

Nutrition and Health 2018: Evaluation of antioxidant activity of wild medicinal plant *Ziziphora tenuior* L and using plant tissue culture to increase its activity- Abdulkarim Dakah- Damascus University

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Abstract

Reactive oxygen species (ROS) are a by-product of ordinary metabolism. ROS are additionally produced in cells as a response to several factors, including oxidative and thermal stresses, ultraviolet light, and ionizing radiation. Oxidative stress can damage DNA, cell functions reserve lipid and protein peroxidation, and trouble of glutathione levels. In addition, ROS contribute to the development of cancer, diabetes, atherosclerosis, inflammatory diseases, and ageing. ROS are divided into free radical species, such as superoxide anion radical, hydroxyl radical, and non-free radical species that are involved in oxidative reactions, such as singlet oxygen. Oxidative damages can be reduced through enzymatic mechanisms such as superoxide dismutase and catalase or by antioxidants presented as natural products.

Medicinal plants played an important role in the treatment of diseases and health illnesses for 1000 of years and are still important in traditional medicine organizations around the world. A sufficient supply of the plant raw material contains a steady quality of valuable natural products becomes so difficult with increasing the need to the consume such a natural products. Therefore, laboratories worldwide are trying to produce secondary metabolites from plant tissue cultures for profitable applications (Wink et al., 2005, Alfermann, 2009) as an alternative or addition to plants produced in fields or in greenhouses.

Plant tissue culture is the procedure whereby small pieces of living tissue. They are isolated in the from where the organism and grown aseptically for unlimited periods on a nutrient medium under controlled conditions (Ali et al., 2007). In vitro farming the plants is a necessary step in many experiments like micropropagation, creation of virus-free plants and genetic transformation. (Georgieva et al., 1996).

Materials and methods

This study was carried out in the Plant Tissue Culture and Molecular Biology Laboratory in Damascus University, Faculty of Science, and Subdivision of Plant Biology. Naphthalene acetic acid (NAA), Indole-3-butyric acid (IBA), kinetin (Kin), benzyladenine (BA), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich

GmbH (Munich, Germany).

Wild plants were gather from Kalamoon Mountains, Assal Al-Ward (Syria), and authenticated by Dr. Imad Alkadi at the Department of Plant Biology,

Aerial parts of wild and in vitro plants were dry to the ground by pistil and mortar to a soft powder. For the aqueous extract, 5 g of powder was immersed in 100 ml of distilled water. The free radical foraging activity of samples was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH), following this method described by Blois (1958) with some changes (Laouini et al., 2012). DPPH is reduced to hydrazine when it reacts with hydrogen donors. Briefly serial dilutions (10, 1, 0.1, 0.01, 0.001 mg/ml) of samples (aqueous and methanol extracts) were verified for radical scavenging activity. 0.2 mM DPPH was ready in methanol and 500 µl of this solution was added to the 1000 µl of a sample at different absorptions.

Results

Auxins are known to exhibit a important effect on a callus development from leaf explants. 1.5 mg/L of NAA was the best absorption for all callus introductions with $70.2\% \pm 0.28$ of leaf explants whereas 2 mg/L of NAA reduced the callus developed up to 60%. The treatment with 2 mg/L of IBA led to an increase in callus formation percentage of 62.1, while the growing regulator free MS average did not show any callus formation to the leaf explants died after 10 days of culture.

Different absorptions of BA induced to shoot the formation from the callus without root formation while MS medium without growth regulator did not show any shoot introduction.

Discussion

Callus is a mass of undistinguishable cells, which are moulded in vitro from an explant tissue cultured on nutrient medium added with suitable plant growth regulators (PGRs) during a dedifferentiation process. The difference in callus to differentiated organs is a complex process controlled by many factors. Most of the antioxidant activity is due to secondary metabolites especially phenolic compounds and some terpenes (Marzouk et al., 2007, Awaad and Al-Jaber, 2010). Our results

showed that the IC₅₀ value of aqueous and methanol abstracts of wild *Z. tenuior* were 0.516 and 9.22 mg/ml, respectively. An increase of the concentration of Kin led to a decrease in the number of sprouts and this corresponds with findings of Ozudogru and his colleagues (Ozudogru et al., 2011). Steephen and his group showed that the increasing concentration of BA above 1 mg/L led to callus formation and decreased shoot development of *Vitex negundo* L. (Steephen et al., 2010).

Water extract exhibited a strong antioxidant activity in solidity with methanol extract which could be due to a better solubility of antioxidant compounds in water. Wong and his colleagues found that water extracts of 23 from 30 therapeutic plants have antioxidant activity higher than those of the methanol abstracts (Wong et al., 2006). The results of the current study showed that the radical searching ability of in vitro propagated plant extracts of *Z. tenuior* was developed than aqueous and methanol extracts of wild plants. The components in *Z. tenuior* responsible for the antioxidant activity are unidentified. Further research is the therefore needed for the identification and isolation of the corresponding antioxidant components are in antioxidant.

Conclusion:

In conclusion is the current investigation that shows the micropropagation of *Z. tenuior* done in vitro is a dependable method for the rapid multiplication of this species. In the current study *Z. tenuior* has been cultured in the vitro for the first time and it was possible to obtain the more than 314 plants from one single explant after four subculture cycles of multiplication. This procedure will be helpful for rapid and large scale propagation. Also we can use plant tissue culture to increase the active material, our results show that the water extracts of in vitro produced plants showed an increase in antioxidant activity as compared to the starting material.

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