CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE, INSTITUTE OF TROPICS AND SUBTROPICS

IN VITRO EVALUATION OF SELECTIVE ANTIMICROBIAL ACTIVITY OF PLANT DERIVED COMPOUNDS AGAINST BIFIDOBACTERIA AND CLOSTRIDIA

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ABSTRACT

There is a need of developing antimicrobial agents against intestinal pathogens, which are not harmful to beneficial intestinal microflora. Based on analysis of the scientific literature, we selected five plant compounds, which we considered perspective to prove selective antimicrobial activity against pathogenic clostridia and beneficial bifidobacteria. Using the broth microdilution method, we performed antimicrobial tests of these chemicals against three standard strains of bifidobacteria and clostridia. The alkaloid 8-hydroxyquinoline (8HQ) and anthraquinone-2-carboxylic acid (AQCA) showed selective antimicrobial activity. The average minimum inhibitory concentrations (MICs) of AQCA for clostridia and bifidobacteria were 192 and 680 μM, respectively. 8HQ showed even more distinct selective properties with average MIC = 298 μM for clostridia and > 2048 μM for bifidobacteria. 8HQ proved to be a potential selective agent, so we carried out further microbial assay against wider range of standard and isolated bacterial strains. Antimicrobial tests against 12 clostridia and 10 bifidobacteria confirmed our hypothesis, that 8HQ possesses significant selective inhibitory action. All clostridia were susceptible with the maximum MIC 128 μg/ml. Standard strains, *C. tertium, C. clostridioforme, C. difficile, C. perfringens,* and *C. ramosum* were among the most sensitive strains exhibiting the MICs 8, 16, 32, 32, and 32 μg/ml, respectively. Interestingly, two isolated strains, which are not associated with infections, have shown the least susceptibility to 8HQ. In contrast to clostridia, bifidobacteria were much less susceptible. MICs for isolated strains *B. bifidum* and *B. dentium* were ≥ 2048 and 1024 μg/ml, for standard strain of *B. breve* it was 1024 μg/ml. Five strains exerted MIC at 512 μg/ml and two remaining strains 256 μg/ml. The average MIC for clostridia was thirteen times lower than for bifidobacteria. These results indicate that 8HQ is a prospective compound for use in pharmaceutical and veterinary preparations. It could also be applied in microbiology for bifidobacteria isolation.

Key words: plant compounds, selective microbial activity, bifidobacteria, clostridia

ABSTRAKT

V poslední době je snaha vyvinout antibakteriální prostředek se selektivním účinkem, který působí proti střevním patogenům, ale nepoškozuje prospěšnou probiotickou mikrofloru. Cílem našeho výzkumu bylo najít rostlinnou látku, která inhibuje růst střevních patogenních bakterií rodu *Clostridium,* ale nepůsobí inhibičně na probiotické bifidobakterie. Na základě rešerše vědecké literatury jsme nejprve vybrali a testovali celkem pět rostlinných látek proti třem kmenům bifidobakterií a třem kmenům klostridií. Použili jsme mikrodiluční metodu a stanovili minimální inhibiční koncentrace (MIC) těchto látek. Alkaloid 8-chinolinol a anthrachinon-2-karboxylová kyselina (AQCA) projevily selektivně inhibiční účinek ve prospěch bifidobakterií. AQCA byla ze všech látek nejúčinnější proti klostridiím, inhibovala je při průměrné koncentraci 192 µM, zatímco průměrná MIC u bifidobakterií byla 680 µM. 8HQ prokázal vyšší selektivitu, kdy průměrná MIC u klostridií byla 298 µM a u bifidobakterií 2048 µM. 8HQ byl na základě těchto výsledků vybrán pro další testování proti širšímu spektru klostridií a bifidobakterií. Tyto testy pak potvrdily naši hypotézu, že 8HQ vykazuje značnou selektivní antibakteriální aktivitu. Všech 12 kmenů klostridií bylo citlivých k této látce, nejnižší MIC byly zjištěny u standardních kmenů *C. tertium* (8 µg/ml) a *C. clostridioforme* (16 µg/ml). Další standardní kmeny *C. butyricum*, *C. perfringens* a *C. difficile* byly inhibovány při koncentraci 32 µg/ml. Nejvyšší naměřená MIC - 128 µg/ml - byla naměřena u dvou izolovaných kmenů. Je zajímavé, že se v těchto případech nejednalo o klostrídie, které jsou spojené s nebezpečím infekce. V porovnání s klostridiemi, všech 10 kmenů bifidobakterií prokázalo mnohem větší toleranci k 8HQ, přičemž izolovaný kmen *B. bifidum* nebyl inhibován ani při nejvyšší testované koncentraci, 2048 µg/ml. Pro další dva kmeny byla naměřena MIC 1024 µg/ml, pro pět kmenů 512 µg/ml a pro zbylé dva 256 µg/ml. Porovnáním průměrů minimálních inhibičních koncentrací jsme zjistili, že 8HQ projevil 13 x vyšší antibakteriální účinek proti klostridiím než proti bifidobakteriím. Na základě výsledků naší práce můžeme tuto látku označit jako perspektivní pro vývoj farmaceutických nebo veterinárních přípravků, které mají potlačit výskyt patogenních klostridií a zároveň nenarušit kolonizaci zdraví prospěšných bifidobakterií. Další možné využití by mohl 8HQ najít jako přídavek do mikrobiálního média pro izolaci bifidobakterií.

Klíčová slova: rostlinné látky, selektivní antimikrobiální aktivita, bifidobakterie, klostridie

CERTIFICATION

I declare that this thesis, submitted for a partial fulfillment of the requirements for the Master degree at the Institute of the Tropics and Subtropics of the Czech University of Life Sciences Prague, is wholly my own work, unless otherwise referenced or acknowledged.

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CONTENT

FOREWORD

The human body contains thousands of species of bacteria, viruses, fungi, and protozoa. The microbes that normally live in or on the body usually cause no ill effects to their host, more to the contrary, it is known, that the host requires their colonization for its development and health. The interaction between them and the host plays a critical role in the host's survival. Some of the commensals have especially beneficial impacts on human health, such as bifidobacteria and lactobacilli. They are a normal part of the human intestinal microflora (HIM) and they play a significant role in the immune system. Their insufficient presence in the intestines is associated with health disorders. They are called probiotic bacteria and are used in many disease treatment and prevention strategies. They behave as pathogens extremely rarely. On the other hand, some intestinal commensals tend to be opportunistic pathogens, and when the microbial ecosystem is disturbed, they often cause infections. For example, *Escherichia coli* and *Clostridium* spp. are very frequent endogenous pathogens. Gastrointestinal (GI) infections can also be caused by pathogenic bacteria getting into the gastrointestinal tract (GIT) from the environment. Certain species of *Salmonella*, *Yersinia*, *Campylobacter*, *Clostridium* and *E. coli* belong among the most frequent exogenous intestinal pathogens. Gastroenteritis (term for various pathologic states of the GIT) causing pathogens are the second leading cause of morbidity and mortality worldwide. In the developed countries, diarrhea is the most common reason for missing work, whilst in the developing world, it is a leading cause of death. Internationally, the mortality rate is 5-10 million deaths each year.

The most common treatment against bacterial infections are antibiotics (ATBs). Although the discovery of ATBs was a great step in treatment of infectious diseases, two serious problems appeared during the era of wide spread usage. Firstly, bacterial resistance, a worldwide health problem that continues to grow. A wide range of bacteria has developed various defense systems against ATBs. Frequent unjustified treatment with the wide spectrum ATBs and usage of ATBs as food supplement for domestic animals worsen this problem significantly. Resistant bacteria strains cause nosocomial infections worldwide. For example, oral vancomycin is being restricted in most hospitals because of vancomycin-resistant enterococci (VRE), which were demonstrated to be able to transfer resistance into

clostridia via plasmids. In LDC countries, ATB resistant bacteria considerably worsen mortality caused by bacterial pathogens. Formerly, first line antimicrobials were both effective and quite affordable, but with the onset of bacterial resistance, newer treatments are getting too costly for the vast majority of people living in LDCs. This negative health trend calls for a global initiative for the development of new prevention and treatment strategies of infectious diseases. After 100 years of synthetic drug era, mainstream medicine has become more receptive to the research and development of plant-based medicines. In the last 15 years, the search for new anti-infection agents has notably risen and has occupied many scientists in the field of ethnopharmacology. Thousands of plant species have been tested against hundreds of bacterial strains in vitro and many medicinal plants were found active against a wide range of bacteria.

Second problem arising with ATB treatment, is that together with pathogens, they indiscreetly reduce the number of beneficial human microbiota. They upset this uniquely balanced gut ratio, allowing pathogens to propagate in a largely unrestrained environment. A typical example of this case is a *Clostridium difficile*-associated diarrhea (CDAD) or pseudomembranous colitis, which often follow prolonged ATB treatment. CDAD causes substantial morbidity, financial cost and, unfortunately, the proportion and severity of CDAD has been markedly increasing. The need of new antimicrobial agents, which would selectively inhibit harmful clostridia without affecting beneficial bacteria, has inspired a number of scientists to examine plant extracts and compounds against intestinal bacteria. Some of these plant-derived products were reported to prove such desired selective antimicrobial action.In my last work in 2010, I collected data about these studies. As a result of this work, several substances were proposed for further research as potential selective antimicrobial agents against pathogenic clostridia and probiotic bifidobacteria. Last year, I decided to continue in my previous work, and I chose the evaluation of five most prospective substances as the subject of my diploma thesis.

1. INTRODUCTION

1.1 Human Intestinal Microflora (HIM)

1.1.1 Characterization

The human's gastrointestinal tract (GIT) is a complex ecosystem that associates resident microflora and cells of various phenotypes lining the epithelial wall. It is inhabited by aerobic, facultative and anaerobic bacteria (Servin, 2004). The composition differs quantitatively and qualitatively along the tract. The microbial profile is typified by the absence of anaerobic microorganisms in the stomach and conversely their overwhelming predominance in the distal colon. Moreover, different microbial communities may be located in the intestinal lumen, in the mucus covering the epithelium, in the crypt spaces or in the different cells lining the epithelium, and in addition, some species adhere whereas others do not (Kasper, 2001). Facultative anaerobes are associated close to the epithelial layer for oxygen (Bhunia, 2008). A total of 400 - 500 different bacterial species exist in the human intestinal tract but only 40 species account for 99 % of all microorganisms (Kasper, 2001). Evidence that the intestinal microbiota is intrinsically linked with overall health is emerging (Davis, et al., 2009). GI ecological investigations have indicated that there are differences in composition of intestinal bacteria between patients and healthy control subjects, between young and elderly people, as well as between breast - and bottle - fed infants (Finegold, et al., 1975; Modler, et al., 1990; Kasper, 2001). The microbiota of patients with Alzheimer's disease or cancer is known to be mainly composed of *Bacteroides, Clostridium* and *Eubacterium*, with low percentage of *Bifidobacterium* species (Finegold, et al., 1975; Fujisawa, et al., 1992; Yamamoto, et al., 1997). Also changes of the gut microflora in elderly appear to involve a reduction in numbers of probiotic bacteria (lactobacilli and bifidobacteria) and an increase in numbers of potentially pathogenic species such as *Clostridium difficile*. These changes are generally described as GI disorders and infections (Malaguarnera, et al., 2011).

1.1.2 Colonization of the GIT

Mammals are born microbiota free. The first bacteria to colonize the gut originate from the birth canal and include aerobic and anaerobic bacteria, such as *Escherichia coli*, *Clostridium* spp., *Streptococcus* spp., *Lactobacillus* spp., *Bacteroides* spp., and *Bifidobacterium* spp. All the components are necessary for the gut to develop its specific intestinal functions (Mccracken, et al., 2001). Microbiota contributes toward postnatal intestinal maturation, development of intestinal architecture and angiogenesis (Bhunia, 2008). Recent studies with preterm born infants support the concept that the early intestinal colonization with organisms such as *Lactobacillus rhamnosus* and *Bifidobacterium infantis* protects infants against high mortality causing Necrotizing enterocolitis (Walker, 2000). The concept of positive health effect of early colonization by *Bifidobacteria* was also supported by studies comparing breast fed and formulae fed infants. With breast –fed infants the colonization starts primarily with *Bifidobacteria* (Kasper, 2001). Formulae fed infants, are more likely to be colonized by possibly harmful clostridia (Smith, et al., 1984). After all, weaning the child to early away predisposes it for food allergies (Bhunia, 2008). The barrier and immunological defense mechanisms of the intestinal mucosa are not fully developed till the age of one year. Therefore small children are at high risk of pathogen invasion and this is one of the main reasons of high infant mortality due to intestinal pathogens. As the infant makes transition to a mixed diet the colonization of their GIT increasingly resembles that typically seen in adults (Kasper, 2001).

1.1.3 Role and Physiological Functions of HIM

1.1.3.1 Metabolic Function

Autochthonous microbiota help in the metabolism of indigestible compounds by synthesizing necessary enzymes. They play a key role in essential metabolic processes, like fermentation, bile acid and steroid transformations, metabolism of xenobiotic substances, mineral absorption and the activation and destruction of potential mutagenic metabolites (Bhunia, 2008). They work symbiotically in carbohydrate and protein digestion, where the host provides dietary residues or endogenously produced substrates for the bacteria, which in turn supply the host with metabolic end products. Microbial carbohydrate fermentation produces generated short-chain fatty acids (SCFA). SCFA - butyrate, acetate and propionate -

can be further metabolized for energy and this way, microbiota create an important part of fat metabolism (Bhunia, 2008).

1.1.3.2 Barrier Function

Microbiota stabilize mucosal barrier and protect the intestinal mucosa against colonization and invasion by pathogenic microorganisms. If the niches of intestinal ecosystem are occupied by commensals, pathogen intruders have relatively little chances to colonize the host. Bacteriocins produced by some natural flora can kill certain pathogens in the gut. There is evidence that the normal GI flora inhibits *C. difficile* growth and toxin release (Borriello, 1990); *Lactobacillus* together with *Bifidobacterium* inhibit invasion by *Salmonella*. In summary, microbiota provide frontline defense by inducing mucus secretion and protecting the host against invading pathogens (Bhunia, 2008).

1.1.3.3 Immunological Function

Gut microbiota help maintain immune homeostasis (Bhunia, 2008), suppress unnecessary inflammatory response, and participate in innate and adaptive immune system. Their components such as lipopolysaccharides, peptidoglycans, and formylated chemotactic oligopeptide translocate actively through mucosal barrier (mucus and epithelium) and are found in *lamina propria* and activate macrophages, dendritic cells, neutrophils, natural killer cells, and T-cells. The amount of IgA (Immunoglobulin - antibody produced by B-cells) production is directly dependent on the number of gut flora. The germ-free animals have low levels of plasma cells and IgA, and decreased number of immune cells (Kasper, 2001).

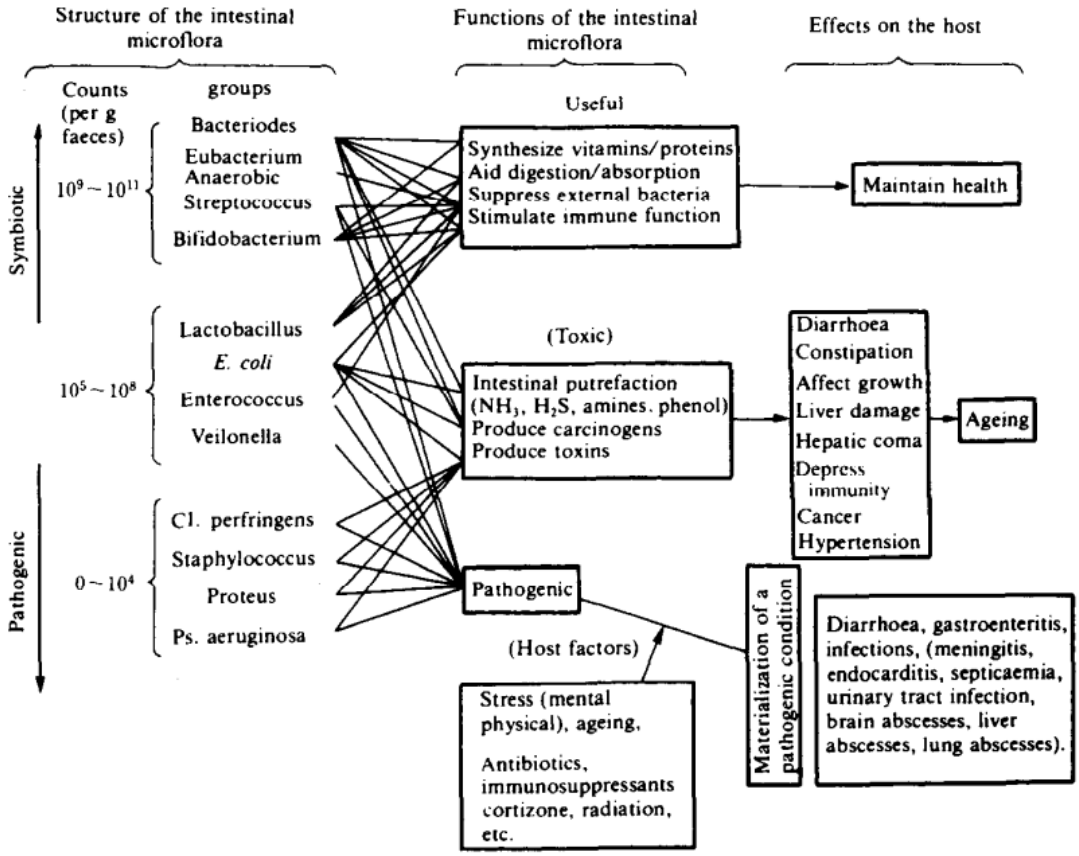
1.1.3.4 Reducing Cancer Risks

Microbiota have a critical role in setting the tone for a healthy bowel including the risk for developing colorectal cancer. They generate bioactive compounds from food components, such as equol from isoflavones, enterodiol and enterolactone from lignans and urolithins from ellagic acid, which retard experimentally induced cancers. The SCFAs' control proliferation and differentiation also reduces colon cancer risk (Davis, et al., 2009). Specific strains of bacteria have been implicated in the pathogenesis of cancer, including *Clostridium* spp., *Streptococcus bovis*, *Bacteroides*, and *Helicobacter pylori* (Gold, et al., 2004; Moore, et

al., 1995; Nakamura, et al., 2002; Peek, et al., 2002). Conversely, some strains of bacteria, including *L. acidophilus* and *Bif. longum*, have been shown to inhibit carcinogen-induced colon tumor development (McCintosh, et al., 1999). Thus, a balance between "detrimental" and "beneficial" bacteria has implications in setting the stage for cancer (Davis, et al., 2009).

1.1.4 Common Commensal Representatives

Host relationships of the intestinal bacteria (Arunachalam, 1999)



G^+ Rods, Anaerobes, Non–Spore Forming

Bifidobacterium

Bifidobacteria live in the intestines as commensals throughout the life and make an important part of the beneficial intestinal microbiota. Although not numerically dominant part of the IM, they are considered as key commensals that promote a healthy GIT.

Newborns are colonized within days after birth. Recent studies with preterm born infants support the concept that the early intestinal colonization with organisms such as *B. infantis* protects infants against high mortality causing Necrotizing enterocolitis (Walker, 2000). Together with other commensal microbiota, bifidobacteria provide frontline defense by inducing mucus secretion and protecting the host against invading pathogens (Bhunia, 2008). By elderly people, reduction in numbers of bifidobacteria and an increase in numbers of potentially pathogenic species such as *Clostridium* are generally described as GI disorders and infections (Malaguarnera, et al., 2011). Moreover, bifidobacteria together with *L. acidophilus* have been documented to inhibit carcinogen-induced colon tumor development (McCintosh, et al., 1999). Although the population of bifidobacteria in the intestine is stable, it is influenced by diet, antibiotics, stress etc. Due to their positive effect and extremely low pathogenicity, some species are widely used as probiotics (live microbial food supplements that beneficially affects the host by improving his intestinal microflora balance), which are discussed in a separate chapter.

Lactobacillus

Lactobacilli are present in the human GIT and vagina as beneficial part of the microflora complex. Together with bifidobacteria they belong to lactic acid producing bacteria. Lactic acid keeps the environment acidic, which helps to inhibit the growth of some harmful bacteria. They are common and mostly benign. They are widely explored for their significant probiotic functions and together with bifidobacteria are commonly contained in probiotic formulas. They were formerly considered non-pathogenic, but they have been reported to be rare opportunistic pathogens in the 1980th (Bayer, et al., 1978; Cox, et al., 1986). Superinfection may be possible only in an immunocompromised host.

Propionibacterium

This commensal is a recognized agent of modulating the immune response to unrelated antigens, which has been well documented (Pulverer, et al., 1990; Roszkowski, et al., 1990). Species from the normal flora can act as primary pathogens, but have never been reported to be involved in significant bacteremia (Salonen, et al., 1998).

Eubacterium

This genus is a part of the normal microflora. It is an important producer of SCFA. The species most observed in IT are *E. lentum* and *E. tortuosum* (Murray, et al., 1999).

Actinomyces

With the exception of *A. humiferus*, which is found exclusively in soil, the natural habitat of *Actinomyces* spp. appears to be the mucous membranes of humans or animals. Actinomycosis caused by *Actinomyces* spp. occurs throughout the world and it is neither a rare nor a common disease (Slack, et al., 1975).

G^+ Cocci, Anaerobes, Non-Spore Forming

Ruminococcus

They are among the first bacteria engaging the IT after birth. They are widely distributed as commensals of the normal flora in humans and animals, pathogenicity is exceedingly rare (Murray, et al., 1999).

Peptostreptococcus

They are widely distributed as members of the normal flora in humans and animals. They are opportunistic pathogens; bacteremia may follow obstetric or gynecologic infections (Murray, et al., 1999).

G^- Anaerobes, Non-Spore Forming

Prevotella & Porphyromonas

They are part of the normal flora of the mouth, upper respiratory tract, IT, and urogenital tract of humans and animals, where they participate on the saccharolytic metabolism. They are opportunistic pathogens, able to cause oral cavity, pleuropulmonary, oral, and genital infections (Civen, et al., 1995) and they are often resistant to the β-lactam ATBs (Murray, et al., 1999).

Veillonella

Veillonella parvula is a part of the normal human fecal flora. The pathogenicity of these anaerobic G^- cocci is rare. However, together with *Clostridium* spp. they belong to bacterial group with gas-producing ability and their population outbreak is associated with Irritable Bowel Syndrome (IBS) (Jiménez, 2009).

Fusobacterium

These G^- bacilli are usually found in the mouth, genital, GI, and upper respiratory tracts without causing an infection. *Fusobacterium* has exceptional ability to adhere with both G^- and G^+ plaque microorganisms. *F. necrophorum* is a rare, but very virulent species that may cause severe infection (Murray, et al., 1999).

G^+ Anaerobes, Spore – Forming

Clostridium

Most clostridia species stain G^+ during the early stage of growth. Most species are motile by means of peritrichous flagella. Principal habitats are the soil and the IT (Smith, et al., 1984). Several species reside in the lower IT of humans as a part of the normal flora. Examples of those commonly found include *C. innocuum, C. ramosum, C. paraputrificum, C. putrificum, C sporogenes, C. tertium, C. bifermentans* and *C. butyricum*. With *Veillonella* species they belong to bacterial group with gas – producing ability and their population outbreak is associated with IBS (Jiménez, 2009). Although most species occur as harmless saprophytes, they often become opportunistic pathogens. Some species cause exogenous diseases, like food-borne botulism, tetanus, and myonecrosis, which are well known historically and they are clinically important. However, **endogenous diseases, involving clostridia that are a natural part of the host's own microflora** are much more common. The development of clostridial disease is usually associated with special circumstances. Common predisposing factors include trauma, operative procedures, vascular stasis, obstruction, treatment of cancer patients with immunosuppressive agents or chemotherapeutic agents, prior treatment with ATBs (as in pseudomembranous colitis), and underlying illness such as

leukemia, carcinoma, or diabetes mellitus. Under the right conditions, clostridia can invade and multiply in essentially any tissue of the body (Murray, et al., 1999).

Clostridium difficile

It was first described in 1935 and by 1978 it had been identified as the pathogen responsible for pseudomembranous colitis (Hall, et al., 1935; Bartlett, et al., 1978). It has been isolated from diverse natural habitats. The feces of infants commonly contain *C. difficile*, but it is seen less frequently in healthy adults (George, et al., 1985). It is the major cause of antibiotic-associated diarrhea and pseudomembranous colitis and also the most frequently identified cause of hospital-acquired diarrhea, also called *C. difficile* associated diarrhea (CDAD) (Gerding, et al., 1995; Johnson, et al., 1990). Although it is usually acquired from the patient's own GI tract, it may be spread exogenously from person to person during outbreaks of hospital-acquired diarrhea or colitis (Kim, et al., 1981). As reviewed by Gerding et al., hospitalized patients frequently become colonized with this organism (Gerding, et al., 1995). McFarland et al. reported that 21 % of 399 patients with negative cultures on admission to a hospital with a high prevalence of CDAD acquired *C. difficile* during hospitalization (McFarland, et al., 1987). Of these patients, 37 % developed diarrhea. Antimicrobial agents of all classes and several anticancer chemotherapeutic agents have been implicated in the development of CDAD or pseudomembranous colitis (George, 1989; Golledge, et al., 1992; Golledge, et al., 1995).

Clostridium perfringens

It is also called *C. welchii*. It produces a variety of biologically active proteins, or **toxins**, that play an important role in its pathogenicity. Five toxin types of *C. perfringens* have been identified, types A through E (Hatheway, 1990). The bacterium can be found in many different habitats, environment, such as sewage and soil, or the normal flora of GIT. It is a cause of many veterinary diseases. In humans, it causes a wide variety of clinical settings ranging from simple contamination of wounds to myonecrosis, clostridial cellulitis, intra-abdominal sepsis, gangrenous cholecystitis, postabortion infection with devastating septicemia, intravascular hemolysis, bacteremia in various clinical settings, aspiration pneumonia, necrotizing pneumonia, thoracic impyema, subdural empyema, brain abscess (Finegold, et al., 1989) (Gorbach, 1998), and clostridial myonecrosis - gas gangrene

(Summanen, et al., 1993) It is one of the most common bacterial causes of food-borne illness in the USA (Shandera, et al., 1983). Almost all U.S. outbreaks and cases *of C. perfringens* food-borne gastroenteritis appear to be due to type A strains (Shandera, et al., 1983). In *C. perfringens* type A food-borne disease, the food vehicle is almost always an improperly cooked meat or a meat product. In humans, the illness is usually mild, and most patients recover within 2 to 3 days after onset. *C. perfringens* may produce a severe necrotizing disease of the small bowel known as *enteritis necroticans*, which can occur sporadically or in an epidemic form. The syndrome has been called Darmbrand in Germany and **Pig-bell** in Papua New Guinea (Allen, 1997).

Clostridium septicum

It is isolated rarely from the feces of healthy individual. Whether *C. septicum* is part of the indigenous microflora, at least in low concentrations, has not been established. However, it has been found in the lumens of 10 – 68 % of appendixes (Bennion, et al., 1990). The portal of entry for *C. septicum* into the bloodstream is believed to be the ileocecal region of the bowel. Of particular interest has been the association of the bacteremia with malignancies, especially leukemia and lymphoma or carcinoma of the large bowel. Recognizing this bacteremia and starting appropriate treatment without delay cannot be overemphasized. Patients with this condition are usually acutely ill, with high temperatures, often metastatic spread of myonecrosis and mortality rates are significant (68 % or greater).

Other clostridia spp. associated with bacteremia are: *C. septicum*, *C. ramosum*, *C. clostridioforme, C. bifermentans, C. sordellii, C. tertium, C. paraputrificum, C. innocuum, C. butyricum, C. cadaveris*, and *C. sporogenes* (Brook, 1989; Gorbach, et al., 1975).

C. acetobutylicum has been found in the human colon, however, it is not known to be a part of normal human flora (Acetone-Butanol Fermentation Revisited, 1986). In addition, because the organism does not appear to be toxic to mammals through the production of intracellular or extracellular substances, the organism would have to be present in enormous quantities to produces any threat (Bacterial Toxins: a Table of Lethal Amounts, 1982).

Aerobes and Facultative Anaerobes, Non Spore - Forming

Escherichia coli

E. coli – a G^- rod, typically colonizes an infant's GIT within hours of life. It is the predominant facultative anaerobe in the human colonic flora, which survives also in an outer environment. Although *E. coli* is a part of the normal intestinal microflora, it may act as an opportunistic pathogen causing intestinal and extra-intestinal infections in immunocompromised patients. On contrary, the strain *Escherichia coli* Nissle was used during the 1st World War against acute shigellosis and salmonellosis. Microcin produced by this strain prevents invasion of *Salmonella*, *Shigella*, and *Listeria* (Arribas, et al., 2009). Anyway, there are *E. coli* strains associated with frequent serious infections even in healthy persons, causing urinary tract infections, bacteremia, meningitis, and infectious diarrhea or dysentery (Murray, et al., 1999).

Enterococcus

The nature permits these G^+ cocci survival in harsh environments. They can be found in soil, food, water, plants, animals, birds and insects. In humans they inhabit the GIT and the female genital tract. The prevalence of the different *Enterococcus* spp. appears to vary according to the host, his age, diet, and other factors (Devriese, et al., 1992). *E. faecalis* and *E. faecium* are the most common bacteria isolated from the GI tract of humans (Mead, 1978). *E. faecalis* is a part of some probiotic formula. There is a variety of infections in which enterococci may be involved. Most common are the urinary tract infections (Moellering, 1992).

Streptococcus

While some of these G^+ cocci are virulent pathogens, other strains live harmoniously with their hosts as commensals. They can be isolated as part of the normal flora of the alimentary, respiratory, and genital tract. *Streptococcus bovis* is associated with malignancies of the GI tract (Ruoff, et al., 1984). Highly pathogenic strains, for example *S. pyogenes* (Group A) or *S. agalactiae* (Group B) represent some of the most impressive

human pathogens. The numerous virulence factors allow it to cause a wide array of serious infections.

Staphylococcus

These G^+ cocci are widespread in nature, although they are mainly found living on the skin glands, and mucous membranes of mammals and birds. They are also a part of GI microflora. They generally have a benign or symbiotic relationship with their host; however, if the natural cutaneous barriers are damaged, these organisms may gain entry into the host tissues or colonize foreign bodies and develop the lifestyle of a pathogen. Nosocomial infections caused by Methicilin resistant *S. aureus* are a major problem worldwide, being a major cause of morbidity and mortality (Murray, et al., 1999).

1.2 Illnesses and Health Disorders

1.2.1 Colonization, Pathogenicity, and Infection

A pathogen is an organism that is able to cause cellular damage by establishing in tissue, which results in clinical signs with an outcome of either morbidity or mortality. More specifically, a pathogen is characterized by its ability to replicate in a host, by its continued persistence of breaching or destroying cellular or humoral barriers that ordinarily restrict it (Bhunia, 2008). The ability to produce disease is determined by the microorganism's virulence properties, as well as by specific factors of the host. Colonization of the host is an infection, but it can be one in which the host and organism evolve a commensal relationship without the development of disease. Although colonization is necessary for infection to take place, not all colonized persons will develop disease. However, an important distinction exists between colonization with a pathogenic organism and the disease state produced by that organism (Murray, et al., 1999).

Certain organisms entering the body will always be associated with pathogenicity, while others appear to cause disease only under certain circumstances. We can divide pathogens according to the mere of their pathogenicity into these groups (Murray, et al., 1999):

Strict pathogens are always associated with disease.

Principal pathogens regularly cause disease in some proportion of healthy individuals

Opportunistic pathogens do not usually cause disease in people with intact defense systems but are capable of devastating disease in immunocompromised individuals (after ATB treatment, chemotherapy).

Facultative pathogens exist between the two extremes of strict and opportunistic pathogens and comprise the majority of organisms found in the body.

Most common pathogenic organisms in the intestine are aerobic *Campylobacter*, *Salmonella*, *Shigella*, *Vibrio*, *Staphylococcus aureus*, *Bacillus cereus*, *Yersinia*, *E. coli*, *Edwardsiella*, *Aeromonas* and anaerobic *Clostridium* and *Bacteroides* (Murray, et al., 1999).

1.2.2 Food Borne Pathogens

Intestinal infections have two possible origins: **endogenous**, in which bacteria normally represented in intestinal flora cause infection, or **exogenous**, where the bacteria enter the GIT orally. Concerning this way of origin we are speaking about "food borne pathogens". Food borne pathogenic microorganisms when grown in a food may not alter the aesthetic quality of products (contrary of the spoilage microorganisms) and, thus may not be easy to assess the microbial safety of a product without performing multiple microbiological tests (Bhunia, 2008).

The foodborne disease is obtained by three possible ways (Bhunia, 2008):

Food Intoxication - ingestion of preformed toxin. It is generally caused by *Staphylococcus aureus*, *Clostridium botulinum*, and *Bacillus cereus*. (We do not focus on this kind of pathogenicity, because these bacteria do not enter the GIT)

Toxicoinfection - toxin is produced inside the host after ingestion of bacteria. It is generally caused by *Clostridium perfringens*, ET *Escherichia coli* and *Vibrio cholerae*.

Food Borne Infection - ingestion of infective pathogen. It is most often caused by bacterial pathogens as *Salmonella enterica*, *Campylobacter jejuni*, enterohemorrhagic *Escherichia coli* (E. coli O157:H7), *Shigella* spp., *Yersinia enterocolitica*, and *Listeria monocytogenes*.

1.2.3 Diseases as Consequences of Microbiota Imbalance

Disturbance of the microbiota may be caused by a variety of diseases or abnormal physiological states (Chae, et al., 1999). ATB treatment, critical illness, age, diet, immunosuppression, exposure to nosocomial microorganisms, and the length of hospitalization are additional factors that contribute to the overgrowth of opportunistic pathogens (Rohde, et al., 2009).

Small Intestine Bacterial Overgrowth Syndrome (SIBOS)

This condition means a chronic recurrent diarrhea with malabsorption, intoxication and increased risk of endogenous infection. These syndromes are caused by increase of overall bacterial burden in biotope, mainly *Enterobacteria*, *Bacteroides*, clostridia and *Fusobacteria* etc. in a small intestine. Such characteristics of the syndrome allow consider it

as dysbacteriosis. Microecological changes are accompanied by B12 vitamin deficiency, anemia, hypovitaminosis, protein deficiency, translocation of bacteria and their toxins from intestine in blood, emergence of endotoxinemia and possible generalization of infection. SIBOS is diagnosed by concentration of hydrogen in expiratory flow (lactulosa load test) or by bacteriological study of aspirate from proximal part of small intestine. Complex treatment includes containing *Lactobacilli* and *Bifidobacteria* probiotics and, in more severe cases, ATB treatment, with following correction of disturbed microbiocenosis by different probiotics (Bondarenko, et al., 2006).

Irritable Bowel Syndrome

The irritable bowel syndrome (IBS) is a functional bowel disorder and is characterized by a number of symptoms including abdominal pain or discomfort and disturbed bowel habits. Other typical IBS symptoms are diffuse abdominal pain, bloating, excessive passing of gas, irregular bowel movements with improvement of symptoms after defecation, and/or the feeling of incomplete evacuation. Morphological abnormalities are missing (Martens, et al., 2010). This condition is caused by microbiota abnormalities (Jiménez, 2009). It is associated with lack of *Bifidobacteria* and *Lactobacilli,* and proliferation of bacterial groups with gas-producing ability (clostridia, *Veillonella* etc.). It can be also a consequence of SIBOS (Jiménez, 2009).

Clostridium difficile - Associated Diarrhea (CDAD)

A practical clinical example of consequences of the microflora ecosystem disturbance is the feared multiplication of the bacterium *C. difficile* during the treatment with broad spectrum ATBs (Kasper, 2001). *C. difficile* colonization rates are roughly 3% in healthy adults; however, this rises to 15–35 % in hospitalized patients treated with chemotherapeutic agents (Aslam, et al., 2005). In severe cases CDAD can lead to pseudomembranous colitis, a severe inflammation of the colon and may have devastating complications. With the increasing use of broad-spectrum ATBs over the two past decades, the incidence of CDAD has risen. The majority of cases are elderly people in prolonged stays in health care settings, however, the evidence suggests that there is an increasing incidence of CDAD in populations previously thought to be at low risk. Although CDAD causes worldwide substantial morbidity

and financial cost, it has previously been associated with low additional mortality of 1-5 %. However, there has been a marked increase in morbidity and mortality since the early 2000s, particularly associated with a new hypervirulent strain NAP1/BI/027. Mortality associated with this strain has been estimated between 6-12 % (Parkes, et al., 2009).

1.2.4 Infectious Diarrhea

Infectious diarrhea is caused by exogenous or endogenous pathogen, which is able to adhere or invade the intestinal mucosa, colonize there, and cause disease state by affecting the intestinal wall function.

Mechanisms of Pathogens Causing Infectious Diarrhea

Pathogenic organisms cause two diarrheal syndromes (Navaneethan, et al., 2008):

1) **Noninflammatory diarrhea** - caused by **enterotoxin**-producing organisms such as *Vibrio cholerae* and ET *E. coli*
2) **Inflammatory diarrhea** - caused by two groups of **cytotoxin -** producing organisms:
    a) **Noninvasive bacteria** which adhere to the mucosa, activate cytokines and stimulate the intestinal mucosa to release inflammatory mediators (e.g. EI *E. coli* , EH *E. coli* and *C. difficile*)
    b) **Invasive bacteria** –invade the intestinal mucosa to induce an acute inflammatory reaction, involving the activation of cytokines and inflammatory mediators (e.g. *Salmonella* spp., *Shigella* spp., *Campylobacter* spp.)

1.2.5 Chronic Diseases Resulting from Intestine Malfunction

Besides acute gastroenteritis, the sequelae of the foodborne infections may result in variety of chronic diseases (Bhunia, 2008):

Autoimmune diseases, such as allergic encephalitis and autoimmune polyneuritis

Guillain–Barré syndrome (GBS), triggered mostly by *Campylobacter jejuni* infection. In this case, the immune responses directed towards the infecting organisms are involved in the pathogenesis of GBS by cross-reaction with neural tissues. Immune reactions result in acute inflammatory demyelinating neuropathy (85% of cases) or cause the acute axonal forms of

GBS (15% of cases). Care for such patients may be challenging, yet the prognosis overall is favorable. Optimal supportive care and anticipation and prevention of complications are the mainstay of therapy. Admission to the intensive-care unit is necessary in 33% of patients. Immunomodulation with infusions of IgG or plasma exchange treatments foreshorten the disease course (Hahn, 1998).

Hemolytic–uremic syndrome develops by approximately 6 % of O157 *ST E. coli* diarrhea patients. This condition is characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure (Murray, et al., 1999).

Atherosclerosis can occur as a consequence of long term alternation of intestinal microflora. Due to altered lipid metabolism, the lipid deposits in arteries.

1.3 Treatment strategies

1.3.1 Symptoms Therapy

Rehydration

Rehydration is the most important with all kinds of diarrhea. It is replacing lost fluid and salts. In non severe cases of diarrhea, it is actually the only treatment needed and it is emergent with every type of diarrhea. There are various electrolytes administered orally or intravenously in severe cases.

Antimotility Agents

Endogenous peptide opiates are the most widely used drugs in treating the symptoms of diarrhea. Most widely distributed is Loperamide (Imodium). It works by decreasing the activity of the myenteric plexus, which, like morphine, decreases the tone of the longitudinal smooth muscles but increases tone of circular smooth muscles of the intestinal wall. This increases the amount of time substances stay in the intestine, allowing for more water to be absorbed out of the fecal matter. There are three types: enkephalins, β-endorphins and dynorphins (Baldi, et al., 2009). Treatment should be avoided in the presence of fever or in the case of dysentery. It is of no value in diarrhea caused by *V. cholerae, Shigella* or *Campylobacter*. If there is a suspicion of diarrhea associated with organisms that can penetrate the intestinal walls, such as EH *E. coli* or *Salmonella*, loperamide is contraindicated (Butler, 2008).

Adsorbents

These drugs work in the physical way by adsorbing gas and toxic substances. There are adsorbents on different bases. For example organic **Active coal**, **Palygorskite** – a magnesium aluminium phyllosilicate, **Diosmectite** - a natural silicate of aluminium and magnesium, or **Tannin albuminate**. **Bismuth subsalicylate** is partially an adsorbent and as a derivative of salicylic acid, it displays also an anti-inflammatory action

1.3.2 Germicidal Agents

Halogenated Hydroxyquinoline – a substance, used as a germicidal agent for various purposes. In humans, 5-chloro-7-iodo-8-hydroxyquinoline (an antidiarrheal drug) was associated with an incidence of subacute myelooptic neuropathy (Anonymous, 1985). **Dichlorchinolinol** (Dichlorchinolinolum or also Cloroxinum, in Czech Republic it is sold as Endiaron, 5, 7 dichlorquinolinol) is a germicidal agent, used for treatment of infectious or protozoal diarrhea and microbiota disturbance after ATB treatment. It reportedly acts against genera *Streptococci*, *Staphylococci*, *Shigella*, *Candida* (Fungi), *Giardia* and *Trichomonada* (Protozoa).

1.3.3 Antibiotical Therapy

1.3.3.1 Antibiotical Agents

Infectious diarrhea cannot be always treated by ATBs. Because of numerous side effects and the insensitive derogation of natural microbiota, they are indicated only in severe cases. Treatment with inappropriate ATBs can actually significantly worsen the course of the disease (Tauxe, et al., 1990) and increase the risk of pathogen recurrence (Sklenickova, et al., 2010).

Penicillins (β-lactam ATBs)

It is a group of natural and semisynthetic ATBs. The natural compound is produced by *Penicillium* spp. They have activity against most G^+ and many G^- and anaerobic organisms. Pivmecillinam is an extended-spectrum penicillin ATB, which is recommended by WHO for the treatment of dysentery in children (Traa, et al., 2010). Penicillin G shows excellent activity against most but not all strains of *C. perfringens* and has traditionally been considered the antibiotic of choice for the clostridia in general (Rosenblatt, 1989). However, resistance among *C. perfringens* isolates is increasing to the extent that alternative antimicrobial agents may have to be considered (Koneman, et al., 1997). Resistance to penicillin is especially common in *C. ramosum*, *C. clostridioforme*, and *C. butyricum* (Finegold, et al., 1989; Rosenblatt, 1989). The principle of β-lactam ATBs activity is inhibition of bacterial enzymes that are essential for peptidoglycan synthesis, which leads to destruction of the bacterial cell wall. This ability usually confers on the activity mainly against G^+

bacteria. Wide range of bacteria produce enzymes β-lactamases and this makes them resistant to β-lactam ATBs. Penicillins are usually well tolerated; their side effects might be diarrhea, rash, drug fever or serum sickness (Murray, et al., 1999).

Cephalosporins (β-lactam ATBs)

They are derivatives of fermentation products of *Cephalosporium acremonium*. Some of the *Enterobacteriaceae*, including many strains of *E. coli*, *Klebsiella* spp. are susceptible to them. The second generation of cephalosporins increased activity against G^- organisms (Murray, et al., 1999). Ceftriaxone, which belongs to younger agents of this group, was recommended by WHO for treatment of dysentery in children (Traa, et al., 2010). They are usually well tolerated, with similar side effects as penicillins (Murray, et al., 1999).

Carbapenems (β-lactam ATBs)

These ATBs represent unique class agents with the widest spectrum of ATB activity of the currently available ATBs. They have excellent in vitro activity against streptococci, *Bacillus* spp., more than 90 % of the *Enterobacteriaceae*, including those resistant to other β-lactams and aminoglycosides (Neu, et al., 1982). Side effects are similar to those of other β-lactams (Murray, et al., 1999).

Tetracyclines

It is a group of broad spectrum bacteriostatic, synthetic ATBs. *E. coli* strains are often susceptible to them. They are also used for *Vibrio* and *C. difficile* infections. Many members of fam. *Enterobacteriaceae* are resistant (Murray, et al., 1999). Side effects include upper GIT irritation, ATB – associated diarrhea; pseudomembranous colitis can develop after prolonged use, discoloration of teeth, and depression of bone growth when given during tooth and skeletal development (Grossman, et al., 1971).

Macrolides

Erythromycin is the oldest member of this ATB group. They are derived from *Streptomyces erythereus*. It is relatively broad spectrum ATB against G^+ and some G^- bacteria. Macrolides are active against many *Clostridium* spp., especially *C. perfringens* (Murray, et al., 1999). They are the most effective agents in the case of *Campylobacter* infection (Butzler, 2004). Side effects of this drug include GI irritation, hypersensitivity,

cholestatis hepatitis, reversible hearing loss, pseudomembranous colitis, superinfection of GIT, or vagina with *Candida* spp. (Murray, et al., 1999).

Metronidazole

This synthetic drug plays an important role in the treatment of anaerobic bacteria and certain protozoa. It exhibits potent activity against almost all anaerobic bacteria including the *B. fragilis*, *Fusobacterium* spp. and *Clostridium* spp. (Barlett, 1982), but has no activity against *Enterobacteriaceae*. It is effective in the treatment of ATB-associated colitis caused by *C. difficile* (Barlett, 1992), with efficacy equivalent to that of oral vancomycin for this indication (Teasley, et al., 1983). Adverse effects are not common.

Vancomycin

This drug belongs to the ATB group of glycopeptides and lipopeptides. It is obtained from *Streptomyces orientales*. It was initially introduced against penicillin resistant *Staphylococci*. It is active mainly against G^+ organisms, including *Clostridium* spp. It can be applied for treatment of *C. difficile* ATB-associated colitis. The most frequent adverse effects of this drug include fever, chills, and histamine release (Murray, et al., 1999).

Streptogramins

These drugs are natural cyclic peptides produced by *Streptomyces* spp. They have two structurally unrelated components, which act synergistically. They are active mainly against G^+ bacteria, and few G^- and anaerobic pathogens. Among the anaerobes, *C. perfringens* and *C. difficile* are the most susceptible. Phlebitis is the most often side effect of these drugs (Murray, et al., 1999).

Quinolones and Fluoroquinolones

This is a group for ATBs related to nalidixic acid, which is the first of the synthetic quinolone ATBs. They prevent bacterial DNA from unwinding and duplicating (Hooper, 2001). This group can be divided into three groups according to the spectrum of activity: narrow, spectrum, and expanded spectrum.

Broad use of fluoroquinolones has been followed by emergence of resistance, which has been due mainly to chromosomal mutations in genes encoding the subunits of the

drugs' target enzymes, DNA gyrase and topoisomerase IV, and in genes that affect the expression of diffusion channels in the outer membrane and multidrug-resistance efflux systems (Hooper, 2001). Fluoroquinolones are still active against many *Clostridium* spp., *Salmonella*, *Shigella*, toxigenic *E. coli*, *Citrobacter* spp., *Serratia* spp., *Campylobacter*, *Vibrio* spp., *Aeromonas* spp. etc. Resistance emerged first in species in which single mutations were sufficient to cause clinically important levels of resistance (e.g., *Staphylococcus aureus* and *Pseudomonas aeruginosa*). Subsequently, however, resistance has emerged in bacteria such as *Campylobacter jejuni, Escherichia coli*, and *Neisseria gonorrhoeae*, in which multiple mutations are required to generate clinically important resistance (Hooper, 2001). *Campylobacter* spp. gain high resistance due ATB additives in poultry feed in USA (Butzler, 2004). Resistance and reduced susceptibility have emerged also in *Salmonella*, *Shigella* and *Campylobacter* (Horiuchi, et al., 1993; Endtz, et al., 1991).

Broad-spectrum fluorouinolines together with cephalosporins and clyndamycin belong to ATBs which increase the CDAD inception (Vonberg, et al., 2008). There is also a clear association between exposure to these ATBs and MRSA isolation (Tacconelli, et al., 2007).

In general, fluoroquinolones are well tolerated, with most side effects being mild to moderate. On occasion, serious adverse effects occur (De Sarro, et al., 2001; Owens, et al., 2005). Some of the serious adverse effects that occur more commonly with fluoroquinolones than with other antibiotic drug classes include CNS and tendon toxicity (Owens, et al., 2005; Iannini, 2007).

Aminoglycosides

Streptomycin was introduced already in 1944. It has played an important role in G^- bacteria infections. They are isolated from *Micromonospora* or *Streptomyces* spp. GI absorption of these agents is unpredictable and always low. Widespread resistance among the *Enterobacteriaceae* and *P. aeruginosa* has limited the usefulness of Streptomycin. The aminoglycosides are not active against anaerobes. Because of its severe toxicity with systemic administration, Neomycin is available only for oral and topical use. By oral way it can be used against small intestinal bacterial overgrowth, because of its minimum absorption (Madrid, et al., 2001). The nephrotoxic potential varies, with neomycin being the

most toxic one and streptomycin the least. All aminoglycosides are capable of causing damage to the eighth cranial nerve (Murray, et al., 1999).

Polymixins

These ATBs belong to the group of polypeptides. They are derived from *Bacillus polymixa*. They have limited spectra of antimicrobial activity and they show significant neurotoxicity and nephrotoxicity. They are active only against G^- bacilli, especially *Pseudomonas* spp. Emergence of resistance during therapy is rare and there is no cross-resistance (Murray, et al., 1999).

Rifampin

It is also known as rifampicin, and it is a semisynthetic ATB, derived from rifamycin B, which belongs to a group of macrocyclic compounds produced by *Streptomyces mediterranei*. Rifampin – resistant bacteria possess an altered RNA polymerase, which arises easily from single-step mutations during monotherapy with this drug. Wide spectrum of *Streptococci*, *Staphylococci*, *Enterobacteriaceae* are highly susceptible bacteria. It is often used for treatment of "traveler's diarrhea" (Steffen, et al., 2003). It has many side effects, including GI discomfort, drug fever, skin rashes, and eosinophilia (Murray, et al., 1999).

Sulfonamides

These are synthetic ATBs, with action to various G^+ and G^- bacteria, fungi and protozoa. *E. coli* strains were initially susceptible to sulfonamides; however, increasing bacterial resistance has limited their efficacy in recent years.

1.3.3.2 Bacterial Resistance and Intestinal Pathogens

It was just a few years after the introduction of penicillin that scientists began to notice the emergence of a penicillin resistant strain of *Staphylococcus aureus*, bacterium that makes up part of normal human bacterial flora (Cowan, 1999). Since then, the problem of antimicrobial resistance has grown into a serious threat to public health worldwide (Cowan, 1999). Due to frequent unjustified treatment with the wide spectrum ATBs and mass usage of ATBs as food supplement for domestic animals, human commensal bacteria are relatively often exposed to these substances and are more likely to develop defense

systems. In hospitals, the resistant bacterial strains, for example *Cl. difficile* causing CDAD have became a worldwide nosocomial problem. A hypervirulent strain of *C. difficile* called NAP1/BI/027, which has been implicated in *Cl. difficile* outbreaks associated with increased morbidity and mortality since 2000, is resistant to fluoroquinolones, which was not before 2001 (Hookman, et al., 2009). In most countries of South-East Asia, almost 98% of all *Gonorrhea* dysentery cases are multi drug-resistant, and in the industrialized world, as much as 60% of hospital-acquired infections are caused by multidrug-resistant microbes (Witte, et al., 2004). For example oral vancomycin is being restricted in most hospitals because of vancomycin-resistant enterococci (VRE), which were demonstrated to be able to transfer resistance into clostridia via plasmids (Peterson, et al., 1996). Since the early 1990s, O157 and other diarrhea causing ST *E. coli* strains have demonstrated slowly increasing levels of resistance to certain ATBs, particularly streptomycin, sulfonamides, and tetracycline (Farina, et al., 1996; Slutsker, et al., 1997). ATB treatment can be still helpful for diarrhea caused by EP *E. coli* (Nataro, et al., 1998), but most strains associated with outbreaks are resistant to multiple antimicrobial agents (Donnenberg, 1995). Because of the widespread ATB resistance among dysentery causing *Shigella* strains, all isolates are recommended to undergo susceptibility testing. Infections caused by these strains are often acquired during international travel to areas where most strains are multidrug resistant (Tauxe, et al., 1990). In certain areas of Africa and Asia, *S. dysenteriae* 1 strains are resistant to all locally available antimicrobial agents, including nalidixic acid. These resistant strains are still susceptible to the fluoroquinolones, but usage of these drugs in LDC is usually precluded by their high cost (Sack, et al., 1997). A strain of *Salmonella* serovar Typhimurium phage type 104, which is pentadrug-resistant, has emerged in the USA and UK as the predominant strain of this serotype. Its incidence has dramatically increased during the past 7 years (Murray, et al., 1999). Resistance among genera *Citrobacter*, *Klebsiella*, *Enterobacter*, and *Serratia* is also becoming increasingly worrisome (Chartrand, et al., 1996; Jones, et al.).

Bacterial resistance is the result of complex interactions among antimicrobial agents, microorganisms, and the environments in which they are brought together. Intrinsic resistance is given by the normal genetic, structural or physiologic state of a microorganism. An acquired resistance results from altered cellular physiology and structure due to changes in the usual genetic makeup of a microorganism. Unlike intrinsic resistance, it may be a trait

associated with only some strains of a particular organism group or species and therefore, its presence is unpredictable. The methods for acquisition are basically those that allow for gene change or exchange: genetic mutation, acquisition of genes from other organisms via gene transfer mechanisms, or a combination of both (Murray, et al., 1999). Multiple antibiotic resistances in bacteria are most commonly associated with the presence of plasmids which contain one or more resistance genes. Transmission of resistance genes from normally more virulent pathogenic species to nonpathogenic organisms is very common with the HIM (Uma, et al., 2009). Bacteria have developed various resistance mechanisms, such as altering the target of the drug, changing outer membrane to decrease its permeability, synthesizing enzymes destroying the drug and many others. The competition between bacterial resistance and researchers developing new antimicrobial agents is a very long distance run with unpredictable result.

1.3.4 Probiotics

Lactic acid bacteria *Lactobacillus* and *Bifidobacterium* and are commonly consumed as part of fermented foods (milks, cheeses, fruit juices, wine, and sausages). The concept of probiotics probably dates back to 1908, when Noble Prize winner Eli Metchnikoff suggested that the long life of Bulgarian peasants resulted from their consumption of fermented milk products (Metchnikoff, 1908). The term "probiotic" was first used in 1965, by Lilly and Stillwell for describing substances secreted by one organism, which stimulate the growth of another (Lilly, et al., 1965). Marteau et al, in 2002 defined them as "microbial preparations or components of microbial cells that have a beneficial effect on health and well-being" (Marteau, et al., 2002). There are many various probiotic formulas on the market these days, as this way of therapy has been gaining more popularity in recent years. It often accompanies or follows ATB therapy, due to gain back the microbiota balance. As single therapeutical agents, they are helpful in prevention, mild infections or chronic diseases of GIT. Probiotics have been shown to be effective in varied clinical conditions, ranging from infantile diarrhea, necrotizing enterocolitis, antibiotic-associated diarrhea, relapsing *C. difficile* colitis, *Helicobacter pylori* infections, inflammatory bowel disease to cancer, female urogenital infection and surgical infections (Gupta, et al., 2009). Single and mixed cultures of live microorganisms are used in probiotic preparations (D'Souza, et al., 2002).

Various bacterial genera most commonly used in probiotic preparations are *Lactobacillus*, *Bifidobacterium*, *Escherichia*, *Enterococcus*, *Bacillus* and *Streptococcus*. *B. adolescentis, B. bifidum, B. breve, B. infantis* and *B. longum* are species used for manufacturing the therapeutic fermented milk products (Arunachalam, 1999). Some fungal strains belonging to *Saccharomyces* are also used as probiotics (Gupta, et al., 2009). Probiotics are live micro-organisms and hence, it is possible that they may result in infection in the host (Gupta, et al., 2009). Different strains of probiotics have different safety profiles. Systemic infection has rarely been reported with *Bifidobacterium*, although many cases of sepsis secondary to *Lactobacillus rhamnosus* GG or *Lactobacillus casei* have been reported (Simhon, et al., 1982; Adlerberth, et al., 1991). The issue of safety becomes more complex when the probiotical organism is *Enterococcus* spp. (Franz, et al., 1999). However, based on different reports, we can conclude that the risk of infection caused by lactobacilli or bifidobacteria is similar to infection with commensal strains, and that consumption of such products presents a negligible risk to consumers including immunocompromised hosts (Ouwehand, et al., 2003). The microorganisms used in probiotic preparations should be generally recognized as safe, they should be resistant to bile, hydrochloric acid and pancreatic juice, have anti-carcinogenic activity and stimulate immune-system, have reduced intestinal permeability, produce lactic acid, able to survive both acidic conditions of the stomach and alkaline conditions of the duodenum (Vimala, et al., 2006).

1.3.5 Prebiotics

Prebiotics are food components, which have beneficial effects resulting from the selective stimulation of growth and/or activity of the gut microbiota, particularly *Lactobacilli* and *Bifidobacteria* (Lim, et al., 2005). Most of the attention in this area has been aimed at indigestible oligosaccharides (Kolinda, et al., 2007). Common prebiotics include inulin, lactulose, resistant starch and other oligosaccharides, that is plant primary metabolites. Dietary fiber has also been shown to convey a prebiotic response (Lim, et al., 2005). Inulin occurs naturally in several foods such as leek, asparagus, chicory, garlic, artichoke, onion, wheat, banana, oats and soybeans (Kolinda, et al., 2007). A functional food approach has been utilized to add inulin to more frequently consumed products, such as cereals, biscuits, infant foods, yogurts breads and drinks, at concentrations at which a prebiotic effect may

occur (Kolinda, et al., 2007). There are also a number of commercially available dietary supplements containing oligosaccharides, primarily inulin. Many formulas contain both prebiotics and probiotics and are called synbiotics.

1.3.6 Plant Secondary Metabolites in Human's Diet

Several findings suggest that the microbial cohort remains relatively constant once adulthood is reached (Volker Mai, 2004), however, it can be significantly influenced by probiotic, prebiotic and other biologically active constituents in our diet (Hill, 1998). Plant secondary metabolites contained in our diet may also act probiotically. These are substances not involved in primary plant's metabolic processes of growth and development. They are biologically active plant compounds fulfilling such functions as protection against pathogens, pollinator's attraction, allelopathy and many others. They are of significant importance with regard to plant's medicinal purposes. Phenolics are a large group of plant secondary metabolites, which is often associated with positive impact on GI microflora, as several studies have documented. The most abundant types of polyphenols found in our diet are the flavonoids, most often conjugated as glycosides. More than 4000 chemically unique flavonoids have been identified in plants, particularly fruits, vegetables, nuts, seeds, spice and flowers, as well as in several beverages, which deliver a complex mixture of polyphenols and phenolic acids to our GIT. The average consumption of polyphenols is about 1 g per day, the main sources being fruits, tea and coffee, and to a lesser extent, vegetables, cereals and legume seeds (Scalbert, et al., 2000). Several polyphenols which are present in regular diet have been demonstrated to inhibit the growth and adhesion of bacterial pathogens and to enhance the proliferation and adhesion of PB (Dolara, et al., 2005; Parkar, et al., 2008). Dietary polyphenols are not completely absorbed from the GIT and are metabolized by the gut microflora so that they and their metabolites accumulate and exert physiological effects. Lee, et al. (2009) was investigating the impact of tea main and minor phenols on the growth of intestinal microflora, and also the ways intestinal bacteria metabolize them. This study indicates that phenolics are likely to benefit the host by inhibiting pathogen growth and regulating commensal bacteria, including probiotics, and could therefore be considered as prebiotics. It is known that tannins and related polyphenols show antibacterial activities against *Helicobacter pylori, S. aureus, E. coli, Salmonella* spp., and streptococci (Yoshida, et

al., 2000; Funatogawa, et al., 2004; Puupponen-Pimia, et al., 2005; Hatano, et al., 2003; Osawa, et al., 1999). The antimicrobial action of tannins is associated with their prevailing capability to form stable complexes with proteins, starch, and physiological metals, thereby disturbing the metabolic activity of bacterial enzymes, nutrient availability, and functionality of biological membranes (Goel, et al., 2005; Chung, et al., 1998). Clinical reports specified that after human consumption the majority of ellagitannins remain in the colon for up to 56 h before excretion (Seeram, et al., 2006). They or their microbial metabolites may also significantly affect intestinal bacterial population by decreasing the pH of the intestinal environment. Generally, lower pH favors probiotic bacteria as compared to pathogenic bacteria. The antimicrobial activity of certain berries and their phenolic extracts has been attributed to the resulting lower pH of the media (Puupponen-Pimia et al., 2005). The most abundant types of polyphenols found in our diet are the flavonoids, most often conjugated as glycosides. More than 4000 chemically unique flavonoids have been identified in plants, particularly fruits, vegetables, nuts, seeds, spice and flowers, as well as in several beverages, which deliver a complex mixture of polyphenols and phenolic acids to our GIT. The average consumption of polyphenols is about 1 g per day, the main sources being fruits, tea and coffee, and to a lesser extent, vegetables, cereals and legume seeds (Scalbert, et al., 2000). There are some food supplement formulas, based on plant phenolic compounds, indicated for intestinal microflora balance improvement. For example, there is bilberry extract in the formula intended for intestinal flora recovery after ATBs treatment and intestinal disorders. Bilberries and other wild berries are known for being rich at phenolic compounds, which have shown selective antimicrobial activity against intestinal pathogens (Puupponen-Pimia, et al., 2005). We can also buy a tea extract as a dietary supplement, with a standardized content of 60 % epigallo-1-katechin-3-gallate (EGCG). Tea contains polyphenols, tannins, caffeine, and catechins, which have been most widely studied for their biological action and among many other activities proved probiotic, and antimicrobial properties (Balentine, et al., 1997; Roedig-Penman, et al., 1997). Tea extract exerts a selective anti-adhesive effect against certain pathogenic bacteria with no adverse effects against probiotic bacteria (Lee, et al., 2009). In addition, EGCG demonstrates a significant synergistic behavior with ATBs (Hemaiswarya, et al., 2008). Plant polyphenols probiotic activity deserves further research.

1.3.7 Medicinal Plants

It is important to remember that for thousands of years the traditional systems of medicine in all countries exclusively employed plant-based medicines (Mahady, 2001). Even though, the synthetic chemistry dominated the 20th century, approximately 25% of all prescription drugs dispensed in the USA were derived from plants in 1970's and in 2000, 11% of 252 drugs on the WHO's essential drugs list were exclusively obtained from flowering plants (Farnsworth, et al., 1976). Furthermore, it has been estimated that between 20-80% of the populations many developing countries use botanical products almost exclusively, and consider them a normal part of primary healthcare until these days. The consumer of the developed world is now very interested in alternative medicines, including medicinal plants (herbal medicines) as they are perceived as being both safe and effective (Mahady, et al., 2003).

1.3.7.1 Herbal Dietary Supplements

There are several dietary supplements including diarrhea or GI disorders among their indications available on the US market (Raskin, et al., 2002):

Hydrastis canadensis L. (goldenseal) - a plant from the family *Ranunculaceae*. Roots or rhizomes are used for medicinal purposes. **Berberine** (Figure 7), a naturally occurring **isoquinoline** alkaloid present in this plant has antibacterial properties, and well documented in several studies concerning its influence on intestinal microflora (Chae, et al., 1999; Xie, et al.). It occurs also in another plant species, such as *Coptis chinensis* and *Berberis vulgaris.* Chae et al., 1999, examined 3 root-derived *Coptis japonica* alkaloids against intestinal bacteria using the disk dilution method. Berberine chloride, palmatine iodide, and coptisine chloride showed antimicrobial action against intestinal bacteria, with slight selective properties in favor to probiotic bacteria. Xie et al., 2011, documented in the trial ex vivo, that *Rhizoma coptidis* and berberine significantly reduce the growth of gut bacteria, whilst focusing on possible anti-obesity strategy. Smith, at al. (2005) described promising growth inhibition of multidrug resistant MRSA caused by synergic action of berberine and 5'-Methoxyhydnocarpin, both products of berberry plants.

Matricaria chamomilla L. (German chamomile), from the fam. *Asteraceae*, is also recommended dietary supplement in the case of intestinal disorders. The main constituents of the flowers include several phenolic compounds, primarily the flavonoids apigenin, quercetin, patuletin, luteolin and their glucosides. The principal components of the essential oil extracted from the flowers are the terpenoids α-bisabolol and its oxides and azulenes, including chamazulene. Chamomile has moderate antioxidant and antimicrobial activities, and significant antiplatelet activity in vitro (McKay, et al., 2006).

Antidiarrheal dietary supplements, which can be found on the EU market, include Tormentil, which is standardized plant extract of *Potentilla erecta* Hampe (fam. *Rosaceae).* A plant of the same genera, *Potentilla pacifica,* is used as an ethnoveterinary medicine against diarrhea in ruminants (Lans, et al., 2007). According to Tomczyk, et al., 2009, genus *Potentilla* extracts of the aerial and/or underground parts have been applied in traditional medicine for the treatment of inflammations, wounds, infections due to bacteria, fungi and viruses, diarrhea, and other ailments. A series of altogether 27 tannins (hydrolysable tannins and related compounds and proanthocyanidins of type A and B including some proanthocyanidins also occurring in *Potentilla* species) were tested for their activity against two G^+ and four G^- bacteria and two yeasts. The compounds showed only weak to moderate antibacterial activity, but fairly high anticryptococcal activities (Kołodziej, et al., 1999). Also, the methanol extract from the aerial parts of *P. erecta* showed moderate antibacterial and antifungal activities against *S. aureus*, *E. coli*, *Candida albicans* and *C. krusei,* respectively (Tosun, et al., 2006).

Scutellaria baicalensis Georgi (*Lamiaceae*) root extract is sold as a dietary supplement, advertised to have an anti-inflammatory, anti-diarrheal and other medicinal properties due to its high flavonoids content. Antimicrobial properties of *S. baicalensis* and *S. planipes* were tested by Zhang, et al. (1998) and significant inhibitory effect on *E. coli* and *S. au*reus were observed. Its flavonoid **baicalin** was reported to prove inhibitory action to *S. aureus* (Ng, et al., 1996). In addition, *Scutellaria* extract showed significant synergistic effect with β-lactam ATBs (Zai-Chang, et al., 2005).

Terminalia chebula, Terminalia bellerica and *Emblica officinalis* – mixed extract called Triphal, is another dietary supplement advertised for anti-infective diarrheal properties. The

genus *Terminalia* (*Combretaceae*) is widely used for dysentery treatment in East and SE Asia (Perry, 1980). Khan, et al. (2002) reported, that ethyl acetate fractions of the stem bark of *T. complanata* showed a range of activity against bacteria and protozoa. Antimicrobial activity was also found in fruit extracts of *Emblica officinalis*, *T. chebula*, *T. belerica* (Ahmad, et al., 1998), and *T. brownie*, which supported traditional medicinal use of these plants (Mbwambo, et al., 2007). In 2004, Rani, et al. reported, that *T. arjuna* and Triphal mixture showed activity against multi-drug resistant *Salmonella typhi*. There has been assased a very prospective MIC 31,3 µg/ml of *T. chebula* ethanol extract against MRSA (Sato, 1997). Another study refers that MIC of *T. catappa* Linn. extract is only 65 µg/ml against *E. coli* and 250 µg/ml against *Salmonella typhi* (Pawar, et al., 2002).

1.3.7.2 Traditional Chinese Medicine Plants

Traditional Chinese medicine uses multiple plant prescriptions and has served the health needs of the Chinese population for over 5000 years. This system of medicine has its own unique methods of diagnosis, and incorporates over 7000 species of medicinal plants into clinical practice (Shen, 1996). Here are the plants from the Chinese medicine handbook, which are used for diarrhea treatment, and might be useful for further pharmacological study (Bensky, et al., 1993):

Alpinia katsumadai Hayata, fam. *Zingiberaceae* (grass cardamon), fruit is together with Cortex of *Cinnamomum cassia* Blume (*Lauraceae*) and *Zingiber Officinale* Roscoe *(Zingiberaceae)* used against attack by cold causing vomiting, pain and sensation of cold in the abdomen, and intense diarrhea. According to the scientific papers, the *Cinnamomum* essential oil showed marked selective antimicrobial properties (Si, et al., 2005). *Z. officinale* (ginger) exerts antioxidative, antitumorigenic, immunomodulatory, antimicrobial, and antiviral effects in vitro, and in vivo studies demonstrates positive effects on the GIT (Chrubasik, et al., 2005). MICs of its extract were promising 30 µg/disk against several pathogenic bacteria (Samy, 2005).

Amomum tsao-ko Crevost & Lemarié (Z*ingiberaceae)* fruit is used against abdominal distention, fullness, and pain together with vomiting and diarrhea and a very greasy tongue coating. Antimicrobial study of Yang, et al., 2008 confirms mild antimicrobial activities of *Amomum tsao-ko* essential oil (Yang, et al., 2008).

Amomum villosum Lour (*Zingiberaceae)* fruit is prescribed in the states of discomfort in epigastrum, nausea, abdominal pain and diarrhea.

Prunus mume Sieb. et Zucc. (*Rosaceae)* fruit is indicated in the case of unremitting chronic diarrhea or dysenteric disorders, binds up the intestines. They are used together with *Terminalia chebula* Retz. and *Schisandra chinensis* K.Koch (*Schisandraceae)* against chronic diarrhea and cough; together with *Coptis chinensis* Franch. (*Ranunculaceae*), and *Scutellaria baicalensis* Georgi (*Lamiaceae*) against dysenteric disorders. *Prunus mume* showed antimicrobial properties, with relatively low MIC to some human pathogens (Choi, et al., 2004). Antimicrobial activity assays about *Scutellaria* and *Terminalia* has been already mentioned in the previous chapter. Selective antimicrobial properties of alkaloids of *Coptis chinensis* has been documented in the assay of Chae et al., 2009 (Chae, et al., 1999).

Terminalia chebula Retz. (C*ombretaceae)* fruit is indicated in cases of chronic diarrhea and dysenteric disorders, it binds up the intestines. With *Galla Rhois Chinensis* it is indicated against purulent, bloody stools associated with chronic dysenteric disorders, with *Coptis chinensis* and *Saussurea lappa* (*Compositae*) also for chronic dysenteric disorders. No significant antimicrobial properties of *S. lappa* have been observed (Parekh, et al., 2007).

Punica granatum L. (*Punicaceae)* – Punigrate fruit binds up the intestines and stops diarrhea. It is used against chronic diarrhea, dysenteric disorders, and rectal prolapse. With *T. chebula* and *Myristica fragrans* Houtt. (*Myristicaceae*) seed is used against chronic diarrhea and dysenteric disorders. *P. granatum* extract exerted very promising antimicrobial activity against serious intestinal pathogens, such as *Vibrio* or *Shigella* (Mathabe, et al., 2006). Punigrate extract was also documented to inhibit the growth of pathogenic *Clostridia* and enhance the growth of bifidobacteria (Bialonska, et al., 2009).

Ailanthus altissima Mill, *Simaroubaceae* (bark of ailanthus), bark binds up the intestines and it is indicated for chronic diarrhea or dysenteric disorders.

Rosa laevigata Michx. (*Rosaceae)* fruit binds up the intestines and stops diarrhea, and is used against chronic diarrhea and dysenteric disorders. Pharmacological research: Decoctions have strong ATB effect against *S. aureus* and *E. coli*, antiviral too (Bensky, et al., 1993).

Galla Rhois Chinensis is the gallnut made by an aphis *Melaphis chinensis* (Bell) on a plant – *Rhus chinensis* Mill. (A*nacardiaceae)*. It binds up the intestines and stops diarrhea, it is used against chronic diarrhea, dysenteric disorders, chronic blood in the stool, and rectal prolapsed. It has been reported to prove mild selective antimicrobial activity against clostridia (Ahn, et al., 1998).

1.3.7.3 Selective Inhibitory Action of Plant-derived Compounds

As the regular ATB agents disturb the health beneficial microflora, there is a need of developing new antimicrobial agents with selective antimicrobial properties against intestinal pathogens. Among traditional sources of antimicrobial compounds, the selective effect of several plant extracts and plant-derived compounds on intestinal bacteria have been studied in the last decade. Plant-products, which have been reported to achieve interesting results, are mainly phenolic compounds (e.g. quinones, naphthoquinones, phenolic acids, tannins etc.) and alkaloids (8HQ, coptisine). There are also few plant extracts and essential oils in the list. Majority of these assays were performed by disc dilution method.

Alkaloids

8-Quinolinol, an alkaloid derived from the roots of *Sebastiana corniculata* (*Euphorbiaceae*) was documented to prove growth inhibitory action against *C. difficile* and *C. perfringens* (at 50 μg/disc), whilst probiotic bacteria were not affected even at much higher concentrations (Kim, et al., 2006b). On the base of this study, we selected this substance for our microbial assay and it proved the most distinctive selectivity (discussed later). Jeon, et al. (2009) tested its derivates and found, that **2-methyl-8-hydroxyquinoline** and **8-hydroxyquinoline** exert even more prospective selective activity against *C. difficile*, *C. perfringens* and *E. coli*. Susceptibility of *C. perfringens* began at 20 μg/disc and strong response occurs at 100 μg/disc. Susceptibility of *C. difficile* began at 50 μg/disc and from the two bifidobacteria, only *B. longum* showed weak response at 1,000 μg/disc. Isoquinoline alkaloid isolated from *Coptis japonica (Ranunculaceae)* also exhibited selective inhibition of clostridia. *C. perfringens* responded to **coptisine chloride** at 62.5 μg/disc, whilst all the six probiotic bacteria kept no response until 125 μg/disc. At concentration 1,000 μg/disc, *C. perfringens* and *C. paraputrificum* were moderately susceptible and two of the six probiotic bacteria showed

weak response. However, selectivity does not seem to be convincing according to these results (Chae, et al., 1999).

Phenolic Compounds

Quinone isolated from the inner bark of *Tabebuia impetiginosa* (*Bignoniaceae*), **anthraquinone-2-carboxylic acid** (AQCA), exerted high inhibitory effect towards *C. paraputrificum*, which exerted response already at 0.1 µg/disc, and a very strong response at 1 µg/disc, whilst the six probiotic bacteria (4 bifidobacteria and 2 lactobacilli) were not sensitive even at 1000 µg/disc, except a weak response of *B. longum*. *C. perfringens* started to be sensitive at 100 µg/disc. AQCA was among selected phytocompounds for our assay and its selective inhibitory properties were affirmed, as discussed later. **Lapachol (furanonaphthoquinone)**, the second isolated compound from *T. impetiginosa*, exerted similar results, but the inhibitory action towards clostridia was lower (Park, et al., 2005). Another plant napthoquinone **(5-hydroxy-1,4-naphthoquinone)**, which also showed selective antimicrobial activity, was isolated from the heartwood of *Caesalpinia sappan* (*Leguminosae*). This compound affected *C. perfringens* at 250 µg/disc moderately, whilst two bifidobacteria were not sensitive up to 5,000 µg/disc (Lim, et al., 2007). Remarkable anticlostridial actions of naphthoquinones were further reported by Park, et al. (2005). Of the eight compounds, the most effective ones toward *C. paraputrificum* and *C. perfringens* were **menadione** and **plumbagin** (a strong response of *C. paraputrificum* at 0.1 µg/disc) followed by **1,4-naphthoquinone, lawsone, naphthazarin, juglone**, and **lapachol**. Two **phenolic acids**, isolated from the root of *Pulsatilla cernua (Ranunculaceae)* by Lee, et al. (2001), showed a potential selective inhibition of clostridia. **3,4-Dihydroxy cinnamic acid** had a moderate effect on *C. perfringens* at 100 µg/disc, whereas two strains of bifidobacteria were not susceptible up to 10,000 µg/disc, and one *Bifidobacterium* showed weak susceptibility at 2,000 µg/disc. The other constituent, **4-hydroxy-3-methoxy cinnamic acid (Ferulic acid)** also exerted inhibitory effect towards *C. perfringens* at 100 µg/disc and none of the three bifidobacteria responded up to 10,000 µg/disc. In contrast to the previous substance, it was active also against *E. coli*. We involved ferulic acid in our assay and these anticlostridial properties were not affirmed with other strains of clostridia. Kim, et al. (2006) derived active compounds from *Terminalia chebula* (*Combretaceae*) fruits. **Ellagic acid**

exerted selective growth inhibition properties against *C. perfringens* and *E. coli*. It had strong and moderate inhibitory effect on *C. perfringens* and *E. coli* at 1,000 μg/disc, whereas no adverse effect was observed by four probiotic bacteria. However, the lowest tested concentration was to low to establish the real anticlostridial inhibitory effect. Growth-inhibiting effects of *Cinnamomum cassia* bark-derived materials on HIM was conducted by Lee, et al. 1999. He reported mild selective inhibitory activity of phenolic substance **cinnamaldehyde** against pathogenic *C. perfringens, Bacteroides fragilis* and two strains of bifidobacteria. **Catechol**, also a phenolic substance and **leptospermone** (triketone), isolated from *Diospyros kaki* (*Ebenaceae*), were reported to exert selective inhibitory effect on clostridia. Leptospermone caused moderate and weak inhibition of *C. perfringens* and *C. difficile* at 250 μg/disc. Catechol showed weak activity against *C. perfringens* and *C. difficile* at 100 μg/disc and 500 μg/disc, respectively. Susceptibility of both clostridia was markedly increasing together with the concentration, whilst three probiotic bacteria did not respond even at 5,000 μg/disc. We involved catechol in our essay and its selective inhibitory properties were not affirmed (Jeong, et al., 2009a). **Flavonoid quercetin** was isolated from *Ginko biloba* (*Ginkgoaceae*) among three active compounds. It performed selective inhibitory action against *C. perfringens*. The lowest concentration tested was 500 μg/disc, and *C. perfringens* exerted strong response at this concentration. Both bifidobacteria tested were not susceptible till the highest concentration 5,000 μg/disc. The activity of quercetin was tested at too low concentrations, to assess the real breakpoint of clostridia inhibition (Lee, et al., 2002). **Biochanin A** is another flavonoid, which was reported to prove selectivity against clostridia. This plant compound, present in several plant species, was tested by Skleničková, et al. (2010) against eight *Clostridium* spp. and six *Bifidobacterium* spp. The average MIC for clostridia was 384 μg/ml, whilst for bifidobacteria it was > 4,096 μg/ml (Sklenickova, et al., 2010). Various **tannins**, a wide group of phenolic plant secondary metabolites, have been many times reported to exert positive effect on intestinal microflora. Kamijo, et al. (2008) investigated the selective antimicrobial properties of petals of *Rosa rugosa*. Two hydrolyzable tannins, which were established as responsible for this action, were isolated and identified. At the concentration of 1 μM/ml, the tannin **tellimagrandin II** enhanced the growth of *B. breve* to 114 %. The growth of *L. salivarium* was slightly decreased, but the growth of *E. coli* was reduced to 52 % and the growth of *Salmonella* spp.

to 43 %. The other tannin, **rugosin D**, exerted similar, but not as marked selective action. This study suggests, that the petals of *R. rugosa* and its constituents show probiotic effects on the IM. Puupponen-Pimia, et al. (2005), investigated the the effect of phenolic extracts and purified phenolic fractions of Nordic berries (*Rosaceae*) on intestinal bacteria. The activity was measured in liquid cultures by plate count method. Selective inhibition of IP was observed. Cloudberry (*Rubus chamaemorus*) and raspberry (*Rubus idaeus*) were the best inhibitors. Their extracts strongly inhibited *S. typhimurium* and *S. infantis* at 1,000 µg/ml, whilst *L. rhamnosus* was not affected. These extracts were found to be the richest in **ellagitannins.** However, *Salmonella* spp. were only partly inhibited by the berry phenolics, and most of the inhibition seemed to originate from other compounds, such as organic acids. Nohynek, et al., 2006, has also investigated the berry phenolics for their antimicrobial action. **The phenolic extracts of cloudberry and raspberry** exhibited strong inhibitory effect on *Campylobacter jejuni* and *C. perfringens* at 1,000 µg/ml, whereas *L. rhamnosus* was not affected. Effect of tea phenolics and their aromatic fecal bacteria metabolites on intestinal microbiota was essayed by Lee, et al. (2009). Fecal homogenates containing bacteria significantly catalyzed the major tea phenolics, including **epicatechin, catechin, 3-O-methyl gallic acid, gallic acid** and **caffeic acid** to generate aromatic metabolites dependent on bacterial species. These major phenolic compounds decreased in concentration after incubation with a fecal bacteria homogenate, whilst minor tea phenolics that were measured, 3-phenylpropionic acid and 4-OH phenylacetic acid, increased. These seven phenols were examined together with 3-(4-OH phenyl) propionic acid that has previously been identified as a polyphenol bacterial metabolite, for their growth effects on intestinal bacterial species. The tested culture was compared to that of the control (without tea polyphenol). The growth of certain pathogenic bacteria such as *C. perfringens*, *C. difficile, Bacteroides* spp., *E. coli*, and *Salmonella typhimurium* was significantly repressed by the presence of tea phenolics. In particular *C. perfringens* was strongly inhibited by 3-O-methyl gallic acid and gallic acid. Isolated strains of bifidobacteria were less severely affected. Growth inhibition of *S. enteridis* was only observed when grown with aromatic tea metabolites. Caffeic acid illustrated the strongest growth inhibition of most intestinal bacteria examined, especially *Salmonella* and *Clostridium* (Lee, et al., 2009). Bialonska, et al. (2009) was investigating the effect of *Punica granatum* (*Lythraceae*) fruit extract and its

tannin constituents on the human intestinal bacteria. **Punicalagins** and **ellagic acid** showed the most potent growth inhibition among studied compounds. The extract slightly decreased the growth of five tested lactobacilli (Ø to 80%), slightly affected, or markedly enhanced (Ø to 160 %) the growth of five tested bifidobacteria, significantly reduced the growth of three tested clostridia (Ø 15 %), and also decreased the growth of *Bacteroides fragilis* (to 73 %). Punicalagins showed following effect to the bacterial growth: lactobacilli Ø 82 %, bifidobacteria Ø 94 %, clostridia Ø - 14 %, and *B. fragilis* Ø 83 %. The effect of ellegaic acid was as follows: lactobacilli Ø 72 %, bifidobacteria Ø 92 %, clostridia Ø 8 % and *B. fragilis* 24 %. The bacterial growth media contained 0,01 % (v/v) of individual compound and 0,05 (v/v) of the extract. According to above studies, tannins seem to have positive impact on HIM. They are often a normal part of our diet and that presumps their low toxicity to humans. They are an interesting field for the research of new food supplements.

Essential Oils

Sil, et al. (2005) reported that **cinnamon and clove essential oil** proved selective inhibitory activity against IP. He tested oils and some isolated oil compounds against pathogenic *E. coli, S. typhimurium* and lactobacilli. Although, he did not involve clostridia and bifidobacteria in his assay, we can observe selective antimicrobial activity against different intestinal pathogens and probiotic bacteria. A simulation of GIT conditions was a part of this trial. Essential **cinnamon oil** exerted very perspective results at 100 μg/ml. *S. typhimurium* and *E. coli* were almost 100 % inhibited, whilst the percentage of probiotic bacteria inhibition was max. 7 %. Less prospective results were also exerted by **clove oil. Carvacol** (phenolic substance) was reported to inhibit 100 % of *E. coli* and 97 % of *S. typhimurium* at the concentration 200 μg/ml, with 3 % inhibition effect on *B. longum*.

Extracts

Curcuma longa (*Zingiberaceae*) extract showed selective inhibition of *C. perfringens* and three bifidobacteria at 10 mg/disc (Lee, et al., 2001). The extract of *Panax ginseng*, a well known adaptogen herb, was reported to positively influence the IM. Ahn, et al. (1990a) tested growth responses of several human intestinal bacteria to the extract of six oriental medicinal plants from the family *Araliaceae*. The extracts enhanced the growth of *B. breve* and *B. longum* in media with or without carbon sources. This effect was most pronounced

with water extract of *P. ginseng.* The growth of 27 bifidobacteria strains belonging to *B. adolescentis*, *B. longum, B. breve and B. infantis* were greatly stimulated, whereas seven *B. bifidum* strains and other bacteria such as clostridia and *E. coli* had little or no ability to utilize the extract for growth. Methanol extracts of *P. ginseng* was found to inhibit the growth of *C. perfringens* and *C. paraputrificum*, but this effect was not observed on other bacteria including bifidobacteria (Ahn, et al., 1990a).

1.4 Hypothesis

Since there has been evidenced a selective antimicrobial activity of several plant extracts and compounds against beneficial and harmful intestinal pathogens, we selected five the most prospective plant-derived compounds, which we expected to bear certain selective inhibitory activity against clostridia and bifidobacteria. We conducted an antimicrobial assay to evaluate our hypothesis.

2. OBJECTIVES

The main objective of this study is to evaluate in detail selective antimicrobial activity of five plant-derived compounds against standard strains and clinical isolates of clostridia and bifidobacteria by comparison of their MIC values with aim to verify the potential of their application as selective pharmaceutical or microbiological agents selectively inhibiting the growth of GIT microorganisms.

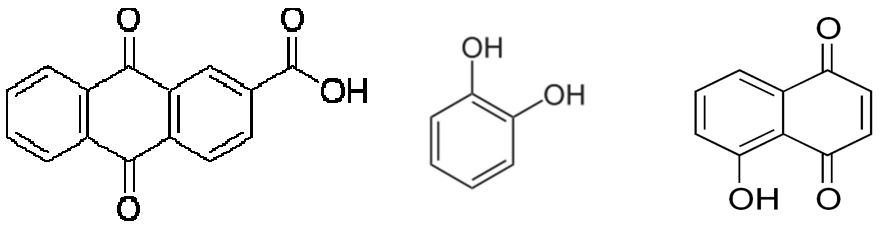
3. MATERIALS AND METHODS

3.1 Bacterial strains and culture media

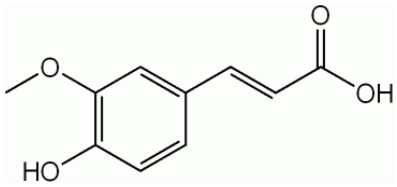
Bacteria were purchased from four different sources. Type strains of *Bifidobacterium* spp. and *Clostridium* spp. were obtained from The American Type Culture Collection (ATCC, Manassas, VA, USA), The Czech Collection of Microorganisms (CCM, Faculty of Science, Masaryk University, Brno, Czech Republic), and The German Resource Centre for Biological Material (DSM, Braunschweig, Germany). Wild strains of bifidobacteria and clostridia were isolated from the fecal samples of healthy infants aged from 1 to 6 months. All the strains we tested are listed in Table 1 and Table 2. Bifidobacteria strains were isolated by using modified Trypticase-Peptone-Yeast extract agar (Scharlau, Spain) supplemented with glacial acetic acid (1 ml/l) and mupirocin (100 mg/l) according to Rada and Petr (2000), and identified according to Vlková et al. Clostridial Reinforced Agar (Oxoid, Basingstoke, United Kingdom) was used for clostridia isolation according to Rada et al. (Rada, et al., 2008). Pure cultures were identified to the genus level using a fluorescence *in situ* hybridization kit with a *Clostridium butyricum* group-specific probe (RiboTechnologies, Netherlands). Isolates were characterized using ANAEROtest 23 (Lachema, Czech Republic) and API 20A (BioMerieux, France). Bifidobacteria were stored in Wilkins-Chalgren broth supplemented with soya peptone and glycerol (20% v/v) at -20°C. Clostridia were stored in Cooked Meat Medium at room temperature. The stock cultures were activated by anaerobic growth at 37°C for 24 hours in Wilkins-Chalgren broth supplemented with 5 g/l of soya peptone and 0.5 g/l of cystein. All media were purchased from Oxoid (Basingstoke, United Kingdom).

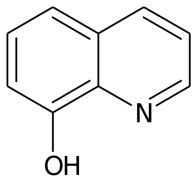
3.2 Chemicals

Anthraquinone-2-carboxylic acid, ferulic acid, juglon, catechol, 8-quinolinol, tetracycline and penicillin G were purchased at Sigma-Aldrich, Prague, Czech Republic. Chemical structures of tested compounds are shown in **Figure 1**.



Anthraquinone – carboxylic acid | Catechol | Juglone





Ferulic acid | 8-Quinolinol

Figure 1

3.3 Antimicrobial assay

In vitro broth microdilution method was used to determine minimum inhibitory concentration (MIC) of tested chemicals. We followed the guidelines of Hecht et al. (Hecht, 1999) and modified the methodology according to the recommendations for more effective evaluation of anti-infective potential of natural products, recently proposed by Cos, at al. (Cos, et al., 2006). We achieved anaerobiosis for our work in anaerobic chamber Bugbox (BioTrace, Bridgend, United Kingdom). The stock solution was prepared in dimethylsulfoxide (final concentration ≤ 1 %), obtained from Lach-Ner, Czech Republic. Twofold dilutions of stock solutions were carried out. Initial concentration for the basic screening of five natural compounds was 2048 µM and 16 µM for tetracycline. For detailed screening of 8H against standard and isolated strains, the initial concentrations were 2048 µg/ml for 8HQ and 16 µg/ml for tetracycline and penicillin G. 96 wells microtiter plates were employed in this assay. The bacterial inoculum was standardized to 1 x 10^6 CFU/ml using McFarland scale. Two ATBs, tetracycline and penicillin G were employed as the positive reference standards. Inoculated microtiter plates were incubated for 48 hours in 37 °C in anaerobic jar (Anaerobic

Plus System, Oxoid, United Kingdom). The growth of bacteria was evaluated by the turbidity in the well, determined spectrophotometrically by Multiscan Ascent Microplate reader (Thermo Fisher Scientific, Waltham, USA) at 405 nm. The MICs were defined as the concentrations resulting in a ≥80% inhibition of growth relative to the growth control. Each test was performed in triplicate as three independent experiments and median values were used to final MICs calculation.

4. RESULTS AND DISCUSSION

4.1 Selective Antimicrobial Effect of Compounds against Standard Strains

The results of screening of the five phytocompounds and tetracycline as the control antibioticum against three clostridia and three bifidobacteria standard strains are summarized in **Table 1**.

Table 1: The selective antimicrobial effect of compounds *in vitro* on standard strains

Bacterial strains	MIC[1] (µM)					
	AQCA[2]	Catechol	Ferulic acid	Juglon	8HQ[3]	Tetracycline
C. butyricum DSM[4] 10702	32	>2048	>2048	2048	128	0,125
C. clostridioforme DSM 933	256	>2048	>2048	2048	512	2
C. perfringens DSM 11778	128	>2048	>2048	2048	256	8
Average for clostridia	140	>2048	>2048	2048	300	5
B. animalis CCM 4988	512	2048	2048	>2048	2048	4
B. catenulatum CCM 4989	1024	>2048	>2048	2048	>2048	4
B. longum ATCC 15 707	512	>2048	>2048	1024	>2048	2
Average for bifidobacteria	680	>2048	>2048	1700	>2048	3,3

[1]MIC = minimum inhibitory concentration; [2]AQCA = anthraquinone-carboxylic acid; [3]HQ = 8-hydroxyquinoline. Data are median values of three independent experiments, each performed in triplicate.

Among the five tested compounds, 8HQ exhibited most distinct selective inhibition of clostridia and bifidobacteria, whereas *Cl. butyricum* was the most sensitive with MIC 128 µM, followed by *Cl. perfringens* and *C. clostridioforme* with MICs 256 and 512 µM, respectively. All three bifidobacteria were considerably less sensitive to 8HQ, with MIC equal to 2048 µM for *B. animalis* and MIC > 2048 for *B. catenulatum* and *B. longum*. Taking into the account the average MICs, the inhibitory activity was 7 x higher against clostridia compared to bifidobacteria. On the base of these results, 8HQ was selected for detailed assay with higher number of bacteria.

AQCA also proved selective inhibition; moreover, it showed as the most potent anticlostridial agent, with the lowest MICs we measured. However, bifidobacteria were also

mildly susceptible to AQCA, and its selectivity came out less significant compare to 8HQ. MICs for clostridia are 32, 128 and 256 μM for *C. butyricum, C. perfringens* and *C. clostridioforme*, respectively. Bifidobacteria were less susceptible, the growth of *B. animalis* and *B. longum* was inhibited at 512 μM and *B. catenulatum* at 1024 μM. The average of MICs for bifidobacteria is 5 x higher than that for clostridia. These results correspond with the study, based on which we selected this compound for our assay. Park et al. (2005) isolated AQCA from the inner bark of *Tabebuia impetiginosa* and documented its effect on intestinal bacteria. He used disc dilution method and different bacterial strains, except *B longum*. Comparing both clostridia, *C paraputrificum* was extremely susceptible (strong response at concentration 1 μg/disc) and *C. perfringens* exhibited strong response at 100 μg/disc. Four bifififidobacteria were not affected up to 1000 μg/disc, except *B. longum* (weakly affected at 10 - 1000 μg/disc). According to the results of both works, AQCA proved as potent agent with selective inhibitory activity against bacteria of GIT.

Catechol was involved in our study on the base of the assay of Jeong et al. 2009. Using the disc dilution method and different bacterial strains, he documented higher inhibitory action towards 2 clostridia (100 and 500 μg/disk) than towards 2 bifidobacteria (>500 μg/disk), which indicated possible selective inhibitory activity. However, we did not affirm this potency in our study, as catechol did not inhibit the growth of neither clostridia, nor bifidobacteria. The MICs were higher than 2048 μM, except *B. animalis*, where MIC = 2048 μM. Ferulic acid (4-hydroxy-3-methoxy cinnamic acid) also did not prove antimicrobial properties, expected on the base of the study documented by Lee, et al. (2001). He isolated this phenolic acid as the active compound from *Pulsatilla cernua* root. Using the disc dilution method, he conducted antimicrobial screening against intestinal bacteria, including *C. perfringens* and 3 bifidobacteria species. As a result, he reported moderate susceptibility of clostridia at concentration 100 μg/disk, whilst three species of bifdobacteria were not affected. Our screening showed inhibition of neither clostridia nor bifidobacteria up to 2048 μM, except *B. animalis*, where MIC = 2048 μM. Not even juglon demonstrated antimicrobial activity towards intestinal bacteria. This naphthoquinone-based compound was formely reported to exert inhibitory action at 10 μg/disk against *C. paraputrificum* and 200 μg/disk against *C. perfringens.* No growth inhibitory activity was observed in our assay, as all the established MICs were 1024 μM or higher. For all compounds mentioned in this paragraph,

the differences between our results and previously reported data can be caused by different methodology and type of bacterial strains used.

Interestingly, we observed opposite selective inhibitory tendency of tetracycline, as bifidobacteria were more susceptible than clostridia.

4.2 Detailed Evaluation of Selective Effect of 8HQ against Standard Strains and Clinical Isolates

The results obtained from the antimicrobial assay of 8HQ, tetracycline and penicillin G against 11 bifidobacteria and 12 clostridia strains are summarized in **Table 2**.

According to our results, 8HQ possesses remarkable anticlostridial effect, as the MICs for all tested clostridia ranged from 8 to 128 μg/ml. The most significant inhibitory effect of 8HQ was observed on *C. tertium* DSM2485 (MIC = 8 μg/ml), *C. clostridioforme* DSM933 (MIC = 16 μg/ml), isolated strain *C. difficile* infant KK4, and *C. perfringens* DSM11778 (MICs = 32 μg/ml for both strains), all of them known as pathogens responsible for various GIT disorders. Interestingly, the non-pathogenic species, isolates *C. acetobutylicum* infant L4 and *C. butylicum* infant AS3, were less suppressed by 8HQ, exhibiting MIC = 128 μg/ml. Compare to clostridia, all bifidobacteria showed lesser susceptibility with the least MIC = 256 μg/ml and the highest MIC ≥ 2048 μg/ml, whereas MIC ≥ 512 μg/ml was observed by 84 % bifidobacteria. Isolate *B. bifidum* infant JKM was the most resistant one, with MIC ≥ 2048 μg/ml. Isolate *B. breve* infant FE and standard strain *B. infantis* were the two most susceptible bifidobacteria with MIC 256 μg/ml. Well-marked selective antimicrobial effect of 8HQ is apparent from the arithmetic mean of MICs, which is 13 x higher for bifidobacteria (≥717 μg/ml) than for clostridia (53 μg/ml). These results correspond with the report of Kim et al., 2006, who conducted an antimicrobial trial of 8HQ and its derivatives against intestinal bacteria and reported 8HQ had proved selective inhibitory activity against 2 clostridia and 2 bifidobacteria strains. He used disk diffusion method and different bacterial strains. He reported strong response of *C. difficile* at 1000 μg/disc and of *C. perfringens* at 250 μg/disc. For these clostridia species, but different strains, we established MICs 32 μg/ml. In both assays, bifidobacteria proved significantly lesser susceptibility to 8HQ. In Kim's experiment, *B. longum* and *B. bifidum* showed weak response at concentration 1000 μg/disc. According

to our results, the growth of two strains of *B. longum* was inhibited by concentration 512 µg/ml and isolate of *B. bifidum* was the most resistant, with MIC ≥ 2048. As a result of both assays, 8HQ exhibited remarkable selective inhibitory action against bifidobacteria and clostridia indicating its application as a new antimicrobial agent against IP. Regarding its possible use as therapeutical drug, its toxicity towards humans should be further explored. It is well known substance, used as a precursor of antimalarial (quinaldine), fungicidal and protozoicidal agents (Ahmed, et al., 2008). Currently, the efficacy of 8HQ and its derivatives in inhibiting the progress of degenerative diseases is being studied intensively (Chobot, et al., 2011). Biological activity of 8HQ has been reported in a number of studies. However the It has been documented to possess broad spectrum of antimicrobial activities, including antibacterial (Kim, et al., 2006b; Jeon, et al., 2009; Shen, et al., 1999; Albert, et al., 1953), antimycobacterial (Darby, et al., 2010; Musiol, et al., 2010), and antifungal (Musiol, et al., 2010). Biocidal properties of 8HQ are most often attributed to the capability for chelating metallic ions, which is caused by hydroxyl group in C8 position in relation to nitrogen in peri position, enabling to chelate bivalent cations and this way to form the complexes with transition metals (Albert, et al., 1953; Shen, et al., 1999). Also toxic effects of 8HQ against various cell types are often explained by interfering with the cellular metallo-enzymes, which leads to metabolic disturbances (Albert, et al., 1953; Fraser, et al., 1975; Chobot, et al., 2011). However, the mechanisms of its antimicrobial activity and its toxicity towards humans are still subjects for further study.

Table 2 - The selective antimicrobial effect of 8-hydroxyquinolinol *in vitro* against bifidobacteria and clostridia.

Bacterial strains	MIC[5] (μg/ml)		
	8HQ[6]	Penicillin G	Tetracycline
B. adolescentis H1 (I[1]1)	512	0.13	0.5
B. animalis CCM[2]4988	512	0.25	2
B. bifidum ATCC 29521	-	0.06	0.25
B. bifidum JKM (I2)	≥2048	2	0.5
B. breve ATCC[3] 14700	1024	0.5	0.5
B. breve FE (I3)	256	0.13	0.5
B. catenulatum CCM 4989	512	0.25	2
B. dentium AP (I4)	1024	-	-
B. infantis ATCC 17930	256	0.06	2
B. longum ATCC 15707	512	0.5	2
B. longum J2 (I5)	512	0.25	16
Average for bifidobacteria	≥717	0.413	2.6
C. acetobutylicum DSM[4] 792	64	0.016	0.5
C. acetobutylicum L4 (I6)	128	0.25	0.25
C. butylicum AS3 (I7)	128	0.13	0.5
C. butyricum CM14 (I8)	32	0.06	0.25
C. butyricum DSM 10702	32	0.25	0.031
C. clostridioforme DSM 933	16	0.5	16
C. difficile KK4 (I9)	32	0.25	0.06
C. paraputrificum DSM 2630	64	0.13	16
C. perfringens DSM 11778	32	0.13	8
C. ramosum DSM1402	32	≤0.015	0.25
C. ramosum HH3 (I10)	64	0.25	0.25
C. tertium DSM2485	8	0.5	0.031
Average for clostridia	52.8	≤0.2	3.5

Data are median values of three independent experiments, each performed in triplicate, [1]I – Infant faeces isolate, [2]CCM - The Czech Collection of Microorganisms (Faculty of Science, Masaryk University, Brno, Czech Republic), [3]ATCC - The American Type Culture Collection (Manassas, VA, USA), [4]DSM - The German Resource Centre for Biological Material (Braunschweig, Germany), [5]MIC - Minimum inhibitory concentration, [6]8HQ - 8 hydroxyquinolinol

5. CONCLUSION

As a result of research focused on development of plant-derived antimicrobial agents effectively eliminating intestinal pathogens, and, at the same time, not harming beneficial intestinal microflora, we identified two prospective phytocompounds, namelly AQCA and 8HQ, which exhibited promising selective inhibitory action against clostridia and bifidobacteria. Although both of them proved desired activity, based on comparison of their selective properties, 8HQ was further evaluated in the series of tests with standard strains and clinical isolates. As a result, it possesses 13-fold higher inhibitory action against clostridia than against bifidobacteria. These findings together with its well-documented bacteriostatic and fungistatic use suggest 8HQ as a prospective agent for further development of antimicrobial preparations with selective activity against clostridia and bifidobacteria. It can also be recommended for application in microbiological media for bifidobacteria isolation.

REFERENCES

Adlerberth, I., Carlsson, B., de Man, P., Jalil, F., Khan, S. R., & Larsson, P. (1991). Intestinal colonization with Enterobacteriaceae in Pakistani and Swedish hospital-delivered infants. *Acta Paediatr Scand*(80), pp. 602-10.

Ahmad, I., Mehmood, Z., & Mohammad, F. (1998). Screening of some Indian medicinal plants for their antimicrobial properties. *Journal of Ethnopharmacology*(62), pp. 183–193.

Ahmed, S. M., & Ismail, D. A. (2008). Synthesis and Biological Activity of 8-Hydroxyquinolin and 2-Hydroxypyridine Quaternary Ammonium Salts. *J Surfact Deterg*(11:231–235).

Ahn, Y. J., Kim, M., Yamamoto, T., Fujisawa, T., & Mitsuoka, T. (1990a). Selective growth responses of human intestinal bacteria to *Araliaceae* plant extracts. *Microb. Ecol. Health Dis.(3)*, 169-175.

Ahn, Y. J., Lee, C. O., Kweon, J. H., Ahn, J. W., & Park, J. H. (1998). Growth-inhibitory effects of Galla Rhois-derived tannins on intestinal bacteria. *Journal of Applied Microbiology*(84), pp. 439–443.

Albert, A., Gibson, M. I., & Rubbo, S. D. (1953). The influence of chemical constitution on antibacterial activity. Part VI: The bactericidal action of 8-hydroxyquinoline (oxine). *The British Journal of Experimental Pathology*(Vol. 34 no. 2).

Allen, S. D. (1997). Pig-bel and other necrotizing disorders of the gut involving *Clostridium perfringens*. In D. H. Connor, F. W. Chandler, D. A. Schwartz, H. J. Manz, E. E. Lack, & E. E. Lack (Ed.), *Pathology of Infectious Diseases* (pp. 717-724). Norwalk, Conn., Conn.: Appelton & Lange.

Anonymous. (1985). TOXICOLOGY AND CARCINOGENESIS STUDIES OF &HYDROXYQUINOLINE (CAS NO. 148-24-3) IN F344/N RATS AND B6C3F1 MICE. *NIH Publication No. 86-2582 NTP-83-029*. New York: U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES.

Arribas, B., Rodriguez-Cabezas, M. E., Camuesco, D., Comalada, M., Bailon, E., Utrilla, P., . . . Zarzuelo, A. (2009). A probiotic strain of Escherichia coli, Nissle 1917, given orally exerts local and systemic anti-inflammatory effects in lipopolysaccharide-induced sepsis in mice. *British Journal of Pharmacology*(157), pp. 1024-1033.

Arunachalam, A. D. (1999). ROLE OF BIFIDOBACTERIA IN NUTRITION, MEDICINE AND TECHNOLOGY. *Nutrition Research*(19), pp. 1559-1597.

Aslam, S., Hamill, R. J., & Musher, D. M. (2005). Treatment of Clostridium difficile-associated disease: old therapies and new strategies. *Lancet Infect Dis*(5), pp. 549–57.

Baldi, F., Bianco, M. A., Nardone, G., Pilotto, A., & Zamparo, E. (2009). Focus on acute diarrhoeal disease. *World J Gastroenterol*(15(27)), pp. 3341-3348.

Balentine, D. A., Wiseman, S. A., & Bouwens, L. C. (1997). The chemistry of tea flavonoids. *Crit Rev Food Sci Nutr.*(37), pp. 693–704.

Barlett, J. G. (1982). Anti-anaerobic antibacterial agents. *Lancer*(2), pp. 478-481.

Barlett, J. G. (1992). Antibiotic - associated diarrhea. *Clin. Infect. Dis.*(15), pp. 573-579.

Bartlett, J. G., Chang, T. W., Gurwith, M., Gorbach, S. L., & Onderdonk, A. B. (1978). Antibiotic-associated pseudomembranous colitis due to toxin producing clostridia. *N Engl J Med*(298), pp. 531–34.

Bayer, A. S., Chow, A. W., Betts, D., & Guze, L. B. (1978). Lactobacillemia - report of nine cases. Important clinical and therapeutic considerations. *Am. J. Med*(64), pp. 808-813.

Bennion, R. S., Baron, E. J., Thompson, J. E., Downes, J., Summanen, P., Talan, D. A., & Finegold, S. M. (1990). The bacteriology of gangrenous and perforated appendicitis - revisited. *Ann. Surg.*(211), pp. 165-171.

Bensky, D., Gamble, A., & Kaptchuk, T. (1993). *Chinese Herbal Medicine.* Seattle, Washington: Eastland Press, Inc.

Bhunia, A. K. (2008). *Foodborne Microbial Pathogens, Mechanisms and Pathogenesis.* New York: Springer Science + Business Media, 233 Spring Street, NY, USA.

Bialonska, D., Kasimsetty, S. G., Schrader, K. K., & Ferreira, D. (2009). The Effect of Pomegranate (*Punica granatum* L.) Byproducts and Ellagitannins on the Growth of Human Gut Bacteria. *J. Agric. Food Chem.*(57), pp. 8344–8349.

Bondarenko, V. M., Lykova, E. A., & Matsulevich, T. V. (2006). Microecological aspects of small intestinal bacterial overgrowth syndrome. *Zh Mikrobiol Epidemiol Immunobiol.*(6), pp. 57-63.

Borriello, S. P. (1990). The infl uence of the normal flora on *Clostridium difficile* colonisation of the gut. *Ann Med*(22), pp. 61–67.

Brook, I. (1989). Anaerobic bacterial bacteremia: 12-year experience in two military hospitals. *J. Infect. Dis.*(160), pp. 1071-1075.

Butler, T. (2008). Loperamide for the treatment of traveler's diarrhea: broad or narrow usefulness? *Clinical Infectious Diseases*(47 (8)), pp. 1015–6.

Butzler, J. (2004). Campylobacter, from obscurity to celebrity. *Clin Microbiol Infect*(10(10)), pp. 868-76.

Civen, R., Jousimies-Somer, H., Marina, M., Borenstein, L., Shah, H., & Finegold, S. M. (1995). A retrospective review of cases of anaerobic empyema and update of bacteriology. *Clin. Infect. Dis*(20(Suppl. 2)), pp. S224-S229.

Cos, P., Vlietinck, A. J., Vanden Berghe, D., & Maes, L. (2006). Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. *Journal of Ethnopharmacology*(106:290-302).

Cowan, M. M. (1999). Plant Products as Antimicrobial Agents. *Microbiol. Rev*(12), pp. 564-582.

Cox, S. M., Phillips, L. E., Mercer, L. J., Stager, C. E., Waller, S., & Faro, S. (1986). Lactobacillemia of amniotic fluid origin. *Obstet. Gynecol.*(68), pp. 134-135.

Darby, C. M., & Nathan, C. F. (2010). Killing of non-replicating Mycobacterium tuberculosis by 8-hydroxyquinoline. *J Antimicrob Chemother*(Vol. 65: 1424 – 1427).

Davis, C. D., & Milner, F. A. (2009). Gastrointestinal microflora, food components and colon cancer prevention . *The Journal of Nutritional Biochemistry*(20), pp. 743-752 .

De Sarro, A., & De Sarro, G. (2001). Adverse reactions to fluoroquinolones. an overview on mechanistic aspects. *Curr. Med. Chem.*(8 (4)), pp. 371–84.

Devriese, L. A., Collins, M. D., & Wirth, R. (1992). The genus Enterococcus. In A. Balows, H. G. Truper, M. Dworkin, W. Harder, K. H. Schleifer, & K. H. Schleifer (Ed.), *The Procaryotes. A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications* (2nd ed. ed., pp. 1465-1481). New York, N. Y.: Springer-Verlag.

Dolara, P., Luceri, C., Fillippl, C. D., Femia, A. P., Giovannelli, L., & Caderni, G. (2005). Red wine polyphenols influence carcinogenesis, intestinal microflora, oxidative damage and gene expression profiles of colonic mucosa in F344 rats. *Mutat Res*(591), pp. 237–246.

Donnenberg, M. S. (1995). Entheropatogenic Escherichia coli. In B. M. J., P. D. Smith, J. I. Ravdin, H. B. Greenberg, & R. L. Guerrant, *Infections of the Gastrointestinal Tract* (pp. 709-726). New York, N. Y.: Raven Press.

D'Souza, A. L., Rajkumar, C., Cooke, J., & Bulpitt, C. J. (2002). Probiotics in prevention of antibiotic associated diarrhea: meta analysis. *BMJ*, p. 324:1361.

Endtz, H. P., Ruijs, G. J., vanKlingeren, B., Jansen, W. H., vanReyden, T., & Mouton, R. P. (1991). Quinolone resistance in campylobacter isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. *J. Antimicrob. Chemother.*(27), pp. 199-208.

Farina, C., Goglio, A., Conedera, G., Minelli, F., & Capriolo, A. (1996). Antimicrobial susceptibility of *Escherichia coli* O157 and other enterohemorrhagic *Escherichia coli* isolated in Italy. *Eur. J. Clin. Microbiol. Infect. Dis.*(15), pp. 351-353.

Farnsworth, N. R., & Morris, R. W. (1976). Higher plants, the sleeping giant of drug development. *Am J Pharm Educ*(148), pp. 46-52.

Finegold, S. M., & George, W. L. (1989). *Anaerobic infections in humans.* (W. L. George, Ed.) San Diego, California: Academic Press, Inc.

Finegold, S. M., Flora, D. J., Attebery, H. R., & Sutter, V. L. (1975). Fecal bacteriology of colonic polyp patients and control patients. *Cancer Res.(35)*, 3407-3417.

Franz, C. M., Holzapfel, W., & Stiles, M. (1999). Enterococci at the crossroads of food safety? *Int J Food Microbiol*(47), pp. 1-24.

Fraser, R. S., & Creanor, J. (1975). Mechanism of inhibition of ribonucleic acid synthesis by 8-hydroxyquinoline and antibiotic lomofungin. *Biochemic Journal*(147: 401-410).

Fujisawa, T., Kuno, M., Kokubu, T., Hirata, R., Sasaki, K., Fujisawa, Y., . . . Mitsuoka, T. (1992). Effects of apple and corn fiber supplemented with bifidobacteria and fructooligosaccharides preparation (A & C) on the fecal microflora and fecal properties in patients with dementia senilis. *Bifidus(5)*, 173-176.

Funatogawa, K., Hayashi, S., Shimomura, H., Yoshida, T., Hatano, T., Ito, H., & Hirai, Y. (2004). Antibacterial activity of hydrolyzable tannins drived from medicinal plants against *Helicobactor pylori. Microbiol. Immunol.*(48), pp. 487–491.

George, W. L. (1989). Antimicrobial agent-associated diarrhea and colitis. In S. M. Finegold, W. L. George, & W. L. George (Ed.), *Anaerobic Infections in humans* (pp. 661-678). New York, N. Y.: Academic Press, Inc.

George, W. L., & Finegold, S. M. (1985). Clostridia in the human gastrointestinal flora. In S. P. Borriello, *Clostridia in Gastrointestinal Disease* (pp. 1-37). CRC Press, Inc., Boca Raton, Fla.

Gerding, D. N., Johnson, S., Peterson, L. R., Mulligan, M. E., & Silva, J. J. (1995). Clostridium difficile-associated diarrhea and colitis. *Infect. Control Hosp. Epidemiol.*(16), pp. 459-477.

Gill, D. M. (1982). Bacterial Toxins: a Table of Lethal Amounts. *MICROBIOLOGICAL REVIEWS. Vol. 46, No. 1*, pp. 86-94. Tufts University School of Medicine, Department of Molecular Biology and Microbiology, Boston, Massachusetts 02111.

Gold, J. S., Bayar, S., & Salem, R. R. (2004). Association of *Steptococcus bovis* bacterium with colonic neoplasia and extracolonic malignancy. *Arch Surg*(139), pp. 760–765.

Golledge, C. L., & Riley, T. V. (1995). *Clostridium difficele*-associated diarrhoea after doxycycline malaria prophylaxis (Letter). *Lancet*(345), pp. 1377-1378.

Golledge, C. L., Carson, C. F., O'Neill, G. L., Bowman, R. A., & Riley, T. V. (1992). Ciprofloxacin and *Clostridium difficile*-associated diarrhoea. *J. Antimicrob. Chemother.*(30), pp. 141-147.

Gorbach, S. L. (1998). *Clostridium perfringens* and other clostridia. In S. L. Gorbach, J. G. Barlett, N. R. Blacklow, & N. R. Blacklow (Ed.), *Infectious Diseases* (2 ed., pp. 1925-1933). Philadelphia, Pa, USA: The W. B. Saunders Co.

Gorbach, S. L., & Thadepalli, H. (1975). Isolation of *Clostridium* in human infections: evaluation of 114 cases. *J. Infect. Dis.*(131), pp. S81-S85.

Grossman, E. R., Walcheck, A., & Freedman, H. (1971). Tetracycline and permanent teeth: the relationship between doses and tooth color. *Pediatrics*(47), pp. 567-570.

Gupta, V., & R., G. (2009). Probiotics. *Indian J Med Microbiol*(27(3)), pp. 202-9.

Hahn, A. F. (1998). Guillain-Barré syndrome. *The Lancet*(352, Issue 9128), pp. 635 - 641.

Hall, I. C., & O'Toole, E. (1935). Intestinal flora in new-born infants. *Am J Dis Child*(49), pp. 390–402.

Hatano, T., Kusuda, M., Hori, M., Shiota, S., Tsuchiya, T., & Yoshida, T. (2003). Theasinensin A, a tea polyphenol formed from -epigallocatechin gallate, suppresses antibiotic resistance of methicillin-resistant *Staphylococcus aureus*. *Planta Med.*(69), pp. 984–989.

Hatheway, C. L. (1990). Toxigenic clostridia. *Clin. Microbiol. Rev.*(3), pp. 66-98.

Hecht, D. W. (1999). Antimicrobial Agents and Susceptibility Testing:. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, & R. H. Yolken, *Manual of Clinical Microbiology* (pp. 1555–63). Washington, DC, USA: ASM Press.

Hemaiswarya, S., Kruthiventi, A. K., & Doble, M. (2008). Synergism between natural products and antibiotics against infectious diseases. *Phytomedicine*(15), pp. 639–652.

Hill, M. J. (1998). Composition and control of ileal contents. *Eur J Cancer Prev*(7), p. Eur J Cancer Prev.

Hookman, P., & Barkin, J. S. (2009). *Clostridium difficile* associated infection, diarrhea and colitis. *World Journal Gastroenterol*(15).

Hooper, D. C. (2001). Emerging mechanisms of fluoroquinolone resistance. *Emerg Infect Dis*(7 (2)), pp. 337–41.

Horiuchi, S., Inagaki, Y., Yamamoto, N., Okamura, N., Imagawa, Y., & Nakaya, R. (1993). Reduced susceptibilities of Shigella sonnei strains isolated from patients with dysentery to fluoroquinolones. *Antimicrob. Agents Chemother.*(37), pp. 2486-2489.

Chae, S. H., Jeong, I. H., Choi, D. H., Oh, J. W., & Ahn, Y. J. (1999). Growth-Inhibiting Effects of *Coptis japonica* Root-Derived Isoquinoline Alkaloids on Human Intestinal Bacteria. *J. Agric. Food Chem.*(47), pp. 934-938.

Chartrand, S. A., Thompson, K. J., & Sanders, C. C. (1996). Antibiotic-resistant, gram - negative bacillary infections. *Semin. Pediatr. Infect. Dis.*(7), pp. 187-203.

Chobot, V., Drage, S., & Hadacek, F. (2011). Redox Properties of 8-Quinolinol and Implications for its Mode of Action. *Natural Product Communications*(Vol. 6 No. 5).

Choi, M., Won, H., & Park, H. (2004). Antimicrobial Activities of Maesil (*Prunus mume)* Extract . *The Korean Journal of Community Living Science, 2004*(15(4)), pp. 61-66.

Chrubasik, S., Pittler, M. H., & Roufogalis, B. D. (2005). Zingiberis rhizoma: a comprehensive review on the ginger effect and efficacy profiles. *Phytomedicine.*(12(9)), pp. 684-701.

Iannini, P. B. (2007). The safety profile of moxifloxacin and other fluoroquinolones in special patient populations. *Curr Med Res Opin, 23 (6)*, pp. 1403–13.

Jeon, J. H., Lee, C. H., & Lee, H. S. (2009). Antimicrobial Activities of 2- Methyl-8-Hydroxyquinoline and Its Derivatives against Human Intestinal Bacteria. *J. Korean Soc. Appl. Biol. Chem.*(52(2)), pp. 202-205.

Jeong, E. Y., Jeon, J. H., Lee, C. H., & Lee, H. S. (2009a). Antimicrobial activity of catechol isolated from *Diospyros kaki* Thunb. roots and its derivatives toward intestinal bacteria. *Food Chemistry*(115), pp. 1006–1010.

Jiménez, M. B. (2009). Treatment of irritable bowel syndrome with probiotics. An etiopathogenic approach at last? *REV. ESP. ENFERM DIG*(101. N.° 8), pp. 553-564.

Johnson, S., Clabots, C. R., Linn, F. V., Olson, M. M., Peterson, L. R., & Gerding, D. N. (1990). Nosocomial *Clostridium difficile* colonisation and disease. *Lancet*(336), pp. 97-100.

JONES, D. T., & WOODS, D. R. (1986). Acetone-Butanol Fermentation Revisited. *Microbiological reviews. 50 no 4*, pp. 484-524. American Society for Microbiology.

Jones, R. N., Kehrberg, E. N., Erwin, M. E., & Abderson, S. C. (1994). Prevalence of important pathogens and antimicrobial activity of parenteral drugs at numerous medical centers in the United States. I. Study on the threat of emerging resistances: real or perceived? *Diagn. Microbiol. Infect. Dis.*(19), pp. 203-215.

KAMIJO, M., KANAZAWA, T., FUNAKI, M., NISHIZAWA, M., & YAMAGISHI, T. (2008). Effects of *Rosa rugosa* Petals on Intestinal Bacteria. *Biosci. Biotechnol. Biochem.*(72 (3)), pp. 773–777.

Kasper, H. (2001). Development and Modification of the Intestinal Flora. *The Balance – Functional aspects of intestinal flora*. Yakult Europe B. V.

Khan, M. R., Kihara, M., & Omoloso, A. D. (2002). Antimicrobial activity of *Terminalia complanata* and Flacourtia zippelii. *Fitoterapia*(73), pp. 737–740.

Kim, K. H., Fekety, R., Batts, D. H., Brown, D., Cudmore, M., Silva, J., & Waters, D. a. (1981). Isolation of *Clostridium difficile* from the environment and contacts of patients with antibiotic-associated colitis. *J. Infect. Dis.*(143), pp. 42-50.

Kim, Y. M., Jeong, E. Y., Lim, J. H., & Lee, H. S. (2006b). Antimicrobial effects of 8-quinolinol. *Food Sci Biotechnol*(15), pp. 817-819.

Kolinda, S., & Gibson, G. R. (2007). Prebiotic capacity of inulin-type fructans. *J Nutr* (137), pp. 2503s–2506s.

Kołodziej, H., Kayser, O., Latté, K., & Ferreira, D. (1999). Evaluation of the antimicrobial potency of tannins and related compounds using the microdilution broth method. *Planta Medica*(65), pp. 444–446.

Koneman, E. W., Allen, S. D., Janda, W. M., Schreckenberger, P. C., & Winn, W. C. (1997). *Color Atlas and Textbook of Diagnostic Microbiology* (5 ed.). Philadelphia, Pa: Lippincott-Raven Publishers.

Lans, C., Turner, N., Khan, T., Brauer, G., & Boepple, W. (2007). Ethnoveterinary medicines used for ruminants in British Columbia, Canada. *J Ethnobiol Ethnomed.*, p. 3:11.

Leanderson, P., & Tagesson, C. (1996). Iron bound to the lipophilic iron chelator, 8-hydroxyquinoline, causes DNA strand breakage in cultured lung cells. *Cardnogenesls*(vol.17 no.3 pp.545-550).

Lee, H. S., & Kim, M. J. (2002). Selective Responses of three *Ginkgo biloba* Leaf-Derived Constituents on Human Intestinal Bacteria. *J. Agric. Food Chem.*(50), pp. 1840-1844.

Lee, H. S., Beon, M. S., & Kim, M. K. (2001). Selective Growth Inhibitor toward Human Intestinal Bacteria Derived from *Pulsatilla cernua* Root. *J. Agric. Food Chem.*(49), pp. 4656-4661.

Lee, J. H., Shim, J. S., Chung, M. S., Lim, S. T., & Kim, K. H. (2009). In Vitro Anti-Adhesive Activity of Green Tea Extract against Pathogen Adhesion. *Phytother. Res.*(23), pp. 460–466.

Lilly, D. M., & Stillwell, R. H. (1965). Growth promoting factors produced by probiotics. *Science*(147), pp. 747-8.

Lim, C. C., Ferguson, L. R., & Tannock, G. W. (2005). Dietary fibres as “prebiotics”: implications for colorectal cancer. *Mol Nutr Food Res*(49), pp. 609-619.

Lim, M. Y., Jeon, J. H., Jeong, E. Y., Lee, C. H., & Lee, H. S. (2007). Antimicrobial activity of 5-hydroxy-1,4-naphthoquinone isolated from *Caesalpinia sappan* toward intestinal bacteria. *Food Chemistry*(100), pp. 1254–1258.

Madrid, A. M., Hurtado, C., Venegas, M., Cumsille, F., & Defilippi, C. (2001). Long-term treatment with cisapride and antibiotics in liver cirrhosis: effect on small intestinal motility, bacterial overgrowth, and liver function. *The American Journal of Gastroenterology* (96), pp. 1251–1255.

Mahady, G. B. (2001). Global harmonization of herbal health claims. *J. Nutr.*(131(3s)), pp. 1120S-1123S.

Mahady, G. B., Parrot, J., C., L., Yun, G. S., & Dan, A. (2003). Botanical dietary supplement use in peri- and postmenopausal women. *Menopause*(10), pp. 65-72.

Malaguarnera, G., Leggio, F., Vacante, M., Motta, M., Giordano, M., Biondi, A., & Salmeri, M. (2011). Probiotics in the gastrointestinal diseases of the elderly. *Journal of Nutrition, Health and Aging*, pp. 1-9.

Marteau, P., Cuillerier, E., Meance, S., Gerhardt, M. F., Myara, A., & Bouvier, M. (2002). *Bifidobacterium animalis* strain DN- 173 010 shortens the colonic transit time in healthy women: a double-blind, randomized, controlled study. *Aliment Pharmacol Ther* (16), pp. 587-93.

Martens, U., Enck, P., & Zieseniß, E. (2010). Probiotic treatment of irritable bowel syndrome in children. *Ger Med Sci* (8), p. Doc07.

Mathabe, M. C., Nikolova, R. V., Lall, N., & Nyazema, N. Z. (2006). Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa. *JOURNAL OF ETHNOPHARMACOLOGY* (105), pp. 286-293.

Mbwambo, Z. H., Moshi, M. J., Masimba, P. J., Kapingu, M. C., & Nondo, R. S. (2007). Antimicrobial activity and brine shrimp toxicity of extracts of *Terminalia brownii* roots and stem. *BMC Complementary and Alternative Medicine.*, p. 7:9.

McCintosh, G. H., Royle, P. J., & Playne, M. J. (1999). A probiotic strain of *L. acidophilus* reduces DMH-induced large intestinal tumors in male Sprague–Dawley rats. *Nutr Cancer*(35), pp. 153–159.

Mccracken, V., & Lorenz, R. (2001). The gastrointestinal ecosystem: a precarious alliance among epithelium, immunity and microbiota. (3), pp. 1-11.

McFarland, L. V., Coyle, M. B., Krmer, W. H., & Stamm, W. E. (1987). Rectal swab cultures for *Clostridium difficile* surveillance studies. *J. Clin. Microbiol.*(25), pp. 2241-2242.

McKay, D. L., & Blumberg, J. B. (2006). A Review of the Bioactivity and Potential Health Benefits of Chamomile Tea (Matricaria recutita L.). *Phytother. Res.*(20).

Mead, G. C. (1978). Streptococci in the intestinal flora of man and other non-ruminant animals. In F. A. Skinner, L. B. Quesnel, & L. B. Quesnel (Ed.), *Streptococci* (pp. 245-261). London, UK: Academic Press, Inc. (London) Ltd.

Metchnikoff, E. (1908). *The prolongation of life.* New York: Optimistic studies New York: Putman's Sons.

Modler, H. W., McKellar, R. C., & Yaguchi, M. (1990). Bifidobacteria and bifidogenic factors. *Can. Inst. Food Sci. Technol. J.*(23), pp. 29-41.

Moellering, R. C. (1992). Emergence of *Enterococcus* as a significant pathogen. *Clin. Infect. Dis*(14), pp. 1173-1178.

Moore, W., & Moore, L. (1995). Intestinal floras of populations that have a high risk of colon cancer. *Appl Environ Microbiol*(61), pp. 3202–3207.

Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C., & Yolken, R. H. (1999). *Mannual of Clinical Microbiology* (7 ed.). Washington, DC 20005: American Society for Microbiology.

Musiol, R., Jampilek, J., Nycz, J. E., Pesko, M., Carroll, J., Kralova, K., & Polanski, J. (2010). Investigating the Activity Spectrum for Ring-Substituted 8-Hydroxyquinolines. *Molecules*(Vol. 15, 288-304).

Nakamura, J., Kubota, Y., Miyaoka, M., Saitoh, T., Mizuno, f., & Benno, Y. (2002). Comparison of four microbial enzymes in Clostridia and Bacteroides isolated from human feces. *Microbiol Immunol*(46), pp. 487–490.

Nataro, J. P., & Kaper, J. B. (1998). Diarrheagenic Escherichia coli. *Clin. Microbiol. Rev.*(11), pp. 142-201.

Navaneethan, U., & Giannella, R. A. (2008). Mechanisms of infectious diarrhea. *Nat Clin Pract Gastroenterol Hepatol.*(5(11)), pp. 637-47.

Neu, H. C., & Lubthavikul, P. (1982). Comparative in vitro activity of N-formimidoyl thienamycin against gram-positive and gram-negative aerobic and anaerobic species and its Beta-lactamase stability. *Antimicrob. Agents Chemother.*(21), pp. 180-187.

Ng, T. B., Ling, J. M., Wang, Z. T., Cai, J. N., & Xu, G. J. (1996). Examination of coumarins, flavonoids and polysaccharopeptide for antibacterial activity. *General Pharmacology: The Vascular System*(27/7), pp. 1237-1240.

Nohynek, L. J., Alakomi, H. L., & Kähkönen, M. P. (2006). *Berry Phenolics: Antimicrobial Properties and Mechanisms of Action Against Severe Human Pathogens* (Vol. 54).

Osawa, K., Saeki, T., Yasuda, H., Hamashima, H., Sasatsu, M., & and Arai, T. (1999). The antibacterial activities of peppermint oil and green tea polyphenols, alone and in combination, against enterohemorrhagic Escherichia coli. *Biocontrol. Sci.*(4), pp. 1-7.

Ouwehand, A., & Vesterlund, S. (2003). Health aspects of probiotics. *IDrugs*(6), pp. 573-80.

Owens, R. C., & Ambrose, P. G. (2005). Antimicrobial safety: focus on fluoroquinolones. *Clin. Infect. Dis.*(41 Suppl 2), pp. 144–57.

Parekh, J., & Chanda, S. (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Research.*(10), pp. 175 - 181.

Park, B. S., Kim, J. R., Lee, S. E., Kim, K. S., Takeoka, G. R., Ahn, Y. J., & KIM, J. H. (2005). Selective Growth-Inhibiting Effects of Compounds Identified in *Tabebuia impetiginosa* Inner Bark on Human Intestinal Bacteria. *J. Agric. Food Chem.*(53), pp. 1152-1157.

Parkar, S. G., Stevenson, D. E., & Skinner, M. A. (2008). The potential influence of fruit polyphenols on colonic microflora and human gut health. *Int J Food Microbiol*(124), pp. 295–298.

Parkes, G. C., Sanderson, J. D., & Whelan, K. (2009). The mechanisms and efficacy of probiotics in the prevention of *Clostridium difficile*-associated diarrhoea. *Lancet infect Dis*(9), pp. 237-244.

Pawar, S. P., & Pal, S. C. (2002). Antimicrobial activity of extracts of *Terminalia catappa* root. *Indian J Med Sci.*(56(6)), pp. 276-8.

Peek, R. M., & Blaser, M. J. (2002). *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. *Nat Rev Cancer*(2), pp. 28–37.

Perry, L. M. (1980). *Medicinal plants of East and Southeast Asia: attributed properties and uses.* Cambridge, Massachusetts and London: The MIT Press.

Peterson, L. R., Kelly, P. J., & Nordbrock, H. A. (1996). Role of culture and toxin detection in laboratory testing for diagnosis of *Clostridium difficile*-associated diarrhea. *Eur. J. Clin. Microbiol. Infect. Dis.*(15), pp. 330-336.

Pulverer, G., Beuth, J., Roszkowski, W., Burrichter, H., Roszkowski, K., Yassin, A.,& Jeljaszewicz, J. (1990). Bacteria of human physiological microflora liberate immunomodulating peptides. *Zentralbl. Bakteriol*(272), pp. 467-476.

Puupponen-Pimia, R., Nohynek, L., Hartmann-Schmidlin, S., Ka¨hko¨nen, M., Heinonen, M., Ma¨a¨tta¨-Riihinen, K., & Oksman-Caldentey, K. M. (2005). Berry phenolics selectively inhibit the growth of intestinal pathogens. *Journal of Applied Microbiology*(98), pp. 991–1000.

Rada, V., & Petr, J. (2000). A new selective medium for the isolation of glucose non-fermenting bifidobacteria from hen caeca. *Journal of Microbiological Methods*(43:127–32).

Rada, V., Nevoral, J., Trojanová, I., Tománková, E., Smeholová, M., & Killer, J. (2008). Growth of infant faecal bifidobacteria and clostridia on prebiotic oligosaccharides. *Anaerobe*(14:205-8).

Raskin, I., Ribnicky, D. M., Komarnytsky, S., Nebojsa, I., Poulev, A., Borisjuk, N., & Fridlender, B. (2002). Plants and human health in the twenty-first century. *TRENDS in Biotechnology*(20 No.12).

Roedig-Penman, A., & Gordon, M. H. (1997). Antioxidant properties of catechins and green tea extracts in model food emulsions. *J Agric Food Chem.*(45), pp. 4267–4270.

Rohde, C. L., Bartolini, V., & Jones, N. (2009). The Use of Probiotics in the Prevention and Treatment of Antibiotic-Associated Diarrhea With Special Interest in *Clostridium difficile*–Associated Diarrhea. *Nutrition in clinical practice* (Vol. 24, No. 1), pp. 33-40.

Rosenblatt, J. E. (1989). Susceptibility testing of anaerobic bacteria. *Clin. Lab. Med.*(9), pp. 239-254.

Roszkowski, W., Roszkowski, K., Ko, H. L., Beuth, J., & Jeljaszewicz, J. (1990). Immunomodulation by propionibacteria. *Zentralbl. Bakteriol* (274), pp. 289-298.

Ruoff, K. L., Ferraro, M. J., Holden, J., & Kunz, L. J. (1984). Identification of *Streptococcus bovis* and Streptococcus salivarius in the clinical laboratory. *J. Clin. Microbiol* (20), pp. 223-226.

Sack, R. B., Rahman, M., Yunus, M., & Khan, E. H. (1997). Antimicrobial resistance in organisms causing diarrheal disease. *Clin. Infect. Dis.*(24(Suppl. 1)), pp. S102-S105.

Salonen, J. H., Erola, E., & Meurman, O. (1998). Clinical significance and outcome of anaerobic bacteremia. *Clin. Infect. Dis* (26), pp. 1413-1417.

Samy, R. P. (2005). Antimicrobial activity of some medicinal plants from India . *Fitoterapia* (76, Issues 7-8), pp. 697-699 .

Sato, Y. (1997). Extraction and purification of effective antimicrobial constituents of *Terminalia chebula* Retz. against methacillin resistant *Staphylococcus aureus*. *Biol Pharmaceut Bull*(20), pp. 401-404.

Scalbert, A., & Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *J. Nutr.*(130), pp. 2073S–2085S.

Seeram, N. P., Henning, S. M., Zhang, Y., Suchard, M., Li, Z., & Heber, D. (2006). Pomegranate juice ellagitannin metabolites are present in human plasma and some persist in urine for up to 48 h. *J. Nutr.*(136), pp. 2481–2485.

Servin, A. L. (2004). Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. *FEMS Microbiology Reviews*(28), pp. 405–440.

Shandera, W. X., Tacket, C. O., & Blake, P. A. (1983). Food poisoning due to Clostridium perfringens in the United States. *J. Infect. Dis.*(147), pp. 167-170.

Shen, A. Y., Chen, C. P., & Roffler, S. (1999). A CHELATING AGENT POSSESSING CYTOTOXICITY AND ANTIMICROBIAL ACTIVITY: 7-MORPHOLINOMETHYL-8-HYDROXYQUINOLINE. *Life Sciences*(64, No. 9, pp. 813-825,).

Shen, Z. (1996). Recent research and developments in traditional Chinese medicine in China. In *Report of the third meeting of directors of WHO Collaborating Centres for Traditional Medicine* (pp. 7-13). Geneva,, Switzerland,: WHO Publications Office.

Si, W., Gong, J., Tsao, R., Zhou, T., Yu, H., Poppe, C., & Johnson, R. a. (2005). Antimicrobial activity of essential oils and structurally related synthetic food additives towards selected pathogenic and beneficial gut bacteria. *Journal of Applied Microbiology*.

Si, W., Gong, J., Tsao, R., Zhou, T., Yu, H., Poppe, C., . . . Du, Z. (2005). Antimicrobial activity of essential oils and structurally related synthetic food additives towards selected pathogenic and beneficial gut bacteria. *Journal of Applied Microbiology*.

Simhon, A., Douglas, J. R., Drasar, B. S., & Soothill, J. F. (1982). Effect of feeding on infants' faecal flora. *Arch Dis Child*(57), pp. 54-8.

Sklenickova, O., Flesar, J., Kokoska, L., Vlkova, E., Halamova, K., & Malik, J. (2010). Selective Growth Inhibitory Effect of Biochanin A Against Intestinal Tract Colonizing Bacteria. *Molecules* (15), pp. 1270-127.

Slack, J. M., & Gerencser, M. A. (1975). *Actinomyces, Filamentous Bacteria: Biology and Pathogenity.* Minneapolis, Minn., USA: Burgess Publishing Co.

Slutsker, L., Ries, A. A., Greene, K. D., Wells, J. G., Hutwagner, L., & Griffin, P. M. (1997). *Escherichia coli* O157:H7 diarrhea in the US: clinical and epidemiologic features. *Ann. Intern. Med.*(126), pp. 505-513.

Smith, E., Williamson, E., Zloh, M., & Gibbons, S. (2005). Isopimaric acid from *Pinus nigra* shows activity against multidrug-resistant and EMRSA strains of *Staphylococcus aureus*. *Phytother. Res.*(19), pp. 538–542.

Smith, L. D., & Williams, B. L. (1984). *The pathogenic Anaerobic Bacteria. 3rd ed.* Charles C. Thomas, Springfield, Ill.

Steffen, R., Sack, D. A., Riopel, L., Jiang, Z. D., Stürchler, M., Ericsson, C. D., & DuPont, H. L. (2003). Therapy of travelers' diarrhea with rifaximin on various continents. *The American Journal of Gastroenterology.*(98), pp. 1073–1078.

Summanen, P., Baron, E. J., Citron, D. M., Strong, C. A., Wexler, H. M., & Finegold, S. M. (1993). *Wadsworth Anaerobic Bacteriology Manual* (5th ed.). Belmont, California, USA: Star Publishing.

Tacconelli, E., De Angelis, G., Cataldo, M. A., Pozzi, E., & Cauda, R. (2007). Does antibiotic exposure increase the risk of methicillin-resistant *Staphylococcus aureus* (MRSA) isolation? A systematic review and meta-analysis. *Journal of antimicrobial Chemotherapy, 61*, pp. 26-38.

Tauxe, R. V., Puhr, N. D., Wells, J. G., Hargrett-Bean, N., & Blake, P. A. (1990). Antimicrobial resistance of Shigella isolates in the USA: the importance of international travelers. *J. Infect. Dis.*(162), pp. 1107-1111.

Teasley, D. G., Gerding, D. N., Olson, M. M., Peterson, L. R., Gebhard, R. L., Schwartz, M. J., & Lee, J. T. (1983). Prospective randomized trial of metronidazole versus vancomycin for *Clostridium difficile*-associated diarrhea and colitis. *Lancet.*(2), pp. 1043-1046.

Tosun, A., Bahadir, Ö., & Altanlar, N. (2006). Antimicrobial activity of some plants used in folk medicine in Turkey. *Turkish Journal of Pharmaceutical Sciences.*(3), pp. 167–176.

Traa, B. S., Walker, C. L., Munos, M., & Black, R. E. (2010). Antibiotics for the treatment of dysentery in children. *Int J Epidemiol.*(39), pp. Suppl 1:i70-4.

Uma, B., Prabhakar, K., Rajendran, S., Kavitha, K., & Sarayu, Y. (2009). Antibiotic Sensitivity and Plasmid Profiles of *Escherichia coli* Isolated from Pediatric Diarrhea. *J Glob Infect Dis*(1(2)), pp. 107–110.

Vimala, Y., & Dileep, P. (2006). Some aspects of probiotics. *Ind. J of Microbiol*(46), pp. 1-7.

Vlková, E., Nevoral, J., Jenčiková, B., Kopečný, J., Godefrooij, J., & Trojanová, I. (2005). Detection of infant faecal bifidobacteria by enzymatic methods. *Journal of Microbiological Methods* (60:365-73).

Volker Mai, M. (2004). Dietary Modification of the Intestinal Microbiota. *Nutrition Reviews*(62), pp. (I)235–242.

Vonberg, R. P., Kuijper, E. J., Wilcox, M. H., Barbut, F., Tull, P., & Gastmeier, P. (2008). Infection control measures to limit the spread of *Clostridium difficile*. *Clin Microbiol Infect*(14).

Walker, W. A. (2000). Role of nutrients and bacterial colonization in the development of intestinal host defenses. *J Pediatr Gastroenterol Nutr*(30b), pp. S2-S7.

Witte, W., Cuny, C., Strommenger, B., Braulke, C., & D., H. (2004). Emergence of a new community acquired MRSA strain in Germany. *Euro Surveill*(9), pp. 1-2.

Xie, W., Gu, D., Li, J., Cui, K., & Zhang, Y. (n.d.). Effects and Action Mechanisms of Berberine and Rhizoma coptidis on Gut Microbes and Obesity in High-Fat Diet-Fed C57BL/6J Mice. *PLoS ONE 6(9): e24520.*

Yamamoto, T., Juneja, L. R., Chu, D. C., & Kim, M. (1997). *Chemistry and Applications of Green Tea.* Boca Raton, USA: CRC Press.

Yang, Y., Yan, R. W., Cai, X. Q., Zheng, Z. L., & Zou, G. L. (2008). Chemical composition and antimicrobial activity of the essential oil of Amomum tsao-ko. *J Sci Food Agric.*(88), pp. 2111–2116.

Yoshida, T., Hatano, T., & Ito, H. (2000). Chemistry and function of vegetable polyphenols with high molecular weights. *BioFactors*(13), pp. 121–125.

Zai-Chang, Y., Bo-Chu, W., Xiao-Sheng, Y., Qiang, W., & Liang, R. (2005). The synergistic activity of antibiotics combined with eight traditional Chinese medicines against two different strains of *Staphylococcus aureus*. *Colloids and Surfaces B: Biointerfaces*(41), pp. 79–81.

ABBREVIATIONS

AQCA	anthraquinone-carboxylic acid
ATB	antibiotic, antibiotical
CDAD	*Clostridium difficile* - associated diarrhea
EGCG	epigallo-1-katechin-3-gallate
G^+ and G^-	gram positive and gram negative
GI	gastrointestinal
GIT	gastrointestinal tract
IBS	irritable bowel syndrome
IP	intestinal pathogens
IT	intestinal tract
LDC	low developed countries
MIC	minimum inhibitory concentration
MRSA	methicillin – resistant *Staphylococcus. aureus*
NE	necrotizing enterocolitis
PB	probiotic bacteria
SCFA	short chain fatty acids
ST	shiga toxin - producing
VRE	vancomycin - resistant *Enterococcus*
8HQ	8-hydroxyquinoline (8-quinolinol)