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TEHNICI CURENTE DE OBȚINERE, CARACTERIZARE ȘI TESTARE A RESURSELOR VEGETALE ȘI PRODUSELOR DERIVATE

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Malignant melanoma (MM) is one of the most challenging skin cancers due to its aggressive behaviour and resistance to treatment in advanced disease stages. In particular, invasive MM remains the major cause of skin cancer-related deaths worldwide owing to the acquired mutations that influence its survival, proliferation, metastasis, and response to therapy. Despite the advancements made in melanoma treatment which has progressed to the development of active molecules specifically targeting BRAF-mutated MM – vemurafenib, ipilimumab, and nivolumab among others - a continuous increase is melanoma incidence is still recorded, announcing the urgent need in improving the currently existing methods for MM treatment [1].

Drug delivery systems (DDS) emerged as efficient carriers of chemotherapeutic agents in cancer treatment since the conventional drug-based therapies result in low treatment effectiveness and high toxicity due to the non-specific distribution of the drugs to both normal and cancer cells. DDS bring numerous advantages – increased drug concentration in cancer cells, diminished cytotoxicity to normal cells, drug protection against degradation and clearance - which advance cancer management and treatment. Through the successful progress of nanotechnology, nanoparticles (NPs) have become promising candidates to be used as DDS. NPs were shown to improve drug efficacy by increasing its half-life and solubility, but also by releasing it in a controlled and sustained manner [2].

Regarding MM treatment, the last years were marked by the emergence of many innovative DDS - from lipid, polymeric and noble metals-based NPs to nanovaccines - able to passively or actively target MM and thus reduce toxic side-effects, increase efficacy, and enhance delivery of therapeutic molecules [3]. Thus, DDS based on NPs are the most promising solution for efficient MM chemotherapy which should be countinuously studied in the future for clinical implementation.

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2. Boswellia Species: *In Vitro* Anticancer Effect of Frankincense Essential Oil

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Frankincense, also known as olibanum, is an oleo-gum-resin obtained through incisions made in the trunk of Boswellia species trees. Although there are many species present in this genus, *B. serrata, B. sacra, B. carterii, B. frereana* and *B. papyrifera* are among the most intense studied, being of an economic and medicinal importance. The oleo-gum-resin is composed of a mixture of constituents, like penta- and tetracyclic triterpenoids, polyphenols, essential oils, sugars, and it is well-known for its wide pallette of actions: anti-inflammatory, antimicrobial, analgesic and anticancerous [1]. The essential oil obtained from frankincense through distillation or hydrodistillation is heavily employed today in aromatherapy, and has also drawn the interest of researchers in the field of cancer. In vitro, *Boswellia sacra* essential oil exerts a cancer cell selective cytotoxic effect in human breast cancer cells. The mechanism behind the cell death seems to be the inducing of apoptosis, the treatment causing DNA fragmentation. Moreover, in 3D multicellular spheroids, the essential oil suppresses spheroid growth and causes the breakdown of breast cancer cellular aggregates [2]. Also, *Boswellia carterii* essential oil reduces cell viability in bladder cancer cells through modulating genes responsible for cell cycle arrest and apoptosis [3]. Boswellia species are a natural source of pharmacological active compounds, the essential oil extracted from the tree exudate being most promising due to its capacity of inducing cancer-cell selective cytotoxicity in various cancer cell lines.

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3. Current Perspectives Regarding the Influence of Melanin on the Behavior of Cutaneous Malignant Melanoma

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Malignant melanoma (MM), characterized by a high metastatic potential and multi-drug resistance, represents one of the most lethal types of cutaneous neoplasms, being resposible for the majority of deaths caused by skin cancers [1]. The aggressive behaviour of MM is influenced by growth factors, neurotransmitters, hormones, peptides, and pigmentation (or melanin presence) [2].

Melanin (MEL), the pigment produced by melanocytes in many areas of the human body (i.e., skin, eyes, hair), has been defined as the most enigmatic polymer existing in nature [3]. At cutaneous level, the primary biological activity exerted by MEL is to confer photoprotection against UVR-induced injury by hidering UV light penetration through the skin and counterating UV-generated reactive oxygen species (ROS). A classification based on the presence of MEL, has divided MM into two main categories: pigmented (melanotic) melanoma and non-pigmented (amelanotic) melanoma [2]. Thus, a question has been raised regarding the impact of pigmentation on the behavior of MM cells.

A previous study showed that pigmented MM cells (B16-F10) exerted a higher resistence to betulinic acid treatment compared to non-pigmented MM cells (SK-MEL-5) explained by the presence of intracellular melanin which can chelate pharmacologically active molecules via covalent or non-covalent bonding [2]. However, other studies demonstrated that melanotic MM cells have a reduced migratory ability and metastatic potential compared to amelanotic cells because MEL reduces the deformation capabilities of MM cells and their ability to penetrate through biological barriers. Other studies indicated that intracellular MEL enables a selective tumor treatment with compounds that possess affinity for the pigment [3]. Extensive research should be further conducted to conclude whether MEL has a beneficial effect on the aggressiveness of MM cells or its presence is detrimental to the successful MM treatment.

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4. Improvement of Anticancer Properties of Essential Oils on Colorectal Cell Lines through the Nanoencapsulation Method

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Essential oils (EOs) are an important source of phytocompounds with anti-oxidant, anti-inflammatory, antibacterial, antiviral, and anticancer properties [1]. Interest in EOs has increased both in the field of scientific research and among consumers. Colorectal cancer (CRC) is one of the most encountered forms of cancer threatening life, with a mortality rate reaching 9 per 100,000 inhabitants [2]. Identifying non-toxic, targeted, and safe treatments for patients affected by CRC would be an enormous achievement in the cancer field. The results of cytotoxicity and genotoxicity of phytoconstituents containing geraniol, nerolidol, thymol, methylisoeugenol, linalool, and eugenol highlighted their potential on colorectal adenocarcinomas [3]. The aim of the present work is to identify natural, safe, targeted, and efficient therapeutic solutions against CRC.

The nano-delivery systems are widely designed to encapsulate EOs to improve their pharmacological properties and usability, control the release kinetics and target their site of action without negative consequences on human health or the environment. The technological process is based on creating new nanocomplexes in which the natural source is framed in submicron-sized capsules or nanoparticles that ensure increasing hydro-solubility and targeting delivery. The potential of EOs as anticancer contenders is continuous studied, but not fully exploited. Thus, EOs with a proven significant effectiveness on cancer cell lines can be introduced in nanoformulations, to enhance the cytotoxic potential and to compare the results with those obtained after treating the cells with simple EO. The most used cell lines are HT-29 and Caco-2 colorectal adenocarcinoma and HCT 116 colorectal carcinoma, due to their biological properties and the high capacity of fast growth.

In conclusion, CRC raises aggravating problems at the global level, and the current treatment requires improvements. EOs seem to be promising treatment/ co-treatment solutions, and their introduction into nanoformulations improves their anticancer potential.

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5. Cytotoxicity Assessment of *Galium Verum* Extract in Melanoma

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Since ancient times, the therapeutic properties of plant species have been highlighted. Plants play an important role in the treatment of various pathologies, including in the fight against cancer due to the vast composition of phytoconstituents, responsible for numerous pharmacodynamic actions. Currently, an impressive number of plants are known for their beneficial effect in the prevention and treatment of cancer, with fewer toxic effects than chemotherapy medication. One of the species studied for its anticancer effect is *Galium verum*, belonging to the *Rubiaceae* family. *Galium* species are known for their antimicrobial, antioxidant, and hemolytic activity and for their beneficial effect in endocrinology [1]. Despite the traditional use of *Galium* species, there are still limited studies on the pharmacological activity and involvement in cancer treatment. The aim of our study was to evaluate the cytotoxic effect of the ethanolic extract of *Galium verum* on the human melanoma cell line RPMI-7951. Furthermore, the antioxidant activity was determined by the DPPH method. The results indicated that the extract possessed a significant antioxidant activity, compared to that of ascorbic acid, used as a positive control. Five concentrations (10 - 150 µg/mL) of the extractive solution were tested using the MTT assay to determine the cytotoxic effect. At 24 hours post-stimulation, *Galium verum* extract induced a dose-dependent decrease in the viability of skin cancer cells. At the lowest concentration, no major changes in cell morphology were observed. The highest concentration produced a significant decrease in the number of cells and the confluence, the cells becoming round and detaching from the plate. The data obtained showed that the ethanolic extract of *Galium verum* has a dose-dependent cytotoxic effect on skin cancer cells and can be an effective preparation in the treatment of melanoma, but further studies are needed to identify the mechanism of action.

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6. The Importance of Probiotics in the Field of Colorectal Cancer

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The human microbiome directly influences homeostasis, inflammation regulation, drug metabolism, and even cancer development. In the cancer field, the colorectal type has been studied because of its pathogenesis and its often inefficient response to treatment. Obesity and reduced physical activity are lifestyle factors associated with colorectal cancer (CRC). The incidence has also been associated with the consumption of specific dietary elements. In this context, it has been suggested that dietary habits high in fruit and low in meat might have a protective effect, reducing the incidence of CRC by modulating and protecting the composition of the normal nonpathogenic commensal microbiota [1].

Thus, the gut microbiome plays a vital role in the daily functions of the body. According to the literature review, probiotics improve enteric microbiota by enhancing the number of beneficial bacteria, including *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Eubacterium*, and *Peptostreptococcus*, while decreasing the number of potentially harmful bacteria, such as *Porhyromonas*, *Fusobacterium*, *Enterococcus* and *Pseudomonas* [2]. Furthermore, probiotic intervention ameliorates intestinal permeability, antimicrobial release, and tight junction function in patients affected by CRC. The cancer development is prevented by some species of bacteria and therapeutic responses, but other species seem to enhance the pathogenesis. In this regard, it is essential to understand the biology mechanism behind these opposing different bacterial species. In vitro models are preferred for identifying the specific mechanisms and the spectrum of action.

In conclusion, the human microbiota plays an essential role in maintaining good health, and recent studies have highlighted its importance in reducing the incidence of CRC. In vitro studies on various species of bacteria are indispensable for deepening the mechanisms of action and the anticancer potential of probiotics.

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7. Green Synthesis of Gold Nanoparticles Using *Galium Sp.* Extract - Promising Candidates in Melanoma

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Melanoma is one of the most aggressive types of skin cancer due to its drug resistance and low survival rate. Conventional treatment is limited by the low response rate, but new targeted therapies have a large impact on overall survival in the population. With advances in nanotechnology, nanoparticle applications will change the landscape of melanoma treatment in the future [1]. Recent studies have focused on the use of gold nanoparticles (AuNP) in the treatment of melanoma, as they increase the effectiveness of antitumor therapy, and improve drug delivery to the target site [2].

Plants, due to the wide variety of phytocompounds and strong therapeutic actions, have a important role in the treatment of various pathologies, including cancer. More recently, gold nanoparticles from plants have gained much attention in this direction. Green synthesized AuNPs are of great interest in the treatment of melanoma but also in its diagnosis due to their advantageous action, such as high stability and permeability but also low cytotoxicity [3].

In recent years, *Galium* species from the *Rubiaceae* family, considered a class of plants with multiple benefits, have been studied regarding their antitumor effect on different cancer lines. Moreover, our research group obtained the first data on the cytotoxicity of the ethanolic extract of *Galium verum* on the human skin cancer line (RPMI-7951).

This first step paves the way to the knowledge of the mechanism of action of the antitumor activity of *Galium* species and indicates that their incorporation into gold nanoparticles can be a promising therapy in the management of skin cancer.

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8. *In Vitro* Biologic Impact of Green Synthesized Magnetic Nanoparticles on Two Different Lung 2D Tumorigenic Cell Lines

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According to global cancer observatory data [1], lung cancer represents the second most commonly diagnosed tumor, and the leading cause of cancer death worldwide, with approximately 1.8 million deaths. Regarding the treatment approaches, a new strategy that may provide a personalized management of patients suffering from lung cancer, involves the use of magnetic nanoparticles (MNPs), especially magnetite – Fe_3O_4 and maghemite – γ-Fe_2O_3, because they possess a superparamagnetic behavior, low toxicity, small dimensions – under 100 nm and a chemistry surface easy to controll and modify [2].

For this purpose, the present study aims to synthesized and characterized the magnetite and maghemite through a single step green synthesis method, which implies the use of two ethanolic extracts based on leaves of green tea (*Camellia sinensis* L.) and basil (*Ocimum basilicum* L.), used as reducing and stabilizing agents for the metal salts precursor. The second aim of the study was to evaluate the antitumoral potential of the preformed MNPs, on two morphologically different lung cancer 2D cell lines: i) human lung carcinoma - A549 cells and ii) large human lung carcinoma - NCI-H460 cells. Due to the fact that the conditions of the synthesis reaction significantly affects the physico-chemically properties of the MNPs, the reactions was conducted at room temperature, as well as 80 °C [3].

The results exhibited that the reaction at 25 °C led to the formation of a mixture of Fe_3O_4 and γ-Fe_2O_3 nanoparticles, while the synthesis at 80 °C led to the formation of Fe_3O_4 nanoparticles as a unique phase. The physico-chemical investigations have demonstrated that the MNPs have nearly spherical shape with narrow size under 8 nm and a strong saturation magnetization, around 60 emu/g (in the case of the samples prepared from green tea ethanolic extract). In addition, the biological investigations revealed that the MNPs are more active on A549 cells compared to NCI-H460 cells.

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9. Boswellic Acids as Potent Proapoptotic Molecules in Cancer Treatment

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The recent cancer statistics are most alarming, just in 2020 19.3 milion new cancer cases were estimated, cancer having caused 10 million deaths [1]. The available therapies are accompanied by severe side-effects and therefore, researchers look for alternatives in the Plantae Kingdom. Natural compounds which seem to be most promising have been found, a group of such molecules being the boswellic acids (BA). BA are the major bioactive constituents of the resin obtained from the *Boswellia* spp. (Burseraceae family) and are part of the pentacyclic triterpene group. Among them, β-boswellic acid (β-BA), acetyl-β-boswellic acid (ABA), 11-keto-β-boswellic acid (KBA), and 3-O-acetyl-11-keto-β-boswellic acid (AKBA) are the central molecules of interest which have shown potent anticancer effect. In vitro, AKBA, ABA and BA decrease cellular viability in breast cancer cells (MDA-MB-231 and MCF-7), among them, AKBA and ABA having the strongest effect. Moreover, AKBA and ABA are capable of inducing apoptosis in MCF-7 and MDA-MB-231 cells through the regulation of P53, P21, BAX, and BCL2 gene expression [2]. But not only breast cancer cells are sensitive to the treatment with BAs. In colon cancer cells (HT-29), AKBA, KBA and BA inhibit cell proliferation and exert a proapoptotic effect through increasing the activity of caspase 8, 9 and 3. In case of prostate cancer cells (PC-3), AKBA produces fragmentation of DNA and induces apoptosis through caspase 3 activation [3]. Coming from a natural source and having the ability to induce apoptosis, boswellic acids are promising molecules in the treatment of various cancers.

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10. Acute Biosafety Screening of Plant-Mediated Magnetic Nanoparticles On 3D Epiairway^{tm} Microtissues

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Plant-mediated biosynthesis of different nanoparticles represents a new eco-friendly approach to develop sustainable compounds. This method can be easily implamentated to obtain green synthetized iron oxide magnetic nanoparticles such us magnetite and maghemite NPs, by using plant extract as reducing agents.

However, the toxicological potential of metal oxide NPs is still a hot topic [1] and must be addressed before developing these NPs for further biological applications.

In this regard, the presents study aims to evaluate the acute biosafety level of four different magnetic nanoparticles (MNPs) obtained through a green synthesis procedure, by using an in vitro three dimensional (3D) human bronchial model – microtissues that present functional human bronchial cells with a ciliated apical surface [2].

The present in vitro method constitutes an alternative approach to classic in vivo tests on animals. Nevertheless, this model is able to provide close results to the human respiratory tract.

The data obtained in the present study showed that the microtisues treated with concentration of 500 ug/mL of all four MNPs elicit viability of more than 80%. Moreover, the histophatological evaluation revealed that no tissue manifested important morphological changes when compared to control.

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11. Bioactive Magnetic Nanoparticles – A Suitable Tool for Hyperthermia Applications

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Magnetic hyperthermia represents an additional antitumor approach besides conventional therapies, which offers many benefits for the treatment of various types of cancer, based on an increased of temperature up to 46°C, until the tumoral cells are destroyed [1]. The most utilized nanomaterials for magnetic hyperthermia are the magnetic nanoparticles (MNPs), more exactly magnetite – Fe_3O_4 and maghemite – γ-Fe_2O_3 due to their unique properties.

Various synthesis techniques were applied for the production of MNPs, but, from all, the green synthesis is by far the most suitable technique. Green synthesis represents the most simple, economic and eco-friendly method for the fabrication of bioactive MNPs due to the fact that implies the use of biocompatible entities such as algae, fungi yeast or plant extracts [2].

In the current study, we want to establish to what extent the MNPs obtained by green synthesis, starting from an aqueous extract of *Artemisia absinthium* L based on leaves and stems, have magnetic hyperthermia properties, so that they can be used in the development of new therapeutic strategies, regarding the treatment of various types of cancer. Therefore, the aims of the current study is: i) the synthesis of Fe_3O_4 and γ-Fe_2O_3 through green synthesis, using aqueous extracts based on leaves and stems of wormwood; ii) a complete physico-chemical screening of the synthesized MNPs and iii) a detailed investigation regarding the hyperthermic capacity of the synthesized MNPs, by determining the specific absorption rate (SAR). Regarding the parameters involved in the chemical reaction, that influence the shape, size or surface chemistry of the final product, it was taken into account two parameters: temperature and precipitation agent.

The outcomes obtained showed that at 80°C and using a mixture of NaOH and $NH_{3(aq)}$ as precipitation agent, one can obtain larger magnetic nanoparticles and implicitly more effective AC magnetic heating agents [3].

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12. Combined Chemo-Thermo-Therapy - An Innovative Approach for Melanoma Treatment

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Skin melanoma is considered the most aggressive type of skin cancer, characterized by a poor prognosis due to the high potential for metastasis [1].
However, anti-melanoma treatment strategy has important drawbacks due to the administration of classic antitumor compounds and cytokines that induce significant side effects, thus altering the well-being of the patient's life; therefore, melanoma treatment strategy imperatively triggers the development of innovative approaches.
Phytocompounds are considered a suitable alternative for the treatment of melanoma showing limited side effects. Nevertheless, their low bioavailability in vivo limits their biological use. Thereby, to address this shortcoming, new pharmaceutical formulations that are administered through an innovative strategy may increase their pharmacokinetics and therapeutic potential.

Since hyperthermic treatment of tumor cells may be induced through magnetic nanoparticles due to magnetic relaxation phenomenon and tumor cells are more likely to suffer cell damage compared to normal healthy cells under these hyperthermic conditions [2], these parameters may induce sensitization of tumor cells, thus making the cells more susceptible to the antitumor activity of the phytocompounds.

In this regard, the current study proposes the development of a new pharmaceutic formulation that may address the melanoma treatment through a combined chemo-thermo-therapy by obtaining magnetolipogels loaded with phytocompounds.

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13. Assessment of *Olea europaea* L. Extract *in Vitro* Effects

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Olea europaea L. is a species belonging to the *Oleaceae* family reported to possess a wide range of therapeutical effects including antioxidant, antibacterial, anti-inflammatory, hypotensive, hypoglycemic and cytotoxic properties. Various extracts obtained from the leaves and the fruits of the plant are currently evaluated in preventing different pathologies [1, 2]. The purpose of the present study was to evaluate an ethanolic extract obtained from olive leaves in terms of the antimigratory potential on a melanoma cell line and the antimicrobial effect on various bacterial strains.

Olive ethanolic extract was obtained from the leaves of the tree by means of ultrasound technique. The antimigratory effect on a melanoma cell line, A375, was determined by Scratch assay. Different concentrations of the extract were tested (10, 25, 50, 100 and 200 µg/mL). The antimicrobial effect was evaluated on four bacterial strains, represented by *Streptococcus pyogenes* (ATCC 19615), *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 51299) and *Escherichia coli* (ATCC 25922). The extract was applied in different concentrations (50 mg/mL, 25 mg/mL, 5 mg/mL, 1 mg/mL, 0.5 mg/mL). The antibacterial activity was evaluated by measuring the diameters of the inhibition zones.

The data obtained showed that the olive ethanolic extract elicited a dose dependent antimigratory effect on A375 tumor cell line. The extract also presented an antibacterial effect at 25 mg/mL for *S. pyogenes, S. aureus* and *E. faecalis* and at 50 mg/mL for all the tested bacterial strains.

The results obtained indicate that olive ethanolic extract reduced melanoma cells migration and possess an antibacterial effect at the highest doses tested.

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14. Preliminary Studies Regarding the Quality and Stability of *Origanum Vulgare* L. Essential Oil Formulated as Polymeric Micelles Drug Delivery Systems

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Origanum vulgare L. is an aromatic herb widely used in the medicinal field as well as in the food industry due to the complex chemical composition of the volatile oil, beeing one of the most commercially important plants in the *Lamiaceae* family [1]. The essential oil was formulated as polymeric micelles drug delivery systems. The formulation was comprehensive characterized and tested *in vitro* for a preclinical assessment as an approach for the management of skin tags [2]. For furtherer *in vivo* evaluation the research line fallowed some qulaity testes like: microbiological test, preservation test, stability and compatibility test. In order to control microbial contamination, the total number of aerobic germs (bacteria, fungi) and the absence of pathogenic or conditionally pathogenic microorganisms (*Salmonella* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus, Candida parapsilosis*) were determined. To test the preservation, five different strains of microorganisms (*Pseudomonas aeruginosa*, *Staphylococcus aureus, Escherichia coli, Candida parapsilosis, Aspergilius brasiliensis*) were inoculated into the cosmeceutical product, and the remaining concentration was determined after 7,14 and 28 days according to ISO 11930:2019. Evaluation of ph, rheological characteristics (rheological behavior and viscosity), formulation consistency and display capacity, as well as organoleptic characters was also determined initialy and at 7, 14, 28 days, respectiveley 2,3 and 6 months after preparation. All the mentioned testes showed that the modern pharmaceutical formulation is sterile, stabile and compatible beeing appropriate for evaluation on human subjects.

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15. Antimelanoma and Antimicrobial Activities of 2,3-Indolo-betulinic Acid and its Glycine Derivatives

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One of the most critical health matters in the developing world is malignant melanoma. A growing number of phytocompounds, either as pure or after undergoing various physicochemical structural changes, have been brought to the forefront, due to their successful outcomes in pre-clinical and clinical research within oncology. Semisynthesis is a pivotal approach for enhancing the bioactivity and medicinal effectiveness of natural product precursors. N-(2,3-indolo-betulinoyl)diglycylglycine (BA1) and N-(2,3-indolo-betulinoyl)glycylglycine (BA2) are two novel semisynthetic derivatives obtained from 2,3-indolo-betulinic acid. Their effectiveness against A375 human melanoma cells in terms of antiproliferative, cytotoxic, and anti-migratory activities were compared to those of existing N-(2,3-indolo-betulinoyl)glycine (BA3), 2,3-indolo-betulinic acid (BA4) and betulinic acid (BI). The five tested compounds exhibited statistically significant cytotoxic and antiproliferative effectiveness when compared to the control (untreated melanoma cells). IC_{50} values spanning from 5.7 to 19.6 µM revealed a dose-dependent antiproliferative effect. The derivatization of 2,3-indolo-betulinic acid considerably increases its anti-melanoma potential. . Compared to the parent cyclic structure BA4 and natural BI, the novel derivatives BA1 (IC_{50}= 5.7 µM) and BA2 (IC_{50}= 10.0 µM) were three and two times, respectively, more efficient. In addition, BA2, BA3, and BA4 exhibited antibacterial activity towards *Streptococcus pyogenes* ATCC 19615 and *Staphylococcus aureus* ATCC 25923 with MIC values between 13 and 16 µg/mL and 26 and 32 µg/mL, respectively. Therefore, can be observed that the attachment of three glycine moieties had no effect on the proliferation of bacterial strains, but the attachment of two glycine moieties caused antibacterial activity. Additionally, compound BA3 with a MIC value of 29 µg/mL exhibited antifungal activity towards *Candida albicans* ATCC 10231 and *Candida parapsilosis* ATCC 22019. These early findings bring up a new avenue for the study of 2,3-indolo-betulinic acid and its derivatives.

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16. Avocado seed and peel extracts: polyphenolic content, antioxidant activity and *in ovo* effects

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Persea americana Mill., known as avocado, is an evergreen tree used since antiquity due to the numerous therapeutic properties of its fruits. Avocado production has increased substantially in recent years due to the benefits and its complex chemical composition. Recent studies revealed the antioxidant potential of avocado extracts, with benefits in various pathologies such as cardiovascular diseases, diabetes mellitus, cancer and liver diseases, as well as in dermato-cosmetology [1,2]. The present study aims to evaluate the byproducts of avocado fruits: peel and seed. Ethanolic extracts were assessed in terms of total polyphenolic content by Folin-Ciocâlteu method, the antioxidant activity by the DPHH assay, as well as the evaluation of the potential effects *in ovo*, influencing angiogenesis using the CAM assay and the evaluation of the irritative potential by the HET-CAM method.
Results showed similar antioxidant capacity in correlation with the polyphenolic content of the peel and the seed extracts. Both extracts expresseed a very good biocombatibility *in ovo* on mucosal tissues with no toxic effects on the vascular net, nevertheless inducing limitation of capillary development at higher concentrations. Avocado byproducts such as seed and peel represent natural resources with therapeutic benefits in cutaneous applications as well as for angiogenesis-related diseases.

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17. Oleanolic acid induces selective cytotoxicity in hepatocarcinoma cells via the mitochondrial pathway

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Cancer remains an enormous threat to health, being considered the second leading cause of death worldwide regardless of the remarkable advances in the medical field [1]. In recent years, researchers have become increasingly interested in mitochondria's role in cancer formation and progression. Therefore, mitochondria play an important role in the regulation of processes related to metabolism, oxidative stress, and apoptosis within cancer cells. The use of plants as a source of biological compounds goes back thousands of years [2]. The antitumor properties of many plant compounds, as well as their reduced toxic effects when used in combination with classical chemotherapy, make them highly desirable to use in antitumor treatments. Plant compounds such as pentacyclic triterpenes display a variety of biological actions, including anti-inflammatory, hepatoprotective, anti-viral, and anticancer properties. As a pentacyclic triterpene compound, oleanolic acid (OA) inhibits tumor cell proliferation by causing them to undergo apoptosis. OA induces apoptosis in tumor cells through complex mechanisms that have not yet been fully elucidated [3].

Consequently, the current study evaluated the antitumor effects of OA both in hepatocarcinoma cells and in healthy hepatocytes. Therefore, OA (1-100 uM) was evaluated for its effect on cell viability, morphology, as well as on the structure of actin filaments, mitochondria and nuclei. In addition, cellular respiration rates were assessed. The results indicated that OA has a selective cytotoxic effect, affecting tumor cells in a dose-dependent manner, with the most intense effects reported at 100 uM. Additionally, signs of cell apoptosis were observed (rounding of cells, condensation of nuclei, actin filaments, and mitochondria). Tumor cells were found to exhibit a decrease in respiratory rates after exposure to OA; in contrast, healthy cells showed an increase in respiratory rates.

In conclusion, these preliminary findings suggest that oleanolic acid may be capable of causing tumor cell apoptosis in addition to affecting mitochondrial morphology and structure in a selective manner.

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18. Eugenol Exerts a Dose-Dependent Cytotoxic Effect in Tumoral Cells

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In spite of sustained efforts by the medical community, colorectal cancer (CRC) ranks third in frequency and is considered the fourth leading cause of death from cancer worldwide. The majority of new cases of colorectal cancer were registered in Western countries, with the incidence increasing continuously. In terms of statistics, the chances of developing colorectal cancer are approximately 4 to 5%. Age, chronic disease history, and lifestyle are most likely factors contributing to the development of this type of neoplasia. Additionally, the intestinal microbiota plays an important role in the pathogenesis of CRC, thus dysbiosis can cause carcinogenesis through a mechanism of chronic inflammation. The survival rate of CRC patients has increased in recent years as a result of advances in antitumor therapy, but side effects and resistance to chemotherapy have not been completely eliminated [1]. In view of this, the plant kingdom offers a large number of compounds that have been proven to be cytotoxic and apoptotic against several types of cancer, including colorectal cancer [2].

In this context, eugenol (Eug), a naturally occurring compound found in many plants, is exploited in a number of medical applications, including cancer treatment [3]. Hence, the present study sought to assess the cytotoxic and pro-apoptotic effects of Eug (0.1 - 1 mM) against colorectal carcinoma cell lines HT-29 and HCT-116.

After 24 hours, Eug exerted a concentration-dependent cytotoxic effect on both cell lines, causing a reduction in cell viability of approximately 34% in HT-29 cells and approximately 41% in HCT-116 cells. Moreover, characteristic signs of cell apoptosis were observed at the level of cell morphology, nucleus, and actin filaments. By examining markers involved in the apoptosis process, Eug also leads to a significant increase in both Bax and Bad, which are pro-apoptotic genes. Consequently, Eug may be a valuable drug candidate for the treatment of CRC, but additional research is necessary to elucidate the biological mechanisms that underlie its action.

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19. Role of *Coriandrum sativum* L. Extract in the Management of Diabetes Mellitus

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Nature has always been and still is an important source of active principles beneficial to human health. Nowadays, plant extracts have gained a considerable scope due to their content in bioactive compounds. The efficient extraction of these compounds from their natural sources and the determination of their activity represent great challenges for researchers in the development of preparations with positive effects on human health [1]. Medicinal plants have a vast potential in the treatment of numerous ailments. Diabetes is a serious metabolic disorder in which several drugs are available that reduce the symptoms of diabetes, but which are expensive and have adverse effects. Thus, herbal medicines are gaining importance as they are cost-effective, with improved therapeutic effects and fewer side effects [2].

The main purpose of this study is to observe the effect of *Coriandrum sativum* L. extract on serum glucose and insulin release capacity from pancreatic beta cells in diabetic mice. In addition, to make a comparison of the effect of the ethanolic extract from the leaves and stem with the extract from coriander seeds in the treatment of diabetes.

Diabetes is the most common endocrine disorder that affects a growing number of people, which has led to the study of plants in this medical sector. Moreover, there are data in the literature that suggest the hypoglycemic and anti-hyperglycemic properties of certain plant species. Therefore, the current study will add to the existing information, so that the plants are considered beneficial in reducing the complications of diabetes.

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20. The benefits of *Commiphora molmol* in the dental field - oral cancer

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Plants have been used for centuries to prevent and control diseases and have been the basis for the discovery of new phytocompounds for pharmaceutical products. Plants and bioactive compounds play a crucial role in the treatment of dental diseases. The use of phytotherapy in the somatological field has been known since ancient times, starting with the use of miswak. Plant products have demonstrated their effectiveness either as dental materials or as active compounds included in the dental clinic. Various plant species have exhibited anti-inflammatory and anti-infectious properties in dental pathologies [1].

Myrrh (*Commiphora molmol*) is known for its antimicrobial activity and for its astringent and calming effect on inflammation in the oral cavity [2]. From these properties, we outlined the purpose of our study, that of highlighting the anticancer potential of the species. Moreover, we want to emphasize the in vitro antitumor activity of the resin on oral cancer cell lines (FaDu and SCC-4) and demonstrate the non-toxic effect on healthy cell lines (HGF and PGK) through viability, morphology and immunofluorescence tests.

Therefore, highlighting the antitumor effect of myrrh will bring benefits in dental practice, but especially in the treatment of cancer. This study aims to discover the usefulness of *Commiphora molmol* as an adjuvant therapy in cancer.

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21. In Vitro Toxicological Profile Using 3D Reconstructed Human Epidermis Tissue of Oleanolic Acid

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Contrary to the enormous efforts made by people in the field, cancer is still a major threat to human health globally. In addition, in terms of the incidence of melanoma, it is on the rise, being one of the leading causes of death worldwide due to cancer. One of the modern targets of antitumor therapy is mitochondria. In the previous study, it was shown that oleanolic acid (OA) is capable of penetrating the mitochondrial membrane and influencing its respiratory function, having a cytotoxic effect on the human melanoma cell line [1]. Compounds that target mitochondria as a therapeutic target are generically called "MITOCANS" [2].

Assuming that OA has a selective cytotoxic effect on human melanoma cells and may be a therapeutic alternative to this pathology by formulating a dermal formulation, the aim of the present study was to evaluate the effect of OA on Reconstructed Human Epidermal Model EpiDerm by In Vitro Application of EpiDerm ™ Skin Irritation Test [3].

EpiDerm skin irritation model (EPI-200-SIT) (Lot 30838) were purchased from MatTek Corporation. A negative control of Dulbecco's phosphate-buffered saline (DPBS) and a positive control of 1% sodium dodecyl sulfate (SDS) were used to perform the EpiDerm Skin Irritation Test. The tissue was prepared as indicated in the MatTek protocol. To evaluate the potential irritant effect on the tissue, the sample was applied in a volume of 30 uL, imprinted with negative control and positive control. The samples were kept in contact with the tissue for 60 minutes, after which the inserts were washed with DPBS, and incubated for 24 hours. After this time, the culture medium was changed to a fresh one, and then the tissues were incubated for a period of 18 ± 2 h. On the third day of the experiment, the MTT viability test was applied by measuring absorbances at 570 nm.

Following the application of the standard protocol for determining the skin irritant potential of the test sample, it was found that this does not cause significant changes in terms of viability. In accordance with the working protocol and OECD Test Guideline 439, a sample is considered irritating if the viability of the sample-treated insert is below 50% after employing the skin irritation test. Thus, in the present case, OA is not considered to be a potentially irritating substance, the viability recorded for each tissue being greater than 50%.

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22. Ursolic Acid Exerts an Antiproliferative and Antimigratory Effect *In Vitro* on Tumoral Cells

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As the second leading cause of death in the world, cancer is a major health concern. Global Cancer Incidence, Mortality, and Prevalence (GLOBOCAN) data show that there were 19 million new cancer cases reported in 2020 and approximately 10 million cancer-related deaths. Despite considerable effort in the field, cancer incidence is predicted to rise by 40% by 2023, primarily due to resistance to treatment. In light of this, efforts are currently being directed toward finding new therapeutic alternatives [1]. The literature has highlighted many therapeutic properties of ursolic acid (UA), including its antioxidant, anti-inflammatory, and anti-tumor properties [2]. Furthermore, it has been proven that UA can induce sensitization to conventional treatment methods [3].

Accordingly, the present study aims to evaluate ursolic acid's cytotoxic and antimigratory capacities in human melanoma cells SK-Mel-28 and in human keratinocytes HaCaT. Cell viability was determined using the MTT method, and biological mechanisms were evaluated through analyses of cell morphology and actin filaments. Regarding the anti-migratory effect, the scratch method was applied.

Ursolic acid has a concentration-dependent cytotoxic effect on tumor cells, according to the study. Additionally, it was found that after stimulation with 100 uM, for a period of 24 hours, the cells change their shape, becoming rounded and separated from the plate. Furthermore, at a concentration of 100 uM, alterations in the structure of nuclei and actin filaments were observed, including signs of apoptosis (condensation of chromatin, reorganization of actin filaments). Additionally, using the scratch method, it was observed that UA inhibits cell migration in a dose-dependent manner, with the greatest migration inhibition observed at 100 uM. On the other hand, UA did not result in a significant decrease in cell viability or alter the morphology of human keratinocytes.

In conclusion, ursolic acid may be a promising anti-tumor agent, exhibiting an apoptotic-like effect on tumor cells.

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23. Evaluation of *Galium verum* Hydro-Alcoholic Extract

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Gallium verum (G. verum, lady's bedstraw, yellow bedstraw) is an herbaceous perennial plant belonging to the *Rubiaceae* family. This medicinal plant was used for many years to treat urinary tract infections, hepatic disfunctions and respiratory diseases. Many studies explored further the potential of *G. verum* and proved it's antiinflamatory, cardioprotective and anticancer properties [1,2].

In this study we prepared two hydro-alcoholic extract of *G. verum herba*. The extracts were prepared with water and ethanol in different ratios, GV-1 – water:ethanol 30:70 (v/v) and GV-2 – water:ethanol 40:60 (v:v). The extracts were characterized by HPLC technique and DPPH assay.

The two extracts presented a dose-dependent antioxidant activity, rosmarinic acid as majoritary constituent and apigenin, gentisic acid, chlorogenic acid, cafeic acid, hyperozid, isoquercitrin, rutin, luteolin, quercitrin as minor constituents.

Furthermore, *in vitro* scratch assay was performed on HaCat cell line. The extracts significantly stimulated cell migration as demonstrated by the narrowing of the scratch area at 48 hours treatment.

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24. The Effectiveness of Phytocompounds Against SARS-CoV-2 Infection

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The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a new strain belonging to the ß-coronavirus family, which produced the disease COVID-19, the reason for the epidemic situation that started in February 2020, which resulted in millions of deaths, and has not ended even at the present time. The novel virus' target is the human respiratory system, producing respiratory symptoms (cough, chest pain, dyspnea, throat, and soreness) digestive symptoms (gastrointestinal pain, vomiting, nausea, and diarrhea), and non-specific neurological symptoms (muscle pain, headache, and fatigue) [1]. Most of the drugs included in the treatment regimens are non-specific, and the multiple adverse reactions have directed the attention of patients, as well as medical personnel, to phytopreparations.

Allium sativum (A. sativum), commonly named garlic is an aromatic herbaceous plant from the *Amaryllidaceae* family. It is a good known herb that exhibits antiviral, antibacterial, antifungal, and even antitumor effects. Garlic presents more than 200 chemical substances, the most abundant being sulfur-containing phytoconstituents: allicin, ajoenes, alliin, and vinyldithiins, but also flavonoids, the most important being quercetin. *A. sativum* presented significant potency against influenza B virus, viral pneumonia, herpes simplex virus type 1 and 2, rhinovirus, cytomegalovirus, and human immunodeficiency virus [2]. *Zingiber officinalis* (*Z. officinalis*), also known as ginger, is rich in polysaccharides, terpenes, organic acids, lipids, and phenolic compounds. Different studies have demonstrated that ginger manifests different biological activities such as antimicrobial, antiviral, antioxidant, anti-inflammatory, neuroprotective, cardioprotective, and anticancer properties. Recent research highlighted that 8-Gingerol, and 10-Gingerol were significantly active against COVID-19 [3].

In conclusion, *A. sativum* and *Z. officinalis* have a demonstrated antiviral effect, so the mechanism of action on SARS-CoV-2 is of current interest for the identification of a therapeutic alternative, without adverse effects.

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25. *In Vitro* Evaluation of the Potential Synergistic Effect Between Eugenol and 5-Fluorouracil in the Treatment of Colorectal Cancer

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Colorectal cancer represents a serious threat to the health and life of patients, especially due to rapid progression and metastasis. Although conventional antitumor therapies have made major progress in the treatment of colorectal cancer, until now they have not succeeded in improving the survival rate. For this reason, recent studies have focused on finding new therapeutic alternatives, and natural compounds seem to have a significant potential both in the prevention and treatment of colorectal cancer. The advantage of natural compounds is mainly represented by the complex biological mechanisms underlying the antitumor activity. For this reason, the association of natural compounds with conventional therapy can become a promising therapy for the treatment and prevention of the progression and metastasis of colorectal cancer [1].

One of the major components of conventional colorectal cancer therapy is represented by 5-Fluorouracil (5-FU) [2]. At the same time, Eugenol (Eug), a compound of natural origin, has proven its effectiveness as an anti-inflammatory, antioxidant, and anti-tumor compound. In addition, the association between Eug and conventional antitumor therapy led to increased effectiveness and prevention of the emergence of resistance to therapy [3]. Thus, the aim of the current study was to evaluate the cytotoxic potential of Eug, but also the association between Eug and 5-FU at the level of colorectal carcinoma cells - HT-29.

Cell viability studies suggested that Eug (0.5 – 2.5 mM) causes a decrease in cell viability, the largest decrease being recorded in the case of a concentration of 2.5 mM (approximately 56%). In addition, by combining with 5-FU (25 µM), cell viability showed a decrease of up to 42% compared to 5-FU alone which led to a decrease of up to 64%. In addition, by evaluating the cell morphology, it was found that the association between Eug and 5-FU determines the alteration of the cell shape, the cells becoming rounded and detached from the plaque.

In conclusion, by combining Eug and 5-FU, an improved cytotoxic effect was obtained, which can represent a solution for reducing the dose of 5-FU in conventional therapy and avoiding the occurrence of toxic reactions associated with it.

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26. Aspirin exerts a dose- and time-dependent cytotoxic effect on colorectal carcinoma cells

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Among the most common forms of neoplasia, colorectal cancer is the second leading cause of death from cancer worldwide. Risk factors like sedentary lifestyle, obesity, and eating habits are associated with a rising number of new cases of colorectal cancer in developed countries. Despite the advent of new therapeutic approaches, such as laparoscopic surgery and radiotherapy, healing and survival rates have not improved significantly. Traditionally, aspirin has been used to prevent cardiovascular diseases [1]. Moreover, recent studies have demonstrated that aspirin can both prevent cancer and reduce its metastases [2]. A recent study demonstrated that aspirin may improve the clinical trial outcomes of patients with metastases receiving conventional chemotherapy [3].

Based on these premises, the present study investigated the cytotoxic potential of aspirin at two-time intervals (24 and 72 hours) on colorectal carcinoma cells HT-29. As part of a more comprehensive analysis of the effect of aspirin on HT-29 cells, cell viability, morphology, nuclei, and actin filaments were evaluated, as well as the expression of the pro- and anti-apoptotic markers Bax and Bcl2.

The results indicate that aspirin is cytotoxic in a time- and concentration-dependent manner. Thus, at 24 hours, cell viability decreased slightly, with the greatest decrease occurring at a concentration of 10 mM (approximately 75%). A more significant decrease in cell viability was recorded after 72 hours, with a value of approximately 45% at a concentration of 10 mM. Further, after 72 hours, morphological changes characteristic of the apoptosis process were observed (rounding of cells, appearance of apoptotic bodies, condensation of chromatin, and reorganization of actin filaments). In addition, anti-apoptotic marker (Bcl2) expression decreased, while pro-apoptotic marker (Bax) expression increased.

These findings indicate that aspirin may be a promising candidate for antitumor therapy, due to its ability to reduce cell proliferation, most likely through the induction of apoptosis in cells. Nevertheless, further studies are necessary to elucidate the mechanism of action.

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27. Insertion of Phytocompounds into Nanostructures to Increase their Anticancer Potential

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Cancer is one of the most devastating diseases of the 21st century. According to the GLOBOCAN database, the highest mortality is in the case of lung cancer, followed by breast, then colorectal cancer forms [1]. The major disadvantages observed in classic therapeutics are aggressive adverse effects, multidrug resistance, and lack of selectivity. The identification of natural alternatives is indispensable, thus phytochemicals are intensively studied in terms of their anticancer profile. However, poor bioavailability imposes great problems on the efficiency of these natural resources.

Nanoemulsions, liposomes, solid lipid nanoparticles, and other nanoparticles are biodegradable and biocompatible nanoparticles that can ameliorate the solubility and stability of phytochemicals, enhance their bioavailability and absorption and, defend them from premature metabolism or enzymatic degradation, grow their circulation time, improve their target specificity to cancer cells, thus enhancing anti-cancer activities [2].

Quercetin is a polyphenol from the flavonoid group encountered plentifully in red onions, berries, and apples. The last studies highlighted, in addition to antioxidant properties, important apoptotic induction properties, reduction of cell viability, and inhibition of migration in breast, non-small cell lung, colon, gastric, squamous cells, and endometrial cancer cells, and leukemia [3]. Its low water solubility makes it the perfect candidate for insertion into nanostructures, with increased therapeutic properties.

In conclusion, quercetin is a known anticancer compound, with promising effects on different forms of cancer, and the identification of an optimal nano-insertion, with the analysis of the mechanism of action, would significantly increase the potential of this compound in the field of oncology.

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28. *In vitro* Evaluation of the Antimelanoma Potential of Natural cardiovascular Drugs

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Among the different types of skin cancers, melanoma is considered to be the most deadly. This type of cancer presents a great deal of difficulty in its treatment. Therefore, in the early stages of the disease, surgical intervention is the only option. In contrast, the survival rate for patients with metastasis is very low. Regarding the treatment of metastatic melanoma, new drug therapies have been introduced in the last decade that have contributed to improving the prognosis. However, patients do not show a lasting response to these treatments, so it is imperative to develop new treatments for this condition [1]. As secondary metabolites, cardiac glycosides are a large family of natural compounds found in both plants and animals. Digoxin, a cardiac glycoside widely used to treat heart failure, is one of the most well-known cardiac glycosides [2]. A number of recent studies have examined digoxin's antitumor properties, indicating that it can inhibit proliferation, induce autophagy and induce apoptosis in cancer cells [3].

As a consequence, the aim of the current study was to evaluate the antitumor potential of digoxin (10-150 nM) at the level of human melanoma cells, especially SK-Mel-28, by examining the viability and morphology of the cells. Moreover, the antiangiogenic potential of digoxin (50 nM) was investigated using the chorioallantoic membrane of hen's eggs as a biological model. The results indicated that after treatment for 72h, digoxin causes a decrease in cell viability in a dose-dependent manner, the concentration of 150 nM causing a decrease in cell viability of approximately 40%. In addition, these effects were associated with morphological alterations, such as the rounding of cells, their detachment from the plaque and the decrease in number. At the level of the chorioallantoic membrane, digoxin 50 nM, determined an inhibition of the formation of blood vessels and, in addition, did not influence the viability of the embryo.

These results indicate that digoxin has an in vitro cytotoxic effect on human melanoma cells, potentially representing a potential antitumor candidate for future studies.

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29. *Mentha x piperita* L. (peppermint) Essential Oil Increases the In Vitro Cytotoxic Activity of Tetracycline on Pharyngeal Cancer Cells

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In the case of head and neck cancer, oral cancer accounts for about 50% of cases. In spite of the advancements in antitumor therapies, the 5-year survival rate for oral cancer is below 50% in most countries. Due to the heterogeneity of head and neck tumors, therapeutic approaches are very challenging. Tobacco, alcohol consumption, chronic inflammation, immunosuppression, and altering the microbiome are among the most implicated factors in the development of oral cancer [1]. The possibility of using tetracycline as an antitumor therapy has been raised in the last 40 years [2]. Furthermore, extracts and essential oils of Mentha piperita have been shown to reduce the viability and proliferation of tumor cells [3].

These premises led to an in vitro evaluation of tetracycline, peppermint essential oil, and the combination of the two to determine their cytotoxic potential and impact on nuclei and actin filaments. According to the results of the viability test, tetracycline (10-100 uM) has a concentration-dependent cytotoxic effect, causing up to 65% decrease in the viability of the cells. Meanwhile, peppermint essential oil, evaluated in five concentrations (5 - 50 uL/mL), decreased cell viability by up to 72%. A significant increase in the cytotoxic effect of peppermint essential oil was observed when tetracycline was combined with peppermint essential oil (37% decrease in the viability). Additionally, the association between these two compounds resulted in a strong condensation of chromatin, fragmentation of nuclei, and reorganization of actin filaments in a peripheral nucleus.

In conclusion, by combining the essential oil of mint and tetracycline, a potent cytotoxic effect is obtained, which can lead to a decrease in the dose of the drug and a reduction in the adverse effects associated with its administration.

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30. *In Vitro* Evaluation of the Cytotoxic Effect of Natural Compounds on Human Melanoma Cell Lines

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Malignant melanomas represent some of the most aggressive forms of cancer, being responsible for over 75% of skin cancer deaths. It is worrying that in the last 50 years, the incidence of these forms of neoplasia has increased more than any other form of cancer. Another major problem related to melanoma is the very high tendency to metastasize, as well as the ineffectiveness of current treatments. With the appearance of metastases, survival at 5 years, with treatment, is approximately 15% [1]. Special attention was given to dietary compounds that are naturally found in fruits and vegetables, due to their preventive or antitumor properties. One such compound is quercetin, which is the main flavonoid in the human diet. The beneficial effects of quercetin include antioxidant, proapoptotic and antiproliferative effects [2].

Based on the data from the literature, the current study aimed to evaluate the antitumor potential of quercetin at the level of two human melanoma cell lines - A375 and SK-Mel28. To determine cell viability, the MTT method was applied, and to determine the impact at the cellular level, cell morphology was analyzed, as well as the structure of actin filaments and nuclei.

The results of the study highlighted the fact that quercetin, evaluated in concentrations between 1 and 40 µM, presented a concentration-dependent cytotoxic effect, causing a reduction in cell viability up to 45%. In addition, at the level of cell morphology, a rounding of them was observed, as well as a massive condensation of chromatin, the appearance of apoptotic bodies and the reorganization of actin filaments in a peripheral ring. All these results led to the conclusion that quercetin represents a dietary compound with potential anti-melanoma effect, however, additional studies are needed to elucidate the possible biological mechanisms.

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31. Insights into the Effectiveness of *Nigella sativa sp.* as Therapeutic Option in the Treatment of Colorectal Carcinoma

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Colorectal carcinoma (CRC) remains one of the leading causes of death globally despite the progress made so far regarding disease biology and therapy. The existing chemotherapeutic options are limited in advanced disease stages due to the occurrence of drug resistance and drug-induced toxic events. Lately, medicinal plants have received considerable attention in the area of cancer research owing to their abundance in phytoconstituents possessing antitumor activity [1]. Multiple studies have also demonstrated the importance of plant-derived compounds in reducing the risk of CRC [2].

Nigella sativa sp. (NS) has been widely studied as natural remedy for various diseases, including cancer. In particular, the plant's oil and seeds contain considerable amounts of active compounds (i.e., alkaloids, saponins, etc.) with great potential in anti-cancer drug discovery and development [1]. Regarding CRC, the crude saponin extract of has been shown to induce apoptosis and cell cycle arrest in HCT-116 cells [1]. Thymoquinone, the most abundant molecule existing in the oil of NS, has been also known as the active principle responsible for the plant's important therapeutic effects such as anti-inflammatory, antioxidant, and anti-tumor activities [2].

Another promising compound for CRC treatment extracted from NS seed coats is herbal melanin (HM). A recent study demonstrated the ability of HM to inhibit the proliferation of HT29 and SW620 CRC cells by enhancing reactive oxygen species (ROS) formation, depleting intracellular glutathione levels, and inducing apoptotic cell death by increasing cytochrome c release, inhibiting Bcl2 proteins and activating caspase□3/□7. These promising results suggest the potential of NS extracts and phytocompounds to be further studied for the treatment of CRC [3].

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32. Evaluation of Soybean (*Glycine max* L.) Phytoestrogens as Active Compounds in Breast Cancer Prevention and Treatment

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Breast cancer (BC), classified as estrogen receptor (ER)-positive and ER-negative, is the main cause of cancer-related deaths in women. Recently, dietary natural products were described as highly efficient in the prophylaxis and treatment of various cancers, BC included. Various mechanisms were associated with the anti-tumor effect of natural compounds in combating BC, such as the downregulation of ER-α expression, inhibition of cancer cell proliferation and metastasis, angiogenesis blockage, generation of apoptosis and cell cycle arrest, as well as the improvement of treatment outcome upon radiotherapy and chemotherapy. Many studies have supported the protective effect of isoflavones (phytoestrogens) derived from *Glycine max* L. (soybean) such as genistein and daidzein against BC [1].

Genistein, possesses a plethora of biological effects, among which the antioxidant, anti-proliferative, and anti-cancer activities were considerably studied. The tumoricidal effect of genistein has been linked to distinct mechanisms. Some strategies by which genistein exerts its beneficial effects in BC treatment are apoptosis induction, interference with estrogen receptors, and prevention of angiogenesis [2].

Daidzein is another isoflavone predominantly found in soy possessing a potent anticancer effect against different tumor types. Daidzein mainly acts through apoptosis and cell cycle arrest induction. Its supplementation was also found beneficial in reducing BC recurrence [3]. The study of soy-derived phytoestrogens in BC prevention and cure needs further evaluation, though genistein and daidzein showed promising results in preclinical studies.

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33. Probiotic bacteria as epigenetic modulators in diabetes treatment and prevention

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It is becoming more evident that probiotic bacteria have the ability to modulate the intestinal microbiota and serve as alternatives and/or complement the activity of antibiotics or anti-inflammatory drugs. As key players in fine tuning of intestinal processes, the disruption of their homeostasis is closely related to the occurrence of metabolic diseases, including diabetes. Within the framework of this project, we aim to investigate the potential of these bacteria to be used in the treatment of diabetes and other conditions related to glucose dysregulation. To this end, we will conduct experiments with selected strains of probiotic bacteria to determine their epigenetic effects (mediated via changes in DNA methylation) on key genes in lipid and carbohydrate metabolism. Wistar rats will be used as animal models for chemically induced diabetes via treatment with alloxan in a study with multiple doses and exposure times. The results of this study will provide a new understanding of the mechanisms underlying the effect of these bacteria, their anti-diabetic potential and clinical applicability.

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34. Nuclear DNA Methylation in plants: a brief overview

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Nuclear DNA (nDNA) methylation, which is quite abundant in plant genomes, is a distinguishing trait. In plant genomes, the CG, CHG, and CHH (H is A, T, and C) sequences are candidates for cytosine DNA methylation. More than 30% of the total 5mC in plant DNA is located at non-CG sites. This epigenetic mechanism plays a crucial role in the regulation of all genetic processes, including transcription, replication, DNA repair, gene transposition, and cell differentiation. Methylation of DNA in plants varies by species, tissue, organelle, and age. DNA methylation influences the expression of transgenes and foreign DNA in cells, in addition to gene silencing and parental imprinting. Plants possess a more complex and sophisticated multi-component genome methylation mechanism compared to animals; DNA methylation in plant mitochondria is performed differently than in plant nuclei. At least three groups of cytosine DNA methyltransferases are involved for nDNA methylation. In contrast to animals, plants continue to develop and survive despite the inactivation of the essential maintenance methyltransferase MET1 (similar to animal DNMT1). This article describes the plant adenine DNA methyltransferase; the same plant gene can be methylated at both adenine and cytosine residues. Therefore, it appears that two separate mechanisms of genome modification based on methylation of cytosines and adenines coexist in higher plants. This analysis is part of a special issue devoted to the epigenetic regulation of cellular and developmental processes in plants, which is the subject of this issue.

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