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The Use of HPLC in Determination of Endogenous Hormones of *Hypericum retusum* Aucher Exposed to UV- B and Grown Under *in vitro* Conditions

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Aim of the study: This study were determined the using high performance liquid chromatography (HPLC) of endogenous hormones in *Hypericum retusum* Aucher exposed to UV-B and grown under *in vitro* conditions. Proliferation of plantlets on MS medium containing 0.5 mgl^{-1} N-6- benzylaminopurine (BAP) was achieved under in vitro conditions. In this study not analyzed BAP, because it is added to the medium. HPLC metod was used for seperation of these hormones (Kinetin, GA_3 IAA, ABA, IBA, NAA, 2,4D).

Material and Methods: The seeds of *Hypericum retusum* Aucher (Clusiaceae) were collected from south-eastern Turkey, in Diyarbakır. For proliferation of shoot, the micro-shoots were transferred in to MS (Murashige & Skoog 1962) medium contained 0.5 mgl^{-1} N-6-benzylaminopurine (BAP). Afterwards the selected seedlings were exposed to UV-B radiation at different durations (15; 30; 45; 60 min.). Control group was not subjected to UV-B radiation. The plantlets were placed approximately. 56 cm from the surface of the lamp at the middle of the light period. Each radiation experiment was carried out at room temperature for 5 days.The HPLC system consisted of Agilent Eclipse XDB C-18 (250mm x 4.6 mm, 5µm) with a methanol gradient in 0.3 % formic acid and UV detector set at 280 nm. The substance to be analyzed is required to determine the retention time, before going into the analysis process by HPLC. Besides research in chromatographic equipment, extraction and purification procedures are also important. The method of Tang et al. (2011) and Chen et al. (2005) a modified method were used for the application of the sample preparation method.

Results: This study were determined the using high performance liquid chromatography (HPLC) of endogenous hormones in *Hypericum retusum* Aucher exposed to UV-B and grown under *in vitro* conditions. At all the doses of UV-B applications were observed on important changes. In this study were determined significant changes at amounts of endogenous hormones in H. *retusum* extracts with the increasing UV-B radiation doses. To best of our knowledge, there is no report especially on determined the using high performance liquid chromatography (HPLC) of effects of ultraviolet-B radiation to endogenous hormones in *Hypericum retusum* plantlets grown under in vitro conditions.

Key words:*Hypericum retusum* Aucher, HPLC, hormones,UV-B.