

predominant of antimicrobial activity of the polymeric on monomeric of flavan-3-ols. In the  $^1\text{H}$  NMR spectrum of the F3-3 was possible to identify two substances as being epigallocatechin and galocatechin.

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**P.13.51** CYTOTOXIC EFFECT OF THE ANTINEOPLASIC DEHYDROCROTONIN IN THE MYELOID LEUKEMIA CELL LINE

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HL60 is the most widely used human myeloid leukemia cell lines for studying the effect of a wide variety of compounds. The dehydrocrotonin is a nor-diterpene lactone isolated from the barks of *Croton cajucara* Benth (Euphorbiaceae), a brazilian medicinal plant. Previous research showed that Dehydrocrotonin had potent antineoplastic properties against human and murine carcinoma cell lines. In order to obtain more information about the mechanism of action of diterpenes in cancer chemotherapy, we studied the viability of HL60 evaluating the following parameters: MTTreduction (mitochondrial function), protein quantification (Lowry's methods) and phosphatase activity.(p-nitrophenol released). The lactone was used in concentrations up to 500  $\mu\text{M}$ . Our results showed a reduction on the cells number according to protein quantification ( $\text{IC}_{50}=500 \mu\text{M}$ ). In relation the mitochondrial function, this drug presented inhibitory effect ( $\text{IC}_{50}=300 \mu\text{M}$ ). In contrast to the other parameters, the phosphatase activity was stimulated. For all the parameters evaluated, the dehydrocrotonin presented more toxic effect when the cells were treated and cultured for 96 h. Our results suggest that the antitumoral activity of DHC could be due to its activator effect on the phosphatase activity. This enzyme presents different physiological roles including cell cycle regulation.

**P.13.52** EFFECTS OF *CALENDULA OFFICINALIS* L. EXTRACT ON PROLIFERATION OF HUMAN SKIN FIBROBLASTS

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Natural products are complex mixtures, rich in potentially active compounds, which are frequently used based on empirical knowledge. *Calendula officinalis* L. is a plant original from Europe and widely distributed around the

world as an ornamental plant. It is used in dermatological products and cosmetics, due to its healing and anti-inflammatory properties. This work evaluates *Calendula officinalis* extract's action on skin fibroblasts proliferation, aiming to clarify its healing activity. Human skin samples, obtained from individuals subjected to cutaneous plastic surgery, were classified by sex, age, and skin color. Fibroblastic cells were isolated in subcultures and cultivated in six-well plates with 9  $\text{cm}^2$  each. Cells were incubated in DMEM medium (Dubelcco Eagle's minimum essential medium) containing 10% fetal calf serum (FCS), for 24 h at 37°C, in a 5%  $\text{CO}_2$  atmosphere. After this period, the medium was substituted by DMEM without FCS and the fibroblasts incubated for another 48 h using different concentrations of the extract: 26.8; 67.0; 128.0; 322.0 and 644.0  $\mu\text{g}/\text{mL}$ . Cells were then removed using a trypsin-EDTA solution (0.05%–0.02%). Viable fibroblasts were stained by trypan blue and counted in a hemocytometric chamber at 100 $\times$  magnification. Extract concentrations of 26.8  $\mu\text{g}/\text{mL}$ , 67.0  $\mu\text{g}/\text{mL}$ , and 128.0  $\mu\text{g}/\text{mL}$  did not significantly alter cell proliferation, when compared to the blank, while concentrations of 322.0  $\mu\text{g}/\text{mL}$  and 644.0  $\mu\text{g}/\text{mL}$  proved themselves toxic to the cells. We conclude that *Calendula officinalis* extract seems to have no mitogenic effect on human skin fibroblast cultures.

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**P.13.53** CYTOTOXICITY OF THE NATURAL NAPHTHOPYRANONE PAEPALANTINE 1 TO MACROPHAGES

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Isocoumarins are widely distributed in the plants kingdom with a variety of biological activities, ranging from antimicrobial, antiviral, antitumoral and antithrombotic activities. The new isocoumarin 9,10-dihydroxy-5,7-dimethoxy-1H-naphtho(2,3c) pyran-1-one, named Paepalantine 1, isolated of the capitula of *Paepalanthus bromelioides*, showed antimicrobial activity against gram positive cocci. Due to the direct involvement of macrophages on mechanisms of host defense the present study evaluated in vitro the cytotoxic effect of the Paepalantine 1 in mouse peritoneal exudate cells using the microculture tetrazolium (MTT) assay. Resident and thioglycolate-induced macrophages were collected by peritoneal lavage with 0.01 M phosphate-buffered saline (PBS, pH 7.4). The suspension cell was washed twice at 250 g for 5 min at 4°C and resuspended in RPMI-1640 medium.  $2 \times 10^6$  cells/mL were seeded into 96-well culture plates and incubated at 37°C in 5%  $\text{CO}_2$ . After 1 h, the cells were washed and treated with concentrations of Paepalantine 1 from 7.5