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PREPARATION AND CHARACTERIZATION OF NATURAL EXTRACTS WITH ANTIMICROBIAL EFFECT

PŘÍPRAVA A CHARAKTERIZACE PŘÍRODNÍCH EXTRAKTŮ S ANTIMIKROBIÁLNÍM ÚČINKEM

DOCTORAL THESIS

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PREPARATION AND CHARACTERIZATION OF NATURAL EXTRACTS WITH ANTIMICROBIAL EFFECT

Doctoral thesis statement for obtaining the academic title of „Doctor“, abbreviated to „Ph.D.“

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ABSTRACT

Worldwide consumption of antibiotics has increased drastically in the past few decades. The application of antibiotics has led to the production of antibiotic resistance genes (ARGs) which represent a growing and serious human health threat worldwide. The use of natural or alternative medicines increased markedly over the last few years. Therefore, much attention is paid to natural products, which could be used as effective drugs to treat human diseases, with high efficiency against pathogens and negligible side effects. Yeast biomass that contains valuable fatty acids, carbohydrates, nucleic acids, vitamins, and minerals was tested in the potential combination with plant extracts as a food supplement.

This doctoral thesis is focused on impact assessment of selected plant extracts such as acai, clove, turmeric, ginger, ginseng and oregano and their antimicrobial activity, antioxidant activity, cytotoxicity and important nutritional benefits. Subsequently, the biological effects of yeast biomass of the strains *Metschnikowia pulcherrima* CCY 029-002-145, C*ystofilobasidium infirmominiatum* CCY 17-18-4, *Phaffia rhodozyma* CCY 77-1, *Rhodotorula kratochvilovae* CCY 20-2-26 were tested. These yeasts were chosen especially for the high content of active substances in the produced biomass. In conclusion, the potential synergistic effect of plant extracts in combination with yeast biomass to increase the overall antimicrobial effect, antioxidant activity and effect on the growth of Caco-2, B16F1 and HaCaT cell lines was assessed. Considering the results achieved, it is appropriate to include immortalized yeast biomass in combination with natural extracts in food supplements in an appropriate form, as it is clear that there is some direct relationship between cell viability and apoptosis. All microbial extracts showed some positive results for the apoptosis of cancer cell lines and at the same time no cytotoxic effects on non-cancer cell lines at identical concentrations.

KEY WORDS:

antioxidants, antimicrobials, plants, yeasts, apoptosis

ABSTRAKT

Celosvětová spotřeba antibiotik v posledních několika desetiletích významně vzrostla, což vedlo ke zvýšenému výskytu genů antibiotické rezistence (ARG), které představují rostoucí vážnou hrozbu pro lidské zdraví na celém světě. Používání přírodních nebo alternativních léčivých přípravků tudíž v posledních několika letech výrazně vzrostlo. Velká pozornost je nyní věnována přírodním extraktům, které by mohly být v jisté formě použity jako jedna z možností léčby bakteriálního onemocnění, a to s vysokou účinností proti patogenům a se zanedbatelnými vedlejšími účinky. Biomasa kvasinek, která obsahuje cenné mastné kyseliny, sacharidy, nukleové kyseliny, vitamíny a minerály, byla testována jako možná alternativa, a to samotná i v kombinaci s rostlinnými extrakty ve formě doplňku stravy.

Tato disertační práce je zaměřena na studium antimikrobiální aktivity a nutriční přínosy vybraných rostlinných extraktů, jako je acai, hřebíček, kurkuma, zázvor, ženšen a oregano. Následně byly testovány biologické účinky kvasinkové biomasy rodu *Metschnikowia pulcherrima* CCY 029-002-145, *Cystofilobasidium infirmominiatum* CCY 17-18-4, P*haffia rhodozyma* CCY 77-1, *Rhodotorula kratochvilovae* CCY 20-2-26. Tyto kvasinky byly vybrány zejména pro vysoký obsah aktivních látek v produkované biomase. Na závěr byl posouzen potenciální synergický efekt rostlinných extraktů ve spojení s kvasinkovou biomasou ke zvýšení celkového antimikrobiálního účinku, antioxidační aktivity a vlivu na růst buněčných linií Caco-2, B16F1 a HaCaT. Vzhledem k dosaženým výsledkům lze konstatovat, že zařazení imortalizované kvasinkové biomasy v kombinaci s přírodními extrakty do doplňků stravy ve vhodné formě může být prospěšné lidskému zdraví. Existuje přímý vztah mezi životaschopností buněk a apoptózou. Všechny mikrobiální extrakty vykazovaly určité pozitivní výsledky na apoptózu u rakovinných buněčných linií a současně nevykazovaly žádné cytotoxické účinky na nezhoubné buněčné linie při stejných koncentracích.

KLÍČOVÁ SLOVA:

antioxidanty, antimikrobialní látky, rostliny, kvasinky, apoptóza

TABLE OF CONTENTS

1 THEORETICAL PART 7

1.1 ANTIMICROBIALS OF ANIMAL ORIGIN 7

1.2 ANTIMICROBIALS OF PLANT ORIGIN 7

1.2.1 Major components of natural substances 8

1.2.2 Sellected plant extacts used in this thesis 8

1.3 ANTIMICROBIALS OF MICROBIAL ORIGIN 10

1.3.1 Antibiotics 10

1.3.2 Antimicrobial peptides 11

1.3.3 Other antimicrobial substances of microbial origin 11

1.3.4 Oleaginous yeasts as a producers of microbial active compounds 12

2 AIM OF THESIS 14

3 MATERIALS AND METHODS 15

3.1 MATERIALS 15

3.2 MICROORGANISMS AND CELL LINES 15

3.3 EXTRACTION AND DETERMINATION OF PLANT ACTIVE COMPOUNDS 15

3.4 DETERMINATION OF NATURAL ACTIVE COMPOUNDS 16

3.4.1 Determination of carotenoids by HPLC 16

3.4.2 Lipid content and fatty acids profile by Gas chromatography 16

3.4.3 Isolation and determination of β-glucans from yeasts 17

3.4.4 Cultivation of microorganisms for antimicrobial tests 17

3.4.5 Cultivation of yeasts strains 18

3.5 CELL CULTURES CULTIVATION AND TESTS 18

3.5.1 Cultivation of Cell lines 19

3.5.2 MTT Test procedure: 19

3.5.3 Apoptosis with B16F1 cell lines 19

3.5.4 Apoptosis with Raji cell lines 20

4 RESULTS 21

4.1 PLANT EXTRACTS CHARACTERIZATION 21

4.1.1 Characterization of plant extracts 21

4.1.2 Antibacterial activity. dilution method 22

4.1.3 Antimicrobial activity. dilution method 23

4.1.4 Tests of plant extracts on the cell lines 24

4.1.5 Apoptosis ... 27
4.2 PRODUCTION OF MICROBIAL EXTRACTS FROM SELECTED YEAST STRAINS ... 31
4.2.1 Characterization of microbial extracts ... 31
4.2.2 Determination of carotenoids ... 31
4.2.3 Yeasts as a fatty acids producents ... 32
4.2.4 Isolation and determination of β glucans ... 34
4.2.5 Antioxidant activity ... 34
4.2.6 Tests on cell lines ... 36
4.2.7 Apoptosis ... 39
4.3 SYNERGIC EFFECT ... 42
5 DISCUSSION ... 44
6 CONCLUSION ... 47
7 REFERENCES ... 48
8 CURRICULUM VITAE ... 52
9 PUBLICATIONS ... 53

1 THEORETICAL PART

The natural antimicrobials are readily available from various natural sources, such as plants, animals, and microorganisms, in which they constitute part of host defense mechanisms against microbial infections. Natural antimicrobials, such as essential oils and herbs, are traditionally known for their antimicrobial properties and used in different indigenous practices [1].

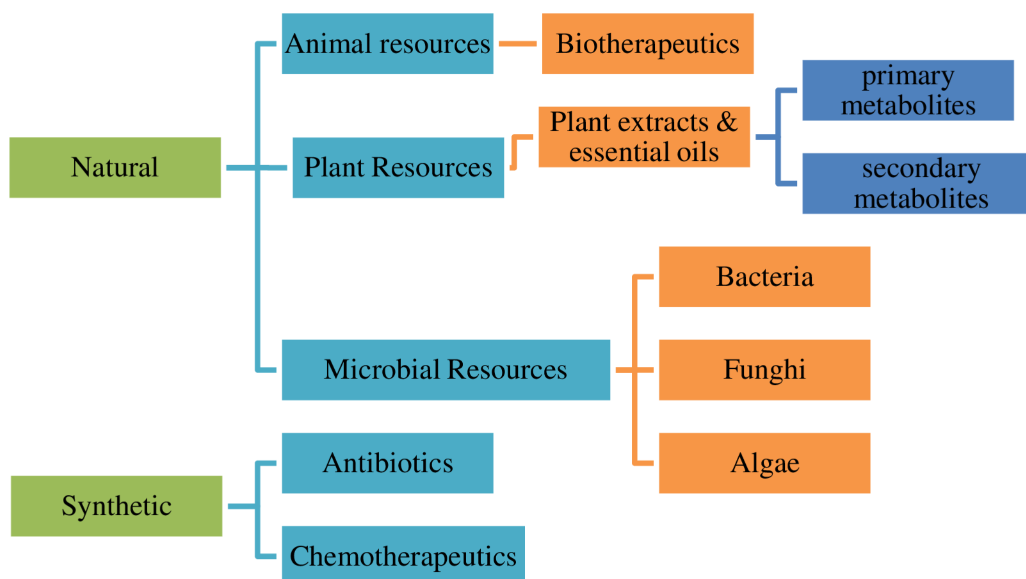

***Figure 1**: Natural and synthetic antimicrobial producents*

1.1 Antimicrobials of Animal Origin

Natural defense systems are reported to exist in animal products, such as milk and eggs, that exhibit strong antimicrobial properties due to well-characterized compounds, such as lactoferrin, lactoperoxidase, and lysozyme. Several polypeptides originating from various animal sources, such as chitosan, megainin, pleurocidin, curvacin A, and spheniscin are reported to exhibit antimicrobial activity. Many of these polypeptides have been studied for their potential application as food preservatives. In this section, some of the most well-characterized antimicrobials of animal origin are described.

1.2 Antimicrobials of Plant Origin

Traditionally, medicinal plants are an important source of compounds with therapeutic potential, and still represent a significant pool for the discovery of new drugs. The medicinal properties of plants are caused mainly due to the presence of active secondary metabolites. Although medicinal plants continue to be an important source of new therapeutic leads, its scientific validation remains inadequate due to the lack of adequate plant material, choice and execution of suitable high-throughput screening bioassays, availability of bioactive compounds in large quantities, and finally their regulatory approval and clinical trials. Although the inherent complication of natural product-based pharmaceutical invention demands integrative research and protocol; the systemic study, latest scientific approaches, and regulatory approvals, but scientific developments unmistakably showed that plant-based products will be the significant upcoming resources of novel therapeutic compounds. [2].

1.2.1 Major components of natural substances

Plants are sunlight-based biochemical manufacturing units, which produce a large collection of bioactive compounds that are collected and extracted. Plant-based molecules are classified as primary or secondary compounds. Primary compounds are extensively distributed in the environment and are required for normal growth and development of plants. In contrast, secondary compounds are biosynthetic derivative of primary compounds but are present in restricted numbers and small quantities among plants. They regularly take part in ecologically important role in the interactions of plants with their environment and help in the continued existence of plants [3].

The important secondary metabolites found in higher plants include alkaloids, flavonoids, phenols, glycosides, saponins, tannins, volatile oils, gums, and resins. Plants-derived molecules are known to have beneficial activities. Some might slow down microbial growth, block metabolic pathways, or alter gene expression and signal transduction (Manson, 2003; Surh, 2003; KrisEtherton et al., 2002). Phyto-compounds can also act as chemotherapeutics or chemopreventives (compounds that slow down, annul, or delay tumorigenesis), and can be used for cancer treatment (Sarkar and Li, 2006; D'Incalci et al., 2005). Many herbal extracts and essential oils revealed diverse antibacterial activity, like the interaction with phospholipids bilayer of the cell membrane resulting in enhanced cell permeability and cell damage, loss of the enzymes associated with cellular respiration and manufacturing of different cellular constituents along with the damage to genetic components of the cell [4,5,6,7,8].

1.2.2 Sellected plant extacts used in this thesis

In presented thesis several plants from different origin were used to evaluate its antimicrobial effect. Plant selection was based on previous results.

1.2.2.1 Acai

The açaí berry is a small round berry (size of a grape) that is green when immature and ripens to a dark purple colour. Açaí pulp (*Euterpe oleracea*) has received much attention in recent years as one of the new 'superfruits'. As a food, açaí berry is consumed raw and as juice. The juice is used commercially in jelly, syrup, ice cream, liquors, energy drinks and a variety of other beverages, and can be used as a natural food colorant. Açaí juice is viscous and contains about 2.4 % protein and 5.9 % lipids. The fruit pulp contains about 4 % protein and 12 % lipids. Nutrients include vitamins A, C, and E, calcium, phosphorus, iron, thiamine, polyphenols, and anthocyanins. The consumption of açaí from the Amazon region has been increasing, mainly owing to the benefits reported in the scientific literature. Açaí fruit possesses two predominant anthocyanins, cyanidin-3-glucoside (C3G) and cyanidin-3-rutinoside (C3R). The *Euterpe* genus has high economic potential, since it is used to prepare açaí beverages which are exported all over the world as energy drinks [9].

1.2.2.2 Clove

Clove is derived from flower buds of the *Eugenia caryophyllata* tree. Several bioactive components are found in clove, including tannins, terpenoids, eugenol, and acetyleugenol. While no studies have been conducted in humans to date to evaluate use of cloves in cancer prevention, a few studies conducted in mice suggest its effectiveness, especially in modifying

cellular detoxification processes. Antimicrobial activities of clove water extract were studied in vitro and in vivo against pathogenic microorganisms (*S. aureus* and *E. coli*, in a model of pyelonephritis) [9]. An in vitro study was conducted with the agar well diffusion method, and the results suggested that clove water extract showed antibacterial activity against S. aureus (minimum inhibitory concentration (MIC: 2 mg/mL) and E. coli (MIC: 2.5 mg/mL). While in vivo, the study was conducted in 40 adult male albino rats, and the results confirmed the efficacy of clove extract as natural antimicrobials. The direct antimicrobial activities of ultra-fine powders of ball-milled cinnamon and clove were tested by Kuang et al. [11,12,13].

1.2.2.3 Curcuma

C. longa is a common spice in the United States and Asian countries which is known to be useful for health. Turmeric has been used to treat coughs, jaundice and other common diseases in Indian Ayurvedic and traditional Chinese medicines. Curcumin, as a member of curcuminoids, is a natural nonflavonoid phenol. The main components of the *Curcuma longa* extract are curcumin (2269.2 ± 12.3 mg/100 g), bisdemethoxycurcumin (1283.5 ± 8.5 mg/100 g), and demethoxycurcumin (1284.6 ± 7.0 mg/100 g), as identified using high-performance liquid chromatography (HPLC) analysis [14].

Turmeric rhizome contains two major classes of secondary metabolites: phenolic curcuminoids and essential oil. These metabolites are largely responsible for the pharmacological effects of turmeric. The composition of both metabolites depends on a genotype, the environment, harvest season, dry process, and storage conditions. Curcuminoids are responsible for the yellow color of the turmeric, and the essential oil that it contains for its aroma and taste. The major and the most studied curcuminoid found in turmeric is curcumin, which is recognized as the most responsible compound for most beneficial effects which this miraculous plant exhibits [15].

Turmeric is used as a dietary spice, coloring agent in foods and textiles, and a treatment for a wide variety of ailments. It is widely used in traditional Indian medicine to cure biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, rheumatism, and sinusitis. There is also significant effect in health benefits with psoriasis, cardiovascular disease, chemopreventive, liver injury, lung fibrosis and etc.

1.2.2.4 Ginger

Ginger was proved to possess antimicrobial activities in several studies. Singh et al. determined the antifungal activities of EO and oleoresin of ginger against *Aspergillus terrus*, *A. niger*, *Aspergillus flavus*, *Trichothecium roseum*, *Fusarium graminearum*, *Fusarium oxysporum* and *Curvularia palliscens*, by food poison and inverted petri-plate technique. The results showed that the EO 100% inhibited *F. oxysporum*, while the oleoresin 100% inhibited *A. niger*. [16] Park et al. compared the ethanol and n-hexane extracts of ginger and five ginger constituents against three anaerobic Gram-negative bacteria, *Porphyromonas, Porphyromonas endodontalis* and *Prevotella intermedia*. The results indicated that ginger extracts exhibited antibacterial activities against three tested bacteria. Two highly alkylated gingerols showed significant inhibition against the growth of these oral pathogens with the MICs ranging from 6 to 30 μg/mL, and also killed the oral pathogens at a MBC range of 4–20 μg/mL. Sa-Nguanpuag et

al. evaluated the in vitro and in vivo antimicrobial activities of ginger oils which were obtained by hydrodistillation and solvent extraction method [17] The results showed that the oils extracted by both methods possessed antimicrobial activities against *B. subtilis*, *Bacillus nutto*, *P. aerugenosa*, *Rhodoturola sp*., *Samonella newport*, *S. enteritidis*, and *Fu*sarium sp.; except *E. coli*, *Campylobactor coli*, and *Campylobactor jejuni* (C. jejuni) *in vitro*. In the case of shredded green papaya, when the package was added with 5 and 10 μL ginger oils the growth of microorganisms was inhibited well, while with 15 μL ginger oil a reduction in growth rate was observed [19]

1.2.2.5 Ginseng

Ginseng is a medicinal plant widely used for the treatment of various conditions. The pharmacological effects of ginseng have been demonstrated in cancer, diabetes, cardiovascular diseases and have been used for promoting immune function, central nervous system (CNS) function, relieving stress, and for its antioxidant activities (Jung and Jin 1996). Korean ginseng contains various functional constituents in addition to saponin, such as phenolic compounds with antioxidant activity, polyacetylene, which exhibits cytotoxicity to cancer cells, sesquiterpene, an essential oil, and acidic polysaccharides, which are currently studied actively in relation to immune response [18,19].

1.2.2.6 Oregano

Oregano belonging to family *Lamiaceae*, has been used as food seasoning and flavouring for a long time. Origanum species are a wide group of plants that are rich in several compounds, such as monoterpene hydrocarbons, oxygenated monoterpenes, phenolic monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and phenolics. Origanum presents a broad variety of properties and uses, such as antifungal, insecticidal, antibacterial, antiparasitic, and antioxidant activities. Babacan et al. [20] evaluated the antimicrobial activities of oregano extract against various Salmonella serotypes by evaluating the bacterial growth with disc diffusion method. The results showed that DIZs of oregano were 15, 19, and 16 mm for *Salmonella gallinarum* (*S. gallinarum*), *Salmonella enteritidis* (*S. enteritidis*), and *S. typhimurium*, respectively. Santoyo et al. [20] observed the antimicrobial activities of EO-rich fractions of oregano which were selectively precipitated in the second separator in different conditions against six microorganism strains (*S. aureus, Bacillus subtilis, E. coli, P. aeruginosa, C. albicans, and Aspergillus niger*) using the disk diffusion and broth dilution methods. [21,23,22,23].

1.3 ANTIMICROBIALS OF MICROBIAL ORIGIN

Plant metabolites and bioactive compounds from chemical synthesis have found their efficacy to be dwindling, despite some of them being developed as drugs and used to treat human infections for several decades. Microorganisms are considered untapped reservoirs for promising biomolecules with varying structural and functional antimicrobial activity.

1.3.1 Antibiotics

Worldwide consumption of antibiotics has increased drastically in the past few decades. The application of antibiotics has led to the production of antibiotic resistance genes (ARGs) which represent a growing and serious human health threat worldwide.

The discovery of first antibiotic occurred by chance when a staphylococci agar plate inoculated by Sir Alexander Fleming got contaminated by a mold. The mold colony displayed a clear zone of bacterial inhibition around itself (Fleming 1980). The compound which was suppressing bacterial growth was identified as penicillin, which was then used as antibiotics to cure many infections and diseases caused by bacteria. Discovery of sulfonamides and β-lactam antibiotics in 1930s leads to an immense improvement in health and medicine services as diseases and bacterial infections which were fatal earlier became curable. Introduction of streptomycin in 1944 was another achievement for the cure of tuberculosis. The golden era for antibiotic discovery was marked till 1970, up to where maximum classes of antibiotics were discovered [24].

1.3.2 Antimicrobial peptides

Many microbial metabolic products act as growth inhibitors against other microorganisms, including spoilage and pathogenic bacteria. Many gram-positive bacteria often produce cationic, amphiphilic, membrane-permeabilizing peptides that are smaller in size and have antimicrobial activity against a wide range of microorganisms. For example, *Lactobacillus spp.* produce one of the groups of antimicrobial peptides known as bacteriocins that can exhibit potent antimicrobial activity. The antimicrobial activities of bacteriocins are due to a heterologous subgroup of ribosomally synthesized cationic peptides (de Vugst & Vandamme 1994a). Various classification schemes have been proposed for bacteriocins (Tiwari et al. 2009). For example, in one such classification, bacteriocins are named after the genus, species,or family of bacteria producing them, such as lantibiotics for the bacteriocins produced by *Lactobacillus spp.* and colicins for bacteriocins from *E. coli* (Riley & Chavan 2006) [30].

Nisin is a ribosomally synthesized polycyclic peptide that contains unusual amino acids, including lanthionine, methyllanthionine, didehydroalanine, and didehydroaminobutyric acid (Hansen 1994). Nisin is commercially produced by fermentation of modified milk medium using several strains of nisin-producing L. lactis and is an effective antimicrobial against many grampositive bacteria. The antibacterial activity of nisin is attributed to its interaction with phospholipids in the cytoplasmic membrane of bacterial cells resulting in disruption of normal membrane function [30].

1.3.3 Other antimicrobial substances of microbial origin

Organisms, such as algae, bryozoans, corals, molluscs, sponges, tunicates, and viruses, are considered potential sources of novel antimicrobials. Their external body structures could serve as an avenue for new bioactive compounds. Additionally, the internal enzymatic machinery of some of these microorganisms enables them to produce secondary metabolites with antimicrobial properties. For example, *Pseudovibrios* species, a marine bacterium of the order Rhodobacterales and class alphaproteobacteria, has bioactive structural composition coupled with harbored polyketide synthases, non-ribosomal peptide synthases, or hybrid enzyme systems that putatively aid them to produce secondary metabolites and new bioactive compounds with antimicrobial activity against varying clinical strains, notably *Staphylococcus aureus, Bacillus subtilis*, and *Escherichia coli* [25]. Psychrophiles, extremophilic organisms that tolerate very low temperatures, were also investigated as a source of new antimicrobials. Given the varying environmental conditions between psychrophiles and temperate regional

dwellers and their adaptive evolution, the bioactive compounds produced by the former might presumably differ from the latter, and that merits its consideration as an antimicrobial source. Tadesse et al. [26] identified synoxazolidinones A and B, oxazolidinone derivative antimicrobial isolates from the Norwegian sea squirt, which showed antibacterial activity against methicillin-resistant *Staphylococcus aureus*. Sanchez et al. also reported the bacteriocin properties of Serraticin A, a bioactive compound produced by *Serratia proteomaculans* and with antimicrobial activity against *Escherichia coli* and *Salmonella enterica*. [27] This compound is putatively considered to exhibit such activity by inhibiting DNA synthesis. [28] Similarly, Phelan et al [29] found subtilomycin, a lantibiotic from the marine sponge *Haliclona simulans*, known to exhibit polymyxin B activity (cell membrane inhibition or pore formation) against strains of *Bacillus cereus, Bacillus megaterium, Clostridium sporogenes, Listeria monocytogenes, Listeria innocua, Staphylococcus aureus, MRSA*, and vancomycin-resistant *Staphylococcus aureus* [30].

1.3.4 Oleaginous yeasts as a producers of microbial active compounds

It has been well documented that yeasts have many applications in fermentation, food, feed, agricultural, biofuel, medical, and chemical industries, as well as environmental protection (Adedayo et al., 2011; Kurtzman and Fell, 2000). They have been used for production of fermented food since 7000 BC (Reed and Nagoda-withana, 1988). Yeast biomass from so-called nutritional yeasts is widely used as a source of nutritional components, such as single-cell protein (SCP). Dried and killed *Y. lipolytica* protein - rich biomass is recognized as safe for human and animal nutrition in accordance with the current food and nutrition safety law. It is worth emphasizing that *Y. lipolytica* is is not causing any allergic reactions to humans. It's a valuable source of bioactive compounds such as proteins, trace minerals, vitamins, and other valuable compounds such as yeast biomass itself [31]. The natural occurrence of *Y. lipolytica* in food, particularly in cheese, other dairy products, and meat, is an additional argument in favor of its safety. The occasional occurrence of opportunistic infections of *Y. lipolytica* in immunocompromised and catheterized patients does not differ from other microorganisms with a history of safe use, such as *S. cerevisiae* [32]. *Rhodotorula* produces pink to red colonies and blastoconidia that are unicellular, lacking pseudohyphae and hyphae. Several authors have isolated *Rhodotorula* in different ecosystems and environments. Previously considered non-pathogenic, *Rhodotorula* species have emerged as opportunistic pathogens with the ability to colonise and infect susceptible patients. Recent studies have demonstrated that the incidence of fungemia caused by *Rhodotorula* was between 0.5% and 2.3% in the USA and Europe. Most cases of infection with *Rhodotorula fungemia* are associated with central catheters in patients with haematologic malignancies [33].

In general yeast biomass contains valuable fatty acids, carbohydrates, nucleic acids, vitamins, and minerals. It is rich in certain essential amino acids, such as lysine and methionine, which are limited in most plant and animal foods. Some of them, so-called "red" yeasts, can produce carotenoids pigments. *Phaffia rhodozyma*, another red yeast, produces in large scale the carotenoid pigment astaxanthin, but this yeast has also the ability to produce torulene and torularhodin in its metabolic pathway. Torularhodin and torulene are two widespread microbial carotenoids. Torularhodin shows a considerable antioxidant activity that helps the

stabilization of membranes under stress conditions. These carotenoids are beneficial because they are precursors of vitamin A and hormones, and they have antiaging and antioxidant capacity. They may also prevent certain types of cancer and enhance the immune system. These possibilities make torularhodin and torulene a hot research topic in carotenoid biotechnology [33].

Some microorganisms are defined oleaginous as a result of their ability to accumulate lipids by as much as 20% of their dry cellular weight. This group includes several eukaryotic microorganisms (such as fungi, yeasts and algae) and some species of autotrophic and heterotrophic bacteria able to accumulate lipids in the form of triglycerides (TAGs) and free fatty acids (FA).

As lipids constitute a major portion of the majority membranes suggest that the presence of massive concentrations of unsaturated FA within membranous structures. In addition, well recognized that the PUFA are bioactive mediators of diverse pathways involved in cellular homeostasis or, in some cases, interact with cellular macromolecules resulting in cell death. These cellular responses may be a consequence of the vulnerability of unsaturated FA to diverse oxidation reactions, or radical reactions, or both. Reactive oxygen species readily bind to unsaturated FA in lipids that contain multiple double bonds (DB), "steal" electrons, and trigger a free radical chain reaction. This oxidative process usually consists of initiation (production of a FA radical), propagation (creation of a peroxyl-FA radical), and termination (production of electrophilic carbonyls. The free radical mediated production of electrophilic products of PUFA proceeds by autocatalysis and is, as a result, not well regulated. SFA are resistant to oxidation. The cellular membranes and lipoproteins containing large amounts of SFA are less active functionally [34,35].

Glucans constitute the cell wall of fungi and yeast, and are the main polysaccharides present in mushrooms, in addition to water (90 %), proteins and amino acids, antioxidants and others. β-Glucans from yeast have similar structures to those from fungi. The biological activities of β-glucans and their applications in healthcare have been investigated for many years [35]. Within the β-glucan health-related effects, the most studied and characterized are the modulation of the immune system and metabolic and gastrointestinal effects [36]. carefully reviewed the information on structure–function relationship of β-glucans in relation to cellular immune modulation and antitumor activities: molecular weight, degree of branching, length of the sidechains, conformation of sugar residues, and consequent tridimensional structure, degree of solubility, and particulate size appear to be the most important features of B -glucans immune recognition, although some literature data are often inconsistent or contradictory [36].

2 AIM OF THESIS

The aim of presented thesis was to prepare and characterize natural extracts with antioxidant and antimicrobial effect.

The thesis is focused on following partial goals:

1. Literary review-overview of medicinal plants and screening of yeast strains with high antimicrobial activity, antioxidant activity and important nutritional benefits.
2. Optimization of methods of characterization and composition of plant extracts
3. Production and characterization of microbial extracts from selected yeast strains.
4. Analysis of active substances, antioxidant and antimicrobial effect of plant extracts, microbial extracts and their mixtures.
5. Testing biological effects and safety of natural extracts by using cytotoxicity and apoptosis tests on human cell lines.
6. Discussion of results and possible applications.

3 MATERIALS AND METHODS

3.1 MATERIALS

Acai, Iswari, CZE
Clove, Sonnentor, CZE
Curcuma, Sonnentor, CZE
Ginger, awashop. CZE
Ginseng, ebio24, CZE
Oregano, lumigreen, CZE

3.2 MICROORGANISMS AND CELL LINES

B16F1- CRL-632 - Murine melanoma cell line
Caco-2 – CRL-2102- Human adenocarcinoma cell line
HaCaT - Human keratinocytes cell line
Raji - CCL-86- Human lymphoblast cell line
Micrococus Luteus, CCM 1569
Candida Rugosa
Cystofilobasidium infirmominiatum CCY 17-18-4
Escherichia Coli CCM 3954
Metschnikowia pulcherrima CCY 029-002-145
Phaffia rhodozyma CCY 77-1
Rhodotorula kratochvilovae CCY 20-2-26
Saccharomyces cerevisiae CCY 6646
Serratia Marcescens, CCM 8587
Stafylococus Aureus CCM 299
Stafylococus dermatitis CCM 4418

3.3 EXTRACTION AND DETERMINATION OF PLANT ACTIVE COMPOUNDS

Based on the bioactive properties of certain compounds, such as antioxidant and anti-inflammatory activities, an interesting subject of research are natural substances present in various parts of plants. The choice of the most appropriate method for separation and quantification of biologically active compounds from plants and natural products is a crucial step of any analytical procedure.

Water extracts were prepared by maceration on shakers [37], essential oils were prepared by soxhlet extraction [37]. Determination of total phenol content was performed spectrophotometrically using the Folin-Ciocaltau reagent [38]. Determination of flavonoides measured spectrophotometrically at 510 nm and catechin is used as a standard [38]. Total antioxidant activity (TAA), was measured using TEAC assay that is based on the reaction with ABTS which is a peroxidase substrate that reacts with peroxyl radicals or other oxidants and forms in the presence of H_2O_2 the metastable radical cation ABTS $^{\cdot+}$[39]. Antimicrobial activity was evaluated by Dilution and diffusion test [40]. Cytotoxicity was measured by MTT assay and is calculated based on the formazan signal generated which has been shown to have good linearity up to 10^6 cells per well and is dependent on the MTT concentration, the incubation time, and metabolically active viable cells [41]. Apoptosis was measured by Exbio kit based on

Annexin V staining, Annexin V binds to phosphatidylserine allowing apoptotic cells to be easily identified by flow cytometry or fluorescence microscopy.

3.4 DETERMINATION OF NATURAL ACTIVE COMPOUNDS

Natural active compounds in biomass, such as carotenoids, fatty acides, glucans, antioxidants etc. were measured.

3.4.1 Determination of carotenoids by HPLC

Into plastic extraction tubes 15 ± 5 mg of freeze-dried biomass was weight and rehydrated by addition of 1 mL distilled water. The water was removed by centrifugation and to the pellet was added 250 ± 50 mg acid-washed glass beads (250 – 500 μm diameter, Roth, Germany) and 1 mL of methanol. To obtain biomass with ruptured cell wall for analysis, it was necessary to vortex for 10 minutes. The content of the PP tube was transferred into a glass reaction tube by washing it with a 2,000 μl of chloroform and the glass tube was vortexed for 10 min. Then, 1 mL of distilled water was added for the phase separation. After centrifugation (3,000 rpm/5 min/4 °C), the separated bottom chloroform phase with extracted pigments was evaporated under nitrogen at 25 °C followed by the addition of 1 mL of mixture ethylacetate:acetonitrile (20:60). The ethylacetate:acetonitrile mixture containing extracted pigments were filtered through syringe filter (0.45 μm, PTFE membrane, 13 mm) and transferred into glass vials for further HPLC analysis.

The conditions of HPLC separation are presented below. Contents of individual pigments were calculated according to calibration standards (beta-carotene, astaxanthin, lycopene, lutein, violaxanthin, neoxanthin, chlorophyll a, chlorophyll b, ergosterol) from regress equation. Composition of mobile phase A is: acetonitrile 840 mL, methanol 20 mL, 0.1M Tris-HCl (pH=8) 140 mL), and mobile phase B: methanol 680 mL, ethylacetate 320 mL.

Table 1: *Conditions for HPLC analysis of carotenoids*

Volume of sample	20 μL
Column	Kinetex, EVO 150 x 4.6 mm, 2.6 μm
Elution	Gradient 0-13 min: from 100% A to 100% B linearly 13-19 min: 100% B 19-20 min: from 100% B to 100% A linearly 20-25 min: 100% A
PDA	285, 435, 450 and 680 nm
Temperature	25 °C
Time of analysis	25 min

3.4.2 Lipid content and fatty acids profile by Gas chromatography

20 mg of biomass was weighed into crimp vials. Subsequently, 1.8 mL of a transesterification mixture containing 0.5 mg/mL of dissolved C17:0 internal standard in 15 % sulfuric acid in methanol was added. The vials were heated to 85 ° C for 2 hours. After cooling, the entire content of the vial was transferred to a 4 mL vial with 0.5 mL of 0.05 M NaOH. 1 mL of HPLC hexane was pipetted into the sample. The resulting mixture was then vortexed vigorously for 5 minutes. After phase separation, 100 μl of the upper hexane phase was collected in a glass vial

for HPLC / GC with pre-pipetted 900 μl of HPLC hexane. Samples with fatty acid methyl esters were then analyzed by a gas chromatograph Thermo Scientific TRACETM Gas Chromatograph with a Thermo Scientific A1 1310 autosampler, Zebron ZB-FAME column (30 m x 0.25 mm x 0.25 μm) and a flame ionization detector (FID) was used.

Table 2: *Mobile phase and temperature program for GC analysis*

Separation parameters	Hydrogen flow	1 mL/min
	sample injection volume	1 μl
	Injector temperature	250 °C with flow divider ratio 10
	Detector temperature	260 °C (air flow 350 mL/min, hydrogen flow 25 mL/min, nitrogen flow 30 mL/min)
Temperature program	80 °C 1 minute	
	140 ° C with a temperature gradient of 15 ° C per minute (t_R = 5 minutes)	
	190 ° C with a temperature gradient of 3 ° C per minute (t_R = 21.7 minutes)	
	260 ° C with a temperature gradient of 25 ° C / min (t_R = 24.5 minutes)	
	Maintaining the temperature at 260 ° C for 1 minute (t_R= 25.5minutes)	

3.4.3 Isolation and determination of β-glucans from yeasts

Dried biomass was weight (25 mg) to a glass centrifuge tube and wetted with 0.2 mL of ethanol (50 % v/v). 4 mL of sodium phosphate buffer (20 mM, pH 6.5) were added, and the content was stirred on vortex mixer. The tube was placed into a boiling water bath and incubated for 3 min. Next, the temperature was decreased to 50 °C and the sample was hydrolysed one hour by lichenase (0.2 mL, 10 U, 50 °C). 5 mL of sodium acetate buffer were added into tube (200 mM, pH 4.0) and the sample was left to equilibrate to room temperature (5 min) and then centrifuge (1,000 g, 10 min). Aliquots of the sample (0.1 mL) were dispensed into the bottom of three test tubes and βglucosidase (0.1 mL, 0.2 U) in 50 mM sodium acetate buffer (pH 4.0) was added to two of these tubes (the reaction). To the third (the reaction blank), 50 mM acetate buffer (0.1 mL, pH 4.0) was pipetted. The samples were incubated at 50 °C for 10 min. In the end, 3 mL of GOPOD Reagent was added to each tube and incubated at 50 °C for a further 20 min. The absorbance was measured at 510 nm against reagent blank. [42]

3.4.4 Cultivation of microorganisms for antimicrobial tests

Plant extracts were prepared by serial one to two dilutions in Luria broth (LB) liquid medium (in the case of fungal strains in, RPMI-1640) with 0.5% Tween 80 at V / V concentration from 10 % to 5 %. The assay was performed in 96-well plates in a final volume of 200 μl per well: 100 μl of each plant extract dilution and 100 μl of microbial suspension at a concentration of 10^6 CFU/mL, were added to each well and then incubated at 37 °C for 24 hours (24/48 for fungal strains).

To determine the MBC value, 10 μL were seeded on solid media, the plates were incubated for 24-48 h at the temperature of 37°C. MBC was considered as the lowest concentration inhibiting bacterial growth. Each strain was tested in duplicate and a positive growth control (the test strain without PE) and a negative one (medium only) is included in each test. The plates were incubated at 37° C for 24/48 h and monitored to detect microbial growth

Different option to determinate MBC is to was perform only in 96-well plates in a final volume of 200 μl per well: 100 μl of each plant/microbial extract dilution and 100 μl of microbial suspension at a concentration of 10^6 CFU/mL, added to each well and then incubated at 37 °C for 24 hours (24/48 for fungal strains). Then read the absorbance at 540 nm of blank (only media), cell control (200 μl of microbial extract) and samples. The decrease in absorbance compared to control samples is equivalent to growth inhibition of a microorganisms

Microorganisms used for the tests: *Candida Rugosa, Stafylococus Aureus, Stafylococus dermatitis, Escherichia coli, Serratia Marcescence, Bacteriococus Luteus.*

3.4.5 Cultivation of yeasts strains

Yeast strains of *Metschnikowia pulcherrima* CCY 029-002-145, *Cystofilobasidium infirmominiatum* CCY 17-18-4, *Phaffia rhodozyma* CCY 77-1, *Rhodotorula kratochvilovae* CCY 20-2-26, and *Saccharomyces cerevisiae* CCY 6646 yeast strains were obtained from Culture Collection of Yeast (Institute of Chemistry, Slovak Academy of Science, Bratislava, Slovakia). First, the yeast from frozen stock were cultured on YPD agar plates (composition see Table 4) for 72 hours at laboratory temperature. Next, the inoculum was prepared by transfering of one biological loop from agar plate into 100 mL of sterile YPD medium (yeast extract, 10 $g{\cdot}l^{-1}$; peptone, 20 $g{\cdot}l^{-1}$, glucose, 20 $g{\cdot}l^{-1}$) in Erlenmeyer flask (250 mL) and cultured under constant shaking regime (120 rpm) for 24 hours. The yeast were then inoculated directly into the production medium in a ratio of 1:5 (inoculum: production medium) and cultivated for 96h at laboratory temperature. Production medium was composed of glucose 69,29 $g{\cdot}l^{-1}$; KNO_3 1,52 $g{\cdot}l^{-1}$, KH_2PO_4 4 $g{\cdot}l^{-1}$, $MgSO_4 \cdot 7H_2O$ 0,7 $g{\cdot}l^{-1}$.

3.5 CELL CULTURES CULTIVATION AND TESTS

B16F1 is a mouse melanocyte cell line. These are skin cancer cells that produce melanin. Melanin is a substance responsible for skin color and protects against the effects of solar UV radiation.

HaCaT a human keratinocyte cell line that has been immortalized. The advantage of this line is easier culture and slower cell aging compared to normal keratinocytes. The HaCaT cell line is used to test skin sensitivity and cytotoxicity.

Caco-2 are epithelial cells isolated from colon tissue derived from a 72-year-old, White, male with colorectal adenocarcinoma. This cell line is a suitable transfection host and has applications in cancer and toxicology research.

Raji cell line of lymphoblast-like cells was established by R.J.V. Pulvertaft in 1963 from a Burkitt's lymphoma of the left maxilla of an 11-year-old black male. This cell line can be used in immunology research.

3.5.1 Cultivation of Cell lines

B16F1, *HaCaT* and *CaCO-2* cell lines were cultivated in DMEM, high glucose medium with 1 % of antibiotics and 10 % of FBS (Fetal Bovine serum), at 37 °C and 5 % of CO_2 atmosphere. *Raji* Cell lines were cultivated in RPMI medium with 1 % of antibiotics and 10 % of FBS (Fetal Bovine serum), at 37 °C and 5 % of CO_2 atmosphere.

3.5.2 MTT Test procedure:

1 DAY: seed $1*10^4$ cell/100µL/ 1 well in 96 well plate, incubate for 24 h

2 DAY: add 20 µL of extract, filtrated and mixed with medium for specific concentration

3 DAY: add 20 µL of MTT (concentration 2.5 mg/ mL PBS)

→ After 3 hours add stop solution which is 10 % SDS in PBS (10g/100mL PBS), 100µL in each, leave it in dark, at room temperature for 20 hours.

4 DAY: Elisa reader 540 nm

3.5.3 Apoptosis with B16F1 cell lines

Murine melanoma cell lines B16F1 were seeded ($5\cdot10^4$ cell/500µL/ 1 well in 6 well plate), incubate for 24 h. Samples were prepared, mixed with media and placed into a wells for 24 hours (to have comparable results according to the cytotoxicity test procedure). Then the samples were proceeded according to the instructions in ExBio kit [43].

3.5.3.1 Reagents

- Annexin V-FITC in stabilizing phosphate buffered saline (PBS) solution containing 15mM sodium azide.
- Propidium Iodide solution in deionized water (0.1 mg/mL).
- Annexin V Binding Buffer filter-sterilized solution (0.1M HEPES/NaOH, pH 7.4, 1.4M NaCl, 25mM $CaCl_2$).

3.5.3.2 Procedure

- Harvest cells intended for analysis by centrifugation (different cells may need different centrifugation conditions), discard supernatant. Resuspend cell pellet in cold PBS and wash cells by gentle shaking or by up and down mixing with a pipette tip. Recentrifuge washed cells again and discard supernatant.
- Resuspend cell pellet in Annexin V Binding Buffer and adjust cell density to $2 – 5x\ 10^5$ cells/ mL, preparing a sufficient volume of cell suspension
- Add 5 µl of Annexin V - FITC and 5 µl of Propidium Iodide to each 100 µl of cell suspension and mix gently.
- Incubate for 15 minutes at room temperature in the dark.
- Centrifuge cells and resuspend pellet in 100 µl of 1x Annexin V Binding Buffer (or in an appropriate volume according to a method of sample acquisition)
- Analyse the stained cells by flow cytometry as soon as possible.

3.5.4 Apoptosis with Raji cell lines

Raji cell were seeded ($5 \cdot 10^4$ cell/500μL/ 1 well in 6 well plate), incubated for 24 hours. Next day samples were prepared, mixed with media for exact concentrations and placed into a wells for 24 hours (to have comparable results according to the cytotoxicity test procedure). Then the samples were proceeded according to the instructions below.

- Cultures, maintained as cell suspension, were harvested by centrifugation, and fixed with 70 % ethanol for 30 min on ice.
- After fixation samples were placed at -20°C until cytofluorometric analysis.
- For cytofluorometric analysis, the cells were harvested by centrifugation and cell pellet resuspended in sodium citrate buffer.
- Fluorochromization was performed by using **Propidium Iodide** and **Fluorescein isothiocyanate** (**FITC**).
- Propidium iodide was used alone for DNA staining or simultaneously with **Fluorescein isothiocyanate** (**FITC**) for the analysis of the DNA/Protein ratio; FITC is reactive towards nucleophiles groups including amine and sulfhydryl groups on proteins.

3.5.4.1 DNA - Propidium Iodide Staining

The cell pellet was gently resuspended in about 5 mL of a solution of propidium iodide (50 μg/mL) containing 4 mM sodium citrate (pH 7.8), 30 units/ mL of (DNAse-free) RNAse, and 0.1% TX-100. After an incubation period of 30 min at 37°C, NaCl was added to a final concentration of 0.15-0.25 M. Flow cytometry fluorescence of individual nuclei was measured on a Flow Cytometer CyFlow Space (Partec), equipped with an argon ion laser tuned to 488 nm at 500 mW, was used to excite the DNA-dye complex.

3.5.4.2 DNA - Propidium Iodide /Protein - FITC - Staining

For bi-parametric analysis (simultaneous labelling with Propidium Iodide and Fluorescein isothiocyanate), after staining with Propidium Iodide, the cells, after centrifugation, were incubated in a FITC solution (20 μg/mL) for 30 min at 37°C.

Fluorescence emissions, at 590 nm (Propidium Iodide) and at 520 nm (FITC), were collected, amplified, and displayed.

4 RESULTS

Presented work is focused on preparation of selected natural extract, namely plant and microbial extract. Both of the extract types were prepared as water-based extracts and organic oil extracts. Plant organic extracts were prepared by Soxtherm. Microbial water extracts were prepared by rehydration of biomass and oil extracts were prepared by Folch extraction. For evaluation of the extracts was necessary to test the set of metabolic parameters and biologically active substances (such as total phenolic content and antioxidant activity) and then choose the most suitable and representative samples for the tests.

4.1 PLANT EXTRACTS CHARACTERIZATION

Firstly, it was important to measure total phenol content, flavonoids and antioxidant activity of selected plant extracts to see potential of health benefits. From water extracts Curcuma and Clove has the highest amount of phenolic compounds. Curcuma and Ginseng has the highest antioxidant activity compared to Trolox equivalent.

4.1.1 Characterization of plant extracts

Natural plant extracts were characterized for antioxidant activity, total phenolic and flavonoids content.

Table 3: *Natural plant extracts characterization*

Extracts	Plants	Polyphenols [mg/g]	Flavonoides [mg/g]	Antioxidant activity [mg/g]
Water	Acai	1.943 ± 0.08	1.082 ± 0.02	1.041 ± 0.04
	Clove	2.724 ± 0.07	1.705 ± 0.03	0.801 ± 0.02
	Curcuma	4.723 ± 0.07	3.656 ± 0.01	5.260 ± 0.06
	Ginger	0.959 ± 0.13	0.600 ± 0.01	1.135 ± 0.01
	Ginseng	1.906 ± 0.02	0.306 ± 0.01	2.260 ± 0.02
	Oregano	0.887 ± 0.09	0.778 ± 0.02	0.760 ± 0.01
Oil	Acai	1.418 ± 0.02	0.822 ± 0.03	0.987 ± 0.04
	Clove	2.181 ± 0.08	1.205 ± 0.03	0.652 ± 0.02
	Curcuma	2.176 ± 0.09	1.589 ± 0.02	3.258 ± 0.04
	Ginger	0.781 ± 0.01	0.560 ± 0.01	1.112 ± 0.01
	Ginseng	0.623 ± 0.02	0.215 ± 0.01	1.152 ± 0.02
	Oregano	0.533 ± 0.03	0.258 ± 0.02	0.650 ± 0.01

The antioxidant effect of the tested natural water extracts. as well as the total contents of phenolic substances and flavonoids are given in the table 3. The highest content of phenolic substances was determined in turmeric extract (4.723 mg/g). while the lowest content was detected in oregano (0.887 mg /g). Turmeric also showed the highest antioxidant effect (5.26 mg /g). For the Oil extracts the best results were obtained from acai, clove and curcuma extracts for total phenol account, for acai 1.418 mg/g, for clove 2.181 mg/g and curcuma 2.176 mg/g. Antioxidant activity of ginseng oil 1.152 mg/g was comparable with ginger 1.112 mg/g and higher result was obtained from curcuma 3.258 mg/g.

4.1.2 Antibacterial activity. dilution method

Antimicrobial activity is tested using standard antimicrobial assays. Testing is performed on microorganisms in suitable culture media and under optimal conditions. Antimicrobial tests are divided into diffusion and dilution. Both types of tests were used in presented study. Thus, various methods for determining inhibitory measures have been tested properties including both visual detection of inhibition zones on solid agar plates and measurements changes in culture concentration during cultivation in liquid media. The selected samples were also monitored for antimicrobial efficacy by changing density during cell lysis. The antimicrobial efficacy of the test substances and extracts was monitored against the bacterial of gram-positive strains - *Micrococcus luteus* and gram-negative strains - *Serratia marcescens*.

Previously this method was performed in a series of test tubes. Today, microtiter plates are mainly used. Test cultures of microorganisms are inoculated with test substances. After 24h incubation the inhibition effect is measured. The evaluation can be performed visually, turbidimetrically, by measurement absorbance or by direct determination of the number of cells in the counting chambers or by flow cytometry. The antimicrobial activity of all tested plant extracts was tested using this method.

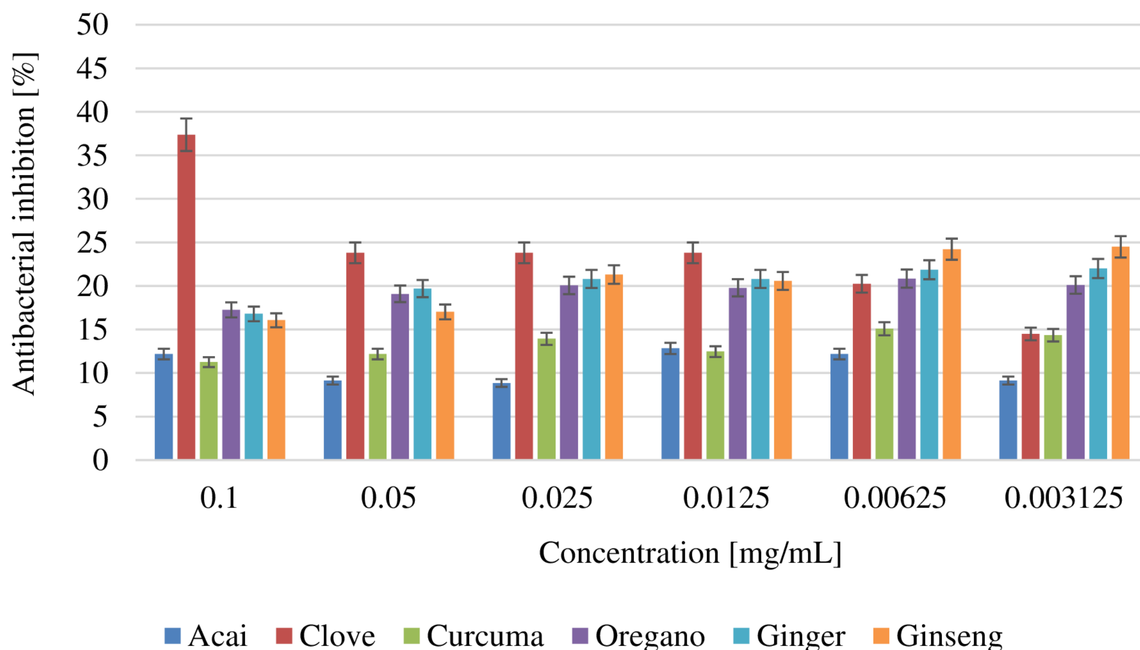

Figure 2: *Antibacterial inhibition of Serratia marcescens with water plant extracts [%]*

In monitoring the growth inhibition of *Serratia marcescence*, antibacterial activity of selected plant extracts was measured. Most of the samples showed similar results in all concentration range from 0.1 mg/mL to 0.003 mg/mL of dry materiál diluted in water. Clove extract showed highest antibacterial effect against gram-negative strains.

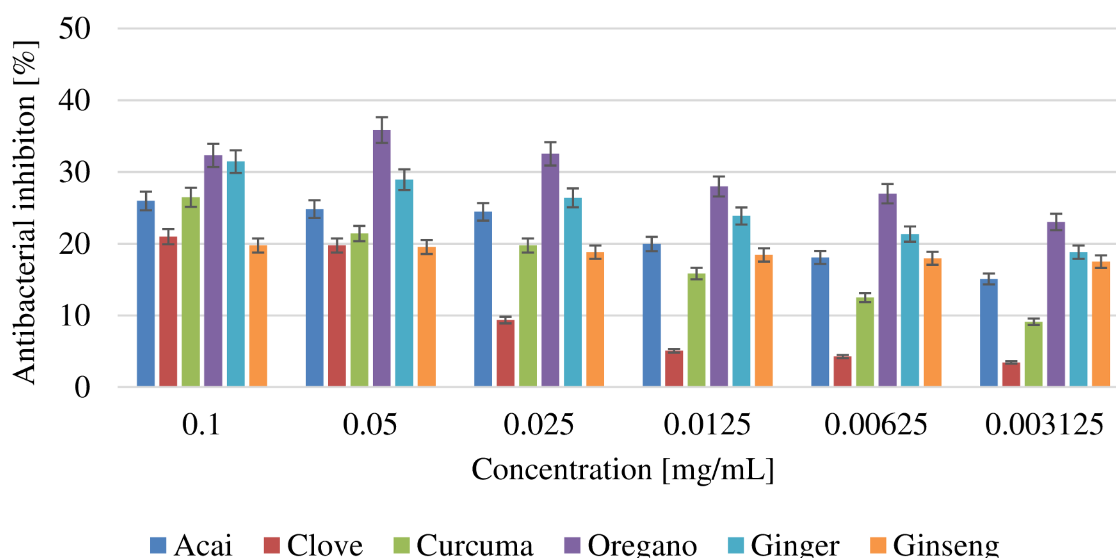

Figure 3: *Antibacterial inhibion of Micrococus luteus with water plant extracts [%]*

For gram- positive strains the most effective was extract from oregano. On the other hand, clove extract wasn't so effective and also showed concentration dependent effect, same as curcuma and ginger.

4.1.3 Antimicrobial activity. dilution method

For better clarity. the following microorganisms were compared with each other in a single table. The percentage efficacy of 2.5 % and 5 % of the extracts was monitored on 3 strains. *Candida glabrata*. *Staphylococcus aureus* and *E. coli*. There were significant differences in the individual extracts when measuring their antimicrobial activity. Water extracts were established 2.5 % and 5 % according to the following tests with oils. Oil extracts were diluted in DMSO and then mixed with media due to a solubility of oils in media.

Table 4: *Antimicrobial activity of selected plant extracts*

Microorganisms		***Candida glabrata***		***Staphylococcus aureus***		***Escherichia coli***			
Concentration [%]		5	2.5	5	2.5	5	2.5		**Inhibition**
Plant Extracts	**Acai**	-	-	-	-	-	-	**+**	**1 - 10 %**
	Clove	+++	+++	+++	++	+	++	**++**	**11 - 25 %**
	Curcuma	+	-	+	-	+	-	**+++**	**≥ 26 %**
	Ginger	++	+	-	-	-	-	**-**	**≤ 1 %**
	Ginseng	-	-	-	-	-	-		
	Oregano	++	-	++	+	+	-		

When measuring antimicrobial activity of selected 2.5 % a 5 % water plants extracts is significant that clove extract showed the highest potential of growth inhibition against microorganisms. On the other hand. ginseng extract was not effective at all.

4.1.4 Tests of plant extracts on the cell lines

The principle of the MTT assay is that for most viable cells mitochondrial activity is constant and thereby an increase or decrease in the number of viable cells is linearly related to mitochondrial activity. The mitochondrial activity of the cells is reflected by the conversion of the tetrazolium salt MTT into formazan crystals. which can be solubilised for homogenous measurement. Thus, any increase or decrease in viable cell number can be detected by measuring formazan concentration reflected in optical density (OD) using a plate reader at 540 nm. Concentration is calculated in percentage that correspond 1:1 to mg/g of dry sample.

B16F1, Raji, HaCaT and Caco-2 cells were treated with different plant extracts in concentration range between 4-28 mg/g for 24h. A considerable reduction in the cell viability was observed in a concentration dependent when compared with the control treated cells.

4.1.4.1 Keratinocytes HaCaT

Immortalized human keratinocytes cell line **HaCaT** from skin has been used as a model for the study of keratinocytes functions.

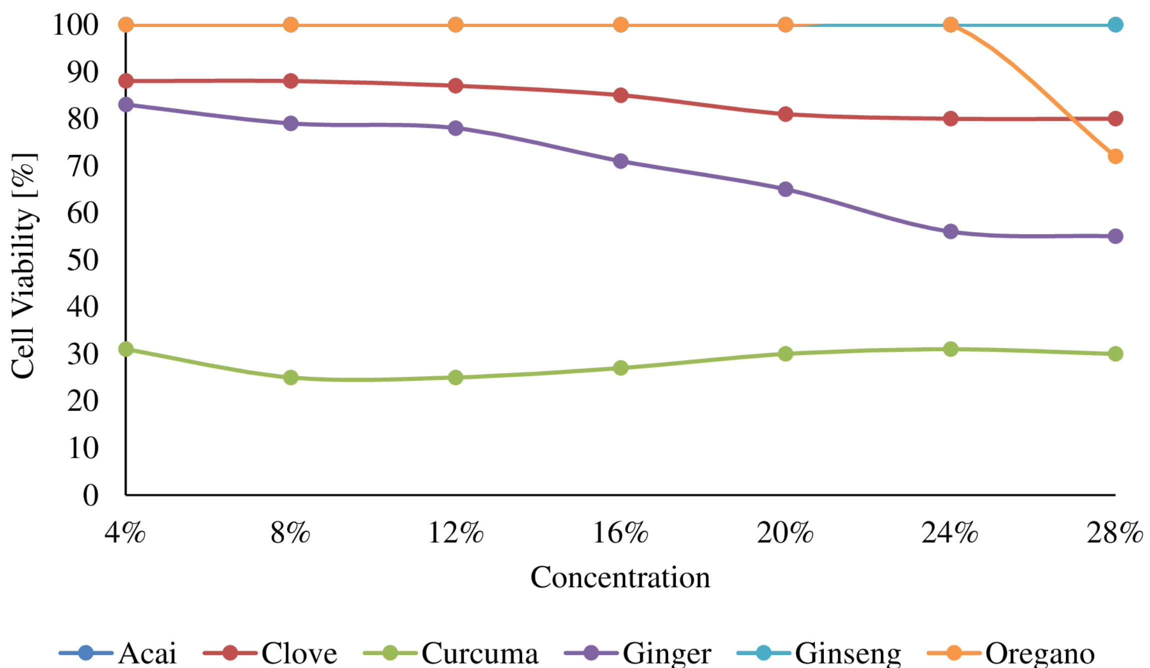

Figure 4*: Cytotoxicity of plant extracts tested on HaCaT cell lines*

Firstly was HaCaT cell line exposed to plant water extracts. In the figure 4 we can see that only curcuma extract was below the 50 % of cell viability and therefore was toxic for the HaCaT cell line. Curcuma was measured in lower concentration ratio and its toxicity was concentration dependent. Below 1 % volume extract which correspond to 1 mg/mL was not toxic. Ginger and oregano extract also showed concentration dependent toxicity and over 30 % which correspond

to 30 mg/g were toxic for HaCaT cell line. On the other hand. ginseng and acai. which is hidden behind sample of ginseng were not toxic at all. Clove extract was in the all-concentration range around 85 - 80 % of viability.

4.1.4.2 Melanoma murine tumor cell line B16F1

B16F1 cell line is usually used for research as a model for human skin cancers. Therefore. it's interesting the correlation between analysis of HaCaT and B16F1. It's required that some extracts with toxic effect on B16F1 would be also safe in the same concentration range for HaCaT cell lines.

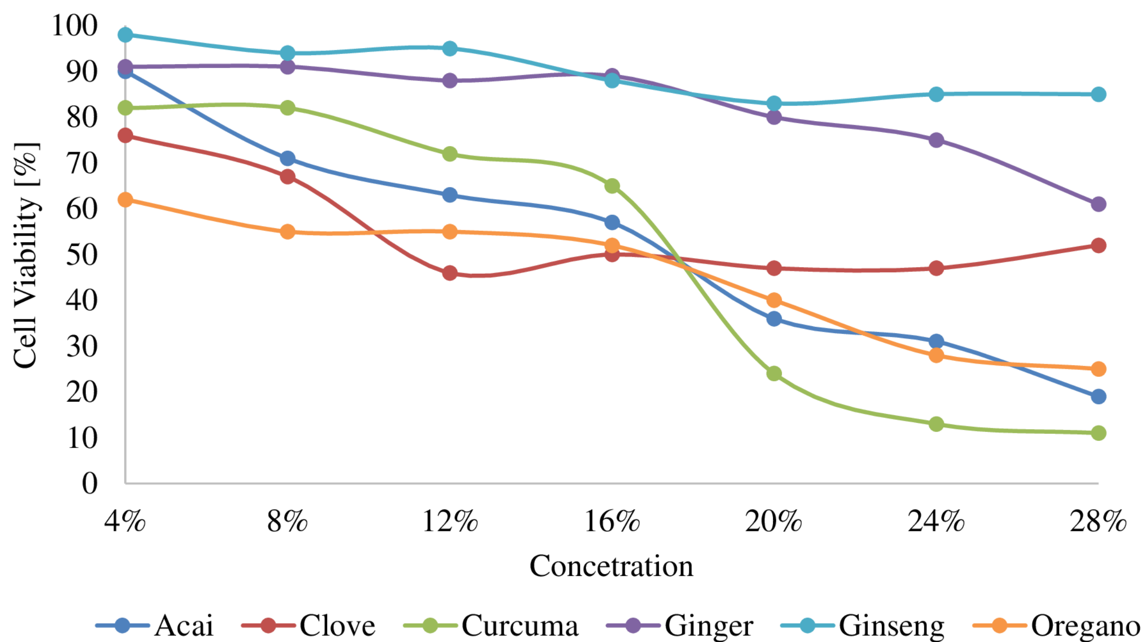

Figure 5: *Cytotoxocity of plant extracts tested on B16F1 cell lines*

Firstly, was B16F1 cell line exposed to plant water extracts. In figure 5 we can see that Ginseng and Ginger extracts were not toxic in all concentration range. but the ginger extract showed concentration depend on trend between 24 % a 28 % so we can expect toxicity at higher concentrations. All the other extracts showed toxicity at higher concentrations. Especially in concentration range from 16 % to 20 % all of the samples except ginger and ginseng have reached the toxicity limit 50 % of viable cells.

4.1.4.3 Raji cell line

These cells have proven to be convenient target cells in studies assessing the effector functions mediated by monoclonal antibodies.

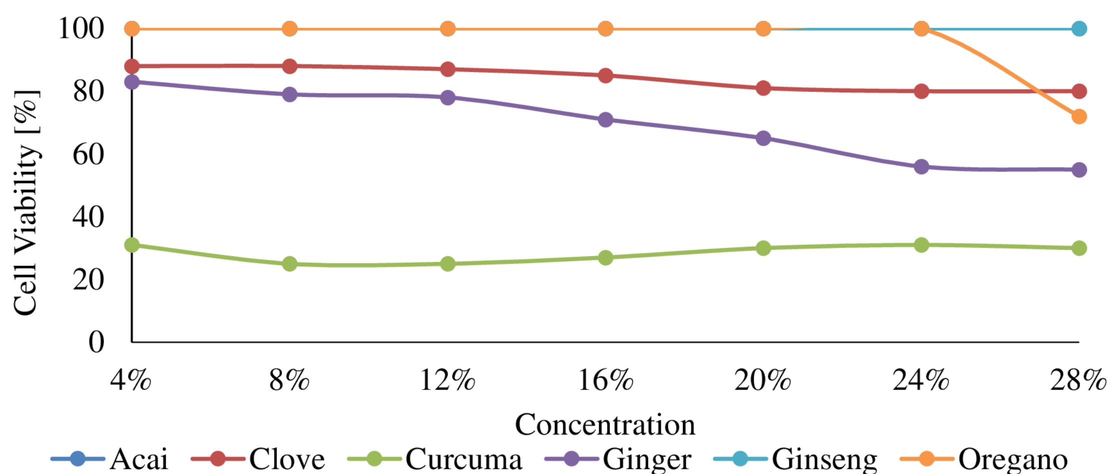

Figure 6: *Cytotoxicity of plant extracts tested on Raji cell lines*

Firstly, was Raji cell line exposed to plant water extracts. In the Figure 6 we can see that Acai extract that is hidden behind Ginseng. because is was not toxic in all concentration range. Clove and Oregano extracts were not toxic in our concentration range. but the oregano extract showed concentration depend on trend between 24 % a 28 % so we can expect toxicity at higher concentrations. Curcuma was measured in lower concentration ratio and its toxicity was concentration dependent. Below 1 % volume extract which correspond to 1 mg/g was not toxic. Toxicity of ginger extract was concentration dependent especially from 24 %.

4.1.4.4 Caco-2

The Caco-2 cells isolated from human colorectal adenocarcinoma are widely used to screen for absorption rate of new compounds in the initial stage of drug discovery.

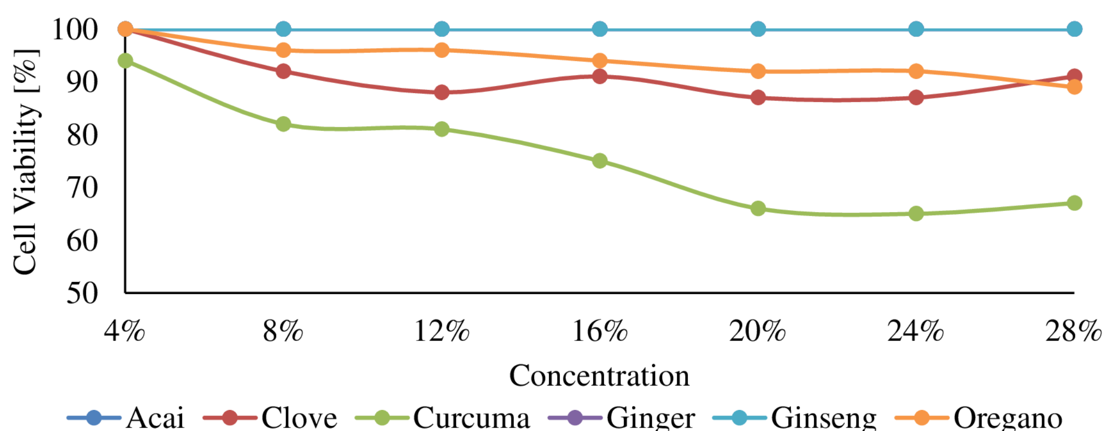

Figure 7: *Cytotoxicity of plant extracts tested on Caco-2 cell lines*

Firstly, was Caco-2 cell line exposed to plant water extracts. In the Figure 7 we can see that acai and ginger extracts that are hidden behind ginseng, because it was not toxic in all concentration range. Clove and oregano extracts were not toxic in our concentration range, but the extracts showed concentration depend on trend so we can expect toxicity at higher concentrations. Curcuma was concentration dependent and showed highest toxicity.

4.1.5 Apoptosis

Apoptosis was tested on two types of cell lines. Raji cell lines were used in combination with plant extracts of oregano. clove. curcuma and ginger. Melanoma murine tumor cell line B16F1 was used in combination with both. plant and microbial extracts.

4.1.5.1 Apoptosis of Raji Cell lines

General evaluation of the results obtained. allows us to underline that treatment of *Raji* cell cultures with plant extracts induces effects on cell distribution in the various phases of the cell cycle (G1-S-G2/M). This is observed in all the experimental conditions with the exception of sample named *Origano 1*.

Percentage values of cell distribution in the cell cycle phases are shown in the table below. in which the more significant changes. in comparison with control condition. are highlighted in red. In green the values less different from control are shown.

CONTROL
Ap = 1,06 ± 0,37
G1 = 49,21 ± 1,85
S = 35,28 ± 2,35
G2/M = 15,53 ± 2,40

CONTROL
(Reference histogram)
Ap = 1,01
G1 = 51,35
S = 32,95
G2/M = 15,70

CLOVE 1
Ap = 1,66
G1 = 69,21
S = 15,88
G2/M = 14,90

GINGER 1
Ap = 1,27
G1 = 58,05
S = 16,02
G2/M = 25,48

CURCUMA 1
Ap = 2,40
G1 = 45,32
S = 36,32
G2/M = 21,67

ORIGANO 1
Ap = 1,01
G1 = 49,04
S = 36,29
G2/M = 14,66

CLOVE 2
Ap = 2,74
G1 = 55,00
S = 15,53
G2/M = 29,48

GINGER 2
Ap = 1,00
G1 = 54,90
S = 35,83
G2/M = 12,62

CURCUMA 2
G1 = 36,44
S = 23,03
G2/M = 40,53
Ap = 8,14

ORIGANO 2
Ap = 1,80
G1 = 42,55
S = 48,12
G2/M = 28,36

CLOVE 3
Ap = 2,31
G1 = 35,97
S = 30,00
G2/M = 34,04

GINGER 3
Ap = 1,49
G1 = 61,90
S = 23,89
G2/M = 14,31

CURCUMA 3
Ap = 1,90
G1 = 50,45
S = 29,64
G2/M = 22,55

ORIGANO 3
Ap = 1,13
G1 = 34,59
S = 30,89
G2/M = 32,90

Figure 8: *Percentage values of cell distribution i the cell cycle phases*

In the Figure 8, cell cycle analyses are reported. Showing the major effects induced by natural plant extracts: *curcuma 2* represents the only condition in which an apoptotic incidence significantly different (even though limited) from controls is revealed.

In general. plant extract treatment (at the conditions here used) do not seem to induce apoptotic effects. Instead. in some conditions particularly cytostatic effects (partly different). caused by a slow-down of the transition of the cells through the cell cycle phases. were observed. Percentage of the cells in the cell cycle phases (G1-S-G2/M) and in apoptosis (Ap). In control. the values ± SD are reported.

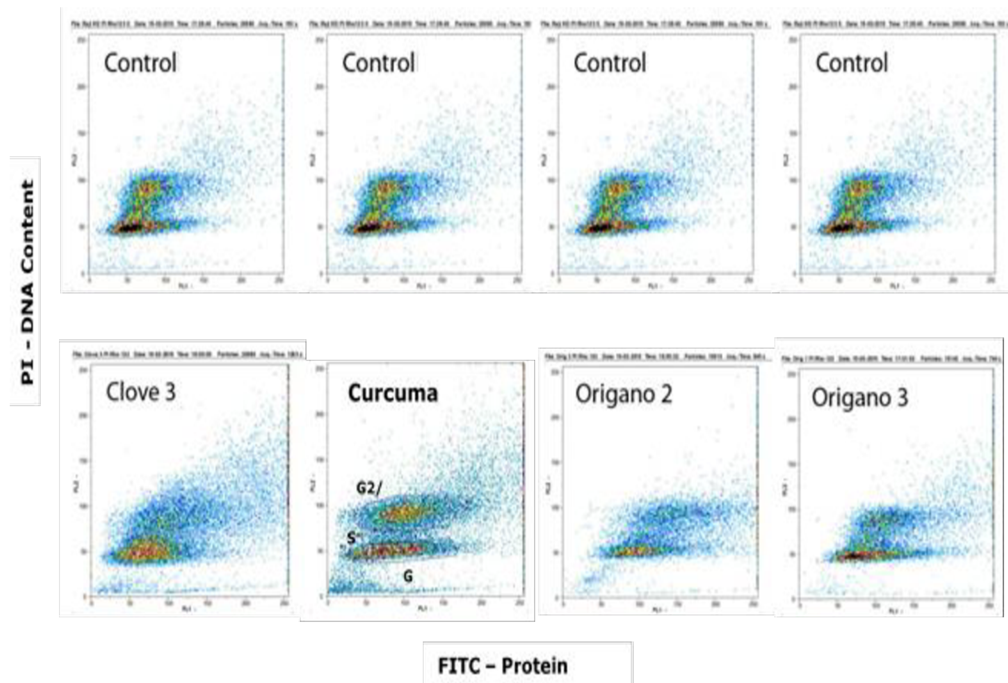

Figure 9: *Delimitation of cells*

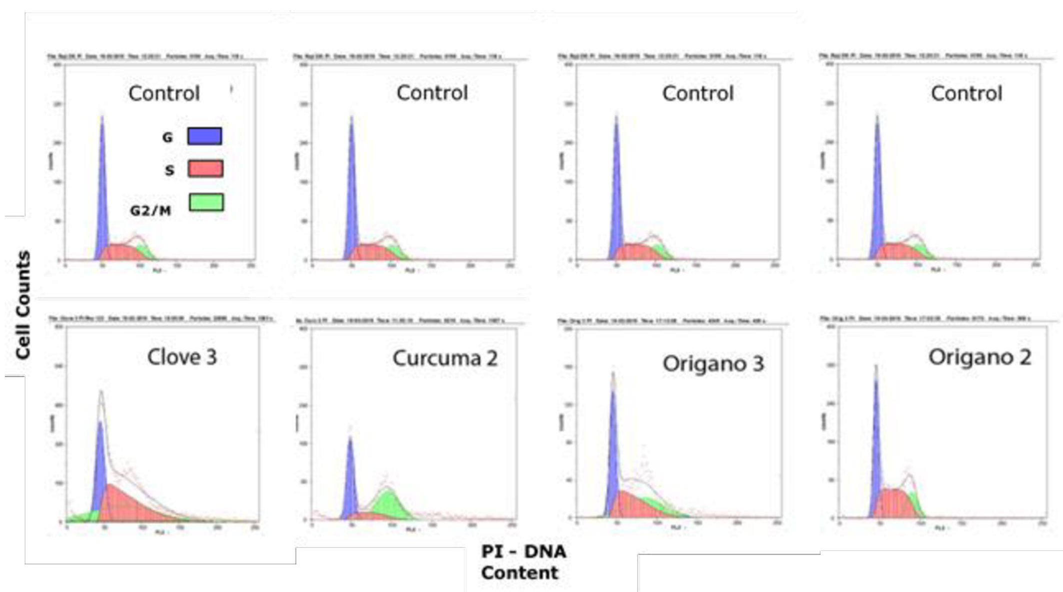

Figure 10: *Histograms of of cell distribution i the cell cycle phases*

4.1.5.2 Apoptosis of B16F1

The purpose of the viability experiments was to observe and compare the impact of selected plant and microbial extracts.

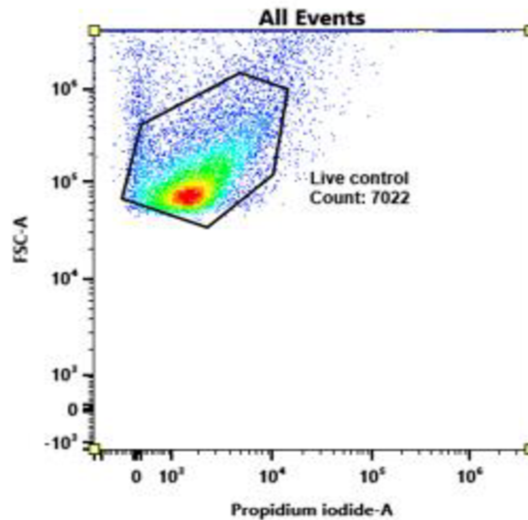

Figure 11: *Delimitation of untreated control cells*

Untreated control cells visualized in the forward-scatter (FSC) versus Propidium iodide dotplot shown in Figure 11 were used as functional control of the method.

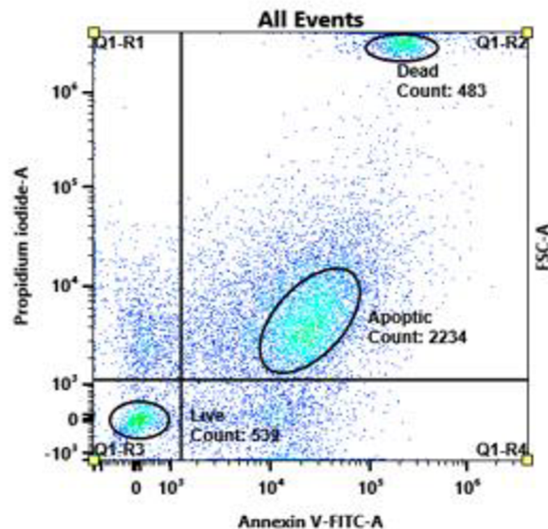

Figure 12: *Stained apoptotic cells (camptothecin treated)*

Camptothecin treated cells visualized in the Propidium iodide versus Annexin V dotplot shown in Figure 12 were used as positive control of apoptotic cells. For negative control we used cell treated with 70 % of ethanol.

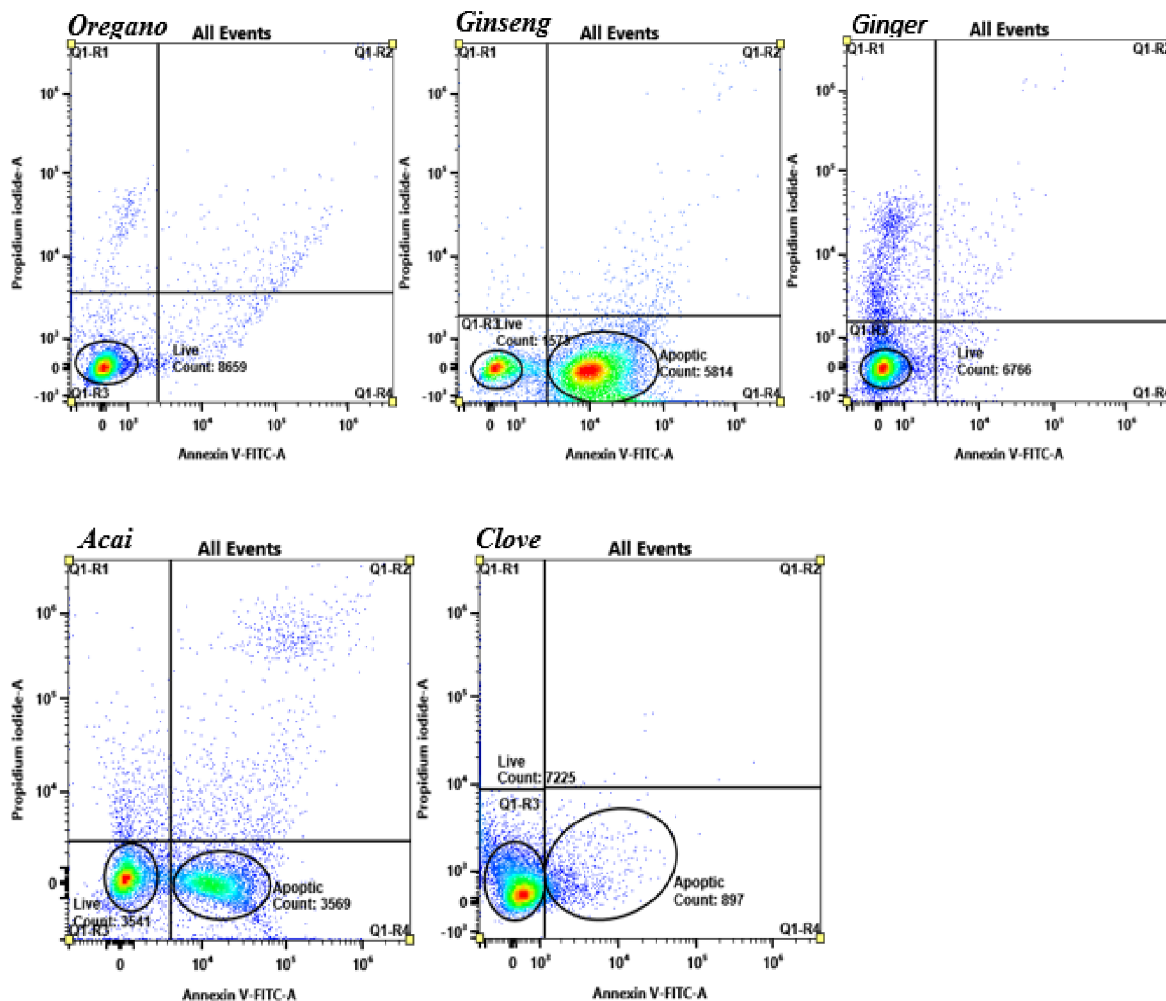

Figure 13: *Stained apoptotic cells (plant extracts treated)*

The cytotoxic effect of selected plant extracts on Raji and murine cancer cell lines was evaluated using MTT assay. We found that plant extract treatment was particularly effective against B16F1 melanoma cells. When measuring apoptosis. in the cytograms we can observe that cells treats with oregano and ginger do not seem to induce apoptotic effects. Instead, treatment with acai, clove and ginseng incidence significantly apoptotic cells in cytograms. Curcumin showed high autofluorescence of itself in cytograms. It was necessary to measure curcumin without PI and Annexin V to obtain better results. Generally, as compared to control cells. there was a significant reduction in cell viability in plant extracts-treated cells. Effect of plant extracts on cell viability was confirmed by crystal violet staining. From these data, we concluded that the cytotoxicity of plant extracts was dose dependent in B16F1 cells and Raji cells. The toxic effects of plant extracts can be correlated to the antioxidant activity and antimicrobial effect. It's obvious that there is correlation between cell viability and the apoptosis.

4.2 PRODUCTION OF MICROBIAL EXTRACTS FROM SELECTED YEAST STRAINS

Yeast strains of *Metschnikowia pulcherrima* CCY 029-002-145. *Cystofilobasidium infirmominiatum* CCY 17-18-4. *Phaffia rhodozyma* CCY 77-1. *Rhodotorula kratochvilovae* CCY 20-2-26. and *Saccharomyces cerevisiae* CCY 6646 were cultivated in Erlenmeyer flasks to obtain biomasses for all the tests below. *S. cerevisiae* CCY 6646 was used as a control strain due to its commercial use as the sole representative of yeast to produce β-D-glucans.

All yeast strains were cultivated under the same conditions. The absence of pathogens was checked microscopically. When viewing the specimen under high magnification yeasts appears as an egg-shaped organism. It is also possible to observe the buds, which can be seen on some of the yeast cells. After 96 hours biomass was centrifuged and prepared for tests.

4.2.1 Characterization of microbial extracts

Health benefits are the most important uptake for this research. Firstly. we measured antioxidant activity. antimicrobial and antibacterial effect for comparison with plant extracts and then prepare some mixture of those extracts. Microbial extract was tested as biomass itself and then as organic Folch extract to measure benefits from carotenoids and other organic substances in biomass.

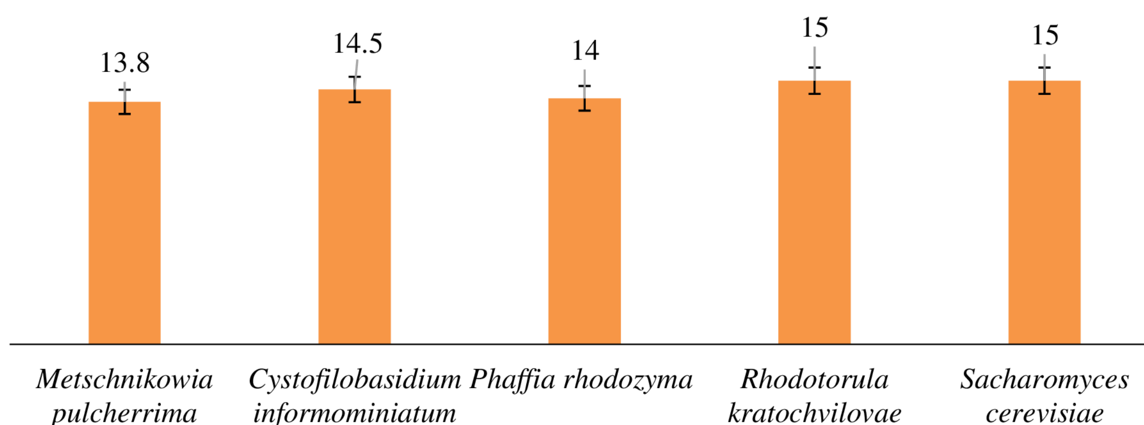

Figure 14: *Biomass yield (g/L) cultivated in Erlenymeyer flasks after 96 hours.*

The biomass yield after the cultivation on the control YPD broth production medium for 96 h in Erlenmeyer flasks. All the strains showed comparable biomass yield from 13.8 g/l to 15 g/l. The biomass was 3x washed with distilled water after cultivation and centrifuged. Biomass was placed in 50mL tubes and store at -80 °C for 48 hours and then lyophilized. After the lyophilization the dry among of biomass was weighted and after rehydration was tested and used for all of the procedures below.

4.2.2 Determination of carotenoids

Carotenoids are an essential group of compounds produced by red yeasts. They are important antioxidants. food colorants. cosmetic ingredients. and feed additives.

Total carotenoids content in presented carotenogenic yeast strain *Rhodotorula kratochvilovae* and *Phaffia rhodozyma* is around 1.3 mg/g of dry biomass. The main

carotenoids occurred in yeast biomass are beta-carotene. thorulene and small amount of lycopene.

Table 5: *Content of carotenoids in biomass [mg/g]*

	CCY 029-002-145	***CCY 17-18-4***	***CCY 77-1***	***CCY 20-2-26***
Ergosterol	0.210	0.940	1.121	2.955
Ubiquinon	0.469	1.350	1.548	2.335
Torularhodin	-	-	0.856	1.063
Lycopene	-	-	-	0.051
Torulen	-	-	0.058	0.103
β- carotene	-	0.006	0.024	0.066
Total carotenoids	-	0.007	1.250	1.374

4.2.3 Yeasts as a fatty acids producents

The amount of lipids and the fatty acid content of lyophilized yeast biomass was determined by gas chromatography in parallel with the production of glucans. Fatty acids were divided into three groups. polyunsaturated fatty acids (PUFA). monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA). The results were graphically evaluated by showing the individual ratios of PUFA. MUFA and SFA in the total amount of fatty acids in the biomass. The total percentage of fatty acids in the biomass feed was also evaluated.

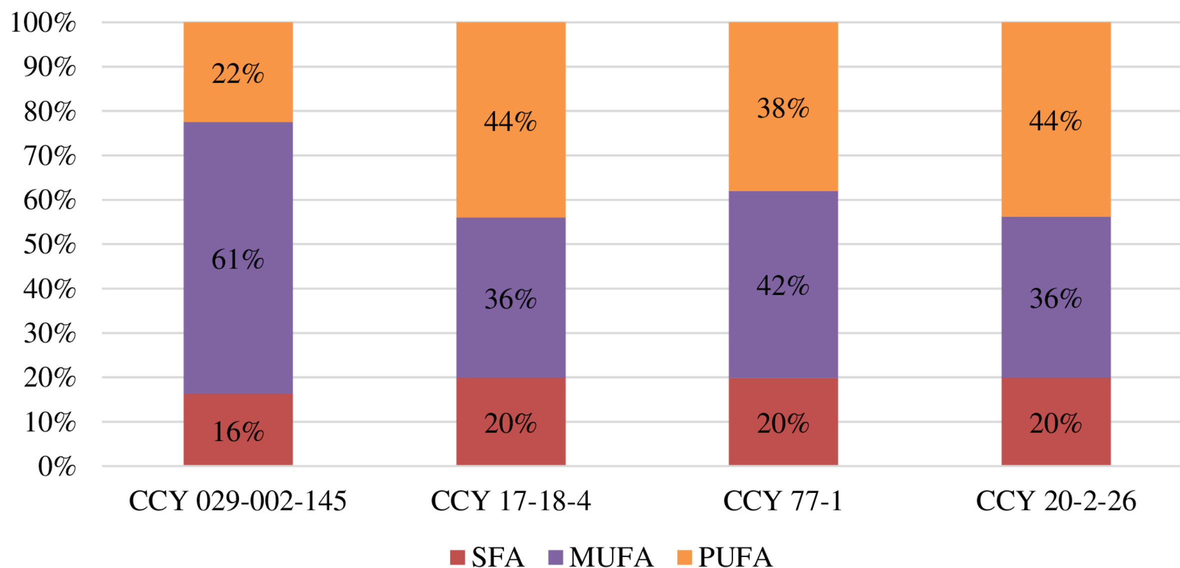

Figure 15*: Production of fatty acids by selected yeast strains*

From the graph shown in figure 15 it’s obvious that there is a big difference in amount of individual ratios between yeast that were used in this thesis. Yeast strain of *Metschnikowia pulcherrima* has the highest amount of monounsaturated fatty acids.

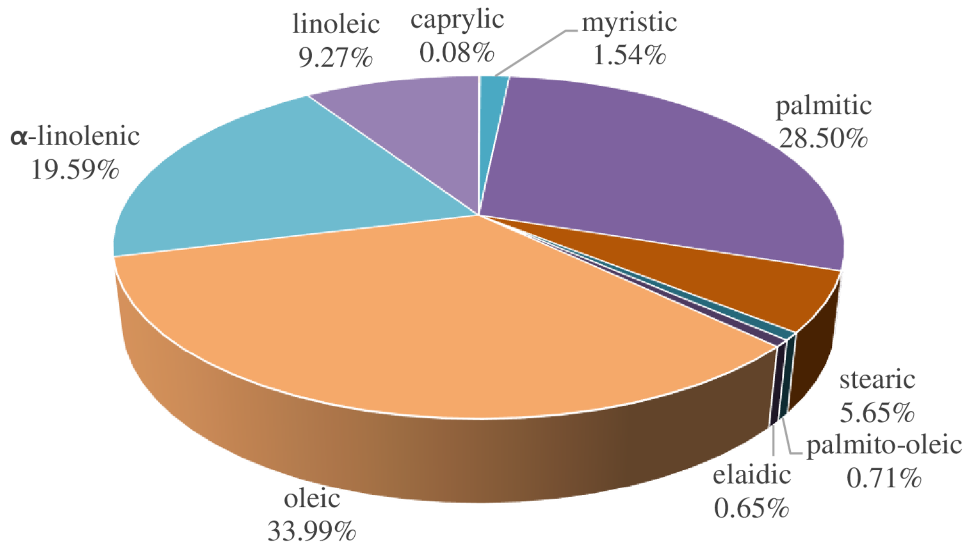

Figure 16: *Percentage of fatty acids in strain Rhodotorula kratochvilovae*

The graph in the figure 16 shows percentage of determinated fatty acids in strain *Rhodotorula kratochvilovae*. Significantly highest amount is of oleic acid (33.99 %), palmitic acid (28.50 %), myristic acid (19.59 %) and α-linolenic acid.

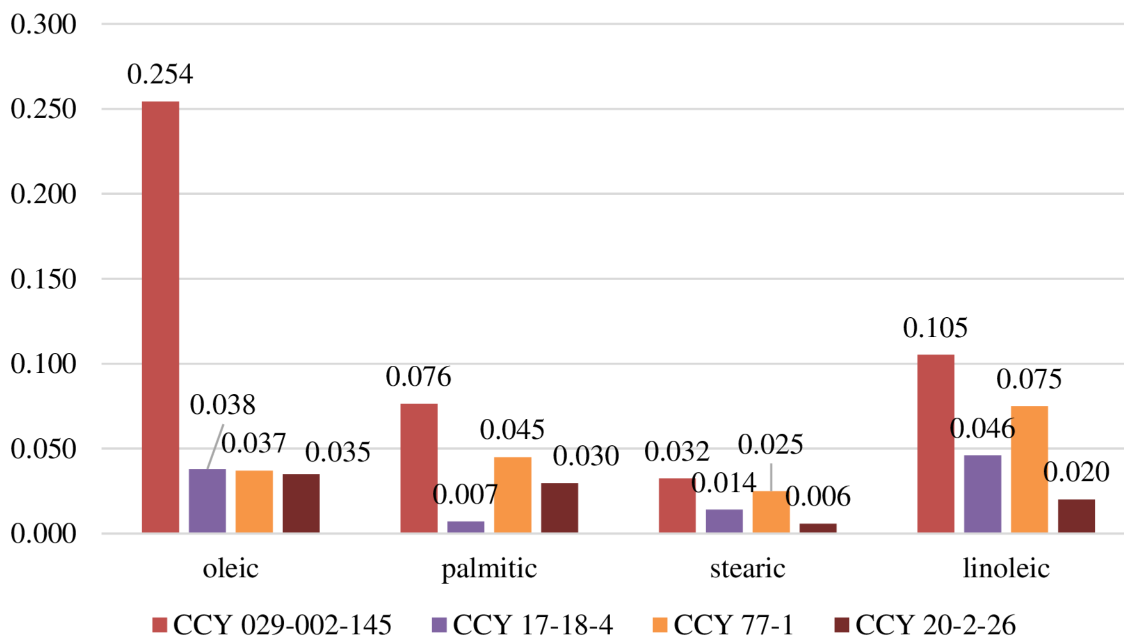

Figure 17: *Volume of the main fatty acids in biomass [mg/g]*

Volume of fatty acids was also measured by gas chromatography. From the figure 17 we can see that yeast strain of *Metschnikowia pulcherrima* has the highest amount of oleic, palmitic, stearic and linoleic acid.

4.2.4 Isolation and determination of β glucans

Several studies have shown a baker's yeast beta-1.3/1.6-D-glucan. extracted from Saccharomyces cerevisiae. is effective in reducing the incidence of cold and flu symptoms. Isolation and determination of beta-glucans in cultivated biomass was described in chapter 3.4.3.

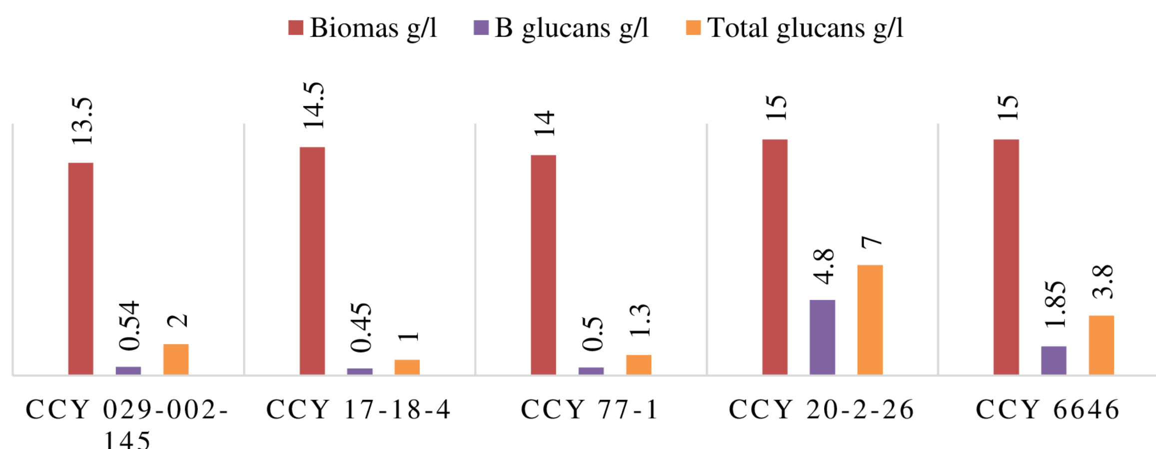

Figure 18: *Production of glucans by selected yeasts strains*

In the Figure 37 we can see the yields of glucans and biomass of all tested yeast strains. From *Rhodotorula katochvilovae* we obtain the highest amount of total glucans which was 7.12 g/L of biomass and from 7.12 of total glucans we obtained 4.75 g/L of beta- glucans. From *Metschnikowia pulcherrima* we obtain 1.92 g/L of total glucans and 0.405 g/L of beta-glucans. From *Cystofilobasidium informominiatum* we obtain 1.12 g/L of total glucans and 0.475 g/L beta-glucans. From *Phaffia rhodozyma* we obtain 1.42 g/L of total glucans and 0.654 g/L beta-glucans.

4.2.5 Antioxidant activity

Microbial extracts were characterized for antioxidant activity.

Table 6: *Antioxidant activity of rehydrated biomass and Folch extract*

	Antioxidant activity [mg/g]	
	Rehydrated biomass	Folch extract
CCY 029-002-145	4.435 ± 0.05	4.107 ± 0.01
CCY 20-2-26	5.807 ± 0.08	5.002 ± 0.02
CCY 77-1	4.215 ± 0.02	4.321 ± 0.03
CCY 17-18-4	4.536 ± 0.07	4.612 ± 0.01

According to the results in Table 6 we can compare antioxidant activity of rehydrated biomass and of folch extract of rehydrated biomass. All the samples showed high potential to be strong antioxidants comparable for example with water extract of curcuma.

Antimicrobial activity is tested using standard antimicrobial assays. Testing is performed on microorganisms in suitable culture media and under optimal conditions. Antimicrobial tests are divided into diffusion and dilution. Both types of tests were used in the work. Thus. various methods for determining inhibitory measures have been tested properties including both visual detection of inhibition zones on solid agar plates and measurements changes in culture concentration during cultivation in liquid media. The antimicrobial efficacy of the test substances and extracts was monitored against the bacterial of gram-positive strains - *Micrococcus luteus* and gram-negative strains - *Serratia marcescens.* To compare plant extracts and microbial extracts. was also prepared biomass of *Rhodotorula kratochvilovae* and mixture in the ration of 1:1 with plant extracts.

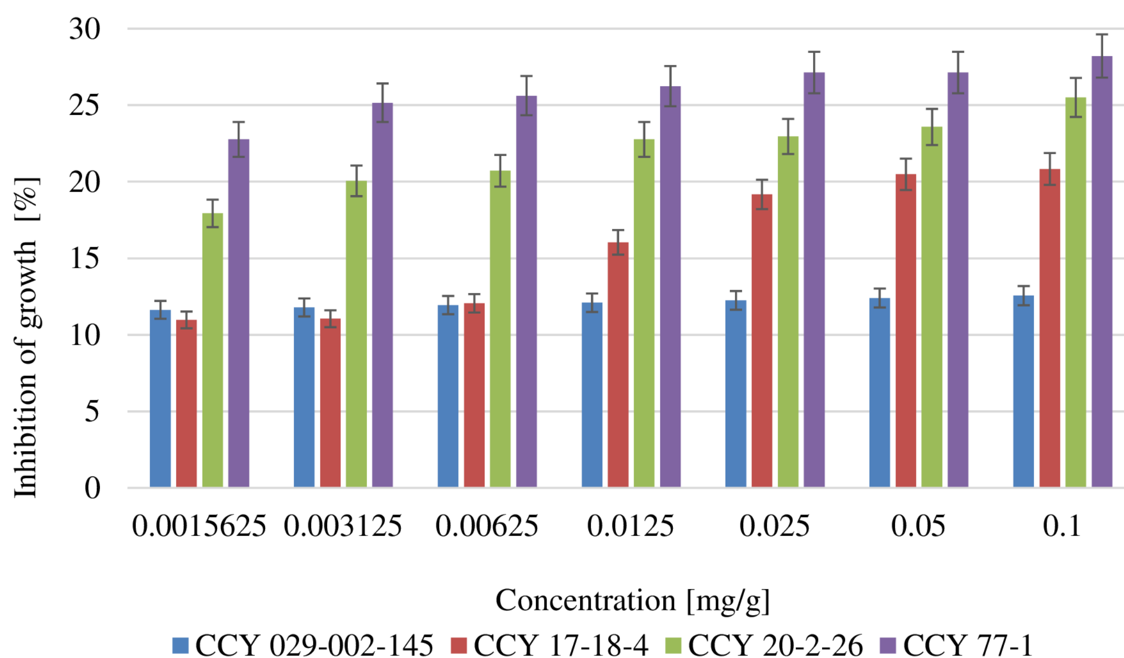

Figure 19: *Antibacterial inhibition of microbial extracts and mixture Serratia marcescens [%]*

According to the test it's obvious that microbial extracts are antimicrobial active substances. The highest potential showed extract of *Phaffia rhodozyma* in all concentration range. Inhibiton of microbial growth showed concentration dependent trend.

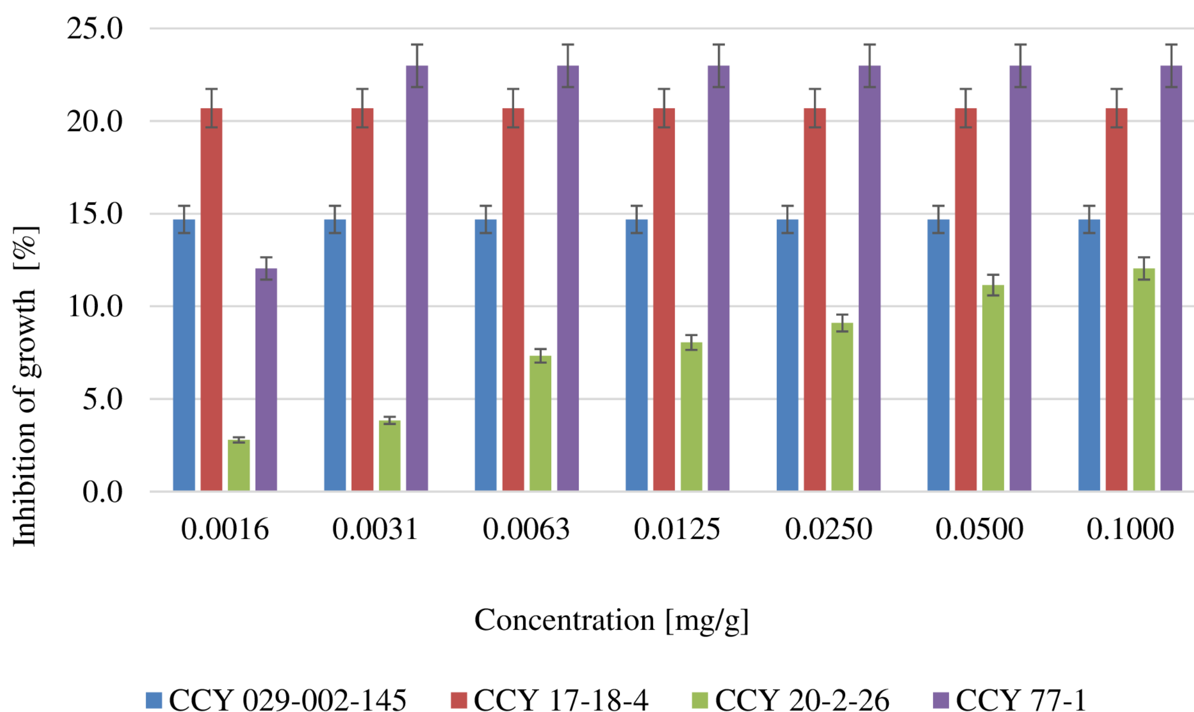

Figure 20: *Antibacterial inhibition of microbial extracts and mixture Micrococcus Luteus [%]*

According to the test it's obvious that microbial extracts are antimicrobial active substances. The highest potential showed extract of *Phaffia rhodozyma* in all concentration range. Inhibiton of microbial growth showed concentration dependent trend. On the other hand extract from *Rhodotorula kratochvilovae* showed less potential as antimicrobial agent.

4.2.6 Tests on cell lines

The principle of the MTT assay is that for most viable cells mitochondrial activity is constant and thereby an increase or decrease in the number of viable cells is linearly related to mitochondrial activity. The mitochondrial activity of the cells is reflected by the conversion of the tetrazolium salt MTT into formazan crystals. which can be solubilised for homogenous measurement. Thus. any increase or decrease in viable cell number can be detected by measuring formazan concentration reflected in optical density (OD) using a plate reader at 540 nm. B16F1, HaCaT and Caco-2 cells were treated with different microbial extracts in concentration range between 4-28 mg/g for 24h. A considerable reduction in the cell viability was observed in a concentration dependent when compared with the control treated cells.

4.2.6.1 Keratinocytes HaCaT

Immortalized human keratinocytes cell line **HaCaT** from skin has been used as a model for the study of keratinocytes functions. According to the results of antimicrobial activity and antioxidant activity it was assumed on slightly higher toxicity than at plant extracts.

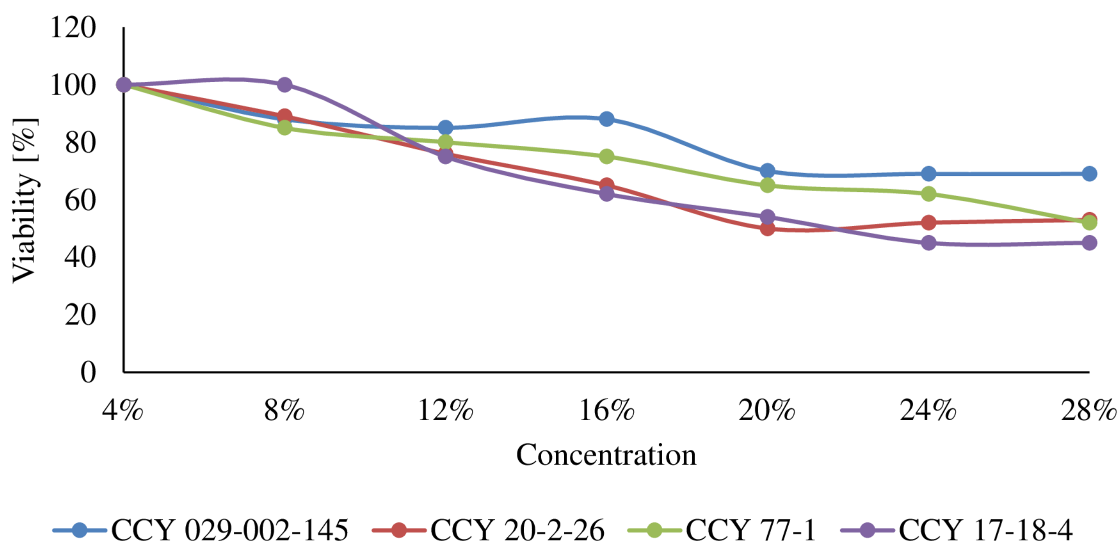

***Figure 21:** Cytotoxicity of microbial extracts tested on HaCaT cell lines*

In the Figure 21 we can see that toxicity of the microbial extracts was concentration dependent and in low concentration it was not toxic at all. From 20 % which correspond to 20 mg of dry biomass diluted in 1 mL of DMEM media. was extract of *Cystofilobasidium informominiatum* and *Rhodotorula kratochvilovae* on the edge of toxicity. On the other hand. extract of *Metschnikowia pulcherrima* and *Phaffia rhodozyma* was in all concentration range above the edge of toxicity.

4.2.6.2 Melanoma murine tumor cell line B16F1

B16F1 cell line is usually used for research as a model for human skin cancers. Therefore, it's interesting the correlation between analysis of HaCaT and B16F1. It's required that some extracts with toxic effect on B16F1 would be also safe in the same concentration range for HaCaT cell lines.

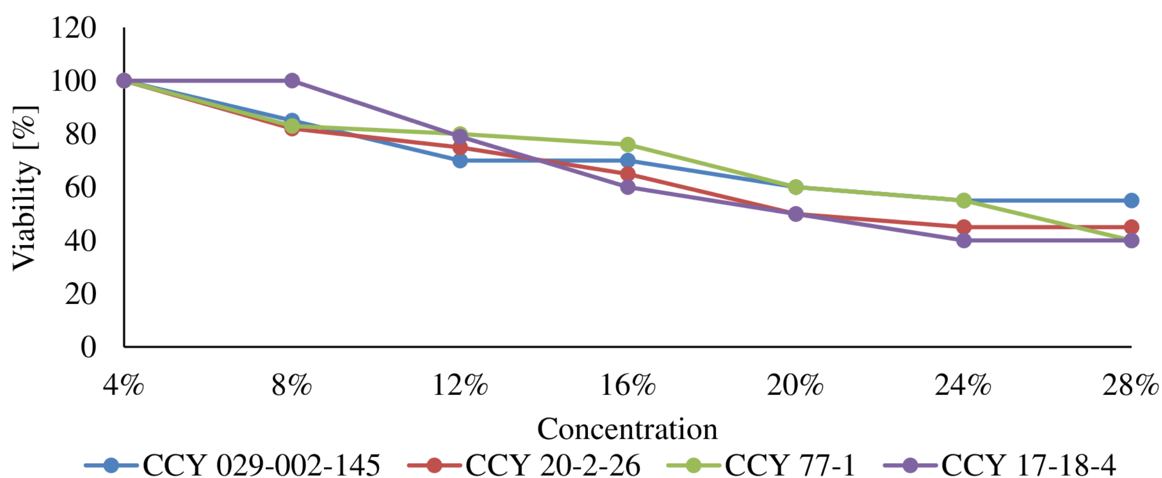

***Figure 22:** Cytotoxicity of microbial extracts tested on B16F1 cell lines*

In the Figure 22 we can see that toxicity of the microbial extracts was concentration dependent in all concentration range. Viability of mouse melanoma cells with microbial extracts in lower concentration about 4 % which correspond to 4 mg of dry lyophilized biomass diluted in 1 mL DMEM media was 100 % or slightly above. From 20 % which correspond to 20 mg of dry lyophilized biomass diulted in 1 mL of DMEM media. were all extracts on the edge of toxicity.

After comparing the cytotoxic effect of natural plant extract and microbial extract on HaCaT cell lines and B16F1 cell lines. we can conclude slightly antitumor effect of microbial extracts in concentrations from 16 % which is on the edge between toxicity for B16F1 and non-toxic for HaCaT. Natural plant extracts showed slightly antitumor effect on the concentration range from 16 % to 28 % except ginger and ginseng. Especially acai extract was remarkable result according to microbial effect. curcuma showed the toxicity from 16 % exponentially. Also oregano extract was toxic for B16F1 but that's what we expect according to the antimicrobial activity and antioxidant activity.

To evaluate possibility of apoptotic effect on B16F1 cell line was necessary prepare organic extract of dry lyophilized biomass. Biomass was rehydrated and then Folch extraction was used to extract all the active compounds from biomass. The organic solvent was evaporated under nitrogen and then dissolved in 1 mL of DMSO. Before testing the toxicity, the sample was diluted in DMEM media. The highest acceptable concentration of DMSO for cell lines was 5%.

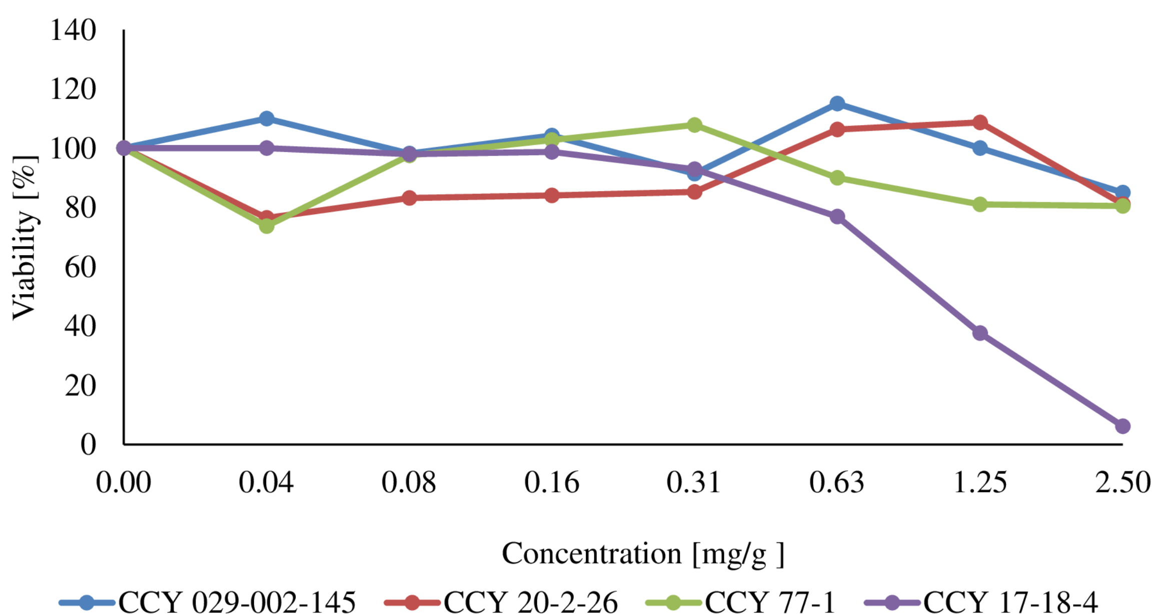

Figure 23: *Cytotoxicity of Folch extracts from lyophilized biomass on B16F1 cell lines*

In the Figure 23 we can see that all the extracts were concentration dependent. Especially from 0.31 mg/g of dry biomass. All of the extracts were toxic at concentration of 5 mg/g of dry biomass and the blank sample of 5 % DMSO was tested too to avoid false positive results.

4.2.6.3 Caco-2

The Caco-2 cells isolated from human colorectal adenocarcinoma are widely used to screen for absorption rate of new compounds in the initial stage of drug discovery.

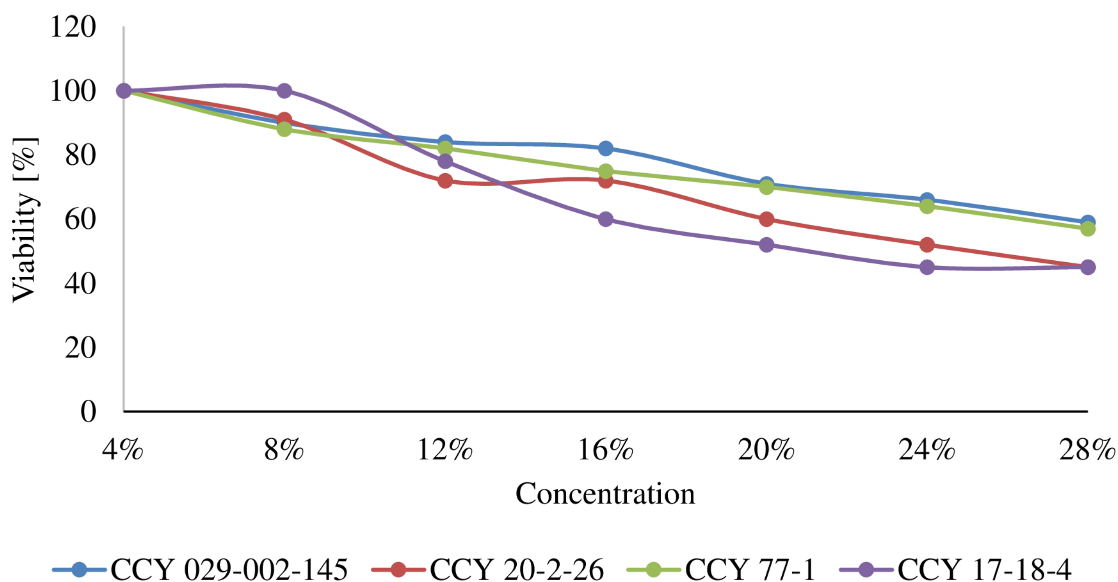

Figure 24: *Cytotoxicity of microbial extracts tested on Caco-2 cell lines*

In the Figure 24 we can see that toxicity of the microbial extracts was concentration dependent and in low concentration it was not toxic at all. From 16 % which correspond to 16 mg of dry biomass diluted in 1 mL of DMEM media. was extract of *Cystofilobasidium informominiatum* on the edge of toxicity. Extracts of *Metschnikowia pulcherrima*, *Phaffia rhodozyma* and *Rhodotorula kratochviloave* were slightly toxic from higher concentrations about 24 % – 28 %.

4.2.7 Apoptosis

Apoptosis was tested on 2 cell lines. Raji cell lines were used in combination with plant extracts of oregano. clove. curcuma and ginger. Melanoma murine tumor cell line B16F1 was used in combination with both. plant and microbial extracts.

4.2.7.1 Apoptosis of B16F1

The purpose of the viability experiments was to observe and compare the impact of selected plant and microbial extracts.

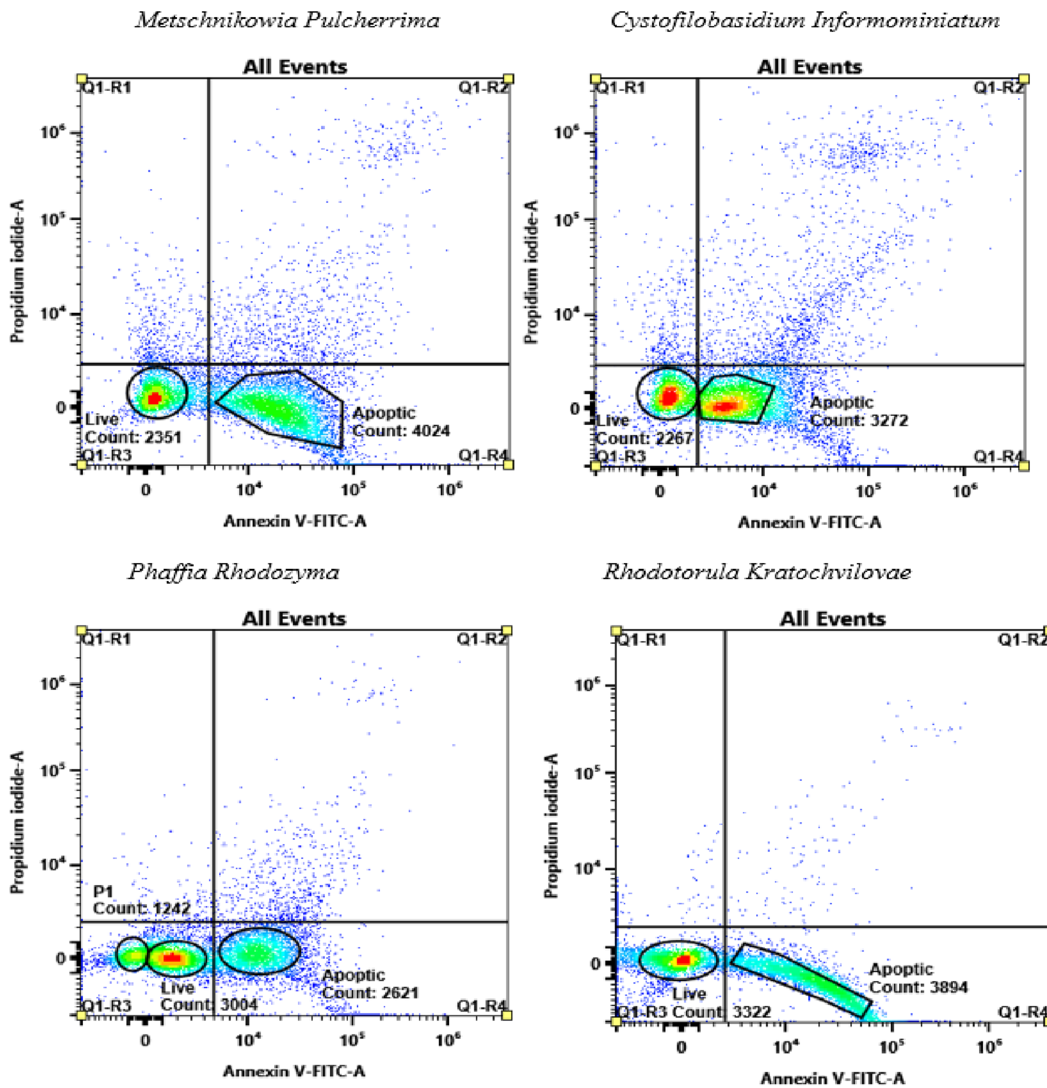

Figure 25: *Stained apoptotic cells (microbial extracts treated)*

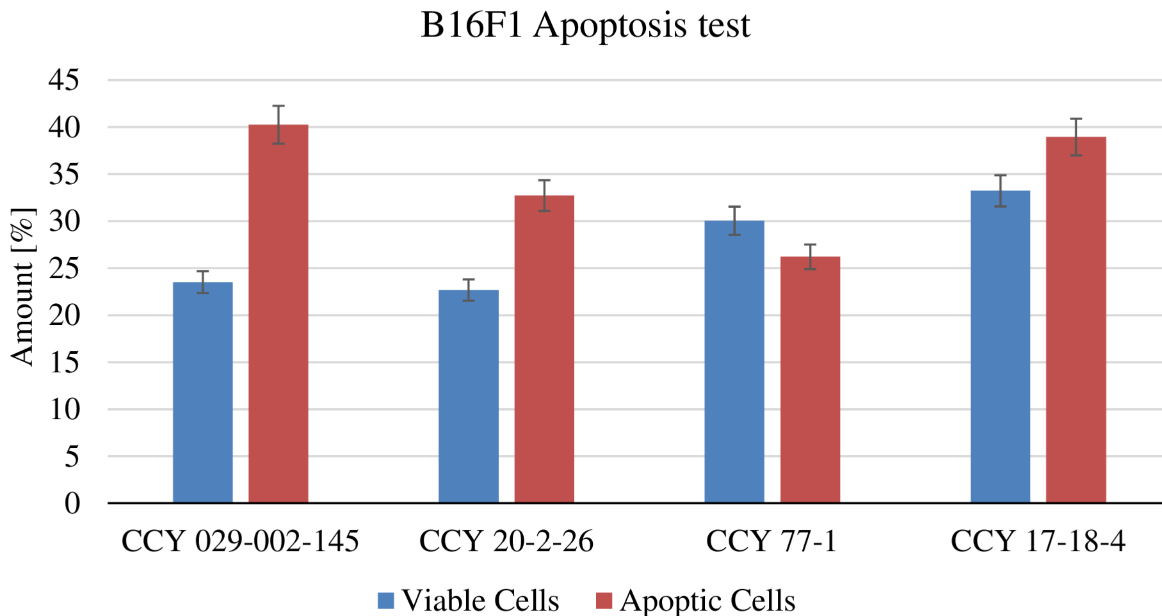

Figure 26: *Amounts of stained apoptotic cells (microbial extracts treated)*

As compared to control cells. there was a significant reduction in cell viability in microbial extracts-treated cells. Effect of microbial extracts on cell viability was confirmed by crystal violet staining. From these data. we concluded that the cytotoxicity of microbial extracts was dose dependent in B16F1 cells. The toxic effects of microbial extracts can be correlated to the antioxidant activity and antimicrobial effect. It's obvious that there is correlation between cell viability and the apoptosis. All of the microbial extracts showed some positive results for apoptosis and also in sample of *Phaffia rhodozyma* there are 3 populations of cells.

Cancer development and progression in most cases is mediated by the suppression of apoptosis. PI/Annexin V detects cellular apoptosis because of differential nuclear staining. The normal and early apoptotic cells are characterized by intact membranes; the plasma membrane undergoes structural changes that include translocation of phosphatidylserine from the inner to the outer leaflet (extracellular side) of the plasma membrane. It has been reported that the translocated phosphatidylserine on the outer surface of the cell marks the cell for recognition and phagocytosis by macrophages.

4.3 SYNERGIC EFFECT

One of the goals of this work was to evaluate the synergistic effect of plant and microbial extracts. The well-known strain of yeast *Rhodotorula kratochvilovae* was selected from the microbial extracts.

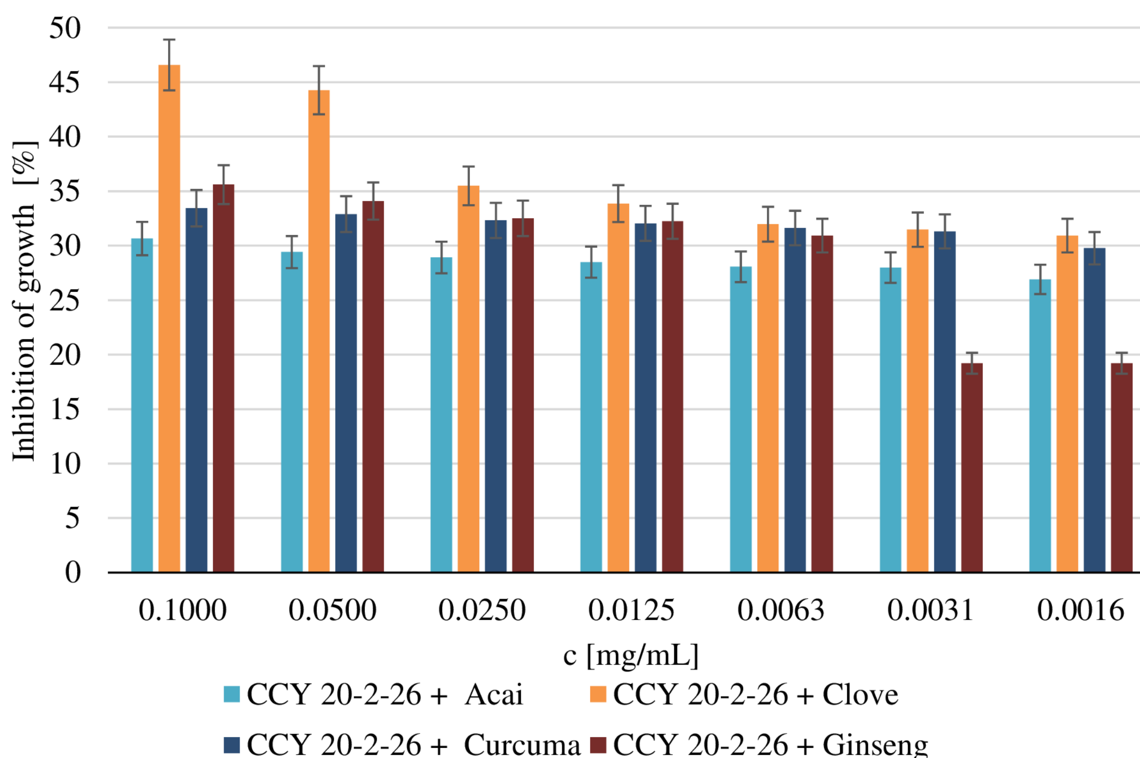

Figure 27: *Antibacterial inhibition of Serratia marcescens by Rhodotorula kratochvilovae extract in combination with plant extracts [%]*

According to the graph in the figure 27 it's obvious that combination of plant extracts and biomass highly increased antimicrobial activity. The highest potential showed extract of *Rhodotorula kratochvilovae* with mixture of clove extract where at 0.1 mg/mL was 48 % of inhibition and at 0.0016 mg/mL was inhibition still around 30 %.

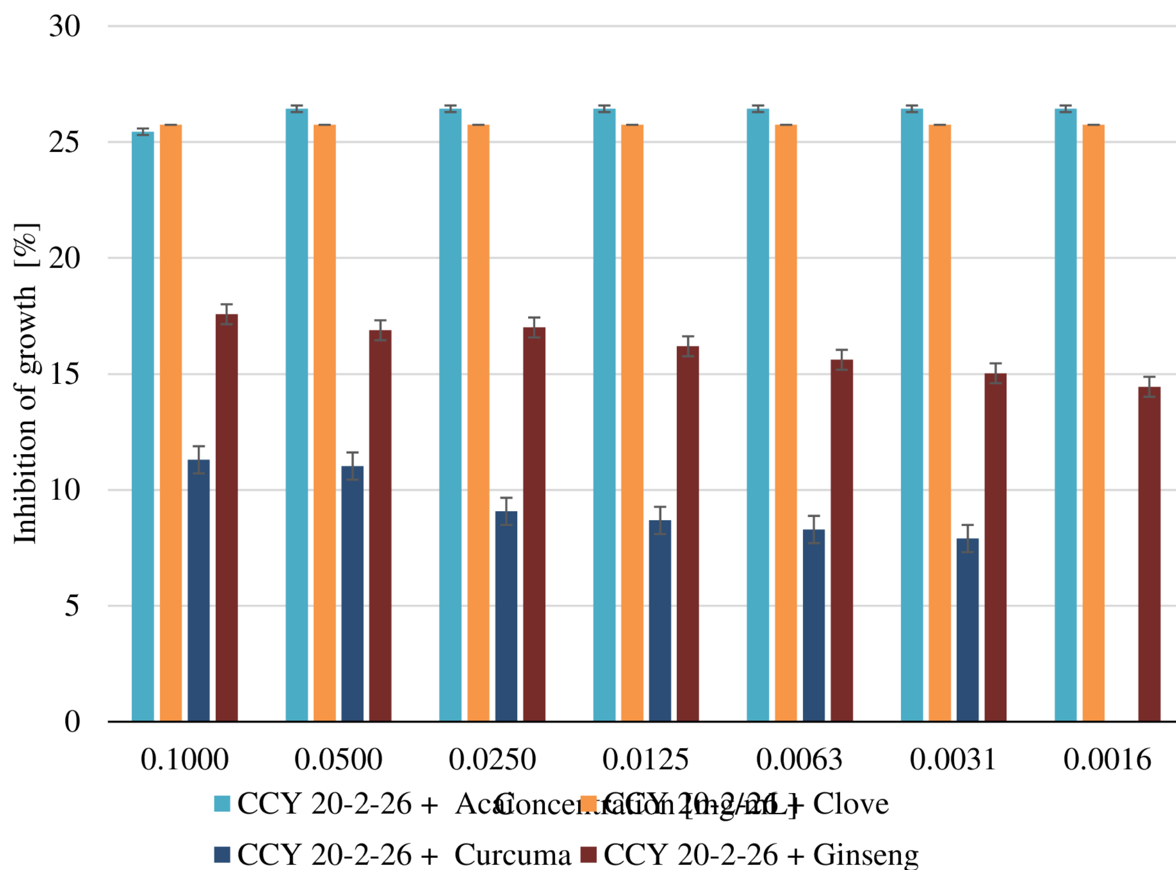

Figure 28: *Antibacterial inhibition of Micrococus luteus by Rhodotorula kratochvilovae extract in combination with plant extracts [%]*

In the figure 28 we can see that combination of plant extracts of acai or clove with *Rhodotorula kratochvilovae and* highly increased antimicrobial activity. Inhibition was around 25 % in all concentration range (0,1 - 0,0016 mg/mL). When we compare the separate results of microbial and plant extracts. we can infer a mutually beneficial increase in microbial activity.

5 DISCUSSION

The presented doctoral thesis is focused on the study of possible synergic effect of natural and microbial extracts on human health. From natural extracts. acai. clove. curcuma. ginger. ginseng and oregano were chosen. prepared and tested. These extracts were prepared both. aqueous (stirring for 24 h in 70° C) and oily (soxtherm extraction with hexane). Microbial extracts were produced by yeast strain of *Rhodotorula kratochvilovae*. *Metschnikowia pulcherrima*. *Cystofilobasidium informominiatum* and *Phaffia rhodozyma*. The microorganisms were cultured under conditions suitable for beta–glucan production. The biomass was also rich in carotenoids and fatty acids that has high benefit in supplementary diet because mammals cannot synthesize carotenoids de novo, and therefore, carotenoids must be obtained from diet. Higher consumption of carotenoid-containing fruits and vegetables and higher plasma concentrations of several carotenoids, including β-carotene. are associated with a lower risk of many different cancers. As lipids constitute a major portion of the majority membranes suggest that the presence of massive concentrations of unsaturated FA within membranous structures. In addition, well recognized that the PUFA are bioactive mediators of diverse pathways involved in cellular homeostasis or, in some cases, interact with cellular macromolecules resulting in cell death, these cellular responses may be a consequence of the vulnerability of unsaturated FA to diverse oxidation reactions, or radical reactions. or both.

First part of the work was focused on the preparation and characterization of natural extracts. Subsequently. their antimicrobial efficacy and influence on mitochondrial activity. The highest content of phenolic substances was determined in curcuma extract (4.723 mg/g), while the lowest content was detected in oregano (0.887 mg/g). Turmeric also showed the highest antioxidant effect (5.26 mg/g). For the oil extracts the best results were obtained from acai, clove, and curcuma extracts for total phenol account, from acai 1.418 mg/g, for clove 2.181 mg/g and curcuma 2.176 mg/g. Antioxidant activity of ginseng oil 1.152 mg/g was comparable with ginger 1.112 mg/g and higher result was obtained from curcuma 3.258 mg/g.

In monitoring the growth inhibition of *Serratia marcescence*. antibacterial activity of selected plant extracts was measured. Most of the samples showed similar results in all concentration range from 0.1 mg/mL to 0.003 mg/mL of dry material diluted in water. Clove extract showed highest antibacterial effect against gram-negative strains. When measuring inhibiton of gram- positive strains *Micrococus luteus*. the most effective was oregano extract. On the other hand, clove extract wasn't so effective and also showed concentration dependent effect. same as curcuma and ginger.

Subsequently, attention was paid to the reactions of plant extracts with cell lines, especially its possibility to induced apoptosis in cancer cell lines. When comparing the cytotoxic effect of natural plant extract on HaCaT cell lines and B16F1 cell lines. we can conclude slightly antitumor effect of natural plant extracts on the concentration range from 16 mg/g to 28 mg/g except ginger and ginseng. Especially Acai extract was remarkable result. Curcuma showed the toxicity from 16 mg/g exponentially. Also, oregano extract was toxic for B16F1 but that's what we expect according to the antimicrobial activity and antioxidant activity.

Raji cell line showed significant toxicity only with curcuma extract. Therefore. this extract was used for cell cycle analysis and also apoptosis. Results showed that curcuma represents the only condition in which an apoptotic incidence significantly different (even though limited) from controls was revealed at Raji cell line. In general. plant extract treatment (at the conditions here used) do not seem to induce apoptotic effects. Instead, in some conditions particularly cytostatic effects (partly different), caused by a slow-down of the transition of the cells through the cell cycle phases are observed. For Caco-2 cells also only curcuma extract showed cytotoxic effect. Curcuma was concentration dependent and showed highest toxicity. We found that plant extract treatment was particularly effective against B16F1 melanoma cells. In the cytograms we can observe that cells treated with oregano and ginger do not seem to induce apoptotic effects. Instead, treatment with acai, clove and ginseng incidence significantly apoptotic cells in cytograms. Curcumin showed high autofluorescence of itself in cytograms. It was necessary to measure curcumin without PI and Annexin V to obtain better results. As compared to control cells. there was a significant reduction in cell viability in plant extracts-treated cells. Effect of plant extracts on cell viability was confirmed by crystal violet staining. From these data. we concluded that the cytotoxicity of plant extracts was dose dependent in B16F1 cells and Raji cells. The toxic effects of plant extracts can be correlated to the antioxidant activity and antimicrobial effect. It's obvious that there is correlation between cell viability and the apoptosis.

The second part of the work was focused on the preparation and characterization of biomass that was cultivated from 4 different strains and was tested for antioxidant activity and antimicrobial activity. Volume of fatty acids was also measured by gas chromatography. *Metschnikowia pulcherrima* has the highest amount of oleic, palmitic, stearic and linolic acid. Volume of beta glucans was tested by enzymatic kit from Megazyme. From the yeast strain *Rhodotorula katochvilovae* we obtained the highest ammount of total glucans which was 7.12 g/L of biomass and from which 4.75 g/L was beta- glucans. From *Metschnikowia pulcherrima* we obtain 1.92 g/L of total glucans from which 0.405 g/L was beta-glucans. From *Cystofilobasidium informominiatum* we obtain 1.12 g/L of total glucans and 0.475 g/L was beta-glucans. From *Phaffia rhodozyma* we obtain 1.42 g/L of total glucans from which 0.654 g/L was beta-glucans. All of the microbial extracts showed some positive results for apoptosis on cancer cell lines. On the other hand, at the same concentration range there was not cytotoxic effect on HaCaT cell lines that is not carcinogenic.

Positive biological effects of pigmented yeast biomass can be related to the content of valuable fatty acids, sterols, beta-glucans and other carbohydrates, nucleic acids, vitamins and provitamins, minerals, quinones, certain essential amino acids and other components. Red yeasts can produce carotenoids pigments. such as astaxanthin. torulene and torularhodin. whose show a considerable antioxidant and antibacterial activity and may also prevent certain types of cancer and enhance the immune system. Another component of oleaginous yeast biomass are unsaturated fatty acids – mainly high portion of oleic acid and up to 40% of PUFA. Recently it was found that both oleic acid and alpha-linoleic acid significantly down-regulated cell proliferation, adhesion and/or migration. Also, it was observed that both these fatty acids positively cross-regulates the expression levels of AMPK/S6 axis. Moreover, they up-regulated

tumor suppressor genes (p53, p21, and p27), have an important role in oesophageal cancer and thus might be useful agents in the management or chemoprevention of oesophageal cancer.

Recent evidence suggests that patients nutritional status plays a major role in immunotherapy outcome. Fatty acids are essential in a balanced diet and well-known to influence the immune response. Moreover, short-chain fatty acids (SCFAs) show beneficial effects in metabolic disorders as well as in cancer and polyunsaturated fatty acids (PUFAs) contribute to body weight and fat free mass preservation in cancer patients. In line with these data. several studies imply a role for SCFAs and PUFAs in boosting the outcome of immunotherapy. Potential roles of omega-9 fatty acids in inflammation and cancer management were discussed. Preclinical and clinical evidence indicating that SCFAs and PUFAs may have the potential to boost immunotherapy efficacy was demon-strated and opportunities for successful application of nutritional interventions focusing on SCFAs and PUFAs to increase the therapeutic potential of immunotherapeutic approaches for cancer was addressed as well.

Last part of this thesis was focused on combination of plant extracts and biomass from *Rhodotorula kratochvilovae* yeast strain. Combinations highly increased antimicrobial activity, the highest potential showed extract with mixture of clove extract where at 0.1 mg/mL was 48 % of inhibition and at 0.0015 mg/mL was inhibition still around 30 %. On the other hand, yeast strain *Metschnikowia pulcherrima* was effective only around 12 % against gram-negative growth. Mixtures of *Rhodotorula kratochvilovae* and acai or curcuma showed the highest potential in inhibition of growth of gram-positive microorganisms, around 30 % in higher concentration extracts. The inhibition was concentration dependent for all extracts.

As part of the work, various projects were solved. one of which was. for example, a project with the company Vincentka. The goal of this collaboration was to develop a new type of energy drink based on Vincentka natural mineral water from Luhačovice. Various natural extracts in several forms were used for this. Lyophilized, filtered, macerated, essential oils or concentrated tinctures.

6 CONCLUSION

The main results could be summarized as follows:

- The highest content of phenolic substances (4.723 mg/g) and antioxidant effect (5.26 mg /g) was determined in turmeric extract.
- Clove extract showed the highest antibacterial effect to Serratia marcescence cells. When measuring the inhibition of *Micrococcus luteus*, the most effective was oregano extract.
- Cytotoxic effect of natural plant extract was tested at HaCaT and B16F1 cell lines. Acai extract was toxic for B16F1 and, at the same time, not toxic for the HaCaT cell line at all tested concentrations. There was no cytotoxicity observed in Caco-2 cell lines.
- Curcuma affected the cell cycle of Raji cell lines by slow-down of the transition of the cells through the cell cycle phases.
- 4 yeast strains were cultivated for biomass and lipids production. *Metschnikowia pulcherrima* has the highest amount of oleic, palmitic, stearic, and linolic acid
- Rhodotorula katochvilovae has the highest amount of total glucans, 7.12 g/L of biomass and from which 4.75 g/L was beta-glucans.
- Apoptotic effect of natural extracts was measured. When comparing viable/apoptotic cells treated with microbial extracts, *Phaffia rhodozyma* effected B16F1 cells in ratio (30:25 viable:apoptotic). The only sample with higher ration of viable cells than apoptotic.
- When mixing biomass of *Rhodotorula katochvilovae* with clove extract, the concentration at 0.1 mg/mL caused 48 % inhibition, and at 0.0015 mg/mL the inhibition was still around 30 % for gram-negative strain.
- Mixtures of *Rhodotorula kratochvilovae* and acai or curcuma showed the highest potential in inhibition of the growth of gram-positive microorganisms.

In order to conclude results from the presented thesis it should be taken into account that to complex activity contribute not only the biological effects of individual natural or microbiological extracts, but also their harmlessness for healthy cell lines. It is advisable to include immortalized yeast biomass in combination with natural extracts in food supplements in a suitable form. Ideally. tablet or capsule form colud be recommended due to the possible taste of biomass or coated microtablets in gel capsules.

7 REFERENCES

1. UNEJA. Vijay K.. Hari P. DWIVEDI a Xianghe YAN. Novel Natural Food Antimicrobials. Annual Review of Food Science and Technology. 2012. 3(1). 381-403. ISSN 1941-1413 doi:10.1146/annurev-food-022811-101241
2. AHMAD KHAN. Mohd Sajjad a Iqbal AHMAD. Herbal Medicine. New Look to Phytomedicine. Elsevier. 2019. 2019. s. 3-13. DOI: 10.1016/B978-0-12-814619-4.00001-X. ISBN 9780128146194.
3. ALTAF. Mohd Musheer. Mohd Sajjad AHMAD KHAN a Iqbal AHMAD. Diversity of Bioactive Compounds and Their Therapeutic Potential. New Look to Phytomedicine. Elsevier. 2019. 2019. s. 15-34. DOI: 10.1016/B978-0-12-814619-4.00002-1. ISBN 9780128146194. https://linkinghub.elsevier.com/retrieve/pii/B9780128146194000021
4. MANSON. Margaret M. Cancer prevention – the potential for diet to modulate molecular signalling. Trends in Molecular Medicine. 2003. 9(1). 11-18. ISSN 14714914. Available z: doi:10.1016/S1471-4914(02)00002-3
5. KRIS-ETHERTON. Penny M. Kari D HECKER. Andrea BONANOME. Stacie M COVAL. Amy E BINKOSKI. Kirsten F HILPERT. Amy E GRIEL a Terry D ETHERTON. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. The American Journal of Medicine. 2002. 113(9). 71-88. ISSN 00029343. Available z: doi:10.1016/S0002-9343(01)00995-0
6. SARKAR. Fazlul H. a Yiwei LI. Using Chemopreventive Agents to Enhance the Efficacy of Cancer Therapy. Cancer Research. 2006. 66(7). 3347-3350. ISSN 0008-5472. Available z: doi:10.1158/0008-5472.CAN-05-4526
7. D'INCALCI. Maurizio. William P STEWARD a Andreas J GESCHER. Use of cancer chemopreventive phytochemicals as antineoplastic agents. The Lancet Oncology [online]. 2005. 6(11). 899-904. ISSN 14702045. Available z: doi:10.1016/S1470-2045(05)70425-3
8. POSCHENRIEDER. Charlotte. Josep ALLU. Roser TOLR. Merc LLUGANY a Juan BARCEL. Trace Elements and Plant Secondary Metabolism: Quality and Efficacy of Herbal Products. Trace Elements as Contaminants and Nutrients. Hoboken. NJ. USA: John Wiley. 2008. 99-120 [cit. 2016-07-30]. DOI: 10.1002/9780470370124.ch5. ISBN 978-047- 0370-124.
9. MARCASON. Wendy. What Is the Açaí Berry and Are There Health Benefits?. Journal of the American Dietetic Association. 2009. 109(11). ISSN 00028223. Available from: doi:10.1016/j.jada.2009.09.017
10. CARVALHO. Ana Vânia. Tayse FERREIRA FERREIRA DA SILVEIRA. Rafaella de Andrade MATTIETTO. Maria do Socorro PADILHA DE OLIVEIRA a Helena Teixeira GODOY. Chemical composition and antioxidant capacity of açaí (Euterpe oleracea) genotypes and commercial pulps. *Journal of the Science of Food and Agriculture*[online]. 2017. **97**(5). 1467-1474 [cit. 2018-10-26]. DOI: 10.1002/jsfa.7886. ISSN 00225142. Available from: http://doi.wiley.com/10.1002/jsfa.7886
11. ASSAN. MA. EH MOHAMED. S ABDELHAFEZ a TA ISMAIL. Effect of clove and cinnamon extracts on experimental model of acute hematogenous pyelonephritis in albino rats: Immunopathological and antimicrobial study. International Journal of Immunopathology and Pharmacology. 2015. 28(1). 60-68. ISSN 2058-7384. Available from: doi:10.1177/0394632015572075

12. KUANG. XUAN. BIN LI. RUI KUANG. XIAODONG ZHENG. BO ZHU. BAOLI XU a MEIHU MA. Granularity and antibacterial activities of ultra-fine cinnamon and clove powders. Journal of Food Safety [online]. 2011. 31(3). 291-296. ISSN 01496085. Available from: doi:10.1111/j.1745-4565.2011.00300.x
13. NUÑEZ. L. a M. D' AQUINO. Microbicide activity of clove essential oil (Eugenia caryophyllata). *Brazilian Journal of Microbiology* [online]. 2012. **43**(4). 1255-1260. DOI: 10.1590/S1517-83822012000400003. ISSN 1517-8382. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1517-83822012000400003&lng=en&nrm=iso&tlng=en
14. VAFAEIPOUR. Zeinab. Bibi Marjan RAZAVI a Hossein HOSSEINZADEH. Effects of turmeric (Curcuma longa) and its constituent (curcumin) on the metabolic syndrome: An updated review. Journal of Integrative Medicine. 2022. 20(3). 193-203. ISSN 20954964. Available from: doi:10.1016/j.joim.2022.02.008
15. LEE. Hwa-Young. Seung-Wook KIM. Geum-Hwa LEE. et al. Curcumin and Curcuma longa L. extract ameliorate lipid accumulation through the regulation of the endoplasmic reticulum redox and ER stress. *Scientific Reports* [online]. 2017. **7**(1). ISSN 2045-2322. Available from: doi:10.1038/s41598-017-06872-y
16. Park M.. Bae J.. Lee D.S. Antibacterial activity of [10]-gingerol and [12]-gingerol isolated from ginger rhizome against periodontal bacteria. Phytother. Res. 2008;22:1446–1449. doi: 10.1002/ptr.2473.
17. Sa-Nguanpuag K.. Kanlayanarat S.. Srilaong V.. Tanprasert K.. Techavuthiporn C. Ginger (Zingiber officinale) oil as an antimicrobial agent for minimally processed produce: A case study in shredded green papaya. Int. J. Agric. Biol. 2011;13:895–901.
18. BENZIE. Iris F. F. a Sissi WACHTEL-GALOR. *Herbal medicine: biomolecular and clinical aspects*. 2nd ed. Boca Raton: CRC Press. c2011. Oxidative stress and disease. 28. ISBN 978-1-4398-0713-2.
19. OH. Ga-Na a Sang-Wook SON. Efficacy of Korean Red Ginseng in the Treatment of Alopecia Areata. *Journal of Ginseng Research* [online]. 2012. **36**(4). 391-395. DOI: 10.5142/jgr.2012.36.4.391. ISSN 1226-8453.
20. ORKUN;CENGİZ. BABACAN. Oregano bitkisinin bazı Salmonella serotipleri üzerine antibakteriyel etkinliğinin belirlenmesi. Ankara Üniversitesi Veteriner Fakültesi Dergisi. 2012. 59(2). 103-106. ISSN 1300-0861. Available from: doi:10.1501/Vetfak_0000002510
21. SANTOYO. S.. S. CAVERO. L. JAIME. E. IBAÑEZ. F. J. SEÑORÁNS a G. REGLERO. Supercritical Carbon Dioxide Extraction of Compounds with Antimicrobial Activity from Origanum vulgare L: Determination of Optimal Extraction Parameters. Journal of Food Protection [online]. 2006. 69(2). 369-375. ISSN 0362-028X. Available from: doi:10.4315/0362-028X-69.2.369
22. ORKUN;CENGİZ. BABACAN. Oregano bitkisinin bazı Salmonella serotipleri üzerine antibakteriyel etkinliğinin belirlenmesi. Ankara Üniversitesi Veteriner Fakültesi Dergisi [online]. 2012. 59(2). 103-106. ISSN 1300-0861. Available from: doi:10.1501/Vetfak_0000002510
23. TALEB. M.H.; Abdeltawab. N.F.; Shamma. R.N.; Abdelgayed. S.S.; Mohamed. S.S.; Farag. M.A.; Ramadan. M.A. Origanum vulgare L. Essential Oil as a Potential Anti-Acne Topical Nanoemulsion—In Vitro and In Vivo Study. Molecules 2018. 23. 2164.

24. HASHMI. Muhammad Zaffar. Vladimir STREZOV a Ajit VARMA. ed. Antibiotics and Antibiotics Resistance Genes in Soils [online]. Cham: Springer International Publishing. 2017. Soil Biology. ISBN 978-3-319-66259-6. doi:10.1007/978-3-319-66260-2

25. BLOND A.. Cheminant M.. Destoumieux-Garzon D.. Segalas-Milazzo I.. Peduzzi J.. Goulard C.. Rebuffat S. Thermolysin-linearized microcin J25 retains the structured core of the native macrocyclic peptide and displays antimicrobial activity. Eur. J. Biochem. 2002;269:6212–6222. doi: 10.1046/j.1432-1033.2002.03340.x.

26. Tadesse M.. Tabudravu J.N.. Jaspars M.. Strom M.B.. Hansen E.. Andersen J.H.. Kristiansen P.E.. Haug T. The antibacterial ent-eusynstyelamide B and eusynstyelamides D. E. and F from the Arctic bryozoan Tegella cf. spitzbergensis. J. Nat. Prod. 2011;74:837–841. doi: 10.1021/np100499c.

27. LO Giudice A.. Bruni V.. Michaud L. Characterization of Antarctic psychrotrophic bacteria with antibacterial activities against terrestrial microorganisms. J. Basic Microbiol. 2007;47:496–505. doi: 10.1002/jobm.200700227.

28. SANCHEZ L.A.. Hedstrom M.. Delgado M.A.. Delgado O.D. Production. purification and characterization of serraticin A. a novel cold-active antimicrobial produced by Serratia proteamaculans 136. J. Appl. Microbiol. 2010;109:936–945. doi: 10.1111/j.1365-2672.2010.04720.x

29. Phelan R.W.. Barret M.. Cotter P.D.. O'Connor P.M.. Chen R.. Morrissey J.P.. Dobson A.D.. O'Gara F.. Barbosa T.M. Subtilomycin: A new lantibiotic from Bacillus subtilis strain MMA7 isolated from the marine sponge Haliclona simulans. Mar. Drugs. 2013;11:1878–1898. doi: 10.3390/md11061878.

30. ERDEM BÜYÜKKIRAZ. Mine a Zülal KESMEN. Antimicrobial peptides (AMPs): A promising class of antimicrobial compounds. Journal of Applied Microbiology [online]. 2022. 132(3). 1573-1596 [cit. 2023-01-19]. ISSN 1365-2672: doi:10.1111/jam.15314

31. JACH M.E.; Malm. A. Yar-rowia lipolytica as a valuable source of bioactive compounds. Molecules 2021. 26. x.

32. GROENEWALD. Marizeth. Teun Boekhout. Cécile Neuveglise. Claude Gaillardin. Piet W. M. van Dijck a Markus Wyss. Yarrowia lipolytica: Safety assessment of an oleaginous yeast with a great industrial potential. Critical Reviews in Microbiology [online]. 2013. 40(3). 187-206. ISSN 1040-841X. doi:10.3109/1040841X.2013.770386

33. WIRTH. Fernanda a Luciano Z. goldani. Epidemiology of Rhodotorula: An Emerging Pathogen. Interdisciplinary Perspectives on Infectious Diseases. 2012. 2012. 1-7. ISSN 1687-708X. doi:10.1155/2012/465717

34. SHRAMKO. Viktoriya S.. Yana V. POLONSKAYA. Elena V. KASHTANOVA. Ekaterina M. STAKHNEVA a Yuliya I. RAGINO. The Short Overview on the Relevance of Fatty Acids for Human Cardiovascular Disorders. *Biomolecules*. 2020. **10**(8) [cit. 2022-02-04]. ISSN 2218-273X. Available from: doi:10.3390/biom10081127

35. CHAICHIAN. Shahla. Bahram MOAZZAMI. Fatemeh SADOUGHI. Hamed HADDAD KASHANI. Marsa ZAROUDI a Zatollah ASEMI. Functional activities of beta-glucans in the prevention or treatment of cervical cancer. *Journal of Ovarian Research*. 2020. **13**(1). ISSN 1757-2215. Available from: doi:10.1186/s13048-020-00626-

36. FARAG. Mohamed A. a Mohamed Z. GAD. Omega-9 fatty acids: potential roles in inflammation and cancer management. Journal of Genetic Engineering and Biotechnology. 2022. 20(1). ISSN 2090-5920. Available z: doi:10.1186/s43141-022-00329-0

37. SOQUETTA. Marcela Bromberger. Lisiane de Marsillac TERRA a Caroline Peixoto BASTOS. Green technologies for the extraction of bioactive compounds in fruits and vegetables. CyTA - Journal of Food. 2018. 16(1). 400-412.

38. SINGLETON, V. L., Jr J. A. ROSSI. Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. American Journal of Enology and Viticulture 1965, 16 (3): 144-158

39. PAULOVÁ. Hana. Hana BOCHOŘÁKOVÁ a Eva TÁBORSKÁ. Metody stanovení antioxidační activity přírodních látek in vitro. Chemické listy. 2004. č. 98. s. 174-179.

40. BALOUIRI. Mounyr. Moulay SADIKI a Saad Koraichi IBNSOUDA. Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis. 2016. 6(2). 71-79. ISSN 20951779. Available from: doi:10.1016/j.jpha.2015.11.005

41. ANGIUS. Fabrizio a Alice FLORIS. Liposomes and MTT cell viability assay: An incompatible affair. Toxicology in Vitro. 2015. 29(2). 314-319. DOI: 10.1016/j.tiv.2014.11.009

42. B-Glucan Assay Kit (Yeast & Mushroom). USA: Megazyme [cit. 2018-07-08]. Available from: https://secure.megazyme.com/Beta-Glucan-Assay-Kit-Yeast-Mushroom

43. ApoFlowEx FITC Kit. Exbio.https://www.exbio.cz/getattachment/f7fa2c4e-9e54-455f-ab69-ed6ad540937d/ED7044_TDS_v4_EN.pdf.aspx

8 CURRICULUM VITAE

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Time:	20. 10. 2018 – 20. 2. 2019
Subject:	Cell lines in biomedical research, cytotoxicity, genotoxicity, apoptosis tests

9 PUBLICATIONS

PAPERS

SZOTKOWSKI. M.; BYRTUSOVÁ. D.; NĚMCOVÁ. A.; **VYSOKÁ. M.;** RAPTA. M.; SHAPAVAL. V.; MÁROVÁ. I. Study of Metabolic Adaptation of Red Yeasts to Waste Animal Fat Substrate. Microorganisms. 2019. 7.11 (Jimp. Q2)

VYSOKA. M.. Martin SZOTKOWSKI. Eva SLANINOVA. Lucia DZURICKA. Paulina STRECANSKA. Jana BLAZKOVA a Ivana MAROVA. Oleaginous Yeast Extracts and Their Possible Effects on Human Health. *Microorganisms* [online]. 2023. **11**(2) [cit. 2023-04-25]. ISSN 2076-2607. Available z: doi:10.3390/microorganisms11020492 (Jimp. Q2)

VYSOKÁ. M.; MÁROVÁ. I. Microbial and plant extracts and their effect on human health based on their antioxidant and antimicrobial activity. The EuroBiotech Journal. The EuroBiotech Journal. 2021. s. 17-17. ISSN: 2564-615X (Jost)

VYSOKÁ. M.. V.L. Mazzarello. G. Delogu. I. Márová. Antimicrobial activity of selected plant extracts and its possibility to effect human health. Biotechnology and Biotechnological Equipment. Biotechnology & Biotechnological Equipment. Volume 35. Issue sup1 (2021). 2021. s. 25-26. ISSN: 1314-3530 (Jimp. Q4)

CONFERENCE ABSTRACTS

VYSOKÁ. M.; MAZZARELLO. V..; SCIOLA. L; MÁROVÁ. I. BIOLOGICAL EFFECTS OF SELECTED PLANT EXTRACTS. 2019.

HOOVÁ. J.; **VYSOKÁ. M.;** DZURICKÁ. L.; MATOUŠKOVÁ. P.; MÁROVÁ. I. THE USE OF BIOACTIVE COMPOUNDS AND THEIR ENCAPSULATION INTO LIPOSOMES TO INCREASE THE EFFECTIVENESS AND CONTROL RELEASE. In NANOCON 2018. 10TH ANNIVERSARY INTERNATIONAL CONFERENCE ON NANOMATERIALS – RESEARCH & APPLICATION. Ostrava. Czech republic: TANGER. 2019. s. 386-391. ISBN: 978-80-87294-89-5.

VYSOKÁ. M.; BYRTUSOVÁ. D.; MATOUŠKOVÁ. P.; MÁROVÁ. I. ANALYSIS OF β-GLUCANS IN SELECTED COMMERCIAL PRODUCTS AND HIGHER FUNGI WITH THEIR POSSIBLE USE IN PHARMACEUTICAL INDUSTRY. 7th Meeting on Chemistry and Life 2018. Book of abstracts. Ing. Petr Dzik. Ph.D. 2018. s. 153-153. ISBN: 978-80-214-5488-0.

VYSOKÁ. M.. M. Szotkowski. K. Bradáčová. J. Holub. P. Matoušková. S. Šimanský. M. Čertík. I. Márová. BIOCONVERSION OF LOW-COST FAT MATERIALS INTO HIGH-VALUE PUFA-CAROTENOID-RICH BIOMASS. 34th Annual Conference on Yeasts. Book of abstracts. 2018. s. 64-64. ISSN: 1336-4839.

HOOVÁ. J.; **VYSOKÁ. M.;** DZURICKÁ. L.; MATOUŠKOVÁ. P.; MÁROVÁ. I. The Use of Bioactive Compounds and Their Encapsulation into Liposomes to Increase the Effectiveness and Control Release. Nanocon - Abstracts. 1. Ostrava: TANGER Ltd.. 2018. s. 103-103. ISBN: 978-80-87294-85-7.

MATOUŠKOVÁ. P.; BOKROVÁ. J.; PAVELKOVÁ. R.; **VYSOKÁ. M.;** MÁROVÁ. I. Organic particles as delivery systems for complex source of antimicrobial and antioxidant component for food applications. Praha: 2017. s. 267-267.

MATOUŠKOVÁ. P.; BOKROVÁ. J.; BENDOVÁ. A.; SOSKOVÁ. S.; **VYSOKÁ. M.**; MÁROVÁ. I. Antimicrobial Activity of Liposomes with Encapsulated Active Components from Plant Extracts. Ostrava: TANGER Ltd.. 2017. s. 137-137.

BOKROVÁ. J.; MATOUŠKOVÁ. P.; SOSKOVÁ. S.; PLACHÁ. M.; HOOVÁ. J.; PAVELKOVÁ. R.; **VYSOKÁ. M.;** MÁROVÁ. I. Candida glabrata - a Model Organism for Studying Antifungal Activity of Plant Extracts. 44th Annual Conference on Yeast Book of Abstracts. 34th Annual Conference on Yeasts. Book of abstracts. 2017. s. 50 (s.)ISSN: 1336-4839.

PROJECTS

CMV: „udržitelnost a rozvoj“ LO1211

LipofungI „Bioconversion of low-cost fat materials into high-value PUFA-Carotenoid-rich biomass“ 268305

Vincentka „ preparation of new type of energy drink with mineral water Vincentka“

Aplikace moderních postupů při výrobě, zpracování a analýze potravin, jejich složek a surovin a při valorizaci odpadů. FCH-S-18-5334

Využití pokročilých metod a postupů v rámci moderních potravinářských věd FCH-S-19-5983

Výzkum a vývoj nové generace inkontinenční pomůcky FW01010649

Aplikace progresivních metod a postupů v rámci moderních potravinářských věd FCH-S-20-6316

Vývoj a využití progresivních postupů při zpracování, analýze a hodnocení potravin, potravinářských surovin a odpadů. FCH-S-21-7483

Příspěvek k rozvoji moderních potravinářských věd FCH-S-22-7961