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Efficacy of Exogenous Abscisic Acid on Cholinesterase Enzyme Activity and Phenolic Compound Variability in *Hypericum perforatum* calli

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ABSTRACT

The aim of this study was to investigate the effect of abscisic acid (ABA) on cholinesterase enzyme activities and bioactive components of *in vitro* calli of *Hypericum perforatum*. Calli were obtained from Linsmaier Skoog (LS)/B5 medium containing 0.5 mg L^{-1} thidiazuron (TDZ) and 0.5 mg L^{-1} indole butyric acid (IBA). Different abscisic acid (0.01 mg L^{-1}, 0.05 mg L^{-1}, and 0.1 mg L^{-1}) applications were performed on the calli obtained in tissue culture and extracted in 80% methanol. Hispidulin, fumaric acid, acacetin, epicatechin, and naringenin compounds were analyzed in Liquid chromatography–high–resolution mass spectrometry (LC-HRMS). While epicatechin (1.09 mg L^{-1}) was found at the highest 0.05 mg L^{-1} ABA in callus samples, fumaric acid (2.30 mg L^{-1}) and hispidulin (0.78 mg L^{-1}) compounds were detected in the highest containing of 0.1 mg L^{-1} ABA. Acacetin (0.10 mg L^{-1}) was produced in only 0.01 mg L^{-1} ABA, whereas naringenin (0.01 mg L^{-1}) was produced in medium containing 0.05 mg L^{-1} ABA. The strongest Acetylcholinesterase (AChE) (0.207±0.012) and Butyrylcholinesterase (BChE) (0.243±0.019) inhibition activity was shown the best with 0.1 mg L^{-1} ABA application. This study has shown that ABA can be used as an elicitor in callus culture of *H. perforatum*, and the amounts of related metabolites can be changed via elicitors.

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Hypericum perforatum L. Kalluslarında Ekzojen Absisik Asidin Kolinesteraz Enzim Aktivitesi ve Fenolik Bileşik Değişkenliği Üzerine Etkinliği

ÖZET

Bu çalışmanın amacı, absisik asitin (ABA) *Hypericum perforatum*'un *in vitro* kalluslarının kolinesteraz enzim aktiviteleri ve biyoaktif bileşenleri üzerine etkisini araştırmaktır. Kalluslar, 0.5 mg L^{-1} thidiazuron (TDZ) ve 0.5 mg L^{-1} indol bütirik asit (IBA) içeren Linsmaier Skoog (LS)/B5 besiyerinden elde edildi. Doku kültüründe elde edilen kalluslara farklı absisik asit (0,01 mg L^{-1}, 0,05 mg L^{-1} ve 0,1 mg L^{-1}) uygulamaları yapıldı ve %80'lik metanolde ekstrakte edildi. Hispidulin, fumarik asit, akasetin, epikateşin, naringenin bileşikleri Sıvı Kromatografisi-Yüksek Çözünürlüklü Kütle Spektrometrisi (LC-HRMS) ile analiz edildi. Kallus örneklerinde en yüksek epikateşin (1.09 mg L^{-1}) 0.05 mg L^{-1} ABA'da bulunurken, fumarik asit (2.30 mg L^{-1}) ve hispidulin (0.78 mg L^{-1}) bileşikleri en yüksek 0.1 mg L^{-1} ABA içerenlerde tespit edildi. Sadece 0.01 mg L^{-1} ABA içeren ortamda akasetin (0.10 mg L^{-1}) üretilirken, 0.05 mg L^{-1} ABA içeren besin ortamında naringenin (0.01 mg L^{-1}) üretildi. En güçlü Asetilkolinesteraz (AchE) (0.207±0.012) ve Bütirilkolinesteraz BChE (0.243±0.019) inhibisyon aktivitesinin 0.1 mg L^{-1} ABA uygulamasıyla olduğu ortaya çıktı. Bu çalışma, ABA'nın *H.perforatum*'un kallus kültüründe uyarıcı olarak kullanılabileceğini ve ilgili metabolitlerin miktarlarının elisitör aracılığıyla değiştirilebileceğini göstermiştir.

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INTRODUCTION

A genus of *Hypericum* comprises nearly 500 species, and *Hypericum perforatum* is the most well-known and investigated species (Al-Khayri et al., 2024; Altıntaş, 2025; Bal et al., 2022; Crockett & Robson, 2011). *H. perforatum* has been traditionally used in the treatment of inflammation, bacterial-viral infections, burns, stomach ulcers, mild depression, diarrhea, pain, fevers, wounds and burns (Kakouri et al., 2023; Sut et al., 2025) Aerial parts of *H. perforatum* have been widely studied because of contains phenolic compounds such naphthodianthrones (hypericin, pseudohypericin), phloroglucinol derivatives (hyperforin, adhyhyperforin) and flavonoids (quercetin, kaempferol, rutin, quercitrin, and hyperoside) (Barnes et al., 2001; Bombardelli & Morazzoni, 1995; Bruneton, 1999). Although naphthodianthrones and phloroglucinols are prestigious in *Hypericum,* flavonoids, xanthones, and tannins are as important as these compounds, and also have synergistic effects (Song et al., 2023; Xiao et al., 2020; Zhang et al., 2020).

H. perforatum extracts have been reported to have anti-inflammatory, anti-angiogenic, anti-fibroblastic, antioxidant, and anti-depressant properties. In recent times, depression is associated with Alzheimer's, and it is a neurodegenerative disease caused by the deficiency of acetylcholine and butyrylcholine, which are hydrolyzed by acetylcholinesterase and butyrylcholinesterase (Altun et al., 2013). It has been reported that cholinesterase inhibition occurred with the methanol extract of *H. capitatum* var. *capitatum*, and the ethyl acetate extract of *H. perforatum* against the butyrylcholinesterase enzyme (Boga et al., 2016). Physiologically, amyloid-beta peptide and tau proteins are produced and expressed in the normal human body. Alzheimer's and Parkinson's diseases occur with the abnormal behavior of these proteins (Abyadeh et al., 2024; Suryawanshi et al., 2024). It has been reported that compounds such as hypericin, hyperforin, and kaempferol have neuroprotective properties in *H. perforatum* (Suryawanshi et al., 2024). Due to its medicinal effects, the production of *H. perforatum* is supported by tissue culture techniques.

Commercial production of bioactive compounds through plant cell, organ, and tissue culture is a very promising technology when natural resources are limited (Karuppusamy, 2009). Thousands of methods can be used to increase efficiency, such as genetic transformation, precursor feeding, media optimization, and elicitation in plant tissue culture (Murthy et al., 2014). Elicitation is stimulating bioactive compounds by adding trace amounts of elicitor to the plant *in vitro* culture systems. Elicitors, such as abscisic acid and salicylic acid, are administered externally through *in vitro* applications and enable the activation of the signal transduction cascade, gene expression, and secondary metabolism (Zhao et al., 2005).

Abscisic acid (ABA) is a growth regulator that governs many physiological steps and developmental times in plants. It makes many regulations such as seed dormancy, germination, lipid synthesis, protein storage, stomatal opening, and closing (Vaičiukynė et al., 2019). It has been reported that the production of abscisic acid in this way activates the antioxidant system. Another investigation (Ellman et al., 1961) the effect of abscisic acid on somatic embryogenesis in cactus. Accordingly, it has been revealed that low ABA concentrations stimulate the elongation of embryos, while high ABA concentrations inhibit growth. (Lema-Rumińska et al., 2013).

According to the literature, this study is the first investigation on the effect of abscisic acid on *H.perforatum* calli in *in vitro*. The aim of the present study was to determine; (i) callus formation in LS/B5 media, (ii) different ABA (0.01 mg L^{-1}, 0.05 mg L^{-1} and 0.1 mg L^{-1}) concentration effect on phenolic compounds amounts of calli, and (iii) anticholinesterase activity of elicited callus of *H.perforatum*, (iv) phenolic compounds were analyzed by Liquid chromatography–high resolution mass spectrometry (LC-HRMS).

MATERIAL and METHOD

Plant Material and Seeds Surface Sterilization

Hypericum perforatum L. seeds were collected from Samsun-Ballıca (41'27'97'01' and 36'33'60'67'), Turkey. Seeds were washed for 15 minutes under tap water, surface sterilized with 70 % ethanol for 1 min, then NaOCl 15 % for 5 min, rinsed 3 times in sterile deionized water. Seeds were cultured LS/B5 medium supplemented with 3 % sucrose and 0.7 % agar without plant growth regulators.

Callus Formation and Abscisic Acid Elicitation

Callus cultures were started with axillary buds of sterile seedlings. The media contained LS (Linsmaier and Skoog)/B5, including 3 % sucrose, 0.7 % agar. Five different TDZ (0.1, 0.25, 0.5, 0.75, 1 mg L^{-1}) and IBA (1,0.75,

0.5, 0.25, 0.1 mg L^{-1}) concentrations were used for callus culture. Calli were obtained from 0.5 mg L^{-1} TDZ and 0.5 mg L^{-1} IBA. After 2 months of callus induction, the friable and green callus as equal fragments were transferred to new LS/B5 media containing abscisic acid (0.01 mg L^{-1}, 0.05 mg L^{-1} and 0.1 mg L^{-1}) elicitor for 15 days. Abscisic acid (Sigma-Aldrich, USA) was dissolved in pure ethanol and filtered using a micro filter of 0.22 µ pore size. All treatments were incubated in the 16 h light/8 h dark photoperiod at 24 ± 2℃ and photon flux of 90 µmol m^{-2} s^{-1} in the culture chamber.

Extraction of Elicited Callus

The calli, which were treated with ABA, were freeze-dried, lyophilized, and powdered. The samples obtained from *in vitro* were weighed (20 mg) and homogenized. After, it was added to these samples 2 ml of 80% methanol and vortexed. Then, the samples were kept in an ultrasonic bath for 30 min at 4°C and centrifuged at 4000 rpm for 15 minutes. The supernatant was used as an extract in the analyses (Gadzovska et al., 2007; Gadzovska et al., 2013).

Statistical analyses

All the experiments were conducted using three biological replicates, and all measurements were analyzed in triplicate. The compliance of the data with normal distribution was evaluated with the Shapiro-Wilk test. In the display of descriptive statistics of variables determined to be normally distributed, mean and standard deviation (SD) values were given; in the display of variables determined to be not normally distributed, median and interquartile range (IQR) values were given. Significant differences in the number of compounds between different plant extracts were evaluated with the ANOVA and Kruskal-Wallis test. IBM SPSS Statistics 26.0 (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.) and OriginPro 2018 (OriginLab Corporation, Northampton, MA, USA) were used for statistical analyses and calculations. Statistical significance level was accepted as $p<0.05$.

Anticholinesterase inhibition

Acetylcholinesterase from Electrophorus electrics (Electric eel) and butyrylcholinesterase from equine serum were obtained from Sigma-Aldrich (St. Louise, MO). The inhibitory effects of extracts against AChE and BChE were measured using a slight modification of Ellman's spectrophotometric method (Ellman et al., 1961) and using commercially available galantamine as the reference compound. Acetylthiocholine iodide (AChI), butyrylcholine iodide (BChI) used as the substrate in the reaction, 5′dithiobis - 2 - nitrobenzoic acid (DTNB) used as Ellman's reagent, were purchased from Sigma - Aldrich (St. Louis, MO).

The absorbance of the reaction mixture was measured at 412 nm three times within 5 min of the start of the reaction on a Thermo Fisher Scientific Multiskan Go, and the results are reported as mean ± standard deviation. Activity (%) was plotted to determine the inhibitory effects of extracts on AChE and BChE. IC_{50} values were obtained by activity (%) versus compound plots.

LC-HRMS device and chromatographic conditions

The analyzes of the extracts were made with LC-HRMS. Epicatechin, acacetin, fumaric acid, hispidulin, naringenin, naringin, chicoric acid, hyperoside, rhamnocitrine, chlorogenic acid, chrysin, isosacuranetin, apigenin 7-glucoside compounds were determined. Hypericin, pseudohypericin and hyperforin compounds were introduced to the device.

LC-HRMS measurements were carried out on a Thermo ORBITRAP Q-EXACTIVE (Bremen, Germany) mass spectrometry-equipped ESI ion source and with Dionex LC system. Scan range was set to m z^{-1} 85-1500 amu, and other mass parameters are used as following: gas flow rate: 45, aux gas flow rate: 10, spray voltage: 3.80 kV, capillary temperature: 320 °C, aux gas heater temperature: 320 °C, and Slens RF is 50. A Fortis C18 column (150 x 3 mm i.d., 3 µm particle size, Istanbul, Turkey) was used for the separation of compounds. The mobile phases A and B were composed of 1% formic acid-water and 1% formic acid-methanol, respectively. The gradient program was 0-1.00 min 50% A and 50% B, 1.01-6.00 min 100% B, and finally 6.01-10 min 50% A and 50% B. The flow rate of the mobile phase was 0.35 mL min^{-1}, and the column temperature was set to 22°C. Environmental conditions were set as temperature 22.0 ± 5.0 °C and relative humidity (50 ± 15) % rh.

Identification of compounds was performed by comparison of the retention time of standard compounds (in the range of purity 95-99%, see section chemicals) and HRMS data of Bezmialem Vakıf University, Drug Application and Research Center Library-ILMER. Dihydrocapsaicin (purity 95%) was used as an internal standard for LC-HRMS measurements in order to reduce the repeatability problem caused by external effects, such as ionization repeatability, in mass spectrometry measurements (Inal et al., 2022).

RESULTS and DISCUSSION

Abscisic acid Induced Phenolic Compounds of Calli

In this study, elicited calli with ABA of *H. perforatum* were used and these calli were extracted with 80% methanol. The phytochemical profile of the extract was determined by LCHR-MS and anticholinesterase inhibition assay was investigated. Epicatechin, acacetin, fumaric acid, hispidulin, naringenin, naringin, chicoric, hyperoside, rhamnocitrin, chlorogenic acid, chrysin, isosakuranetin, and apigenin 7-glucoside compounds were determined with the LCHR-MS (Figure 1,2,3). Hypericin, pseudohypericin and hyperforin compounds were introduced to the device, but these compounds could not be detected in callus.

Initially, as shown in Table 1, epicatechin (1.09 mg L^{-1}) and naringenin (0.01 mg L^{-1}) amounts were found to be the highest at 0.05 mg L^{-1} ABA in callus of *H. perforatum*. As well as naringenin was just found at 0.05 mg L^{-1} ABA. Likewise, acacetin (0.10 mg L^{-1}) was produced only in the medium containing 0.01 mg L^{-1} ABA. On the other side, fumaric acid was produced at 0.1 mg L^{-1} ABA (2.30 mg L^{-1}) and 0.05 mg L^{-1} ABA (1.09 mg L^{-1}). Hispidulin compound had close values in 0.1 mg L^{-1} ABA and 0.05 mg L^{-1} ABA applications. Finally, naringin, chicoric, hyperoside, rhamnocitrin, chlorogenic acid, chrysin, isosakuranetin, and apigenin-7-Glucoside compounds did not detect in elicited calli (Table 1).

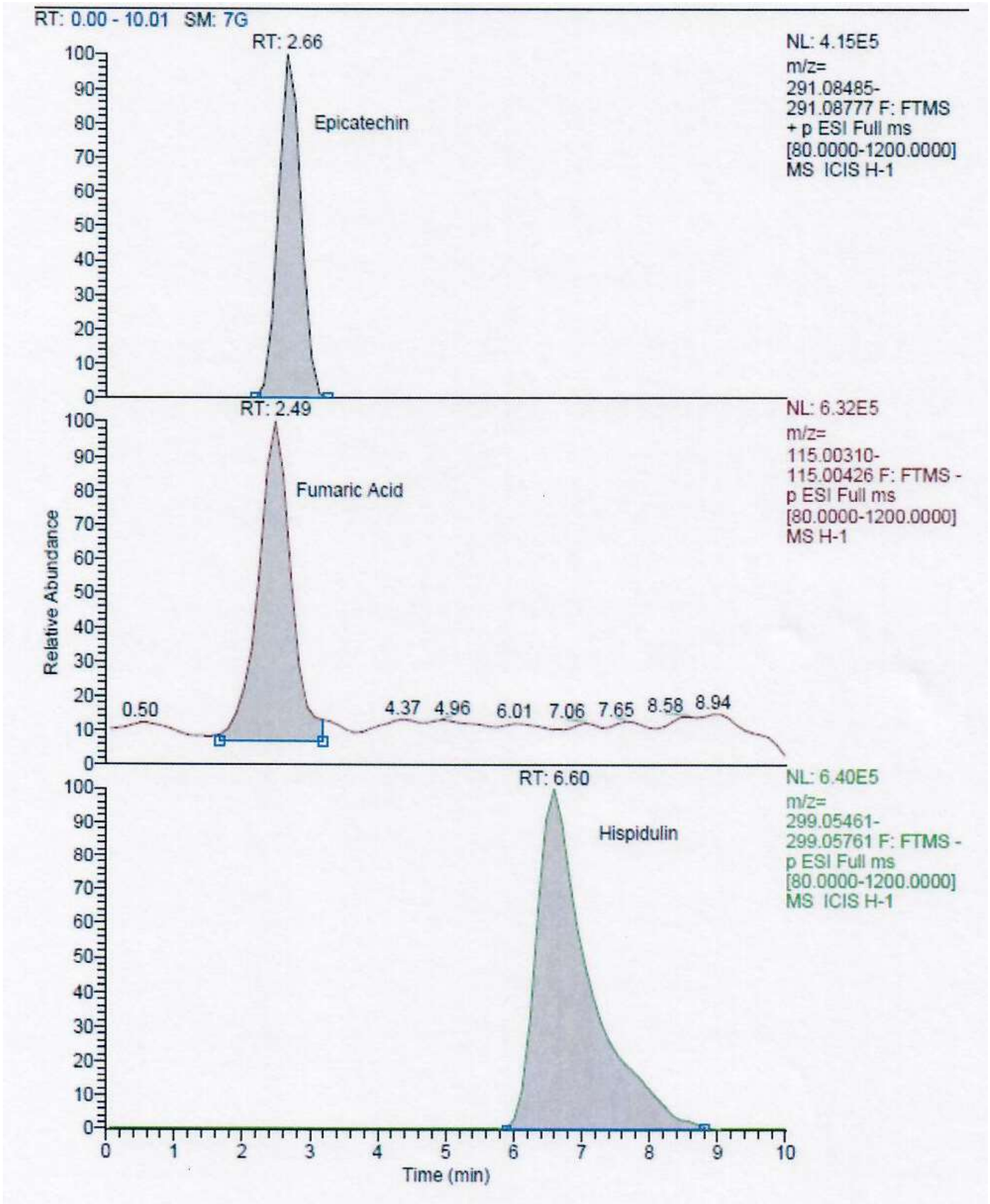
Table 1. Amounts of phytochemical compounds analysed by LC-HRMS in calli of *H. perforatum* L.
Çizelge 1. H. perforatum L. kalluslarında LC-HRMS ile analiz edilen fitokimyasal bileşik miktarları

No	*Compounds*	*R.T.*	*Quantification (mg L^{-1} ± SD)*			*Mother Ion (m z^{-1})*	*Ion mode*	*LOD/LOQ (mg L^{-1})*
			0.1 mg L^{-1} ABA	*0.01 mg L^{-1} ABA*	*0.05 mg L^{-1} ABA*			
1	Fumaric acid	2.49	2.30±3.15	1.45±3.15	1.09±3.15	115.0037	Negative	0.26/0.88
2	Epicatechin	2.66	0.46±3.62	*	1.09±3.62	291.0863	Negative	0.23/0.76
3	Naringenin	5.89	*	*	0.01±4.15	271.0612	Negative	0.2/0.67
4	Hispidulin	6.60	0.78±1.73	0.57±1.73	0.76±1.73	299.0561	Negative	0.14/0.46
5	Acacetin	7.88	*	0.10±1.5	*	283.0612	Negative	0.13/0.42

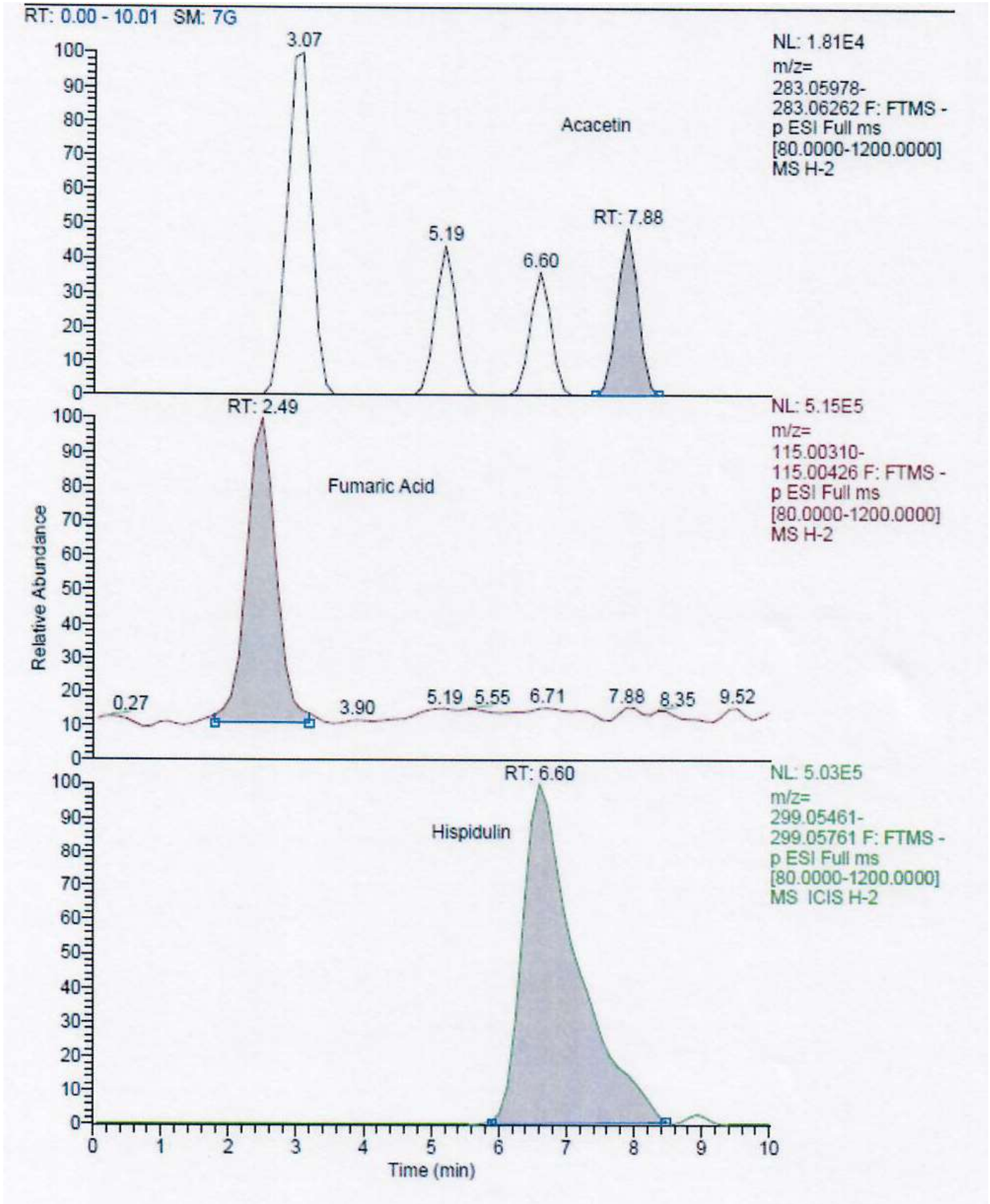
*Not Detected, SD: Standard deviation, R.T: Retention time, LOD: Limit of detection, LOQ: Limit of quantification

According to the LCHR-MS results of jasmonic acid (JA) application of *H. perforatum in vitro* samples, chlorogenic acid was at the highest value in the *in vitro* plantlet control group, while it was not produced in calli (Önlü, 2023). Accordingly, it is observed that there is no production of chlorogenic acid in *H. perforatum* calli in both JA and in our ABA applications. While epicatechin could not be detected in 0.01 mg L^{-1} ABA, acacetin, fumaric acid and hispidulin were produced. Similarly, there was no epicatechin production in 0.01 mg L^{-1} JA calli, and acacetin, fumaric acid, and hispidulin compounds were determined by Önlü (2023). As a result, it is observed that the most effective rate for epicatechin production is 0.05 mg L^{-1} ABA, and no production occurred at lower ABA rates. Epicatechin is a derivative of catechin, and studies have reported that it does not exhibit hepatoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity effects (Pathania & Singh, 2021). Moreover, it has been reported that epicatechin inhibited the replication of Mayaro and hepatitis C diseases (Ferreira et al., 2018; Lin et al., 2013).

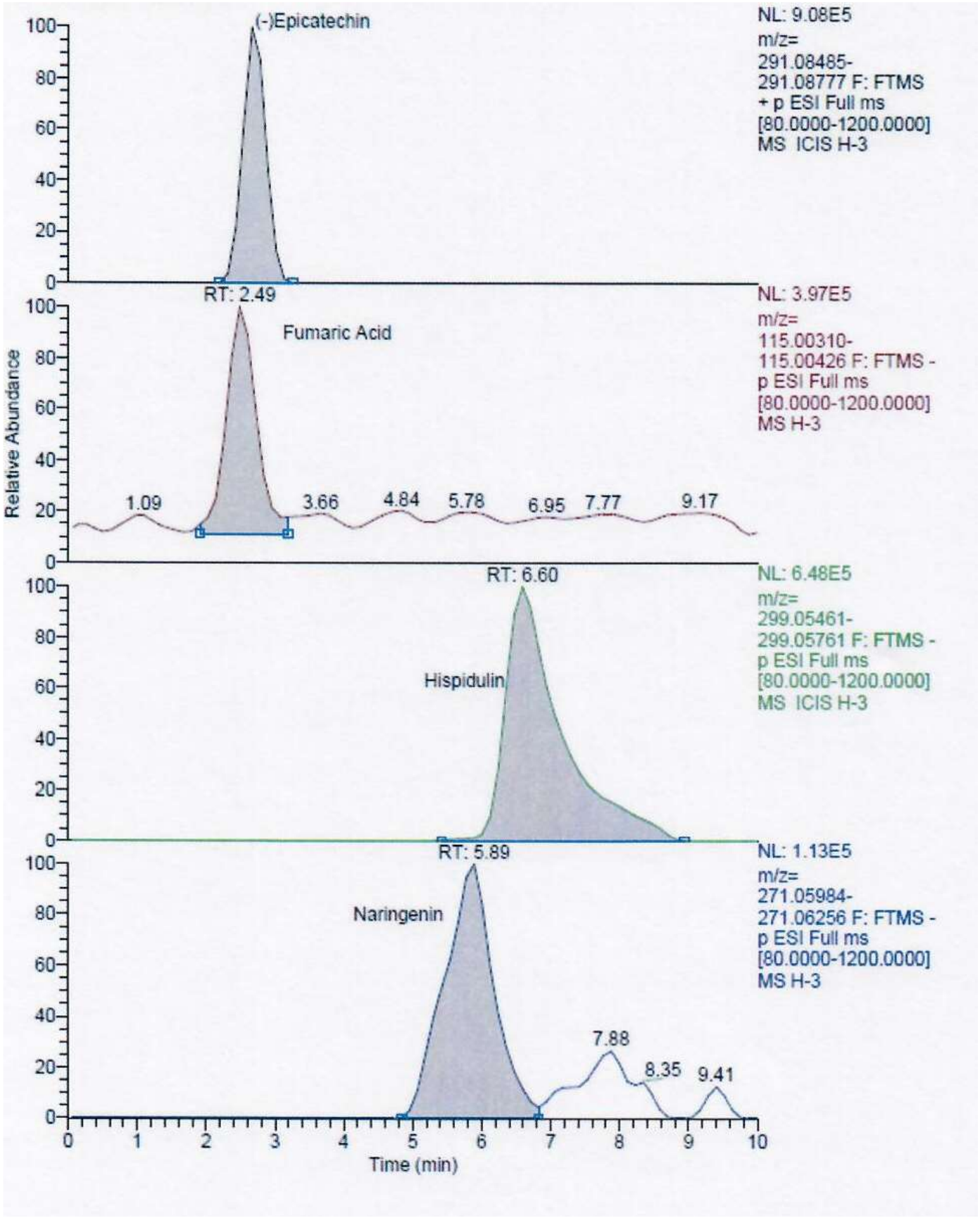
On the other hand, acacetin was produced at only 0.01 mg L-1 ABA. Acacetin, which has various therapeutic effects such as anticancer, antidiabetic, antipyretic, antiperoxidative, anti-inflammatory, antiplasmodial, and antiproliferative activities, stops the cell cycle by inducing apoptosis. As well as, the root part of Scrophularia kakudensis was detected in in vitro samples (19.52 μg·g−1 FW) more than ex vitro (18.23 μg·g−1 FW) (Manivannan et al., 2015). Similar to acacetin, naringenin was determineted at just 0.05 mg L-1 ABA. It has been reported that the naringenin compound has various effects such as antidiabetic, anticancer, antimicrobial, antiobesity, gastroprotective, immunomodulatory, cardioprotective, nephroprotective, and neuroprotective (Uçar & Göktaş, 2023). Low amounts of ABA elicitation appear to be more effective for hispidulin production. Furthermore, it has been reported that hispidulin, mostly found in Artemisia and Salvia species and has antioxidative, antifungal, anti-inflammatory, and antimutagenic effects (Chulasiri et al., 1992; Gil et al., 1994; Kavvadias et al., 2004).



Şekil 1. 0.1 mg L^{-1} ABA uygulaması yapılan kalluslarda LCHR-MS analizi ile elde edilen bileşiklerin kromatogramı
Figure 1. Chromatogram of compounds obtained by LCHR-MS analysis in 0.1 mg L^{-1} ABA-treated calli



Şekil 2. 0.01 mg L^{-1} ABA uygulaması yapılan kalluslarda LCHR-MS analizi ile elde edilen bileşiklerin kromatogramı
Figure 2. Chromatogram of compounds obtained by LCHR-MS analysis in 0.01 mg L^{-1} ABA-treated calli



Şekil 3. 0.05 mg L^{-1} ABA uygulaması yapılan kalluslarda LCHR-MS analizi ile elde edilen bileşiklerin kromatogramı
Figure 3. Chromatogram of compounds obtained by LCHR-MS analysis in 0.05 mg L^{-1} ABA-treated calli

Table 2. Statistical comparison of total phenolic compounds obtained from callus extracts by ABA elicitation
Çizelge 2. ABA elisitasyonu ile kallus ekstrelerinden elde edilen toplam fenolik bileşiklerin istatiksel karşılaştırılması

	Amount of Compound (mg L^{-1})		
	Average±SD	*F*	*p*
0.1 mg L^{-1} ABA	0.71±0.95		
0.01 mg L^{-1} ABA	0.42±0.62	0.192	0.828
0.05 mg L^{-1} ABA	0.59±0.55		

SD: Standard Deviation, a,b,c Values within a row with different superscripts differ significantly p>05
Different letters show significant p>.05 from ANOVA and KW.

Statistical results and comparisons of total phenolic content of *H. perforatum* calli are given at Figure 4 and Table 2 and all groups of ABA (*p> 0.05*) were not found to be significant. The average of the compounds obtained from 0.1 mg L^{-1} ABA was 0.71±0.95; 0.01 mg L^{-1} ABA was 0.42±0.62; and 0.05 mg L^{-1} ABA was 0.59±0.55 (*p>0.05*), respectively. The highest total compound rate was determined in calli containing 0.1 mg L^{-1} ABA. In the literature, no study has been found regarding the effect of ABA on *H. perforatum* calli. When other elicitation studies were examined in *H. perforatum* that investigated the effect of 50, 100, and 250 µM jasmonic acid in cell suspension for 21 days and established that 50 µM JA was more effective in biomass production (Gadzovska et al., 2007). Furthermore, it was reported that total phenolic production in the presence of 100 µM salicylic acid (SA) was 4 times higher than the control calli (Gadzovska et al., 2013). In another study, *in vitro* plantlets of *H. heterophyllum* produced chlorogenic acid and kaempferol compounds only in medium containing 0.01 mg L^{-1} ABA. In addition, hyperoside, quercetin, and catechin compounds were determined at the highest level in 0.01 mg L^{-1} ABA medium. Furthermore, low ABA (0.01 mg L^{-1}) and SA (0.01 mg L^{-1}) induced higher catechin contents in *in vitro* plantlets and callus cultures, respectively (Önlü et al., 2025).

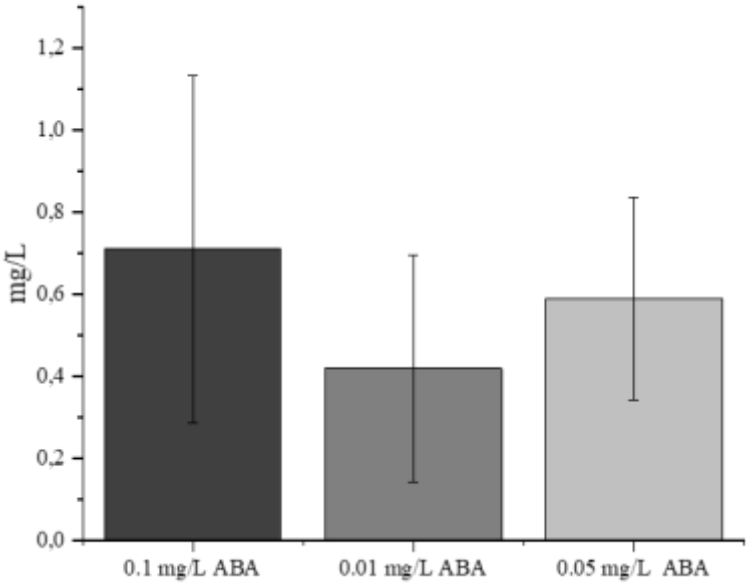


Figure 4. Comparison of total phenolic compounds from *H.perforatum* L. calli. Respectively; 0.1 mg L^{-1} ABA, 0.01 mg L^{-1} ABA, 0.05 mg L^{-1} ABA. Each bar represents the percentage ± standard deviation (SD) for indicated total compounds.
Şekil 4. H.perforatum L. kalluslarının toplam fenolik bileşiklerin karşılaştırılması. Sırasıyla; 0.1 mg L^{-1} ABA, 0.01 mg L^{-1} ABA, 0.05 mg L^{-1} ABA. Her çubuk belirtilen toplam bileşikler için yüzde ± standart sapmayı (SD) temsil eder.

However, there are few reports on the production of bioactive components in *H. perforatum* by direct callus culture that such as; production of pseudohypericin using MS/B5 and 4.0-5.0 mg L^{-1} BA (Gadzovska et al., 2005); pseudohypericin, flavanols and flavonols with 50 µM salicylic acid (SA) (Gadzovska et al., 2013); and hypericin with perlite nanoparticles (25 or 100 L^{-1}) (Ebadollahi et al., 2019; Shasmita et al., 2023). On the other side, several studies have increased the amounts of hypericin, hyperforin, quercetin, rutin, and other phenolic compounds by adding different growth regulators, acetic acid, methyl jasmonate to MS medium through callus-mediated organogenesis (Gjureci et al., 2025; Shasmita et al., 2023).
In a recent work, phenylpropanoid and naphthodianthrone production of *H. perforatum* transgenic shoot clones regenerated from related hairy root lines; it was determined that transgenic shoots produced 2.4 times hypericin and 3 times more pseudohypericin than non-transgenic shoots (Tusevski et al., 2024). Moreover, in extracts of *H.*

perforatum treated separately with 100 µM SA and 100 µM JA in hairy root culture, SA promoted the production of quercetin-6-C-glucoside and epicatechin (Gjureci et al., 2025).

Anticholinesterase activity of Calli's total compounds

As shown in Table 3, the best anticholinesterase activity was exhibited against acetylcholinesterase 0.207±0.012 and butyrylcholinesterase 0.243±0.019 with 0.1 mg L^{-1} ABA in enzyme studies performed on extracts.

According to (Altun et al., 2013); due to the high phenolic compounds contained in the methanol extract of the aerial part of *H. perforatum*, its antioxidant and anticholinesterase activities were highest. On the other hand, *H. neurocalycinum* (85.78±4.11) and *H. malatyanum* (62.24 ±1.81) exhibited stronger AChE inhibitory activity (Ozkan et al., 2018). In a recent hairy root study, compared to non-transformed shoots, transformed shoots were reported to have a superior capacity to inhibit AchE (Tusevski et al., 2024). Furthermore, *H. heterophyllum in vivo* leaves were extracted with methanol, ethanol, acetone, and chloroform solvents. The IC_{50} ratio of AChE enzyme showed the highest value in methanol extracts (Yaman et al., 2024). Similarly, in this study, methanol was used as an extraction solvent. Consequently, in this research, epicatechin, fumaric acid, and hispidulin may be effective in AChE and BchE enzyme inhibition at 0.1 mg L^{-1} ABA. There is no statistically significant difference between the means of the extracts.

Table 3. *Acetylcholinesterase* and butyrylcholinesterase *activities of H. perforatum calli elicited by ABA*
Çizelge 3. ABA tarafından elisite edilen H. perforatum kalluslarının asetilkolinesteraz ve bütirilkolinesteraz aktiviteleri

Extracts	*AChE (IC_{50} value)*	*BChE (IC_{50} value)*
0.1 mg L^{-1} ABA	0.207±0,012	0.243±0,019
0.01 mg L^{-1} ABA	0.212±0,012	0.256±0,021
0.05 mg L^{-1} ABA	0.234±0,017	0.289±0,022
*Galantamine	0.072±0,009	0.196±0,010

Values were the means of three replicates ±SD. *Galantamine was used as reference compound. AChE: Acetylcholinesterase, BchE: butyrylcholinesterase, *IC_{50}: Half maximal inhibitory concentration.*

CONCLUSION

Chemical composition results showed that ABA applications can change phenolic compounds. In particular, 0.1 mg L^{-1} ABA resulted in enriched high levels of fumaric acid and hispidulin. For acacetin production, only 0.01 mg L^{-1} ABA dose should be used. Besides, epicatechin and naringenin contents have the potential to increase by 0.05 mg L^{-1} ABA. The results showed that 0.1 mg L^{-1} ABA exhibited ACHe and BCHe activity after galantamine. Consequently, the highest amount of phenolic compound production was induced in the medium containing 0.1 mg L^{-1} ABA. It has been shown that if the abscisic acid elicitor is 0.1 mg L^{-1} or around in calli, the total phenolic amount, especially fumaric acid and hispidulin, can be increased. Further studies, with different elicitor treatments, can be applied to calli to increase cholinesterase activity. This research may be a guide for *in vitro* elicitation studies to be carried out in different species of *Hypericum*.

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Conflict of Interest

The authors declare that they have contributed equally to the article.

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