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**THE EFFECT OF EXOGENOUS ASCORBIC ACID ON GENE
EXPRESSION OF PHENYLALANINE AMMONIA LYASE AND
ACCUMULATION OF PHENOLIC COMPOUNDS IN *SALVIA VIRGATA***

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Salvia spp. (Labiatae) are important sources of antioxidants that are used as preservatives in food industries, as well as pharmaceuticals for protecting the body against oxidative stress, free radical damages, angiogenesis, inflammation, bacterial and virus infections. In the present study, the effects of exogenous ascorbic acid (AsA) as an elicitor were investigated on *PAL* gene expression and production of three active phenolic compounds in the leaves of *Salvia virgata* Jacq. The mature seeds were collected from the wild plants and were sterilized and germinated in peat moss pots. Plantlets at six- leaf stage were treated with four concentrations (0, 50, 100 and 150µM) of aqueous solution of AsA. Leaf samples were harvested after 0, 12, 24 and 48 h treatment for RNA isolation. Total RNA was isolated from the leaf samples using BIOZOL reagent, cDNA was generated using the iScript cDNA synthesis kit (BIO-RAD) and Real Time PCR was used to investigate the expression profiling of *PAL* in the treated leaf tissues. A reliable and rapid HPLC method was used for simultaneously determination of rosmarinic acid (RA), salvanolic acid A (Sal A) and salvanolic acid B (Sal B) in the methanolic extracts using calibration equations. Real Time PCR analysis revealed that exogenous application of AsA led to up-regulation of *PAL* expression in the leaf samples, with the maximum value at 50µM concentration of AsA after 12 h of treatment. Further analysis showed that in *S. virgata*, the highest contents of Sal A (9.6 µg/g DW), S al B (355.3 µg/g DW) and RA (37.5 µg/g DW) were achieved at 50, 100 and 150 µM of AsA, respectively. Based on the obtained results, it could be concluded that there was no positive correlation between the intensity of *PAL* transcription and the phenolic compounds accumulation in the treated leaf samples with different concentrations of AsA. This is the first report on the effect of AsA as an elicitor on *PAL* enzyme in *S. virgata*. Consequently, the results approved that *PAL* is not the rate-determining step in phenolic compounds biosynthesis and probably some other key enzymes and factors involved in the synthesis of phenolics in *Salvia* species [1].

References

- [1] Ejtahed, Roghayeh Sadat.; Radjabian, Tayebeh; Hoseini Tafreshi, S. A. *Appl. Biochem. Biotechnol.* **2015**, *176*, 1846–1858.