytochemistry reviews*, *2*(3), 307–320. DOI: 10.1023/B:PHYT.0000045500.56476.f5

[14] Javadian, N., Karimzadeh, G., Sharifi, M., Moieni, A., & Behmanesh, M. (2017). In vitro polyploidy induction: changes in morphology, podophyllotoxin biosynthesis, and expression of the related genes in Linum album (linaceae). *Planta*, *245*(6), 1165–1178. DOI: 10.1007/s00425-017-2671-2

[15] Samari, E., Sharifi, M., Ghanati, F., Fuss, E., & Ahmadian Chashmi, N. (2020). Chitosan-induced phenolics production is mediated by nitrogenous regulatory molecules: NO and PAs in linum album hairy roots. *Plant cell, tissue and organ culture*, *140*(3), 563–576. DOI: 10.1007/s11240-019-01753-w

[16] Wawrosch, C., & Zotchev, S. B. (2021). Production of bioactive plant secondary metabolites through in vitro technologies-status and outlook. *Applied microbiology and biotechnology*, *105*(18), 6649–6668. DOI: 10.1007/s00253-021-11539-w

[17] Tashackori, H., Sharifi, M., Ahmadian Chashmi, N., Behmanesh, M., Safaie, N., & Sagharyan, M. (2021). Physiological, biochemical, and molecular responses of Linum album to digested cell wall of Piriformospora indica. *Physiology and molecular biology of plants*, *27*(12), 2695–2708. DOI: 10.1007/s12298-021-01106-y

[18] Samari, E., Ahmadian Chashmi, N., Ghanati, F., Sajedi, R. H., Gust, A. A., Haghdoust, F., … Fuss, E. (2022). Interactions between second messengers, SA and MAPK6 signaling pathways lead to chitosan-induced lignan production in Linum album cell culture. *Industrial crops and products*, *177*, 114525. DOI: 10.1016/j.indcrop.2022.114525

[19] Soltani, M., Samari, E., Vazirifar, S., Ahmadian Chashmi, N., Sharifi, M., & Fotovat, R. (2023). Putrescine induces lignans biosynthesis through changing the oxidative status and reprogramming amino acids and carbohydrates levels in Linum album hairy roots. *Plant cell, tissue and organ culture*, *153*(2), 387–402. DOI: 10.1007/s11240-023-02479-6

[20] Wu, X. H., Fan, M. Z., Li, X. F., Piao, X. C., Gao, R., & Lian, M. L. (2021). Involvement of putrescine, nitric oxide, and hydrogen peroxide in methyl jasmonate-induced ginsenoside synthesis in adventitious root cultures of panax ginseng C.A. Meyer. *Journal of plant growth regulation*, *40*(4), 1440–1449. DOI: 10.1007/s00344-020-10199-w

[21] Sagharyan, M., Sharifi, M., & Samari, E. (2023). Methyl jasmonate redirects the dynamics of carbohydrates and amino acids toward the lignans accumulation in Linum album cells. *Plant physiology and biochemistry*, *198*, 107677. DOI: 10.1016/j.plaphy.2023.107677

[22] Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum*, *15*(3), 473–497. DOI: 10.1111/j.1399-3054.1962.tb08052.x

[23] Owen, R. W., Haubner, R., Mier, W., Giacosa, A., Hull, W. E., Spiegelhalder, B., & Bartsch, H. (2003). Isolation, structure elucidation and antioxidant potential of the major phenolic and flavonoid compounds in brined olive drupes. *Food and chemical toxicology*, *41*(5), 703–717. DOI: 10.1016/S0278-6915(03)00011-5

[24] Zafari, S., Sharifi, M., Ahmadian Chashmi, N., & Mur, L. A. J. (2016). Modulation of Pb-induced stress in Prosopis shoots through an interconnected network of signaling molecules, phenolic compounds and amino acids. *Plant physiology and biochemistry*, *99*, 11–20. DOI: 10.1016/j.plaphy.2015.12.004

[25] Keinänen, M., Oldham, N. J., & Baldwin, I. T. (2001). Rapid HPLC screening of jasmonate-induced increases in tobacco alkaloids, phenolics, and diterpene glycosides in Nicotiana attenuata. *Journal of agricultural and food chemistry*, *49*(8), 3553–3558. DOI: 10.1021/jf010200+

[26] Chashmi, N. A., Sharifi, M., Yousefzadi, M., Behmanesh, M., Rezadoost, H., Cardillo, A., & Palazon, J. (2013). Analysis of 6-methoxy podophyllotoxin and podophyllotoxin in hairy root cultures of Linum album Kotschy ex Boiss. *Medicinal chemistry research*, *22*(2), 745–752. DOI: 10.1007/s00044-012-0067-1

[27] Yousefzadi, M., Sharifi, M., Chashmi, N. A., Behmanesh, M., & Ghasempour, A. (2010). Optimization of podophyllotoxin extraction method from Linum album cell cultures. *Pharmaceutical biology*, *48*(12), 1421–1425. DOI: 10.3109/13880209.2010.489564

[28] Karuppusamy, S. (2009). A review on trends in production of secondary metabolites from higher plants by in vitro tissue, organ and cell cultures. *Journal of medicinal plants research*, *3*(13), 1222–1239.

[29] Gonçalves, S., & Romano, A. (2013). In vitro culture of lavenders (Lavandula spp.) and the production of secondary metabolites. *Biotechnology advances*, *31*(2), 166–174. DOI: 10.1016/j.biotechadv.2012.09.006

[30] Esmaeili, S., Sharifi, M., Ghanati, F., Soltani, B. M., Samari, E., & Sagharyan, M. (2023). Exogenous melatonin induces phenolic compounds production in Linum album cells by altering nitric oxide and salicylic acid. *Scientific reports*, *13*(1), 4158. DOI: 10.1038/s41598-023-30954-9

[31] Esmaeilzadeh Bahabadi, S., Sharifi, M., Behmanesh, M., Safaie, N., Murata, J., Araki, R., … Satake, H. (2012). Time-course changes in fungal elicitor-induced lignan synthesis and expression of the relevant genes in cell cultures of Linum album. *Journal of plant physiology*, *169*(5), 487–491. DOI: 10.1016/j.jplph.2011.12.006

[32] Tahsili, J., Sharifi, M., Safaie, N., Esmaeilzadeh-Bahabadi, S., & Behmanesh, M. (2014). Induction of lignans and phenolic compounds in cell culture of Linum album by culture filtrate of Fusarium graminearum. *Journal of plant interactions*, *9*(1), 412–417. DOI: 10.1080/17429145.2013.846419

[33] Tashackori, H., Sharifi, M., Chashmi, N. A., Behmanesh, M., & Safaie, N. (2018). Piriformospora indica cell wall modulates gene expression and metabolite profile in Linum album hairy roots. *Planta*, *248*(5), 1289–1306. DOI: 10.1007/s00425-018-2973-z

[34] Takó, M., Kerekes, E. B., Zambrano, C., Kotogán, A., Papp, T., Krisch, J., & Vágvölgyi, C. (2020). Plant phenolics and phenolic-enriched extracts as antimicrobial agents against food-contaminating microorganisms. *Antioxidants*, *9*(2). DOI: 10.3390/antiox9020165

[35] Swanson, B. G. (2003). Tannins and Polyphenols. In *Encyclopedia of food sciences and nutrition*. DOI: 10.1016/b0-12-227055-x/01178-0

[36] de la Rosa, L. A., Moreno-Escamilla, J. O., Rodrigo-García, J., & Alvarez-Parrilla, E. (2018). Phenolic compounds. In Yahia, E. M. B. T. P. P. and B. of F. and V. (Ed.), *Postharvest physiology and biochemistry of fruits and vegetables*. Woodhead Publishing. DOI: 10.1016/B978-0-12-813278-4.00012-9

[37] Dias, M. C., Pinto, D. C. G. A., & Silva, A. M. S. (2021). Plant flavonoids: chemical characteristics and biological activity. *Molecules*, *26*(17). DOI: 10.3390/molecules26175377

[38] Samanta Amalesh, Das Gouranga, D. S. K. (2011). Roles of flavonoids in plants. *International Journal of Pharmaceutical Science and Technology*, *6*(1), 12–35. https://www.researchgate.net/publication/279499208%0D

[39] Saini, N., Gahlawat, S. K., & Lather, V. (2017). Flavonoids: A nutraceutical and its role as anti-inflammatory and anticancer agent. In Gahlawat, S. K. … Kaur, P. (Eds.), *Plant biotechnology: recent advancements and developments*. Singapore: springer singapore. DOI: 10.1007/978-981-10-4732-9_13

[40] Šamec, D., Karalija, E., Šola, I., Vujčić Bok, V., & Salopek-Sondi, B. (2021). The role of polyphenols in abiotic stress response: The influence of molecular structure. *Plants*. DOI: 10.3390/plants10010118

[41] Canel, C., Moraes, R. M., Dayan, F. E., & Ferreira, D. (2000). Molecules of interest: podophyllotoxin. *Phytochemistry*, *54*(2), 115–120. DOI: 10.1016/S0031-9422(00)00094-7

[42] Mukhija, M., Joshi, B. C., Bairy, P. S., Bhargava, A., & Sah, A. N. (2022). Lignans: a versatile source of anticancer drugs. *Beni-suef university journal of basic and applied sciences*, *11*(1), 76. DOI: 10.1186/s43088-022-00256-6