INVESTIGATION OF ANTIOXIDANT PROPERTIES OF HYPERICUM HETEROPHYLLUM VENT. AS AN ENDEMİC ST. JOHN'S WORT (YELLOW CENTAURY) SPECIES IN TURKEY

[1]OMER HAZMAN, [2]MUSTAFA KARGIOGLU

[1]Department of Chemistry, Afyon Kocatepe University, Turkey.
[2]Department of Molecular Biology and Genetics, Afyon Kocatepe University, Turkey
E-mail: [1]omerhazman@hotmail.com, [2]kargi@aku.edu.tr

Abstract - The use of plants for pharmaceutical, food and cosmetic purposes has started with the existence of humans and has continued until today. Phytochemicals are considered as one of the cornerstones of pharmacy in the past. Today, more than 20,000 plants are included in drug formulations and are used for complementary treatment as well as clinical treatments. One of the plants used for this purpose is Hypericum perforatum (St. John's Wort or yellow centaury). Studies on the effects of many types of endemic species have not yet been made. In this study, it is aimed that investigation of antioxidant properties of Hypericum Heterophyllum Vent. as an endemic yellow centaur species in Turkey. Total antioxidant capacity (TAC), total oxidant capacity (TOC) of the plant species (Hypericum Heterophyllum Vent.) were analyzed. The oxidative stress index (OSI) was calculated to interpret the possible effect of the plant on the oxidative stress that may occur in organisms. TAC and TOC analyzes were carried out on the crude samples obtained after drying and homogenous decomposition of the leaves, branches and flower parts of the plant. The TAC levels of Hypericum Heterophyllum Vent. were found to be 2.22 mmolTroloxEquivalent/L and TOC levels were 42.41 µmol H_2O_2 Equiv./L. OSI levels were calculated as 1924 AU. These data indicate that Hypericum Heterophyllum Vent. have high antioxidant capacity. For this reason, antioxidant power can be shown as the reason for its use in various diseases.

Keywords - Hypericum Heterophyllum Vent., St. John's Wort, antioxidant and oxidant activity

I. INTRODUCTION

The genus Hypericum (St. John's Wort) includes more than 450 species distributed in Europe, North America, North Africa and West Asia (1). These plants have been used in traditional folk medicine for thousands of years. Hypericum perforatum, which is one of the most common species of Hypericum species, is still widely used among the people in Anatolia. During the flowering period of this species, flowers and buds or all above ground parts are collected and dried immediately. In this way, tea is prepared and consumed. It is said to be consumed because it is good for diseases and depression among the public. Tinctures of the same species are prepared with olive oil. Tincture used for wound healing are especially prepared by collecting the flowers of Hypericum perforatum after drying or by placing them in olive oil without drying and waiting in the sun. The flowers placed in olive oil in a closed glass bottle are kept in the sun (15-30 days) until the color of the olive oil turns into pink-red color. Then it becomes ready to use by filtration. The oily tinctures prepared in this way are used for healing the wounds among the people and are used by drinking (2-3 teaspoons a day) in patients with ulcers. As a matter of fact, scientific studies show us that this information, which has been filtered through traditions in hundreds or even thousands of years, is not wrong. In fact, it is stated that Hypericum perforatum may be useful as an antiinflammatory (7,8) I treatment of wound healing (2,3) and burns (4,5,6). However, it is stated that similar effects can be obtained from different Hypericum genera (9,10). This study for the first time with antioxidant properties designated Heterophyllum Hypericum species, is one of the endemic species grown in Turkey. It is thought that the information obtained from the study will contribute to the literature.

II. MATERIALS AND METHODS

Hypericum heterophyllum and Hypericum perforatum plant species were collected in Turkey, Afyonkarahisar province Bayat district Köroğlubeli region on July, 2017. Identification of the plant was conducted by Dr. Mustafa Kargıoğlu, who is a faculty member in Afyon Kocatepe University, Faculty of Arts and Sciences, Department of Molecular Biology and Genetics. The plant specimen is kept in Afyon Kocatepe University herbarium. To determine the antioxidant effects of Hypericum heterophyllum species, total antioxidant capacity (TAC), total oxidant capacity (TOC) and oxidative stress index (OSI) levels were determined in crude samples of plants. For this purpose, the leaves, branches and flower parts of the powdered plant are mixed thoroughly after 1 g. weighed into a tube. Ice-cold PBS (pH: 7.4) was added at a ratio of 1:10 (w / v). Sonicated in ice with vortexing. Thus, it was aimed to make the samples more homogenous by breaking down the plant thoroughly at the cellular size. In this

way, 5 samples were prepared from the plant and the analysis was started.

Determination of Total Antioxidant Capacity (TAC)

TAC levels was measured by spectrophotometrically using commercial kits (Rell Assay, Bursa, Turkey). TAC levels in the samples were determined by comparison with torolox, a water soluble form of vitamin E. Thus, the total antioxidant amount in the samples was determined as Equivalent torolox. In determining TAC levels, a calibration curve was established using standards prepared with torolox antioxidant at different concentrations (2, 1, 05, 0.25, 0.125 mmol Trolox / L). TAC levels were determined using the calibration curve and absorbance of the samples. TAC levels were expressed as mmol Trolox Equivalent / L.

Determination of Total Oxidant Capacity (TOC)

There are oxidant molecules that are produced in organisms during metabolism and produced at certain levels. Their levels increase when conditions that adversely affect the organism. The increase in the amount of oxidant in the organism can lead to an increase/decrease of antioxidant levels in the acute period and decrease in the chronic period, which causes them to become harmless by reducing them. Therefore, determination of the levels of oxidant molecules in the plant may be useful to explain the state of the antioxidant system in the organism. So, total antioxidant capacity (TOC) was determined in this study.

TOC levels of samples was measured by spectrophotometric method using commercial kits (Rell Assay, Bursa, Turkey). 20 µmol/L hydrogen peroxide (H_2O_2) was used as the standard in determining TOS levels. The TOS results were expressed as hydrogen peroxide equivalent liters (µmol H2O2 Equiv./L). The absorbance values of the supernatants obtained from the crude plant samples were calculated by the formula given in the commercial kit used to determine the TOS levels.

TOC = [(ΔAbs Sample/ΔAbs standart) x 20]

Calculation of oxidative stress index (OSI)

Oxidative stress index was calculated using TAC and TOC data obtained from the analyzes. The formula used in the calculation is presented below (11, 12).

OSI=[(TOC/TAC) x 100]

Preparation of Oily Tinctures of Hypericum perforatum and Hypericum Heterophyllum Vent. Species

In the present study, Hypericum perforatum and Hypericum Heterophyllum Vent. tinctures of olive oil were prepared under the same conditions. For this purpose, the flowers of the plant species were collected and put into a glass jar without drying. The jar was then filled with olive oil to cover the flowers. The jar was closed and kept in the sun for 30 days. During the sun waiting period, the contents of the jar were mixed once a week with the help of a spoon. It was observed that the olive oil in the jar which was placed in Hypericum perforatum started to be colored after a week. But, Hypericum Heterophyllum Vent. showed no discoloration in olive oil.

III. RESULTS AND DISCUSSION

As a natural source of antioxidants, products of plant origin are frequently used. Some types of spices, herbal teas, oils, some seeds, fruits and vegetables are among the most prominent antioxidant sources. Researchers are focusing on plant extracts of plants that contain bioactive molecules such as flavonoids, catechins, phenols and phenolic acid, as well as antioxidant vitamins such as vitamin C, tocopherols (vitamin E) and carotenoids (vitamin A precursor). Antioxidant activity of many Hypericum species, especially Hypericum perforatum, has been determined in scientific studies on Hypericum species. In the present study, Hypericum Heterophyllum Vent. genus antioxidant capacity was determined. The findings are presented in Table 1.

Analyzed Parameters	Results* (AU)
TAC (mmol Trolox Equivalent/L)	2,22 ± 0,15
TOC (µmol H_2O_2 Equiv./L)	42,41 ± 5,45
OSI (arbitrary Unıt)	1924,02 ± 297,56

Table 1. TAC, TOC and OSI levels of Hypericum Heterophyllum Vent.
*** Datas are presented as mean ± standard deviation (n=5).**
Abbreviations: TAC; Total antioxidant capacity, TOC; Total antioxidant capacity, OSI: Oxidative stress index, AU; Arbitrary Unit

According to the results of Hypericum Heterophyllum Vent. TAC levels were found to be 2.22 mmol Trolox Equivalent/L and TOC levels were 42.41 µmol H2O2 Equiv./L. OSI levels were calculated as 1924 AU. These data show that plant species have high antioxidant capacity.

TAS, TOS and OSI parameters are frequently used to express the antioxidant activity of plants (13, 14). For example, studies with Nepeta viscida from the Lamicaceae family and Thermopsis turcica from the Thermopsis family, which are endemic species of Anatolia. In the study conducted with Thermopsis turcica, it was stated that TAC levels were 2.06 mmol Trolox Equivalent/L. Similarly, in the study conducted with Nepeta viscida TAC levels were determined as 1.16 mmol Trolox Equivalent/L and TOC levels were 6.48 µmol H_2O_2 Equiv./L (14). These

data in the literature are compared with the data in Table 1, it can be said that the antioxidant capacity of the Hypericum Heterophyllum Vent. species (2.22 mmol Trolox Equivalent/L) may be higher than other plants. Therefore, the antioxidant power of the Hypericum Heterophyllum Vent. species can be shown as the reason for its usefulness in various diseases.

Turkey has a total of 89 Hypericum species, including 43 endemic (15). Due to the pharmacological activity, Hypericum perforatum is one of the best known Hypericum species in Turkey. However, little has been studied to date for the phytochemical compositions and biological activities of other species used in traditional medicine (16,17). In the present study, olive oil tendurs of Hypericum perforatum and Hypericum Heterophyllum Vent. species were prepared under the same conditions. While the color of the olive oil tinctures prepared with Hypericum perforatum, a color change approaching to red (Figure 1). But there was no such change in the tinctures prepared with Hypericum Heterophyllum Vent. species.

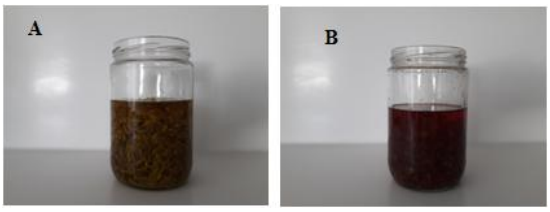


Figure 1. Olive oil tinctures of Hypericum perforatum and Hypericum Heterophyllum Vent. Species. A) Hypericum Heterophyllum Vent.'s tincture, B) Hypericum perforatum's tincture

The reason for this may be related to the different color pigments contained in the anthers of the stamens of the plant species. Because the anthers of the stamens of the Hypericum Heterophyllum Vent. species are yellow in color. And the anthers of the Hypericum perforatum stamens are dark (brown, purple). Presumably, the color of the tendons prepared with the Hypericum perforatum species is given by the bioactive content of this dark anther in the stamens of the flowers.

IV. CONCLUSION

Hypericum species contain biologically active secondary metabolites of at least ten different classes. These metabolites can be listed as naphthodianthrons (hypericin and pseudohipericin), fluoroglucenes (hyperforin), flavonoids (routine, hyperocyte, isocarcinrite, quercitrin quartetin, amentoflavone) and phenylpronoids (chlorogenic acid). However, large differences in contents have been reported for wild populations growing in different regions (1). Therefore, in order to determine the possible therapeutic effects of Hypericum species growing in different regions, it is important to investigate the contents and possible effects of endemic species. In recent years, studies on Hypericum species in Anatolia have been started (15-20). In this study, the antioxidant activity that can mediate the pharmacological effects of the Hypericum Heterophyllum Vent. species was determined. The antioxidant capacity of the Hypericum Heterophyllum Vent. species was determined to be high. This feature of the plant explains the reason for use against diseases. And In order to understand the possible benefits of Hypericum Heterophyllum Vent. species, it is useful to investigate the effects of different extracts of plants, content analysis, antimicrobial effects and antioxidant properties.

Conflict of interest: The authors have declared that there is no conflict of interest

Funding: We declare that, this study was financially supported by Scientific Research Projects Committee (project number: 18.KARİYER149), Rectorate of Afyon Kocatepe University, Afyonkarahisar, Turkey.

REFERENCES

[1] M. Marrelli, G. Statti, F. Conforti, F. Menichini, "New Potential Pharmaceutical Applications of Hypericum Species", Mini Rev Med Chem. 16(9):710-20, September 2016.

[2] M. Hajhashemi, Z. Ghanbari, M. Movahedi, M. Rafieian, A. Keivani, F. Haghollahi, "The effect of Achillea millefolium and Hypericum perforatum ointments on episiotomy wound healing in primiparous women", J Matern Fetal Neonatal Med., 31(1):63-69, January 2018.

[3] S. Gunpinar, O.A. Kılıc, I. Duran, M. Tosun, T. Firat, G. Soyler, "Evaluation of the Effect of Topical Hypericum perforatum Oil on Excisional Palatal Wound Healing in Rabbits", J Invest Surg., 1:1-10, June 2018.

[4] U. Yılmaz, H. Kaya, M. Turan, F. Bir, B. Şahin, "Investigation the effect of Hypericum perforatum on corneal alkali burns", Cutan Ocul Toxicol., 19:1-4, June 2019.

[5] M.A. Akay, M. Akduman, A.Ç. Tataroğlu, C. Eraldemir, T. Kum, Ç. Vural, G.E. Yıldız, "Evaluation of the efficacy of Hypericum perforatum (St. John's wort) oil in the prevention of stricture due to esophageal corrosive burns", Esophagus., Vol. 11, doi: 10.1007/s10388-019-00671-2, April 2019.

[6] A. Yücel, Y. Kan, E. Yesilada, O. Akın, "Effect of St.John's wort (Hypericum perforatum) oily extract for the care and treatment of pressure sores; a case report", J Ethnopharmacol. 20;196:236-241, January 2017.

[7] A.C. Sevastre-Berghian, V.A. Toma, B. Sevastre, D. Hanganu, L. Vlase, D. Benedec, I. Oniga, I. Baldea, D. Olteanu, R. Moldovan, N. Decea, G.A. Filip, S.V. Clichici, "Characterization and biological effects of Hypericum extracts on experimentally-induced - anxiety, oxidative stress and inflammation in rats", J Physiol Pharmacol., 69(5). doi: 10.26402/jpp.2018.5.13, October 2018.

[8] D.X. Jin, J.F. He, K.Q. Zhang, X.G. Luo, T.C. Zhang, "EtOAc extract of H. attenuatum Choisy inhibits inflammation by suppressing the NF-κB and MAPK pathways and modulating the gut microbiota", Phytomedicine, 57:292-304, April 2019.

[9] F. Antognoni, M. Lianza, F. Poli, M. Buccioni, C. Santinelli, G. Caprioli, R. Iannarelli, G. Lupidi, E. Damiani, D. Beghelli, A. Alunno, F. Maggi, "Polar extracts from the berry-like fruits of Hypericum androsaemum L. as a promising ingredient in skin

care formulations", J Ethnopharmacol., 4;195:255-265, January 2017.

[10] F. Vafi, R. Bahramsoltani, M. Abdollahi, A. Manayi, A. Hossein Abdolghaffari, N. Samadi, G. Amin, G. Hassanzadeh, H. Jamalifar, M. Baeeri, M. Heidari, M. Khanavi, "Burn Wound Healing Activity of Lythrum salicaria L. and Hypericum scabrum L. Wounds", pii: WNDS20160929-2, September 2016.

[11] Ö. Hazman, S. Ovalı, "Investigation of the Anti-Inflammatory Effects of Safranal on High-Fat Diet and Multiple Low-Dose Streptozotocin Induced Type 2 Diabetes Rat Model", Inflammation, 38(3):1012-9, 2015.

[12] Ö. Hazman, M.F. Bozkurt, "Anti-inflammatory and Antioxidative Activities of Safranal in the Reduction of Renal Dysfunction and Damage that Occur in Diabetic Nephropathy". Inflammation, 38(4):1537-45, 2015.

[13] L. Aksoy, E. Kolay, Y. Ağılönü, Z. Aslan, M. Kargıoğlu, "Free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status of endemic Thermopsis turcica", Saudi J Biol Sci. 20(3):235-9, July 2013.

[14] Y.S. Karafakıoğlu, L. Aksoy, "Evaluation of Minerals, Phenolics, Radical Scavenging Activity, Total Oxidant Status and Total Antioxidant Statüs of Nepeta Viscida Boiss.", International Journal of Agriculture, Forestry and Life Science, 3 (1), 98-105, 2019.

[15] A.K. Ayan, C. Çırak, " In vitro Multiplication of Hypericum heterophllum an Endemic Turkısh species", American Journal of Plant Pathology, 1 (1):76-81, 2006.

[16] N. Eruygur, E. Ucar, H.A. Akpulat, K. Shahsavari, S.M. Safavi, D. Kahrizi, "In vitro antioxidant assessment, screening of enzyme inhibitory activities of methanol and water extracts and gene expression in Hypericum lydium", Mol Biol Rep., 46(2):2121-2129, April 2019.

[17] S. Keser, F. Keser, O. Kaygılı, S. Tekin, E. Demir, I. Turkoglu, S. Turkoglu, A.E. Parlak, O. Yılmaz, M. Karatepe, S. Sandal, S. KIrbag, "Phytochemical compounds and antiradical, antimicrobial, and cytotoxic activities of the extracts from Hypericum scabrum L. Flowers", Nat Prod Res., 10:1-6, Nov 2018.

[18] E.E. Ozkan, N. Ozsoy, T.Y. Ozden, G. Ozhan, A. Mat, " Evaluation of Chemical Composition and In-vitro Biological Activities of Three Endemic Hypericum Species from Anatolia (H. thymbrifolium, H. spectabile and H. pseudolaeve)", Iran J Pharm Res., 17(3):1036-1046, September 2018.

[19] C. Cırak, J. Radusiene, V. Jakstas, L. Ivanauskas, F. Seyis, F. Yayla, "Secondary metabolites of seven Hypericum species growing in Turkey", Pharmaceutical Biology, 54:10, 2244-2253, 2016.

[20] M. Sarimahmut, N. Balikci, S. Celikler, F. Ari, E. Ulukaya, G. Guleryuz, M.Z. Ozel, "Evaluation of genotoxic and apoptotic potential of Hypericum adenotrichum Spach. in vitro", Regul Toxicol Pharmacol., 74:137-46, February 2016.

★★★