In Vitro Anti Proliferative Effects of *Ocimum Basilicum*

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Abstract
Nowadays, worldwide neoplastic pathology is a major public health problem. Despite the many therapeutic strategies currently available in chemotherapy and radiotherapy, the number of new cancer cases and cancer deaths is increasing. An inexpensive alternative without adverse effects is the development of therapeutic strategies using herbal compounds obtained from various natural sources. On the other hand, the development of therapeutic alternatives involves testing their cytotoxic effects on healthy human cells. In this study, we tested *Ocimum basilicum* extracts (in butanol and acetonitrile), in concentrations between 5mg/ml and 40mg/ml, in order to evaluate the cytotoxic potential on colon (Caco2), liver (HepG2) and blood cancer (K-562). We analysed the biocompatibility of the products in vitro on healthy cell lines (MSC-mesenchymal STEM cells and HDFA- human fibroblasts). Cell proliferation was analyzed by the MTT assay. The obtained results revealed that the tested extracts do not significantly modify the multiplication of healthy human cells. Instead, it causes a decrease in the cell growth rate for liver and colon neoplastic cells, and less so for leukemic cells.

Keywords: *Ocimum Basilicum, buthanolic and acetonitrile extracts,* antiproliferative potential

1. Introduction

In present, worldwide, cancer remains a major public health issue, with high morbidity and mortality rates. Although medical biotechnologies with diagnostic, prognostic, and therapeutic utility have seen revolutionary progress in recent decades, the management of neoplastic diseases remains a continued challenge [1]. Developing new adjuvant therapies for treating neoplasms is one of the primary concerns of the pharmaceutical and medical industry. Phytotherapy represents a viable alternative to support therapeutic efforts in meeting the medical needs and improving the prognosis of patients diagnosed with this type of disease. Recent specialized literature encompasses various studies on the biological activities of compounds extracted from plant sources on biological systems. The advantages of this therapeutic approach, in comparison to standard chemotherapy, lie in the fact that it produces minimal side effects, acts on multiple target cells, induces cytotoxicity, and is economically advantageous [2]. *Ocimum basilicum* (OB) is an aromatic annual plant that belongs to the *Lamiaceae* family. Over time, OB has been extensively used both in gastronomy and for medicinal purposes, having beneficial effects in the treatment of digestive, parasitic, anti-infectious, nervous system, and renal conditions. Currently, specialized studies have reported various therapeutic applications for OB, including its detoxifying, hepatoprotective, anti-inflammatory, immunostimulatory, antibacterial, antifungal, antiviral, antihyperglycemic,

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antilipemic, antiproliferative, and antioxidant properties. These properties are attributed to its chemical composition. *Ocimum basilicum* contains various biologically active compounds, with the most significant ones being linalool, eugenol, α-terpinene, and ρ-cymene [3,4,5,6,7]. In Romania, the uses of basil are comparable to those worldwide, as it is employed for culinary, pharmaceutical, and cosmetic purposes. Within the OB species, there are quantitative and qualitative differences in the main bioactive compounds, which vary depending on the region of origin, genetic factors, and the environment. The aim of this study was to evaluate the in vitro antiproliferative capacity of OB extracts prepared in butanol and acetonitrile solvents. In order to evaluate the cytotoxic effect on human cells, we used different neoplastic cells lines, but also healthy cells. We used MSC (mesenchymal Stem cells) and HDFa (human dermal fibroblasts) cell lines to analyze the biocompatibility of the extracts on healthy cells. To quantify the antitumor effect, we used two different adherent cell lines (Caco2 - human colorectal adenocarcinoma, respectively HepG2 - human hepatocellular carcinoma) and one cell line in suspension (K562 - human chronic myelogenous leukemia).

2. Materials and methods

Materials. Cell lines were purchased from CLS (Cell line services), and cell culture reagents and supplies, including the MTT proliferation kit, were obteined from Invitrogen (Thermo Fisher Scientific, Inc.). DMSO (dimethylsulfoxides) and the solvents were purchased from Sigma-Aldrich.

Extracts preparation. The aerial parts of the plant were are dried and crushed to powder. 10 g of powder was suspended in 100 ml of 70% butanol and 100 ml of acetonitrile, respectively. The extraction was performed at room temperature with stirring at 100 rpm. OB extracts in acetonitrile (A) and butanol (B) were weighed and evaporated, then resuspended in DMSO stock solution 50 mg/ml). Stock solutions were diluted in culture media to a final test concentration (5mg/ml-10mg/ml-20mg/ml-40mg/ml).

Cytotoxicity analysis. The cell line were seeded in 96-well plates ($9x10^4$cells/ml, $9x10^3$cells/well), in complete medium (DMEM, high glucose, GlutaMAX™ Supplement, pyruvate, 10% fetal bovine serum, and 1% antibiotics/antimicotics), and incubated 24h in the humidified atmosphere (90%), 5%CO_2 at 37ºC. Then, they were treated with the OB extracts in the previously mentioned concentrations. Cells treated only with vehicle (0.1% DMSO) were used as a control to calculate the proliferation rate (%). The experiments were run in triplicate. After 24h, the culture medium was replaced with MTT solution (0.5 µg/mL). After incubation at 37°C, 4h, the MTT solution was replaced with DMSO for 15 minutes in order to form the formazan granules. Subsequently, the absorbance (Abs) of the samples and control was mesured at 570 nm, using a plate reader (BioTek Instruments Inc.). Cell viability (%) was calculated after the ecuation:

Cell proliferation (%) = [(Abs_sample-Abs_blank)/(Abs_control-Abs_blank)]/100

3. Results and discussion

OB extracts in butanol and acetonitrile had no significant effect on the proliferation of healthy MSC and HDfa cells. However, in the case of the extract in butanol, the proliferation rate at higher doses was lower than that of the extract in acetonitrile, in both cell types (fig.1).

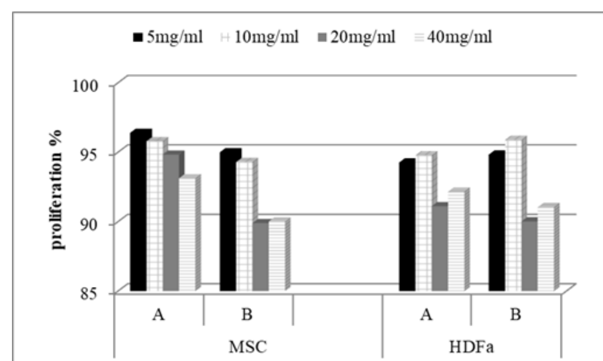

Figure 1. HDFa and MSC cell proliferation rate: (A) acetonitrile; (B) butanol

For Caco2 and HepG2 cell lines, a significant and dose-dependent inhibition of cell proliferation was demonstrated. The butanol extract caused a significant inhibition of cell proliferation at high applied doses (20mg/ml, respectively 40 mg/ml) (fig.2). The action of the extracts on the K562 cell line determined a slight decrease in cell proliferation. At the doses tested, both types of extracts inhibited moderate cell multiplication, only at the high doses applied (fig.3).

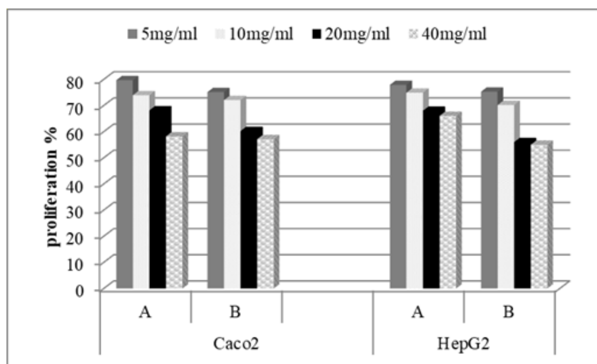

Figure 2. Caco2 and HepG2 cell proliferation rate: (A) acetonitrile; (B) butanol

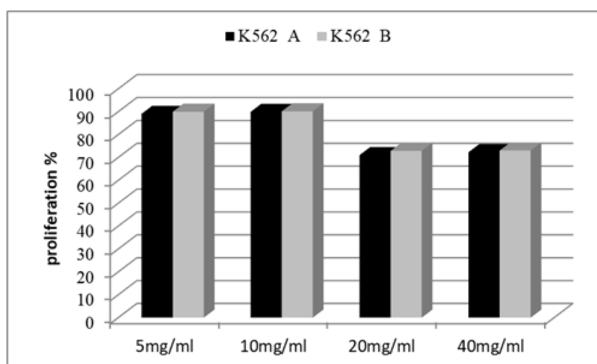

Figure 3. K562 cell proliferation rate: (A) acetonitrile; (B) butanol

Several reports point to the antigenotoxic and chemopreventive activities *of* variou*s Ocimum* species extracts. *Ocimum basilicum* has demonstrated a robust antioxidant effect, primarily attributed to one of its major chemical components, namely linalool. Current experimental evidence suggests that oxidative stress on human DNA is a root cause of aging and numerous pathologies, including cancer. Furthermore, antioxidants possess the ability to inhibit carcinogenic substances, repair DNA, and regulate gene expression. This antioxidant effect of *Ocimum basilicum* extract also confirms its lack of mutagenic impact [8,9,10].

Our objective was to assess the cytotoxicity of alcoholic and acetonitrile-based *Ocimum basilicum* extracts on neoplastic cells. We used neoplastic cell lines derived from the colon (Caco2), liver (HepG2), and the leukemia cell line K-562. Our results indicated that OB extracts obtained in butanol solvent demonstrated slightly higher cytotoxic effects compared to those obtained in acetonitrile. However, these differences did not reach statistical significance. The cytotoxicity observed was dependent on the dosage of the extract applied. Furthermore, the anti-proliferative effects were more pronounced in adherent culture cells (Caco2, HepG2) as opposed to suspension cells (K-562). Simultaneously, we assessed the compatibility of these extracts with healthy human cells, including mesenchymal stem cells and fibroblasts. Notably, the OB extracts did not exhibit cytotoxicity towards MSC and fibroblast cells.

Studies in the specialized literature have provided evidence of the therapeutic potential of *Ocimum basilicum* in cancer. The antiproliferative and cytotoxic effects of OB on cancer cells from various cell lines (HeLa, MCF-7, Jurkat, HT-29, T24, MIAPaCa-2) and a normal cell line (HEK-293) were analyzed. It was found that the antiproliferative and cytotoxic potential varied depending on the cell line type. Additionally, OB influenced the progression of the cell cycle and stimulated apoptosis in most cancer cells. It was concluded that OB extracts not only enhance taste but also exhibit anti-tumor activity against various cancer cells, attributed to the presence of compounds such as rosmarinic acid, cichoric acid, and caffeic acid [11]. Extracts obtained from the leaves of this plant inhibit the proliferation, migration, and invasion of tumor cells and induce apoptosis in pancreatic cancer cell lines, including AsPC-1, MiaPaCa, and Capan-1 [12].

Different research groups have reported in vitro cytotoxic effects of *Ocimum* species, with the extent of cytotoxicity varying depending on the extraction technique and the type of compound tested (be it alcoholic extract, various solvents, essential oil, or nanoparticles with extracts). The tested doses have ranged from 50 µg/mL to 599 mg/ml [13,14,15,16].

Concerning the mechanisms behind OB's cytotoxic impact on neoplastic cells, several molecular processes have been suggested, including apoptosis and cell cycle arrest [17], inhibition of DNA synthesis [18], anti-inflammatory and antioxidant actions [19,20], and the inhibition of matrix metalloproteinases [21]. For instance, in a study involving the Caco2 human colon carcinoma cell line, researchers examined different concentrations of basil's aqueous extract combined with sircenubin syrup. The study concluded that this combination serves as an effective adjuvant for ultrasound treatment due to the presence of bioactive aromatic

compounds such as phenolic acids and flavonoids [16].

4. Conclusions

The buthanolic extracts of *Ocimum basilicum* showed superior cytotoxicity to those obtained in acetonitrile. The antiproliferative effect was evident in adherent cellular cultures Caco2 and HepG2 cell line. The extracts had no toxic character on MSC and HDFa healthy cells, or leukemic cell line. In conclusion, the tested solutions obtained from *Ocimum Basilicum* extracts can be used as adjunctive treatment in adenocarcinoma-type neoplasias**.**

References

1. Ngoua-Meye-Misso, R.L., Sima-Obiang, C., n De La Croix, J., Ndong-Atome G.R., Privat Ondo, J., Ovono Abessolo, F., and Obame-Engonga L.C., Medicinal plants used in management of cancer and other related diseases in Woleu-Ntem province, Gabon, European Journal of Integrative Medicine, 2019, 29, 100924
2. Regassa, H., Sourirajan, A., Kumar, V., Pandey, S., Kumar, D., and Dev, K., A Review of Medicinal plants of the Himalayas with anti-proliferative activity for the treatment of various cancers, Cancers, 2022, 14(16), 3898
3. Shahrajabian, M.H., Sun, W., and Cheng, Q., Chemical components and pharmacological benefits of Basil (Ocimum basilicum): A review, Int. J. Food Prop, 2020, 23, 1961–1970
4. Al-Subhi, L., Waly, M.I., Two cultivars of Ocimum basilicum leaves extracts attenuate streptozotocin-mediated oxidative stress in diabetic rats, Pak. J. Biol. Sci., 2020, 23, 1010–1017
5. Stan, C., Nenciu, F., Muscalu, A., Vlăduț, V.N., Burnichi, F., Popescu, C., Gatea, F., Boiu-Sicuia, O.A., and Israel-Roming, F., Chemical composition, antioxidant and antimicrobial effects of essential oils extracted from two new Ocimum basilicum L. varieties, Diversity, 2022, 14(12), 1048
6. Vázquez-Fresno, R., Rosana, A.R.R., Sajed, T., Onookome-Okome, T., Wishart, N.A., and Wishart, D.S., Herbs and spices—biomarkers of intake based on human intervention studies—a systematic review. Genes Nutr., 2019, 14:18
7. Ghasemzadeh, A., Ashkani, S., Baghdadi, A., Pazoki, A., Jaafar, H.Z.E., and Rahmat, A., Improvement in flavonoids and phenolic acids production and pharmaceutical quality of sweet basil (Ocimum basilicum L.) by ultraviolet-B irradiation, Molecules, 2016, 21, 1203
8. Chaudhary, A., Sharma, S., Mittal, A., Gupta, S., and Dua, A., Phytochemical and antioxidant profiling of Ocimum sanctum. J Food Sci Technol, 2020, 57(10), 3852-3863
9. Kurniasih, N., Latief, M., Supriadin, A., and Junitasari, A., Antioxidant activity of essential oil from basil (Ocimum sanctum L.), AIP Conference Proceedings, 2023, 2646(1)
10. Berić, T., Nikolić, B., Stanojević, J., Vuković-Gacić, B., and Knezević-Vukcević, J., Protective effect of basil (Ocimum basilicum L.) against oxidative DNA damage and mutagenesis, Food Chem Toxicol, 2008, 46(2), 724-732
11. Elansary, H.O., Mahmoud, E.A., In vitro antioxidant and antiproliferative activities of six international basil cultivars, Nat Prod Res., 2015, 29(22), 2149-2154
12. Shimizu, T., Torres, M., Chakraborty, S., Souchek, J., Holy Basil leaf extract decreases tumorigenicity and metastasis of aggressive human pancreatic cancer cells in vitro and in vivo: Potential role in therapy, Cancer Lett., 2013, 336, 270–280
13. Mahmoud, G.I., Biological effects, antioxidant and anticancer activities of marigold and basil essential oils, J. Med. Plants Res., 2013,7, 561–572
14. Lin, B.W., Gong, C.C., Song, H.F., and Cui, Y.Y., Effects of anthocyanins on the prevention and treatment of cancer, Br. J. Pharmacol., 2017, 174, 1226–1243
15. Hanachi, P., Fakhrnezhad, F.R., Zarringhalami, R., and Orhan, I.E., Cytotoxicity of Ocimum basilicum and impatiens walleriana extracts on AGS and SKOV-3 Cancer Cell Lines by flow cytometry analysis, Int. J. Cancer Manag., 2021, 14, e102610
16. Doguer, C., Yıkmış, S., Levent, O., and Turkol, M., Anticancer effects of enrichment in the bioactive components of the functional beverage of Turkish gastronomy by supplementation with purple basil (Ocimum basilicum L.) and the ultrasound treatment, J. Food Process. Preserv., 2021, 45, e15436
17. Thangaraj, K., Balasubramanian, B., Park, S., Natesan, K., Liu, W., and Manju, V., Orientin induces G0/G1 cell cycle arrest and mitochondria mediated intrinsic apoptosis in human colorectal carcinoma HT29 Cells, Biomolecules, 2019, 9, 418
18. Perna, S., Alawadhi, H., Riva, A., Allegrini, P., Petrangolini, G, Gasparri, C., Alalwan, T.A., and Rondanelli, M., In Vitro and In Vivo anticancer activity of Basil (Ocimum spp.): current insights and future prospects, Cancers (Basel), 2022, 11, 14(10), 2375
19. Eftekhar, N., Moghimi, A., Mohammadian Roshan, N., Saadat, S., and Boskabady, M.H., Immunomodulatory and anti-inflammatory effects of hydro-ethanolic extract of Ocimum basilicum leaves and its effect on lung pathological changes in an ovalbumin-induced rat model of asthma, BMC Complement. Altern. Med., 2019, 19, 349

20. El-Nekeety, A.A., Hassan, M.E., Hassan, R.R., Elshafey, O.I., Hamza, Z.K., Abdel-Aziem, S.H., Hassan N.S., and Abdel-Wahhab, M.A., Nanoencapsulation of basil essential oil alleviates the oxidative stress, genotoxicity and DNA damage in rats exposed to biosynthesized iron nanoparticles, Heliyon, 2021, 7, e07537
22.

21. Nangia-Makker, P., Raz, T., Tait, L., Shekhar, M.P.V., Li, H., Balan, V., Makker, H., Fridman, R., Maddipati, K., and Raz, A., Ocimum gratissimum retards breast cancer growth and progression and is a natural inhibitor of matrix metalloproteases, Cancer Biol. Ther., 2013, 14, 417–427.