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EVALUATION OF THE MITODEPRESSIVE EFFECT OF *HYPERICUM HAPLOPHYLLOIDES SUBSP DEVOLLENSE* EXTRACTS

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Abstract: Although numerous species within the genus *Hypericum* have been investigated for their biological and ethnopharmacological activities, the cytotoxic and genotoxic profiles of the endemic *Hypericum haplophylloides* subsp. *devollense* (HHD) have not yet been characterized. The present study assessed the extract's mitodepressive effects, its capacity to inhibit primary root elongation, and the range of chromosomal aberrations induced by three aqueous extract concentrations (2000, 4000, and 8000 mg/mL), utilizing the *Allium cepa* model as a sensitive and widely accepted cytogenotoxicity assay. The results showed a concentration-dependent decline in the mitotic index and root tip length. Chromosomal abnormalities frequency —such as laggard chromosomes, sticky, c-mitosis, anaphase bridges decreased at higher concentrations. Overall, *HHD* exhibited clear antiproliferative activity with higher concentration, inducing only minimal genotoxic effects.

Keywords: mitodepressive, genotoxic, root elongation, *Hypericum haplophylloides* subsp. *devollense*, *Allium cepa*.

INTRODUCTION

Plants remain an indispensable and valuable source for the treatment of diseases and the development of drug (Cooper, 2008). This importance is reflected in the sustained involvement of World Health Organization (WHO, 2019) Member States in traditional and complementary medicine. Between 2005 and 2018, a total of 98 countries—more than 50% of the 194 Member States—had established a national policy on Traditional and Complementary Medicine.

Plant-derived medicines have been utilized throughout human history across diverse cultures and remain a crucial source of remedies. The development of modern pharmacology in the eighteenth and nineteenth centuries was largely supported by plant-based compounds. Despite the established contributions of existing botanical therapeutics, plants continue to play a key role in contemporary medicine. With the increasing global demand for medicinal plants for pharmaceutical and dietary supplement use, the discovery of new plant resources has become an important strategy for both conservation and therapeutic advancement (Nobilli et al., 2009; Yang et al., 2010).

The genus *Hypericum* (family *Hypericaceae)* consists of over 400 species spread out frequently in mild areas both as medicinal or nonmedicinal plants, in Europe, North America, North Africa and West Asia (Guner et al., 2000; Marrelli et al., 2016; Meyer., 2011, Balikci N., 2020). Plant species regarding the genus *Hypericum* has been utilized from primordial times to cure diverse diseases and, lately, scientific studies highlighted their focus on the phytochemicals contained in these plants, confirming the high content in polyphenols (Stojanovic et al., 2013) whose antitumor potential have already been documented. Several medicinal *Hypericum* are potential sources of bioactive phytochemicals, reported in numerous biological studies (Bender et al., 2018; Zhang et al., 2020) with a certain cytotoxic and genotoxic effects against different cancer cells pointed out as well in a number of studies (Keskin et al., 2017; Mirmalek et al., 2015; Sarimahmut et al., 2016).

Extensive research has highlighted the diverse health benefits of *Hypericum perforatum* L. (Common St. John's wort) (Hammer et al., 2007; Napoli et al., 2018; Valletta et al., 2018, Zheng et al., 2019) used since ancient times. Similarly, other *Hypericum* species (Guedes et al., 2012; Keskin et al., 2017; Marrelli et al., 2020) are recognized for their therapeutic potential, however, the boundary between a beneficial and a toxic extract depends largely on the administered dose. Even though interest in this topic has increased in recent decades, the cytotoxicity profiles of certain members of this genus remain insufficiently characterized (Babotˇa et al., 2022; Saˇcková et al., 2011; Allegra et al., 2020, Agostinis et al., 2002, Gislaine et al., 2017, Grafakou et al., 2022).

Our study centers on the endemic *Hypericum haplophylloides* subsp. *devollense*, a subshrub native to temperate regions and largely unexplored in the scientific literature. The native range of this endemic species in Albania is scattered in south and southeastern part of the country mainly in Librazhd, Devoll-Tal, Gramsh, at 400 till 500 m (Meyer, 1978), Gjergjevica valley in the city of Korça from 1100 till 1200 m (Shuka, 2008), situated on limestone and serpentine rock at 0-1750m, cultivated to a limited extent (Barina et al., 2018).

According to Moerman (1991), approximately 79% of medicinal plants exhibited some cytotoxicity, while 75% of nonmedicinal plants demonstrated bioactivity. To date, no data are available regarding the cytotoxic or genotoxic activity of the endemic Hypericum *haplophylloides subsp. devollense*. The findings of this study aim to provide scientific evidence to support its

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inhibitory and genotoxic potential, which warrants further detailed evaluation.

MATERIALS AND METHODS

Plant material and extraction

The Branch and aerial parts of the plant were collected in the Librazhdi region during May 2021 and botanically identified by a botanist from the Department of Biology, Faculty of Natural Sciences, University of Elbasan "Aleksandër Xhuvani," where the voucher specimen is deposited in the Laboratory Herbarium. The collected plant material was washed with distilled water and shade-dried in the laboratory at 20 °C for 14 days. The dried material was then ground into a fine powder using a blender

A total of 14.0 g of *H. haplophylloides* subsp. *devollense* plant powder was used to prepare aqueous infusions at three concentrations (2000, 4000, and 8000 mg/mL) by mixing in water (4% w/v) at 100 °C for 5 minutes using a mechanical mill. The mixtures were subsequently sterilized through a filter and stored at 4°C.

Allium cepa L. root-tip cells and slide preparation

Allium cepa (2n = 16) bulbs were successively placed for rooting in bottles containing the plant extract infusions and tap water as a control to compare the results. Bulbs of equal size, aged over five months, were selected from the city market, and experimental conditions were identical for both treatment and control groups, as previously described (Rank and Nielsen, 1993; Smaka-Kincl et al., 1996). For each treatment, 3–5 bulbs were placed in container. They were well-fixed and in contact with the different extract concentrations, as well as with control samples.

After 96 hours, the four most developed roots from each bulb were measured, then cut to 1–2 cm lengths and washed for further microscopic evaluation of the mitotic index (MI) and chromosomal aberrations (CA), as previously described by Fiskesjo (1985). Root tips were fixed in 1:3 acetic acid/ethanol (v/v) for 24 hours at 4–6 °C. The roots were hydrolyzed with 1 N HCl for 5 minutes and subsequently washed with distilled water. Each root tip was stained with 2% aceto-orcein and the meristematic region was excised, then treated with 45% acetic acid to remove excess stain while leaving the chromosomes deeply colored. Gentle tapping of the cover slip ensured an even spread of cells from the root tip, forming a monolayer free of air bubbles. This preparation facilitated scoring of normal and aberrant cells at different stages of the cell cycle.

Proliferation activity

Proliferative activity was assessed by determining the mitotic frequency in root tip cells. The mitotic index (MI) was calculated by analyzing approximately 400 cells per root, using four slides per concentration (n = 4), for a total of 1600 cells per sample, according to the formula: $MI = (P + M + A + T)/N \times 100$. Here, P, M, A, and T represent the numbers of cells in prophase, metaphase, anaphase, and telophase, respectively, and N is the total number of scored cells (Lamberti et al., 1983). The mitotic index is a widely used parameter for evaluating cell proliferation (Fiskesjö, 1985), and reduction in mitotic divisions is commonly interpreted as an indicator of potential cytotoxic components in the plant (Cantor et al., 1992; Turkoglu, 2007; Celik TA, 2020).

Slides were examined under a microscope at up to 100X objective lens with oil immersion to assess both dividing cells and cells exhibiting chromosomal aberrations. Photomicrographs were captured using a Moticam digital camera with Images Plus 2.0 ML software.

Genotoxicity

Chromosomal aberrations, including c-mitosis, chromatin bridges, stickiness, lagging, vagrant chromosomes, and fragments, were evaluated in metaphase, anaphase, and telophase cells throughout the entire meristematic region of each root. The frequency of chromosomal abnormalities was calculated as the number of aberrant cells divided by the total number of examined cells, multiplied by 100. To minimize observer bias, each sample slide was coded by one researcher before being analyzed by another, ensuring that the assessment of cytotoxicity and genotoxicity remained unbiased.

Statistical Analysis of Data

The length and number of roots grown by each bulb were accounted after 96 hours and the means were calculated. The percentage root growth restriction (RGR) was determined in relation to the control, for each sample. Growth inhibition was scored according to the mean values of five longest roots compared to the control value.

Data are expressed as mean ± standard deviation. A smaller SD reflects less variability and values clustered near the average. Validation of significant differences between the means of two defined groups was carried out by t-test. Whilst statistically significant differences of MI among samples of treatment group compared with the negative control were determined by using anova, one way analysis of variance. The minimum level of significance chosen was $p < 0.05$ (Agresti, 1992). The statistical analysis was accomplished by utilizing the Excel software set (Microsoft Corp, Redmond, WA, USA) accompanied with a device for statistical analysis.

RESULTS

Root growth inhibition

Root elongation under the effect of different concentrations of *H. haplophylloides subsp. devollense* on *Allium cepa* compared with the negative control is summarized in Table 1. Root growth was almost completely inhibited at the highest concentration (8000 mg/mL), corresponding to a 96.33% growth inhibition rate (Table 1). Increasing extract concentrations caused progressive inhibition, with mean root lengths decreasing from 8.2 mm to 2.1 mm, compared with 57.2 mm in the control samples.

Table 1.

Root growth restriction values of *Allium* bulbs treated with different concentrations of *Hypericum haplophylloides* subsp. *devollense* extracts (day 4) with p<0.05

Samples	Concentration mg/ml	Mean root length ±SD (mm)	Root Growth Restriction %
Control	-	57.2 ±15.549	
Hypericum haplophylloides s. *devollense*	2000	8.2 ±1.976	85.67
	4000	3.4 ±0.841	94.06
	8000	2.1 ±0.482	96.33

*All the values were significant at p<0.05 in comparison with the control.

Mitodepressive activity of the plant extracts

The mitotic activity results of the treatments are presented in Table 2. The control group displayed the highest number of dividing cells (734), while also showing the lowest number of interphase cells (889) compared with all treatment groups. With increasing concentrations of the plant extract, the mitotic cells progressively declined, from 451 at the lowest dose to 337 at the highest, whereas interphase cells increased from 1184 to 1363. Treatment with 8000 mg/mL reduced the mitotic index (MI) to 15.15%, representing a 66,5% decrease compared with the control. Notably, even the lowest concentration, although relatively high, still suggested selective bioactivity. As the extract concentrations increased, MI values reflected progressively stronger inhibition of the cell cycle.

Changes in the cell-cycle phase distribution of meristematic *Allium* cells treated with the extracts for 96 hours were examined to clarify the mechanisms underlying the observed cytotoxicity. Compared with the treatments, the water control resulted in significantly higher proportions of cells in prophase and telophase (Table 2). In contrast, the extracts induced an accumulation of interphase cells, suggesting inhibition of cell-cycle progression. At this time point, cell-cycle assessment indicated that only a small fraction of cells were dead or undergoing cell death. Morphological features of apoptosis were observed to a minor extent, including cell shrinkage, nuclear condensation and fragmentation, orange-red staining in late-stage apoptotic cells, or the presence of apoptotic bodies.

Table 2.

The effects of different plants concentrations extracts of *Hypericum haplophylloides* subsp. *devollense* on cell cycle stages

		Cells in division stages							
Samples	Dose	Interph	Proph	Metaph	Anaph	Teloph	Dividing cells	Total cell	MI
Control	-	889	527	36	39	132	734	1623	45.2248
HHS	2000	1184	395	20	5	31	451	1635	27.7538
	4000	1250	257	56	27	50	390	1640	23.7804
	8000	1363	226	3	4	14	247	1610	15.1533

Interph = Interphase; Proph = Prophase; Metaph = Metaphase; Anaph = Anaphase; Teloph = Telophase; MI= Mitotic Index;p <0.05

Chromosomal aberration

Genotoxic effects, as reflected by the total number of aberrant cells, were significantly increased ($p < 0.05$) in *Allium* cells treated with 4000 mg/mL of the extracts (Table 3). In the control, only 0.12% of cells were abnormal, whereas three abnormal cells (0.18%) were observed at 2000 mg/mL. Regarding chromosomal aberrations, sticky chromosomes, Figure 1(f) and c-mitosis, Figure 1(c) were the most frequent.

Table 3.

Cytogenetic analysis of *A. cepa* root tips chromosome aberration analysis

Samples	Doses mg/ml	S	C-M	B	L	Total CA%
Control	-	-	2	-	-	0.12
H. haplophylloides subsp. devollense	2000	2	1	0	0	0.18
	4000	15	13	4	3	2.13
	8000	0	2	1	1	0.25

CA % = Cells with chromosome aberration as %; S = Stickiness; C-M = C-Mitosis; B = Bridge; L = Laggard. p <0.05

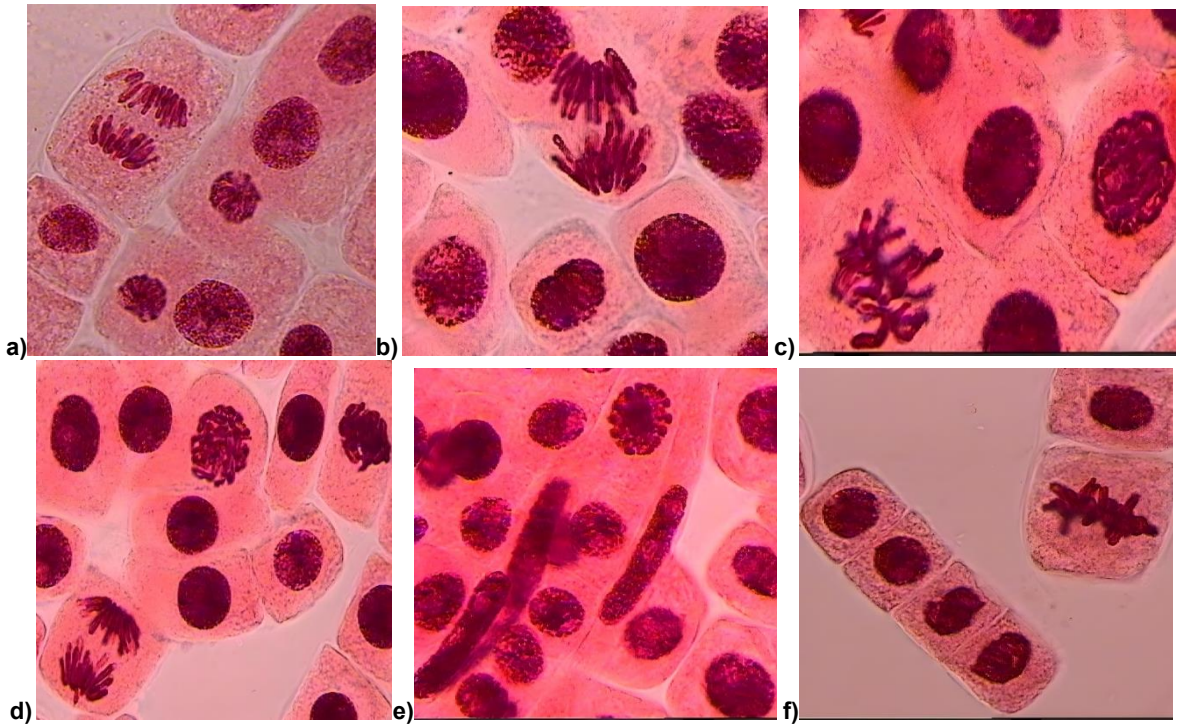

Fig.1. Chromosomal aberrations observed in root tip cells of *Allium cepa*: a) normal anaphase; b) anaphase bridge; c) c-mitosis; d) laggard e) abnormal shape of nuclei; f) sticky metaphase, 780x.

DISCUSSION

Recently, research on therapeutic approaches has increasingly focused on major diseases worldwide (Cooper, 2008; Newman, 2007; Amin, 2009). In this context, natural products continue to play a key role in drug discovery, particularly in the search for compounds with distinctive and potent mitodepressive activities (Kirakosyan et al., 2006; Pajenga et al., 2020, Aztopal et al., 2016), thereby providing new and valuable insights for therapeutic applications. To our knowledge, this is the first study to show that aqueous extracts of *H. haplophylloides* subsp. *devollense* possess selective cytotoxic activity. Previously, no cytotoxic activity had been documented for this plant, nor has it been traditionally used in cancer treatment. This study lays the groundwork for further investigation of cytotoxic compounds from this Albanian plant

Within this framework, the present research valuated the mitodepressive activity of aqueous extracts from the endemic HHD. The cytotoxic potential of aqueous extracts at concentrations of 2000, 4000, and 8000 mg/mL was assessed, with tap water serving as the control. The extracts exhibited a clear concentration-dependent inhibitory effect, as evidenced by a progressive reduction in the mitotic index with increasing concentrations.

Assessment of longitudinal root growth revealed significant inhibition in all treated samples compared with the control, with reductions ranging from 85.67% to 96.33%. The extracts of *HHD* exhibited a concentration-dependent inhibitory effect on *Allium cepa* root growth. Moreover, treatment led to a significant accumulation of prophase and telophase cells, a reduction in anaphase frequency, and comparatively fewer metaphase cells in the control.

The most pronounced cytotoxic effect was observed with the 8000 mg/mL aqueous extract of *HHD*, which inhibited 66.5% of cells. Moderate antiproliferative effects were observed with the other aqueous extracts, resulting in 38.64–47.41% cell inhibition, below the level of the control reference. These findings suggest that the plant extracts may serve as a source of novel mitodepressive compounds, given their pronounced cytotoxic activity and high selectivity against *Allium* cells compared with the control. The antiproliferative effects of the plant constituents may be attributed to mechanisms such as activation of carcinogen-detoxifying enzymes, reduction of free radicals, cell-cycle arrest or induction of apoptosis, (Mehta et al., 2010, Udo et al., 2014).

Hypericum perforatum is the most well-known for medicinal applications in humans, although it is not necessarily the most biologically potent (Stojanovic et al., 2013). Other species within the genus have been reported to possess significant antiproliferative properties, highlighting their potential as biologically active agents. Nevertheless, evidence regarding their anticancer activity remains limited, emphasizing the need for further research. Among these, certain studies have reported significant cytotoxic activity for *H. olympicum subsp. Olympicum, H. sampsonii*, *H. ascyron*, *H. foliosum, H. geminiflorum, H. androsaemum* and, *H. scabrum* etc. (Ramos et al., 2013, Keskin et al., 2017, Marrelli et al., 2020, Balikci N., 2020).

Extensive research on the chemopreventive potential of *Hypericum* constituents is important, not only for evaluating their therapeutic effects but also for assessing their safety and potential genotoxicity. Some studies have reported genotoxic activity in *Hypericum* species, although the findings are not yet consistent. For example, Ramos et al. (2013) reported that *H.*

perforatum exhibited protective effects against oxidative damage in the HT29 colon adenocarcinoma cell line. However, water extracts of the same species were shown to increase the frequency of abnormal metaphases and chromosomal aberrations (Saadat, 2006). In contrast, water extracts from a different species, *H. heterophyllum*, were found to elevate micronucleus (MN) levels in human lymphocytes.

Examination of mitotic abnormalities in *H. haplophylloides* subsp. *devollense* indicated that its aqueous extracts exerted only slight genotoxic effects in *Allium cepa* cells at concentrations up to 4000 mg/mL (Table 3). Based on these observations, the tested concentrations appear not to induce significant genotoxicity in *Allium cepa* cells. This represents a favorable outcome if this species is considered for its mitodepressive potential.

CONCLUSIONS

Data from this study clearly demonstrate the prominent mitodepressive potential of the three extracts prepared from the endemic species *Hypericum haplophylloides* subsp. *devollense*, which can be attributed to their selective and pronounced antiproliferative effects. The prospective anticancer properties of these extracts warrant further *in-vitro* evaluation, considering that this plant has not previously been investigated for such activity.

AUTHORS CONTRIBUTIONS

Conceptualization, E.P. and S.V.; methodology, R.S.; data collection R.S.; data validation, E.P., S.V. and R.S.; data processing E.P.; writing—original draft preparation, E.P.; writing—review and editing, E.P., S.V. and R.S.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Agostinis P, Vantieghem A, Merlevede W et al, Hypericin in cancer treatment: More light on the way. Int J Biochem Cell Biol, 34, 221–241, 2002.

Agresti A, A Survey of exact inference for contegency tables statistical. Science, 131-153, 1992.

Allegra A, Tonacci A, Spagnolo EV, Musolino C, Gangemi S, Antiproliferative Effects of St. John's Wort, Its Derivatives, and Other *Hypericum* Species in Hematologic Malignancies. Int J Mol Sci, 22,146, 2020.

Amin R, Kucuk O, Khuri RF, Shin DM, Perspectives for Cancer Prevention With Natural Compounds. Journal of Clinical Oncology, 27, 2712-2725, 2009.

Aztopal N, Erkisa M, Celikler S, Ulukaya E, Ari F, Antigrowth and apoptosis inducing effects of *Hypericum olympicum L.* and *Hypericum adenotrichum Spach.* on lung cancer cells in vitro: Involvement of DNA damage. J Food Biochem, 40(4), 559–566, 2016.

Babot˘a M, Frumuzachi O, Mocan A et al, Unravelling phytochemical and bioactive potential of three *Hypericum* species from Romanian spontaneous fora: *H. Alpigenum,H. perforatum and H. Rochelii.* Plants. 11, 2773–20, 2022.

Balikci N, Sarimahmut M, Ari F, Aztopal N, Özel MZ, Ulukaya E, Celikler S.Toxicity assessment of *Hypericum olympicum* subsp. *olympicum* L. on human lymphocytes and breast cancer cell lines. Journal of Applied Biomedicine, 18, 123–131, 2020.

Barina Z, Somogyi G, Pifkó D & Rakaj M, Checklist of vascular plants of Albania. Phytotaxa, 378 (1), 1–339, 2018.

Bender O, Martínez EJ, Zengin G, Mollica A, Ceylan R, Molina-García L et al, Integration of in vitro and in silico perspectives to explain chemical characterization, biological potential and anticancer effects of *Hypericum salsugineum*: A pharmacologically active source for functional drug formulations. Plos One, 13(6), e0197815, 2018.

Cantor KP, Blaır A, Everett G, Gıbson R, Burmeıster L F, Brown LM, Schumann L, Dıck FR, Pesticides and other agricultural risk factors for non-Hodkin's lymphome among men in lowa and Minnesote. Cancer Research, 52, 2447-2455, 1992.

Celik TA, Aslantruk OS, Evaluation of cytotoxicity and genotoxicity of Inula viscosa leaf extracts with *Allium cepa* test. J. Biomed. Biotechnol.8, 2020.

Cooper L, "eCAM: an emerging linkage with ethnopharmacology?" Evidence - Based. Complementary and Alternative Medicine, 5(4), 365–366, 2008.

Fiskesjo G, The *Allium* test as a standard in environmental monitoring. Hereditas, 102, 99–112, 1985.

Gislaine PA, Tania MM, Emilene A N, Gustavo MV, Giselle C, Anderson OR, Comparative in vitro study of photodynamic activity of hypericin and hypericinates in MCF-7 cells. Journal of Photochemistry and Photobiology B. Biology,175, 89-98, 2017.

Grafakou ME, Barda C, Karikas GA, Skaltsa H, "*Hypericum* essential oils—composition and bioactivities: an update (2012–2022)". Molecules, 27, 5246, 2022.

Guedes AP, Franklin G and Ferreira MF, "*Hypericum* sp.: essential oil composition and biological activities." Phytochemistry Reviews, 11(1), 127‑152, 2012.

Guner A, Ozhatay N, Ekim T, Başer KHC, Flora of Turkey and the East Aegean Islands (Suppl. 2), 11th ed. Edinburgh, Edinburgh University Press, 2000.

Hammer KD, Hillwig ML, Solco AK, Dixon PM, Delate K, Murphy PA, Wurtele ES, Birt DF, Inhibition of prostaglandin E(2) production by anti-inflammatory *Hypericum perforatum* extracts and constituents in RAW 264. Mouse

Macrophage Cells. J Agric Food Chem, 55(18), 7323–7331, 2007.

Keskin C, Aktepe N, Yukselten Y, Sunguroglu A, Boğa M, In-vitro Antioxidant, Cytotoxic, Cholinesterase Inhibitory Activities and Anti-Genotoxic Effects of *Hypericum retusum* Aucher Flowers, Fruits and Seeds Methanol Extracts in Human Mononuclear Leukocytes. Iran J Pharm Res, 16(1), 210‑220, 2017.

Kirakosyan A, Duke JA, Kaufman PB, Warber S, Cseke LJ, Natural Products from Plants, Taylor & Francis, New York, NY, USA, 2006.

Lamberti L, Ponzetto BP, Ardito G, Cell kinetics and sister chromatid exchange frequency in human lymphocytes. Mutat. Res, 319, 193-199, 1983.

Marrelli M, Statti G, Conforti F, Menichini F, New Potential Pharmaceutical Applications of *Hypericum* Species. Mini-Rev Med Chem, 16, 710–720, 2016.

Marrelli GM, Statti, Conforti F, "*Hypericum spp*.: an update on the biological activities and metabolic proAles," Mini-Reviews in Medicinal Chemistry, 20(1), 66‑87, 2020.

Mehta RG, Murillo G, Naithani R, Peng X, Cancer chemoprevention by natural products: how far have we come? Pharm Res, 27, 950–961. 2010.

Meyer FK, Beiträge zur Flora von Albanien. Haussknechtia, Beih, 15, 1-220, 2011.

Mirmalek SA, Azizi MA, Jangholi E, Yadollah-Damavandi S, Javidi MA, Parsa Y et al, Cytotoxic and apoptogenic effect of hypericin, the bioactive component of *Hypericum perforatum* on the MCF-7 human breast cancer cell line. Cancer Cell Int, 16, 1‑9, 2015.

Moerman DE, "The medicinal flora of Native North America: an analysis." Journal of Ethnopharmacology, 31(1), 1–42, 1991.

Napoli E, Siracusa L, Ruberto G, Carrubba A, Lazzara S, Speciale A, Cimino F, Saija A, Cristani M, Phytochemical profiles, phototoxic and antioxidant properties of eleven *Hypericum* specie. Phytochemistry, 152, 162-173, 2018.

Newman DJ and Cragg GM, "Natural products as sources of new drugs over the last 25 years," Journal of Natural Products, 70(3), 461–477, 2007.

Nobilli S, Lippi D, Witort E, Donnini M, Bausi L, Mini E, Capaccioli S, Natural compounds for cancer treatment and prevention. Pharmacol Res, 59, 365–378, 2009.

Pajenga E, Pisha A, Rapi A, Cytotoxic and genotoxic effects of aqueous extracts of *Helichrysum arenarium* and *Ceterach officinrum*. International Journal of Ecosystems and Ecology Science (IJEES),10 (3), 515-520, 2020.

Ramos AA, Marques F, Fernandes FM et al, Water extracts of tree *Hypericum sps*. protect DNA from oxidative and alkylating damage and enhance DNA repair in colon cells. Food Chem Toxicol, 51, 80–86, 2013.

Rank J, Nielsen MH, A modified *Allium* test as a tool in the screening of the genotoxicity of complex mixtures. Hereditas, 118, 49-53, 1993.

Saadat M, Effects of *Hypericum perforatum* L. and *Matricaria chamomilla* L. extracts on the human chromosomes. J Pharmacol Toxicol, 1(3), 289-292, 2006.

Saˇcková V, Kuliková L, Kello M, Uhrinová I, Fedoroˇcko P, Enhanced Antiproliferative and Apoptotic Response of HT-29 Adenocarcinoma Cells to Combination of Photoactivated Hypericin and Farnesyltransferase Inhibitor Manumycin. A. Int J Mol Sci, 12, 8388–8405, 2011.

Sarimahmut M, Balikci N, Celikler S, Ari F, Ulukaya E, Guleryuz G, Ozel MZ, Evaluation of genotoxic and apoptotic potential of *Hypericum adenotrichum* Spach. in vitro. Regul Toxicol Pharm, 74, 137‑146, 2016.

Shuka L, New taxonomic data for the flora of Albania recorded on the serpentine substrate (Southeast Albania). Natura Montenegrina, 8(1), 5-10, 2008.

Smaka-Kincl V, Stegnar P, Lovka M, Toman M J, The evaluation of waste, surface and groundwater quality using the *Allium* test procedure. Mutation Research, 368, 171-179, 1996.

Stojanovic G, Dordevic A, Smelcerovic A, Do Other *Hypericum* Species Have Medical Potential As St. John's Wort (*Hypericum perforatum*)? Curr Med Chem, 20, 2273–2295, 2013.

Turkoglu S. Genotoxicity of five food preservatives tested on root tips of *Allium cepa*. Mutation Research/ GeneticToxicology and Environmental Mutagenesis, 626, 4- 14, 2007.

Udo I J, Akpan G A, Esenowo I K, Cytotoxic effects of (5) medicinal plants on mitosis in *Allium cepa* root tips. Curr. Res. J. Biol. Sci, 6 (2), 71–75, 2014.

Valletta E, Rinaldi A, Marini M, Franzese O, Roscetti G, Distinct *Hypericum perforatum* L. total extracts exert different antitumour activity on erythroleukemic K562 cells. Phytother Res, 32(9), 1803–1811, 2018.

WHO global report on traditional and complementary medicine 2019– report of a WHO global survey. Geneva: World Health Organization (WHO), 2019.

Yang L, Stockigt J, Trends for diverse production strategies of plant medicinal alkaloids. Nat Prod Rep, 27(10), 1469–1479, 2010.

Zheng M, Hou L, Ma Y, Zhou L, Wang F, Cheng B, Wang W, Lu B, Liu P, Lu W, Lu Y, Exosomal let-7d-3p and miR-30d-5p as diagnostic biomarkers for non-invasive screening of cervical cancer and its precursors. Mol Cancer, 18(1), 76, 2019.

Zhang R, Ji Y, Zhang X, Kennelly E J, Long C, Ethnopharmacology of *Hypericum* species in China: A comprehensive review on ethnobotany, phytochemistry and pharmacology. J Ethnopharmacol, 254, 112686, 2020.