EVALUATION OF LARGE THYME AND CREEPING BENTGRASS ALCOHOLIC EXTRACTS ON HUMAN HEPATOCYTE CARCINOMA CELL LINES

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Abstract. *Romania meadows are home to a great diversity of species. Some species are known to have some pytho-properties such as Thymus sp. varieties, but there are many other species that have been studied only as fodder plants such as of Agrostis sp. varieties. In this study, our aim is to analyze the antiproliferative characteristics of certain plant extracts. For this, were tested Thymus pulegioides L. and Agrostis stolonifera L. The plants alcoholic extracts were evaporated and then re-suspended in dimethyl sulfoxide. To assess the antitumor effect were used hepatic carcinoma cell line HepG2. The cells were cultivated in 90 micro liters of medium supplemented with 10 micro liters of extract in a final concentration of 25 and 50 mg/ml. The anti-proliferative effect was observed after 48 hours through spectrophotometric technique of MTT cellular proliferation. The following have been highlighted: the inhibition of cellular proliferation, dose dependency for Agrostis stolonifera products both in the case of extracts from leaves as well as those from the root, the inhibition rate being of 45% and 48% respectively. In the case of large thymus (Thymus pulegioides L.) extracts, cellular proliferation was observed only for the 50mg/ml dose, the rate of inhibition being of 37% for extracts from leaves, 39% for extracts from flowers and 26% for extracts from the stem. In conclusion, alcoholic extracts of the studied plants present therapeutic antitumor effects through the inhibition of in vivo multiplication of neoplastic cells. Further studies should be carried out for the biochemical characterization and the highlighting of the benefic effect in vivo. We believe that Locvei meadows mountains (Banat Mountains) constitute a reservoir of species with possible herbal effects and it is not yet sufficiently exploited.*

Key words: *Thymus pulegioides L., Agrostis stolonifera L., alcoholic extracts*

INTRODUCTION

Romanian Banat meadows are characterized by a particularly high floristic diversity. The two species *Agrostis stolonifera* L. and *Thymus pulegioides* L. meadows were reported in Banat, along with other species [4, 5]. The grassland biodiversity is studied mostly based on chemical composition and forage production [6] and less based on therapeutically effects of herbs.

Varieties of *Agrostis sp.* are found in most of the Banat Mountains grasslands [8]. Creeping bentgrass meadows (*Agrostis stolonifera* L.) occupy the wetter areas, less sunny slopes at the base while *Thymus pulegioides* L. species occupy larger areas, mostly on sunny coasts.

The varieties of *Thymus* sp. fresh and dried and their processed products were widely used from ancient times [1]. However, in recent decades, they have become a subject for many studies in the search for natural antioxidants and antimicrobials. Recent studies have shown that varieties of *Thymus sp.* have strong antimicrobial and antioxidant activities [1, 2, 6, and 7].

„Thymus pulegioides from Romania are important sources of essential oils, the yield of essential oil being 0.7–1.0 % (v/d.w. herbal drug). The main constituents of the essential oil are monoterpenoid phenols (especially carvacrol) in *T. pulegioides"* [9].

Recent efforts follow the identification of natural products from plants with therapeutic effects. The development of such therapeutic strategies is characterized by low production costs and reduced side effects. At the same time, the recent interest of numerous specialty studies is focused on the identification of products that may be used in neoplastic pathology.

MATERIAL AND METHODS

The studied biological materials is represented by two species, large thyme (*Thymus pulegioides* L.) and creeping bentgrass (*Agrostis stolonifera* L.) and were collected from a meadow from Mountains Locvei, a subdivision of the Banat Mountains.

The *Thymus pulegioides* L. (leaves, flower, and stem) and *Agrostis stolonifera* L. (leaves, root) samples were used fresh to obtain alcoholic extract (1:5). The alcoholic extracts of the studied samples were evaporated and then resuspended in DMSO (dimethyl sulfoxide). To assess the antitumor effect were used hepatic carcinoma cell line HepG2. The cells were cultivated in 90 micro liters of medium supplemented with 10 micro liters of extract in a final concentration of 25 and 50 mg/mL. The number of viable cells was determined by using blue trypan 0, 4% exclusion test [10].

Cell viability is calculated as the number of viable cells divided by the total number of cells within the grids on the hemacytometer. If cells take up trypan blue, they are considered non-viable. After coloring, dead cells lose membrane integrity and they appear colored in blue and living cells not stained.

The cell suspension 1:1 with dye is introduced into 10μL Neubauer counting room and the entire cell were counted: viable ones (colorless) and dead ones (colored). After counting those in five dials and cell density and the number of viable cells were determined using the formulas [10]:

$$\% \text{ viable cells} = [1.00 - (\text{Number of blue cells} \div \text{Number of total cells})] \times 100 \quad [10]$$

$$\text{Number of viable cells} \times 10^4 \times 1.1 = \text{cells/mL culture} \quad [10]$$

$$\text{Cell density} = A \times B \times C \times 10^4$$

Where: A – Volume of cells (10 μl); B – Dilution factor; C – Number of cells and 10^4 – Correction factor;

Statistical analysis was performed using PAST (version 2.14), [3].

RESULTS AND DISCUSSIONS

The anti-proliferative effect of alcoholic extracts *of large thyme* was observed at the concentration of 50 mg/mL extract. Inhibition rate (Figure 1) was 50% for leaves extracts (Thy.L), 51 % for flower extracts (Thy.F) and 51% for stem extracts (Thy.S). At the

concentration of 25 mg/mL extract, the inhibition rate (Figure 1) was lower, 37% for leaves extracts (Thy.L), 39 % for flower extracts (Thy.F) and 26% for stem extracts (Thy.S).

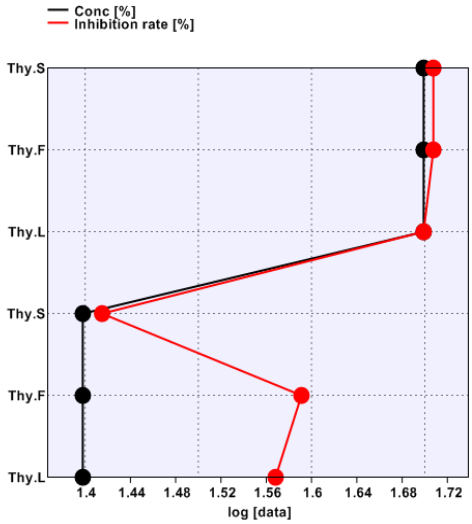

Figure 1. Graphic representation of inhibition rate of the anti-proliferative effect of large thyme (25 mg/mL and 50 mg/mL)
Legend: Thy.L=leaves extract; Thy.F = flower extracts, Thy.S = stem extracts

The Barchart representation of inhibition rate of the anti-proliferative effect of large thyme (Figure 2) is recommending the use of 50% concentration of the alcoholic plant extract

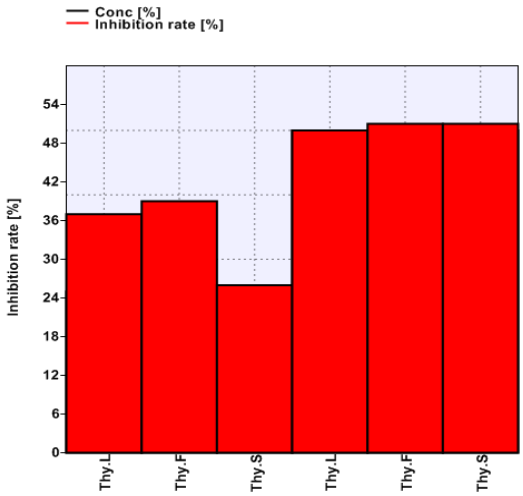

Figure 2. Barchart representation of inhibition rate of the anti-proliferative effect of large thyme (25 mg/mL and 50 mg/mL)
Legend: Thy.L=leaves extract; Thy.F = flower extracts, Thy.S = stem extracts

For *Agrostis stolonifera* L. extracts were determined the inhibition rate of the anti-proliferative effect to be 45% for leaves extract (Ag.L) and 48 % for root extract (Ag.R) at the concentration of 25 mg/mL alcoholic plant extract (Figure 3). Using the concentration of 50 mg/mL alcoholic plant extract (Figure 3) the inhibition rate was 51% for leaves extracts (Ag.L) and 54% for root extracts (Ag.R).

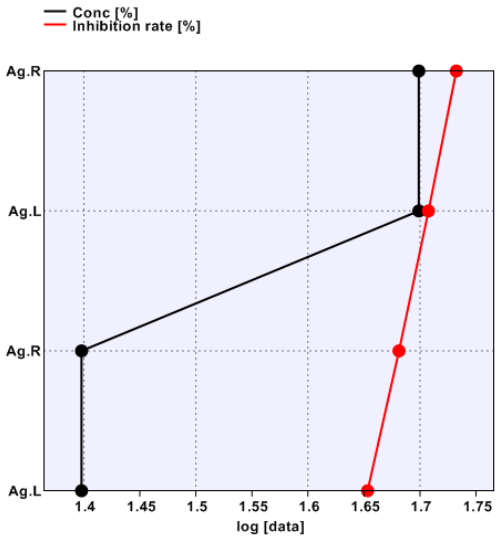

Figure 3. Graphic representation of inhibition rate of the anti-proliferative effect of of *Agrostis stolonifera* L. alcoholic extract (25 mg/mL and 50 mg/mL)
Legend: Ag.L=leaves extract; Ag.R = root extracts

The Barchart representation of inhibition rate of the anti-proliferative effect of creeping bentgrass (Figure 4) is recommending the use of 50% concentration of the alcoholic plant extract

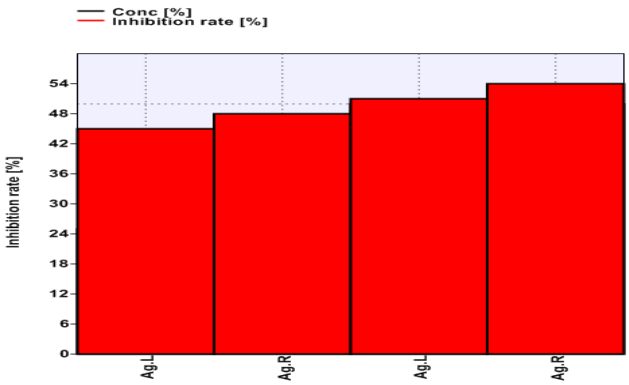

Figure 4. Barchart representation of inhibition rate of the anti-proliferative effect of of *Agrostis stolonifera* L. alcoholic extract (25 mg/mL and 50 mg/mL)
Legend: Ag.L=leaves extract; Ag.R = root extracts

CONCLUSIONS

Both studied plants present antitumor effects. *Large thyme* is having anti-proliferative effect with an inhibition rate of the hepatic tumor cells of over 50% at a concentration of 50 % alcoholic extract, while *creeping bentgrass* presents anti-proliferative effect with an inhibition rate of the hepatic tumor cells of almost 50% even for a concentration of 25% alcoholic extract.

Based on this observation we suggest developing larger studies regarding the antitumor effects of common grasses and we believe that the meadows from Locvei Mountains (Banat Mountains) constitute a reservoir of species with possible antitumor effects and it is not yet sufficiently well exploited.

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