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(20172) *IN VITRO* CYTOTOXIC AND ANTIBACTERIAL ACTIVITIES OF COMBINATION OF 5,7-DIHYDROXY-4-METHYLCOUMARINE AND SELECTED EXTRACTS FROM MEDICINAL PLANTS

Petia Genova-Kalou¹, Maya Zaharieva², Daniela Batovska³, Nadezhda Markova³, Venelin Enchev³, Maya Zaharieva²

¹ National Center of Infectious and Parasitic Diseases (NCIPD)

² Institute of Microbiology "Stephan Angeloff", Department of Infectious Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

³ Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria

petia.d.genova@abv.bg

Medicinal plants have been acknowledged as potential sources of new lead compounds of therapeutic value for drug design and development. These include coumarins, which have attracted the attention because of their diverse pharmacological properties, structural variability and substitutions in their basic structure. Many coumarin compounds have been identified from natural sources. The current study was designed to investigate the cytotoxicity and antibacterial activity of combination of 5,7-dihydroxy-4-methylcoumarine and crude extracts from three selected medicinal plants (*Trigonella foenum graecum*, *Matricaria recutita*, *Silybum marianum*).

The cytotoxicity of tested substances was evaluated on 24h and 48h by three methods: cell morphology characterization by inverted light microscopy and cell viability tests using the Trypan blue dye exclusion method and the MTT assay. Two monolayer cell lines were used in our experiments: larynx carcinoma cell line (HEp-2) and monkey kidney cells (Vero). Based on the data of cytotoxicity were determined maximal nontoxic concentration (MNC) and cytotoxic concentration, which reduce cell viability by 50% (CC50). The antibacterial activity of the combination was tested on methicillin sensitive or resistant strains from the Gram-positive species *Staphylococcus aureus* (MSSA and MRSA, respectively). The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were evaluated following ISO 20776-1:2006(E). In parallel, the redox activity of treated bacteria at MIC was measured using the MTT dye (Abs550nm) versus untreated control.

The results obtained revealed that the combination of tested substances express concentration-dependent cytotoxic and antiproliferative activities. The data presented here showed that the tested herbal combination exhibit low cytotoxicity. It was found to reduce cell viability by 50% when applied at concentration > 30 mg/ml for 48h. The combination showed a moderate bacteriostatic effect against both types of strains tested (MIC=2.5 mg/ml). The redox activity at MIC ranged negligible depending on the strain.

The present study was the first report related to the cytotoxic effect and antibacterial activity of combination of 5,7-dihydroxy-4-methylcoumarine and crude extracts from three selected medicinal plants (*Trigonella foenum graecum*, *Matricaria recutita*, *Silybum marianum*). In particular, these substances exhibit low cytotoxicity against several mammalian cell lines and a moderate antimicrobial effect on MSSA and MRSA strains.

Keywords: Cytotoxicity, Antibacterial activity, 5,7-dihydroxy-4-methylcoumarine, medicinal plants

(19691) ANTI-HERPES SIMPLEX VIRUS AND CYTOTOXIC ACTIVITIES OF THE SUCCULENT PLANT *GRAPTOPETALUM PARAGUAYENSE* E. WALTHER

*Nadezhda Markova*¹, *Petia Genova-Kalou*², *Ivayla Dincheva*³, *Daniela Batovska*¹, *Ilian Badjakov*³, *Stefka Ivanova*², *Venelin Enchev*¹

¹ *Institute of Organic Chemistry with Centre of Phytochemistry, Sofia, Bulgaria*

² *National Center of Infectious and Parasitic Diseases (NCIPD), Bulgaria*

³ *Agrobiointitute, Sofia, Bulgaria*

nadya@orgchm.bas.bg

About 90% of infectious diseases have viral etiology. Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) are the most common human pathogens, infecting about 90% of the world population. Unlike antimicrobial drugs against bacteria and fungi, only a few effective antiviral drugs are available. The toxic side effects and the emergence of virus strains that are resistant to the drugs, enhance the need for new effective compounds against viral infectious diseases. Medicinal plants are known to be a source of abundant of chemical compounds and traditionally used in healthcare in many countries.

The aim of our study is to evaluate *in vitro* anti-herpes simplex virus effect and cytotoxicity of *Graptopetalum paraguayense* E. Walther (*Crassulaceae*) extracts using colorimetric assay. To evaluate the main organic groups, which had the aqueous leaf extract of the tested ethno plant GC/MS analysis was performed. Next three main fractions were obtained - A (lipids), B (amino and organic acids, carbohydrates) and C (phenolic acids) and the composition of each were determined by GC/MS analysis. The first important step of antiviral experiment is determining of cell proliferation and viability. Both maximal nontoxic concentration (MNC) and cytotoxic concentration for inhibiting 50% (CC50) values were evaluated simultaneously by morphological (microscopically) and by cell survival criteria (MTT-test). To determine the capacity of the whole extract, as well as the three main fractions to inhibit the lytic activity of HSV-1, strain Victoria and HSV-2, strain Bja we use MTT colorimetric assay. The results are expressed as concentrations, which have 50% inhibitory effect on the viruses (IC50) and are possible to calculate selectivity indexes (SIs).

The aqueous extract of *Graptopetalum paraguayense* E. Walther has not cytotoxic effect on RD 64 and Lep cells. It effectively inhibits HSV replication in dose-dependent manner. Furthermore, the aqueous extract was effective inhibitor of HSV-1 replication (97%), whereas its effect to HSV-2 was significantly lower *in vitro*. IC50 values are not so significantly lower than that of ACV. Fraction C has not CPE on human cell lines and inhibits HSV replication in dose-dependent manner. A and B fractions showed no antiviral effect. The mechanism of the action of fraction C is not yet completely identified. Further studies are needed in order to verify which compounds could be responsible for this activity.

Keywords: Anti-herpes simplex virus activity, *Graptopetalum paraguayense* E. Walther, cytotoxicity, MTT assay, GC/MS