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Faculty of Tropical AgriSciences

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Assessing the adhesion of probiotic lactobacilli in the presence of kolaviron-rich *G. kola* extract to human colonic epithelial cells using static *in vitro* digestion model

BACHELOR'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled Assessing the adhesion of probiotic lactobacilli in the presence of kolaviron-rich *G. kola* extract to human colonic epithelial cells using static *in vitro* digestion model independently, all texts in this thesis are original, and all the resources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 15. 4. 2022

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Eva Vacíková

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Abstract

Garcinia kola belonging to the Clusiaceae family is a tree growing in Central and Western Africa. Local people chew the raw seeds of this tree to treat fever, digestive problems, malaria or as an aphrodisiac. Current studies have also demonstrated its analgesic, anticancer, immunomodulatory, antidiabetic, anti-inflammatory, antimalarial, antimicrobial, hepatoprotective, and neuroprotective effects on human health.

The seeds contain a wide range of biochemically active molecules, among which kolaviron, a complex of bioflavonoids, seems to possess many biological activities and health benefits. The aim of this work was to determine the adhesion of probiotic lactobacilli in the presence of kolaviron-rich *G. kola* extracts to human colonic epithelial cells. The first step was to determine the cytotoxicity of kolaviron-rich extract and in the second step, selected probiotic strains commonly found in the human gut microbiome were tested for their ability to adhere to epithelial cells in the presence of *G. kola* seed extracts. The MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-Diphenyltetrazolium Bromide) cytotoxicity assay was used to measure the cell viability; 6 common human lactobacilli (*L. acidophilus, L. fermentum, L. gasseri, L. plantarum, L. reuteri, L. rhamnosus*) and two human intestinal epithelial cells lines (Caco-2 and HT29) were used in the adhesion experiment. The results show that kolaviron-rich samples did not significantly improve the ability of lactobacilli to adhere to intestinal epithelial cells; on the contrary, in a limited number of cases, adhesion was reduced. No lactobacilli species reduced adhesion to both cell lines, only one species, *L. acidophilus*, did not show reduced adhesion in any of the cases. Kolaviron-rich *G. kola* extract affects the adhesion of lactobacilli to the human intestinal epithelial cells; nevertheless, the effect is neither unilaterally positive nor negative.

Key words: adhesion, bitter kola, gut microbiota, multipurpose tree

Abstrakt

Garcinia kola patřící do čeledi Clusiaceae je strom rostoucí ve střední a západní Africe. Místní lidé žvýkají syrová semena tohoto stromu k léčbě horečky, zažívacích potíží, malárie nebo jako afrodiziakum. Současné studie prokázaly také jeho analgetické, protinádorové, imunomodulační, antidiabetické, protizánětlivé, antimalarické, antimikrobiální, hepatoprotektivní a neuroprotektivní účinky na lidské zdraví. Semena obsahují širokou škálu biochemicky aktivních molekul, z nichž kolaviron, komplex bioflavonoidů, má zřejmě mnoho biologických aktivit a zdravotních přínosů. Cílem této práce bylo zjistit adhezi probiotických laktobacilů v přítomnosti extraktů z *G. kola* bohatých na kolaviron k epiteliálním buňkám lidského tlustého střeva. V prvním kroku byla stanovena cytotoxicita extraktu bohatého na kolaviron a ve druhém kroku byla testována schopnost vybraných probiotických kmenů běžně se vyskytujících v lidském střevním mikrobiomu adherovat k epiteliálním buňkám v přítomnosti extraktů ze semen *G. kola*. K měření životaschopnosti buněk byl použit test cytotoxicity MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-difenyltetrazolium bromid); v adhezním experimentu bylo použito 6 běžných lidských laktobacilů (*L. acidophilus, L. fermentum, L. gasseri, L. plantarum, L. reuteri, L. rhamnosus*) a dvě linie lidských střevních epiteliálních buněk (Caco-2 a HT29). Výsledky ukazují, že vzorky bohaté na kolaviron významně nezlepšily schopnost laktobacilů adherovat ke střevním epiteliálním buňkám, naopak v omezeném počtu případů došlo ke snížení adheze. U žádného z druhů laktobacilů, nebyla adheze snížena k oběma buněčným liniím. Pouze jeden druh, *L. acidophilus*, nevykazoval sníženou adhezi v žádném z případů. Extrakt z *G. kola* bohatý na kolaviron ovlivňuje adhezi laktobacilů k lidským střevním epiteliálním buňkám, nicméně tento účinek není ani jednostranně pozitivní, ani negativní.

Klíčová slova: adheze, hořká kola, střevní mikrobiota, víceúčelový strom

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List of the abbreviations used in the thesis

ATCC – American Type Culture Collection

BMI – Body mass index

CFU – Colony-forming units

EMEM – Eagle's minimal essential medium

FBS – Fetal bovine serum

G. kola – *Garcinia kola*

KV – Kolaviron

LAC – Lactobacilli

m.a.s.l. – Metres above sea level

MTT – 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide

PBS – Phosphate buffer solution

RFU – Relative fluorescence units

1. Introduction

Plant-based medicines have many advantages, such as relative safety, low toxicity, and availability. About 80% of the population still uses herbal medicine as primary health care, which appears to be effective (Ekene 2014). This work focuses on *Garcinia kola* Heckel (Clusiaceae) which is an important medicinal tree species with a natural occurrence in Western and Central Africa. It is used in traditional herbal medicine worldwide because every part of this plant has medicinal properties. The seeds are an important product of this tree. Many people in Africa chew these seeds to cure cough, chest colds, dysentery, liver disorders, laryngitis, bronchitis, diarrhoea, gonorrhoea (Ekene 2014), and also claim that chewing these seeds daily can prevent malaria infection (Konziase 2015). Last but not least, the seeds can be used to treat headaches and gastric disorders (Usunomena 2012).

One of the main active substances in the seeds of *Garcinia kola* are biflavonoids. (Menezes & Campos 2021). Biflavonoids are natural antioxidants with high antioxidant activity (Konziase 2015). These natural antioxidants exhibit low toxicity to human cells and therefore have great potential in medicine (Menezes & Campos 2021). One of the properties of biflavonoids is to search free radicals and convert them into harmless molecules, as well as influence various aspects of the activation of immune cells of the human body. Another property is the protection of the central nervous system from oxidative and excitotoxic stress (Maňourová et al. 2019).

The dominant biochemical component in the seeds of *Garcinia kola is* kolaviron, a complex of bioflavonoids. Kolaviron offers numerous pharmacological activities, such as gastroprotective activities, antihepatotoxicity, radio-protective vasodilation, hypolipidemics (Wang et al. 2020) and according to published reports it also exhibits antioxidant and anti-inflammatory properties (Ochuko et al. 2021).

Given these facts, many medicines or dietary supplements may contain kolaviron in the future. Therefore, the effect of kolaviron on the intestinal microbiota should be analysed to avoid possible secondary problems. The aim of this work was to determine the effect of kolaviron-rich extracts isolated from *Garcinia kola* seeds on the ability of

lactobacilli commonly found in the human gut microbiota to adhere to epithelial cells of the intestinal mucosa.

2. Literature Review

2.1. Garcinia kola

2.1.1. Taxonomy

Garcinia kola Heckel, commonly known as bitter kola, is medium-sized tree, belonging to the family Clusiaceae (Dadjo et al. 2020), formerly Guttiferae, Juss (1789). The family Clusiaceae has 18 genera, the best known of which are *Calophyllum, Clusia*, or *Garcinia* ("Neotropical Clusiaceae - Neotropikey from Kew" n.d.). The genus Garcinia includes approximately 260 species (Sosef & Dauby 2012), 140 native to the African region (Dah-Nouvlessounon et al. 2016). The genus was named after Swiss botanist Laurent Garcin (1683-1757), who published the first description of *Garcinia mangostana,* the most popular fruit species in the genus *Garcinia*. The genus can be divided into African and Asian groups of species according to its natural occurrence. Some species are also found in South America, where they have been introduced (Guedje & Fankap 2001).

The family Clusiaceae includes hemiepiphytic trees, lianas, or shrubs, often with a tendency to form adventitious roots. Species of this family exude white-yellow latex from various parts of the plant. The leaves are simple, entire, usually growing opposite, and contain glands or laticifers. Flowers are unisexual or hermaphroditic, sepals (2-) 4-many, usually free but can be joined at the base, petals 4-9, the colour may be white or creamy, less often pink, green, red, orange, purple or blackish. The androecium is variable, often secreting oils or resin. The family Clusiaceae has ovary superior and syncarpous, typically surrounded by staminodes. The seeds are often arillate, and the fruit can be berry, fleshy capsule or rarely drupe. ("Neotropical Clusiaceae - Neotropikey from Kew" n.d.).

2.1.2. Distribution and Ecology

Garcinia kola is a perennial tree growing in the forests of Central and Western Africa (Figure 1). We can also find this tree in the forests of Ghana, Cameroon, Sierra Leone and other countries of Western Africa (Ekene 2014). Even though this species is said to be predominant in coastal areas and lowlands up to 300 m.a.s.l., trees are also successfully grown in hilly areas around 750 m.a.s.l. (Agyili et al. 2007; Maňourová et al. 2019). *G. kola* is commonly found in areas classified by Köppen-Geiger as "tropical rainforest climate, tropical monsoon climate or tropical savanna climate" (Kottek et al. 2006). These areas are characterised by daily temperatures between 21 to 31 °C and average annual rainfall from 1000 to 3000 mm; these factors provide a relatively high humidity of around 75% (Babalola & Agbeja 2010; "Climate-Data.org" n.d.). This species is adaptable, tolerating a variety of soil types, and thrives best on sandy loams (Bechem et al. 2014).

Figure 1. Distribution of *Garcinia kola*. Source: MapChart, Author (2022).

2.1.3. Botanical description of *Garcinia kola*

Garcinia kola (Figure 2) is a multipurpose tree species with great medical potential, thanks to which is called as “wonder plant”. It is known in English as bitter kola, and in the African local language Yorubo, this plant is called “Orogbo” (Usunomena 2012).

It is an evergreen, medium-sized tree. It usually grows to a height of 15-17 metres. The leaves contain resin glands, are shiny, simple and approximately 6-14 cm long and 2-6 cm wide (Ekene 2014); they grow in opposition and the colour of the leaves is dark green. The trunk has smooth dark-brown bark, it is straight and cylindrical. The bark is pinkish below the surface and secretes yellow, water-proof, sticky latex when wounded (Eyog-matig et al. 2006; Matig et al. n.d.). The tree’s crown is relatively narrow, and when the tree is in bloom, the flowers are small, covered with short red hair. The fruit is a drupe with 1- 4 seeds (Figure 3). The surface of the fruit is smooth, and the colour of the fruit changes during maturation: unripe fruit is green, changing to orange as it ripens (Ekene 2014). Inside the fruit is yellow-red pulp. The pulp is edible but is usually not consumed because of its sour taste. The seeds have light-coloured pericarp, darken after drying or when aged. The kernel is white, contains branched lines producing red resinous globules. The tree usually blooms once a year, and it is primarily dioecious (“*Garcinia kola*” n.d.).

Figure 2. Morphological features of *Garcinia kola*. (a) bark with a fresh cut; (b) seedlings; (c) leaf collection from one tree; (d) seeds obtained in Yaoundé market; (e) branches with ripening fruits; (f) trunk with typical irregular branching pattern. Source: Manourova et al. (2019)

Figure 3. Softened fruit pulp for easier seeds removal. Source: Maňourová et al. (2019)

2.1.4. Use of *Garcinia kola*

Garcinia kola is an important plant in traditional herbal medicine worldwide. Every part of this plant is used for healing effects (Ekene 2014). The seeds of bitter kola are chewed to cure cough, chest colds, dysentery, liver disorders, laryngitis, bronchitis, diarrhoea, gonorrhoea, or as an aphrodisiac (Ekene 2014). The seeds can also be used to treat headaches and gastric disorders (Usunomena 2012). An extract from the seeds is used as a cure for various types of inflammation or liver cirrhosis. People living in Africa also prepare a traditional paste against cough from dried kernels and honey (Maňourová et al. 2019). The roots of *Garcinia* sp. are commonly used in Western Africa as favourite bitter chew sticks, and in Sierra Leone, the bark and roots are taken as a tonic to cure sexual dysfunction in men (Ekene 2014). The bark is also used to treat malaria and abdominal pain. In some African countries such as Ghana, roots and branches are sold in bundles. These branches and roots are chewed and serve as basic dental hygiene (Blay 2004). Natives in Central Africa also claim that chewing *G. kola* seeds daily can prevent malaria infection (Konziase 2015). An infusion from the leaves of *Garcinia kola* can be used to treat fever (Adebisi 2004). Latex is used to heal fresh wounds. It is administered externally at the site of injury, preventing sepsis. Latex is also popular in Nigeria because it induces nervous alertness and cures insomnia (Ekene 2014). *Garcinia kola* also has the potential to combat viral diseases, including Ebola, by stopping viral replication (Iwu 1993).

2.1.5. Biochemical characterization

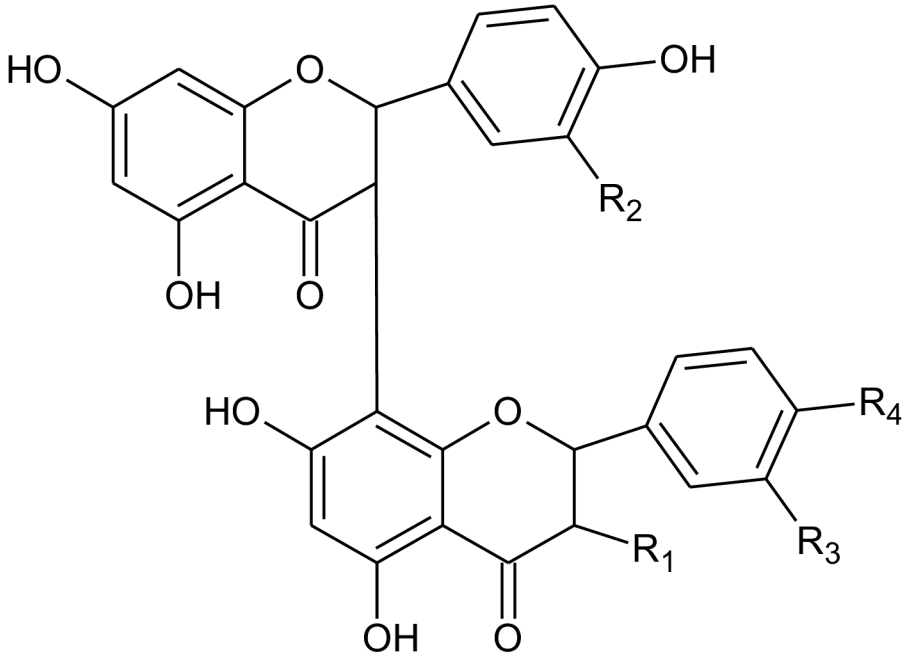
The main active substances in the seeds of *Garcinia kola* are biflavonoids, oleoresins, tannins, saponins, alkaloids and cardiac glycosides (Menezes & Campos 2021). Although antinutritional substances such as oxalates and phytates have been detected, the seeds are safe to eat, and there are no reports of harmful overdoses so far (Konziase 2015). Biflavonoids exhibit low toxicity to human cells and thus have great protentional in medicine (Menezes & Campos 2021). They are natural antioxidants with high antioxidant activity (Konziase 2015). These natural antioxidants are found in various parts of plants, such as stem bark, leaves, fruits, and flowers. In these parts of plants,

biflavonoids are distributed a lot. These substances belong to the class of low molecular weight phenolic compounds with a C6-C3-C6 carbon skeleton (Marais et al. 2006; Treml & Šmejkal n.d.). Biflavonoids are able to search for free radicals and convert them into harmless molecules, as well as influence various aspects of immune cell activation in the human body. These compounds play a useful role in protecting the central nervous system from oxidative and excitotoxic stress (Maňourová et al. 2019).

2.1.5.1. Kolaviron

The dominant biochemical component in *Garcinia kola* seeds is kolaviron (KV) which is a complex of biflavonoids (Wang et al. 2020). KV from the seeds of *Garcinia kola* offers numerous pharmacological activities, such as gastroprotective activities, anti-hepatotoxicity, radio-protective vasodilation, hypolipidemic, etc. (Wang et al. 2020).
KV (Figure 4) is consisting of biflavanones GB1, GB2 and kolaflavanone (Konziase 2015; Michel et al. 2016). According to published reports, KV isolated from *Garcinia kola* has potent antioxidant and anti-inflammatory attributes. These attributes have been studied in several disease models comprising cardiotoxicity, diabetes mellitus, reproductive toxicity, hepatotoxicity and gastrotoxicity (Ochuko et al. 2021). KV also has ability to protect the liver from CCl_4-induced hepatotoxicity (Adaramoye & Akinloye 2000). Previous assays focusing on the hepatoprotective properties have shown that the KV compound prevents liver damages associated with tetrachloromethane (Iwu et al. 1987; Braide n.d.) Recent findings suggest that KV could also prevent the neuro-destructive effects of methamphetamine on hippocampal neurons, thus providing some protection to the hippocampus as well (Ijomone et al. 2012). For its ability to combat oxidative and inflammatory damage induced by cuprizone, KV has demonstrated therapeutic potential against degenerative changes associated with demyelination and neurotoxicity. These abilities could later be exploited in the treatment of multiple sclerosis (Omotoso et al. 2018). In addition to its anti-inflammatory, antioxidant, and hepatoprotective effects, KV exhibits high antimalarial activity by suppressing *Plasmodium berghei* in infected mice (Oluwatosin et al. 2014; Tshibangu et al. 2016). In the future, KV could also be used for clinical benefit in patients with immune

deficiency diseases such as acquired immunodeficiency syndrome (AIDS). In addition, KV has potential in the treatment of benign prostatic hyperplasia by weakening infected prostate tissue in rats, which acted similarly to the conventional drug treatment with finasteride (Nworu et al. 2008; Kalu et al. 2016). In terms of gastroprotective properties, KV was found to reduce basal acid secretion and also simultaneously increase the pH level, reduce the number of ulcers and alleviate oxidative stress in the stomach of rats exposed to ischemia-reperfusion reaction (Odukanmi et al. n.d.).



	R1	R2	R3	R4
GB1	OH	H	OH	H
GB2	OH	H	OH	OH
kolaflavanone	OH	H	OMe	OH

Figure 4. Chemical structure of kolaviron (KV). Source: Author (2022)

2.2. The human gastrointestinal tract

Digestion in the human gastrointestinal tract is a dynamic and continuous process that begins in the oral cavity, with chewing and salivation of food. During mastication, food is mechanically processed into smaller parts by slicing, grinding, pressing and crushing with the teeth (Li et al. 2020). These processes break down food and prepare the food bolus for swallowing (Salles et al. 2010).

Food is salivated before swallowing. Saliva is a complex viscous aqueous medium. This medium contains 99.5 per cent water and 0.3 per cent protein, enzymes, and electrolytes such as calcium, potassium, sodium, magnesium, phosphate, and bicarbonate. There are 30 different enzymes present in human saliva. One of them, ptyalin, ensures the digestion of starch in the oral cavity (Salles et al. 2010).

After mechanical and chemical processing in the oral cavity, food passes through the oesophagus to the stomach (Boland 2016). The stomach is divided into four parts: fondus, body, antrum, and pylorus, and its total resting volume (fasting) is roughly 1 litre but can expand to 1.5- 4 litres (Norton et al. 2014). The proximal part of the stomach, including the fondus and body, acts as a reservoir of undigested material, while the distal stomach (antrum) crushes and mixes solid food. The stomach has three functions: mixing, storage and emptying (Kong & Singh 2008). The rate of food breakdown in the stomach is affected by gastric motility, changes in strength, pressure, and changes in gastric flow (Norton et al. 2014). The stomach secretes on average of 2–3 l of gastric fluid (pH 2) consisting of salt, gastric acid (hydrochloric acid) and digestive enzymes (pepsin, lipase). Because these gastric juices are aggressive, the stomach wall is covered with a mucosal layer that protects it against self-harm from acids present in the digestive juices. This layer is produced by foveolar cells, which are in the gastric pits where they produce mucus and bicarbonate ions. Parietal cells are responsible for the secretion of hydrochloric acid. The primary gastric cells are cells that produce the inactive precursor pepsinogen, which is activated by the hydrochloric acid to form pepsin, and they also produce gastric lipase, which is responsible for 10-30% of hydrolysis of triglycerides in the diet (Bornhorst & Paul Singh 2014). The gastric juices penetrate and dilute the food bolus and, together with stomach contractions, insure

homogenizing and mixing (Kong & Singh 2008). The trigger for the secretion of gastric juice by cells is the presence of food in the stomach (Boland 2016). Gastric juice and stomach contractions convert content into a multiphase mixture called chyme. Chyme contains separate phases of an aqueous solution, solids and fats (Kong & Singh 2008). Peristaltic waves cause the chyme to be propelled back into the body of the stomach by retropulsion, where the chyme is thoroughly mixed and emulsified by the excreted gastric juices. The contraction forces can exert a force of 0.2 to 1.89 newtons (Norton et al. 2014).

The main digestion process takes place in the small intestine consisting of the duodenum, jejunum, and ileum. The small intestine performs a digestive, secretory and absorption function. The small intestinal surface area is noticeably increased by the presence of numerous mucosal folds that contain villi (Figure 5). There are absorptive cells on these villi. Each of these cells has a microvillus border that increases surface area and contains glycocalyx containing the digestive enzymes. The epithelial cell line is on the membrane of the subjacent. This subjacent includes mesenchymal lamina propria, containing blood vessels (Gelberg 2014).

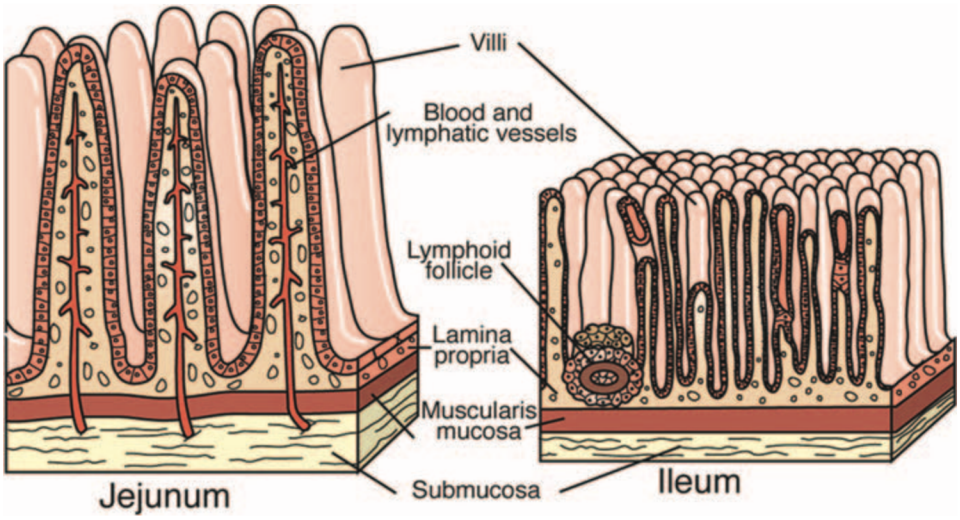


Figure 5. Schematic diagram of the anatomic and histologic organization of the digestive tube. Source: Kierszenbaum (2002)

Digested nutrients are absorbed in the ileum and jejunum, while most digestion occurs in the duodenum. The digestive enzymes and pancreatic enzymes (lipases, amylases, a complex mixture of proteases) produced by inner wall of the small intestine work together to break down food components. The optimal pH for these enzymes is around 6-7, which is provided by the pancreas by the secretion of bicarbonate into the duodenum. These enzymes allow the digestion of proteins by splitting them into dipeptides, tripeptides and amino acids, fats into free fatty acids and 2-monoglycerides and carbohydrates into monosaccharides (galactose, fructose, glucose). Bile from the gallbladder also reaches the duodenum and plays a vital role in the digestion of lipids, as bile enables the emulsification of dietary fats into small droplets, thereby also supporting the activity of pancreatic lipase. Motor activity in the small intestine propels undigested material into the large intestine, where it is fermented (Li et al. 2020). The colon, linking the ileum, is relatively short with no villi. The colon plays a vital role in water, minerals and vitamins reabsorption (Denbow 2015).

2.3. The human gut microbiota

The human gut microbiota is a unique ecosystem that is stretched throughout the gastrointestinal tract. It consists of bacteria, representatives of archaea and eukaryotes. The first gastrointestinal bacteria discovered and identified was *Escherichia coli* in 1885 in a child. The most widely represented genera of bacteria are Firmicutes, Bacteroidetes and Acinetobacter. The total diversity of bacteria colonizing the human digestive tract is estimated at 400-800 species (Bäckhed et al. 2005). Many studies (Arumugam et al. 2011; Clemente et al. 2012) have already tried to divide the gastrointestinal microbiota, settlement of the digestive tract in healthy people, into species. However, there are significant differences in gut microbiota composition between individuals. Therefore, the composition of the gut microbiota in humans remains incompletely described (Rajilić-Stojanović et al. 2007; Wang et al. 2017a). The gut microbiota is very diverse. Changes in gut microbiota composition are described in many studies (Radilla-Vázquez et al. 2016) dealing with obesity, diabetes, liver disease, cancer, and even neurogenerative diseases (Cani 2018). Nowadays, thanks to the use of genetic tools and the metagenomic revolution, we are already able to characterize

the composition and function of the gut microbiota and consequently associate this knowledge with potential diseases, risks, or specific clinical manifestations (Cabinian et al. 2018).

2.3.1. Changes in the composition of the gut microbiota

The type of our diet dramatically influences the composition of the gut microbiota. If we consume a purely plant-based or purely meat-based diet, our microbiota changes entirely within 24 hours. Recovery then takes up to 48 hours (David et al. 2014). As in animals, it has been shown in humans that if there is severe stress occurs in the body, for example, due to inflammation, acute changes in the gut microbiome can occur within a single day (Singh et al. 2017). Other factors such as antibiotic intake, food composition, and physical stress may cause gut dysbiosis in the gut microbiome. Dysbiosis is likely to interfere with the normal functioning of the gut microbiota and digestion. This situation leads to the development of certain microbes, including pathobionts, and can lead to the unregulated production of metabolites that may be harmful to the host, resulting in a range of diseases. (Kho & Lal 2018).

2.3.2. The function of the gut microbiota

The human microbiota is established after birth and plays an irreplaceable role in several metabolic, nutritional, physiological, and immunological processes (Ottman et al. 2012). The microbiota plays an essential role in the development of the intestinal mucosa and immune system of the host. The microbiota has the potential to increase energy extraction from food, contains much more versatile metabolic genes than are found in the human genome, and provides humans with unique and specific enzymes. In addition, the human gut microbiota is involved in nutrient extraction, providing the metabolism of undigested carbohydrates and vitamin biosynthesis, as well as providing a physical barrier to protect host from foreign pathogens through the production of competing antimicrobials (Wang et al. 2017a). Human intestinal microbiota affects basic biological processes (Figure 6). Is involved in the regulation of epithelial development and modulation of metabolic phenotype. Also, chronic diseases such as obesity,

diabetes mellitus, inflammatory bowel diseases, atherosclerosis, metabolic syndrome, alcoholic liver disease, cirrhosis, hepatocellular carcinoma and nonalcoholic fatty liver disease are affected by the human gut microbiota (Wang et al. 2017).

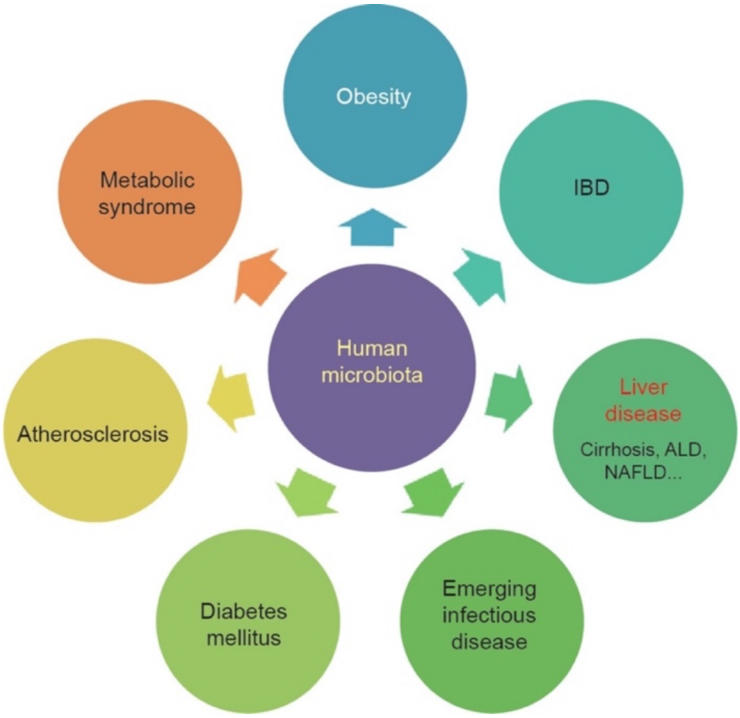


Figure 6. Human microbial symbiosis has a close relationship with diseases of different systems. Source: Wang et al. (2017)

2.3.3. Probiotics

The definition of probiotics is complex. However, they are mainly described as living microorganisms occurring naturally in the human gastrointestinal tract, which in balanced quantities have a positive effect on human health. The World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO) define probiotics as living microorganisms that can have a positive effect on human health in certain quantities. They are able to regulate the intestinal environment and prevent certain diseases (McFarland 2015). If we refer to food as a probiotic, these are foods enriched with modulation microorganisms (Matsumoto et al. 2010). The most important species used as probiotics include *Lactobacillus* spp., *Bifidobacterium* spp., a subspecies of

E. coli, and certain species of yeast *Saccharomyces* spp.(Hudson et al. n.d.). Probiotic organisms can favourably regulate gut health and even treat or prevent inflammatory bowel disease (Shen et al. 2014). Other benefits of probiotics include inhibition of pathogen adhesion in the intestinal mucosa (Collado et al. 2007), reduction of gastrointestinal intolerance symptoms (del Campo et al. 2014), and reduction of abdominal distension and ascites in patients with chronic liver disease (Liu et al. 2010). Probiotics are able to inhibit the adhesion of pathogenic microorganisms due to a number of mechanisms (Figure 7) such as their increased adhesion to the intestinal mucosa compared to pathogens, increased integrity of the epithelial barrier and production of antimicrobials (Hidalgo-Cantabrana et al. 2014; Yousefi et al. 2019).

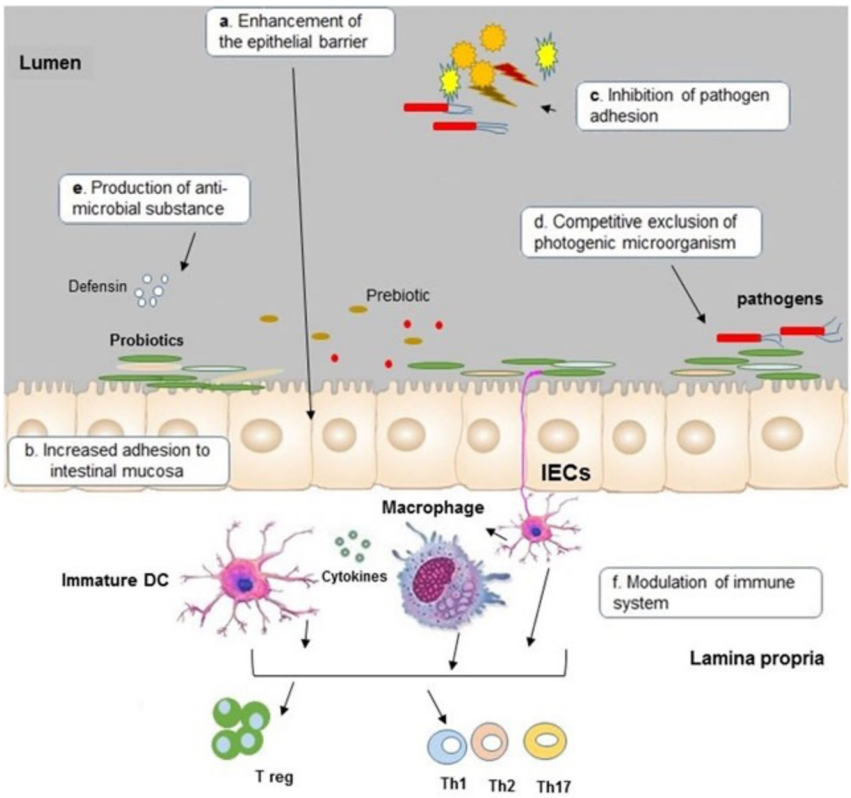


Figure 7. Major mechanisms of action of probiotics. DC: denditic cells, IEC: intestinal epithelial cells. Source: Yousefi et al. (2019)

2.3.4. Lactobacilli

The genus *Lactobacillus*, which we focused on in this work,% roughly represents 1% of the human gut microbiota (Sghir et al. 2000). This genus consists of more than 170 species (Kleerebezem & Vaughan 2009). The amount of lactobacilli (LAC) found in the gastrointestinal tract varies depending on the condition of the intestine, age of the host and other parameters (Lebeer et al. 2008). This genus is a widespread group occupying a wide range of habitats (Heilig et al. 2002). LAC are used in the production of fermented foods of both plant and animal origin. Nowadays, they are one of the most exploited probiotically active microorganisms. The predominant species of the genus *Lactobacillus* found in infant faeces are *L. plantarum, L. salivarius, L. rhamnosus, L. paracasei, L. fermentum, L. gasseri, L. delbrueckii* and *L. reuterii.* In the case of adults, it is *L. gasseri, L. reuteri, L. crispatus, L. salivarius* and *L. ruminis* (Lebeer et al. 2008). In the gastrointestinal tract, the most common representatives are *L. casei, L. plantarum, L. fermentum* and *L. rhamnosus*. In the case of the stomach, it is *L. antri, L. gastricus, L. kalixensis, L. reuteria* and *L. ultunensi* (Turroni et al. 2014). The genus has many positive impacts on hosts. LAC inhibit the growth of other bacteria (Das et al. 2016), favourably affect growth, body weight and host size (Hamdan et al. 2016). Lactobacilli are capable of producing B vitamins, essential for hosts such as vitamin B2, B9 and B12 (Zhongwang et al. n.d.). Some representatives of this genus can also improve the bioavailability of macro- and micronutrients that the host cannot metabolize, by modifying the physiology of the intestines, by regulating the synthesis of growth factors such as B vitamins, calcium and spermidine (Turpin et al. 2010). The genus *Lactobacillus*, in particular the increased presence of *L. helveticus*, has a positive effect on the distribution of red blood cells in adults (Le Roy et al. 2015). Consumption of probiotic lactobacilli can effectively treat infectious diseases caused by rotavirus, adenoviruses, and herpes virus simplex type 1 (Todorov et al. 2008). The high number of individuals and the diversity of this genus in the microbiota are mainly associated with the production of essential fatty acids and short-chain fatty acids (Stsepetova et al. 2011). The high diversity of lactobacilli in the colon (primarily *L. casei* and *L. gasseri*) corresponds positively with lower BMI, and lower incidence of diabetes (Kadooka et al.

2010). Antifungal properties have also been discovered in LAC, particularly in the gastrointestinal tract, where LAC are able to effectively fight severe fungal diseases (Black et al. 2013).

2.3.5. Adhesion

As for adherence to the intestinal mucosa, research on pig intestinal cells shows that lactobacilli exhibit strong adhesion due to the presence of proteins on their surface. This glycoprotein surface and the so-called S-layer proteins forming the crystalline layer around the bacterium bind to the lectins of the intestinal epithelium. Also, the hydrophobic surface of lactobacilli facilitates adherence (Kos et al. 2003; Das et al. 2016). Adherence of probiotic bacteria is commonly evaluated through *in vitro* experiments where human tumorigenic cell lines such as Caco-2 and HT-29 are used to mimic adhesion to intestinal epithelial cells (Lebeer et al. 2012; Monteagudo-Mera et al. 2012; Tuo et al. 2013). However, *in vitro* experiments do not account for a number of factors, such as the host immune system, peristaltic movements, or competition between resident microbiota (Park et al. 2015). Thanks to the ability of LAC to adhere to the intestinal mucosa, a barrier is created that prevents colonization by pathogens (Ehrmann et al. 2002). Therefore, medicines and dietary supplements should not contain ingredients that impair the adhesion process of LAC.

3. Aims of the Thesis

The aim of this thesis was to determine the adhesion of probiotic lactobacilli in the presence of kolaviron-rich *G. kola* extract to human colonic epithelial cells. The specific aims were:

- to determine the cytotoxicity of kolaviron-rich extract from *G. kola*

and

- to test selected probiotic species commonly found in the human gut microbiome for their ability to adhere in the presence of *G. kola* seed extract to epithelial cells.

4. Methods and materials

4.1. Methods

To select appropriate concentrations of kolaviron, cytotoxicity was tested. Subsequently, an adhesion experiment was performed. Lactobacilli (6 species of lactobacilli) were stained with fluorescein isothiocyanate dye. A medium containing stained LAC and kolaviron (sample 5 and sample LME) were pipetted onto the cell monolayer (HT19 cell line and Caco-2 cell line were tested separately). Each lactobacillus species was tested at three different concentrations of the test samples (LME, 5). Fluorescence was then measured on Reader (FITC method). The percentage of adhesion was calculated in Excel.

4.1.1. Plant material

Fresh *Garcinia kola* seeds were obtained on Mfoundi market in Yaoundé, Cameroon (3°51’ 58.52” N; 11°31’28.87” E). They were identified and authenticated by Anna Maňourová, *G. kola* expert (Maňourová et al. 2019). Seeds were pre-dried at 40 °C, transported to the Czech Republic and further processed in the Laboratory of Ethnobotany and Ethnopharmacology of FTA and in the laboratories of the Department of Microbiology, Nutrition and Dietetics, which is a part of the Faculty of Agrobiology, Food and Natural Resources of the Czech University of Life Sciences. Seeds with peels were homogenized using laboratory blender Grindomix GM 100 (Retsch, Germany) and microfine grinder MF 10 Basic (IKA, Germany). Subsequently, they were defatted by separating the petroleum ether extract using the Soxhlet method with the Soxhlet-like extractor SER 148 (Mezos s.r.o., Hradec Králové, Czech Republic). The temperature was set at 70 °C for 120 minutes. The defatted samples were then dried at 103 °C and weighted until a constant sample weight was reached. The samples were then extracted with methanol followed by chloroform using modified methods described elsewhere (Iwu 1985; Iwu et al. 1990; Adaramoye & Akinloye 2000). Briefly, the defatted methanolic extract was partitioned between chloroform and water. The dark yellow-brown chloroform extract was evaporated and used as kolaviron-rich extract from *G. kola.* Two samples were used for the experiments; these samples were called sample 5 and sample LME.

All laboratory analyses were performed at least in duplicates based on Commission Regulation (EC) No 152/2009 (European Commission, 2009). The final result is then the arithmetic average of those measurements (per tree) in complying with standard deviation.

4.1.2. Cytotoxicity

Cell viability was measured using the (MTT) 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (Sigma-Aldrich s.r.o., USA) cytotoxicity assay developed initially by Mosmann (1983) with modification. Briefly, the cultures (ATCC, USA) of two cell lines of human colorectal adenocarcinoma HT-29 (ATCC HTB-38) and Caco-2 (ATCC HTB-39) was seeded in 96-well plates at a density of 2.5×10^3 (Caco-2, HT29). After 24 h, the cells were treated with two-fold serial diluted samples (16.152 to 516 µg/mL) for 72 h. Subsequently, MTT reagent (1 mg/mL) in Eagle's minimal essential medium (EMEM) was added each well and incubated for additional 2 h at 37 °C with 5% CO_2. Medium with MTT was removed and the intracellular formazan product was dissolved in 100 µL of dymethylsulfoxid. The absorbance was measured at 555 nm using a Tecan Infinite M200 spectrometer (Tecan Group, Männedorf, Switzerland) and the percentage of viability (IC_{50}) was calculated when compared to untreated control. Statistical analysis was performed using MagellanTM software (Tecan Group, Männedorf, Switzerland) and Microsoft M365 (Microsoft, Redmond, WA, USA), from the data of 3 independent experiments.

4.1.3. Adhesion

For the determination of adhesion, modified method described by Krausova et al. (2019) was used. Two human colorectal carcinoma cell lines, HT-29 (ATCC HTB-38) and Caco-2 (ATCC HTB-39). All cell lines were cultivated in EMEM supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich), 1% penicillin/streptomycin (10 000 units of penicillin and 100 mg of streptomycin, (Sigma-Aldrich), and 1% sodium bicarbonate (Sigma-Aldrich), were cultivated in humidity atmosphere at 37 °C and 5% CO_2. Before the experiment, cell lines Caco-2 and HT29 1×10^5 cells/well were seeded in a 96-well fluorescent white microtiter plate. The cells were incubated in EMEM until a minimum of 95% confluence was achieved (usually 72 h).

All tested human bacterial species (ATCC, USA) were cultivated for 24 h in the standard culture broth before the adhesion experiment. The bacterial suspension was then washed 2× in phosphate buffer solution (PBS). This suspension was then fluorescently

marked by adding 25 µg/mL of fluorescein (Thermo Fisher Scientific, USA) dissolved in 1ml sodium bicarbonate and incubating for 1 hour at 37 °C in the dark. Then bacterial species were washed 2× in PBS a prepared final bacterial concentration of 1×10^7 CFU (colony-forming units). The old medium was removed from the microtiter plate and 90 µL of new medium without supplements and 10 µL bacterial suspension with testing samples (in concentration 256; 128 and 64 µg/mL) were added.

The plates were incubated for 1.5 h at 37 °C and 5% CO_2 in the dark. All wells (with and without tasting samples) were two times washed with 100 µl PBS and then 100 µl of PBS were added. Fluorescence was measured at 478/510 nm using the TECAN Infinite M200 reader (Tecan Infinite M200 reader). All experiments were done in triplicate.

Percentage of adhesion was calculated as:

$$X\ (\%) = (RFU\ sample / RFU\ control)\ X\ 100$$

where X (%) is % of fluorescence in the well; RFU sample = well fluorescence in relative fluorescence units, with tasting sample. RFU control = well fluorescence in relative fluorescence units, without tasting sample.

4.1.4. Statistical evaluation

The results obtained are expressed as average and ± deviation. The resulting data are processed using a two-way analysis of ANOVA variance in Statistics with a materiality level of $\alpha = 0.05$.

5. Results

This study examined the effect of *Garcinia kola* seed extract (sample 5 and sample LME) on the adhesion of selected species of LAC: *L. acidophilus, L. fermentum, L. gasseri, L. plantarum, L. reuteri, L. rhamnosus*. Three different concentrations of each kolaviron-rich extract were tested on two human colorectal carcinoma cell lines (Caco-2 and HT29). The results were expressed in % as mean and ± standard deviation and were compared with the control (100% adhesion).

Table 1 and Table 2 show the results of the effect of kolaviron-rich extracts on the adhesion of selected lactobacilli species. Our results show that kolaviron-rich samples generally did not significantly improve the ability of selected lactobacilli species to adhere to intestinal epithelial cells. In limited cases, adhesion was statistically significantly reduced. If there was a decrease in adhesion, it occurred at all three concentrations. There was a trend that the greatest reduction in adhesion was observed at the highest concentrations of the kolaviron-rich extract.

Sample 5 reduced statistically significantly adhesion in the case of three lactobacilli species. In the case of the Caco-2 cell line, it was *L. fermentum* that showed statistically significantly reduced adhesion at all three concentrations. Adhesion of other lactobacilli species (*L. acidophilus, L. gasseri, L. plantarum, L. reuteri, L. rhamnosus*) to the Caco-2 cell line was not statistically significantly affected. In the case of the HT29 cell line it was *L. gasseri* and *L. plantarum* that showed statistically significantly reduced adhesion at all three concentrations. In the case of *L. gasseri* and *L. plantarum*, it was evident that the ability of these two species to adhere to the HT29 cell line decreased with the increasing concentration of the kolaviron-rich extract (sample 5). Adhesion of other lactobacilli species (*L. acidophilus, L. fermentum, L. reuteri, L. rhamnosus*) to the HT29 cell line was not statistically significantly affected.

Table 1. Adhesion of selected species of lactobacilli to the cell line in the presence of kolaviron-rich extract.

Sample	Concentration	*L. acidophilus*		*L. fermentum*		*L. gasseri*		Control
		Caco-2	HT29	Caco-2	HT29	Caco-2	HT29	
	µg/ml	% mean ± SD						
5	**256**	110,9 ± 37,3	91,8 ± 17,6	**71,0 ± 19,1***	69,8 ± 23,4	94,8 ± 5,6	**53,0 ± 7,2***	100
	128	90,2 ± 30,0	108,3 ± 38,6	**63,3 ± 21,0***	79,8 ± 13,1	78,3 ± 4,5	**70,5 ± 8,1***	100
	64	114,8 ± 14,9	88,8 ± 13,4	**87,3 ± 3,1***	106,4 ± 8,0	114,2 ± 16,7	**85,1 ± 7,1***	100
LME	**256**	86,5 ± 17,2	98,9 ± 12,2	100,5 ± 9,8	81,3 ± 6,9	107,7 ± 5,6	**71,6 ± 12,8***	100
	128	62,3 ± 11,9	97,1 ± 14,9	113,3 ± 9,8	91,6 ± 13,9	98,6 ± 14,0	**80,6 ± 6,3***	100
	64	120,4 ± 24,1	104,0 ± 19,1	85,6 ± 12,8	96,8 ± 14,4	112,6 ± 20,7	**85,6 ± 11,4***	100

Asterisks show significance of the mean values of three measurements compared to the control *p<0.05.

Table 2. Adhesion of selected species of lactobacilli to the cell line in the presence of kolaviron-rich extract.

Sample	Concentration	*L. plantarum*		*L. reuteri*		*L. rhamnosus*		Control
		Caco-2	HT29	Caco-2	HT29	Caco-2	HT29	
	µg/ml	% mean ± SD						
5	**256**	108,6 ± 23,0	**62, 4 ± 12,1***	113,7 ± 10,8	106,0 ± 1,6	83,6 ± 27,4	79,9 ± 13,3	100
	128	101,9 ± 7,8	**80,5 ± 10,0***	90,4 ± 13,1	102,4 ± 6,6	90,3 ± 7,8	108,4 ± 4,5	100
	64	148,9 ± 33,6	**92,3 ± 1,8***	98,6 ± 4,6	97,1 ± 12,9	87,1 ± 27,1	91,8 ± 16,9	100
LME	**256**	**39,3 ± 3,7***	73,3 ± 11,3	112,1 ± 26,7	**51,9 ± 5,2***	**56,49 ± 7,8***	82,0 ± 14,6	100
	128	**78,3 ± 9,2***	96,3 ± 18,1	84,5 ± 17,6	**50,7 ± 24,0***	**80,3 ± 7,5***	98,5 ± 26,4	100
	64	**71,6 ± 12,7***	92,8 ± 13,8	134,2 ± 18,1	**83,9 ± 1,9***	**70,9 ± 18,0***	90,2 ± 7,7	100

Asterisks show significance of the mean values of three measurements compared to the control *p<0.05.

For sample LME, adhesion was statistically reduced in the case of four lactobacilli species. In the case of the Caco-2 cell line, it was *L. plantarum* and *L. rhamnosus* that showed statistically significantly reduced adhesion at all three concentrations. Adhesion of other lactobacilli species (*L. acidophilus, L. fermentum, L. gasseri, L. reuteri*) to the Caco-2 cell line was not statistically significantly affected. In the case of the HT29 cell line, it was *L. gasseri* and *L. reuteri* that showed statistically significantly reduced adhesion at all three concentrations. In the case of *L. gasseri* it was evident that the ability of this species to adhere to the HT29 cell line decreased with increasing concentration of the kolaviron-rich extract (sample LME). Adhesion of other lactobacilli species (*L. acidophilus, L. fermentum, L. plantarum, L. rhamnosus*) to the HT29 cell line was not statistically significantly affected.

In the case of both kolaviron-rich extracts (sample 5, sample LME), no lactobacillus species showed reduced adhesion to both cell lines.

Only one lactobacilli species, *L. gaserri*, showed reduced adhesion to the HT29 cell line in both the sample LME and sample 5. Only one species of lactobacilli, *L. acidophilus*, did not show reduced adhesion in any of the cases.

A more detailed analysis of the effect of kolaviron-rich extracts on the adhesion of selected lactobacilli species is attached in the appendices.

6. Discussion

The ability to adhere is one of the most fundamental properties of probiotic organisms and is essential for colonization of the digestive tract. It brings some of the many health benefits that these organisms provide. The main site of action is the intestinal mucosa, which is covered with a layer of mucus that provides binding sites for intestinal bacteria (Zheng et al. 2013). To monitor the ability to adhere in vitro, different models are applied, mainly using intestinal cell lines Caco-2, HT29. These cell lines differ in mucin production, with the Caco-2 cell line not producing mucin and representing enterocytes, whereas HT29 produces mucin and represents goblet cells (Kadlec et al. n.d.). When testing the adherence of probiotics, it is important to consider the characteristics of the species, the intestinal cells and factors that might influence the testing. One of the factors influencing the ability of adhesion are binding proteins on the surface of some probiotic species (Jensen et al. 2012).

By their ability to adhere to the intestinal mucosa, probiotic organisms prevent the penetration of undesired substances and pathogenic bacteria. They compete with them for binding sites in the mucosa by various mechanisms of action, thereby regulating the immune system and preventing intestinal diseases (Jensen et al. 2012).

Our results show that kolaviron-rich samples generally did not significantly improve the ability of selected lactobacilli species to adhere to intestinal epithelial cells, but in limited cases, adhesion was statistically significantly reduced. One influencing factor was the cell line and, in this case, according to our results the adhesion was species-specific.

Another influencing factor was kolaviron-rich extracts - biflavonoid complexes, which were the main focus of this work. The only one of the selected lactobacilli species that did not show reduced adhesion in a presence of kolaviron-rich extracts was *L. acidophilus*. A positive effect of plant polyphenols, which include the biflavonoid complexes tested in this study, on the adhesion of *L. acidophilus* to the HT-29 cell line has already been demonstrated in a study (Celebioglu et al. 2018). Although our study did not confirm increased adhesion in *L. acidophilus*, this species was the only one in which adhesion was not negatively affected in either case.

No species of lactobacilli had reduced adhesion to both cell lines. One exception was *L. plantarum* which had reduced adhesion to the Caco-2 cell lines in case of sample LME while in case of the HT29 cell line had reduced adhesion in case of sample 5. This may indicate that both of kolaviron-rich extracts differed in content of non kolaviron substances. Both extracts were produced by the same process from seeds, suggesting that the differences may be also due to different seed origins, tree age, growing conditions, or storage.

These results provide a rough estimate of how these Lactobacilli species would behave in the human gut in the presence of a kolaviron-rich extract. The results of *in vitro* experiments regarding the ability of bacteria to adhere to epithelial cell lines are difficult to translate to the situation in the human gastrointestinal tract, where host defence systems, mucosal shedding, peristaltic flow and competition with resident microbiota influence bacterial adhesion (Lebeer et al. 2008).

7. Conclusion and recommendation

The aim of this study was to test the ability of selected lactobacilli species to adhere to colonic epithelial cells in the presence of a kolaviron-rich extract from the seeds of the *Garcinia kola* tree using a static *in vitro* digestion model. Six strains of lactobacilli (*L. acidophilus, L. fermentum, L. gasseri, L. plantarum, L. reuteri, L. rhamnosus)* were tested on two colorectal carcinoma cell lines: HT29 and Caco-2. Two kolaviron-rich samples were examined: sample LME, sample 5. The ability of selected lactobacilli species to adhere in the presence of kolaviron-rich extracts was not affected in most cases. However, in limited cases, adhesion was statistically significantly reduced. There was the trend of higher reduction in adhesion at the highest concentrations of kolaviron-rich extract which was 256 μg/ml. But if there was reduction of adhesion in given species and cell line it was statistically significant in all concentrations of kolaviron-rich extract. From achieved results it is not possible to determine which kolaviron-rich extract had more profound effect on lactobacilli adhesion.

Father research is needed to test the adhesion of lactobacilli in the presence of the kolaviron-rich extract to the co-cultures of mixed HT29 and Caco-2 cell lines using an *in vitro* static digestion model. This model of would simulate more realistically the conditions in the gut. Most important would be to test the gut microbiota composition of people who regularly chew *Garcinia kola* seeds and compare these results with the gut microbiota composition of people on a similar diet but who do not consume these seeds regularly.

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Appendices

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Appendix 1. Adhesion of selected species of lactobacilli to the cell line (HT29) in the presence of kolaviron-rich extract (sample LME).

Sample LME (HT29)		L. plantarum (Caco-2)			L. acidophilus (Caco-2)			L. rhamnosus (Caco-2)			L. gasseri (Caco-2)			L. reuteri (Caco-2)			L. fermentum (Caco-2)		
		256	128	64	256	128	64	256	128	64	256	128	64	256	128	64	256	128	64
L. plantarum (HT29)	256			**			*												
	128																		
	64																		
L. acidophilus (HT29)	256						**							***					
	128														***				
	64															***			
L. rhamnosus (HT29)	256									**									
	128																		
	64																		
L. gasseri (HT29)	256					*	***						**						*
	128						**												
	64																		
L. reuteri (HT29)	256			**		****	****		*	**						**		*	***
	128			*			***			*									*
	64																		
L. fermentum (HT29)	256																		**
	128																		
	64																		

Asterisks show significance of the mean values of three inter-day measurements compared to the control **** (p<0,0001), *** (p<0,001), **(p<0.01), *(p<0.05).

Appendix 2. Adhesion of selected species of lactobacilli to the cell line (Caco-2) in the presence of kolaviron-rich extract (sample LME).

Sample LME (Caco-2)		L. plantarum (Caco-2)			L. acidophilus (Caco-2)			L. rhamnosus (Caco-2)			L. gasseri (Caco-2)			L. reuteri (Caco-2)			L. fermentum (Caco-2)		
		256	128	64	256	128	64	256	128	64	256	128	64	256	128	64	256	128	64
L.plantarum (Caco-2)	256			**		*	**					****	****	****	****	****		***	****
	128						*						****		****	****			****
	64															****			
L. acidophilus (Caco-2)	256						**						*			*			
	128																		
	64																		
L. rhamnosus (Caco-2)	256						*			**		****	****		****	****		**	***
	128												****			****			**
	64																		
L. gasseri (Caco-2)	256	****	**					***					**						
	128		****	**					***										
	64			****						***									
L. reuteri (Caco-2)	256		**	*				***	*							**			
	128			**					***	*									
	64									***									
L. fermentum (Caco-2)	256	***						*											**
	128		***						*										
	64			***						*									

Asterisks show significance of the mean values of three inter-day measurements compared to the control **** (p<0,0001), *** (p<0,001), **(p<0.01), *(p<0.05).

Appendix 3. Adhesion of selected species of lactobacilli to the cell line (HT29) in the presence of kolaviron-rich extract (sample 5).

Sample 5 (HT29)		L. plantarum (Caco-2)			L. acidophilus (Caco-2)			L. rhamnosus (Caco-2)			L. gasseri (Caco-2)			L. reuteri (Caco-2)			L. fermentum (Caco-2)		
		256	128	64	256	128	64	256	128	64	256	128	64	256	128	64	256	128	64
L. plantarum (HT29)	256									*				*		***			
	128									*					*	***			
	64															*			
L. acidophilus (HT29)	256																		
	128																		
	64																		
L. rhamnosus (HT29)	256																		
	128																		
	64																		
L. gasseri (HT29)	256						*			***				***	**	****			*
	128						*			***					***	****			*
	64															***			
L. reuteri (HT29)	256											**							
	128																		
	64																		
L. fermentum (HT29)	256															**			
	128															*			
	64																		

Asterisks show significance of the mean values of three inter-day measurements compared to the control **** (p<0,0001), *** (p<0,001), **(p<0.01), *(p<0.05).

Appendix 4. Adhesion of selected species of lactobacilli to the cell line (Caco-2) in the presence of kolaviron-rich extract (sample 5).

Sample 5 (Caco-2)		L. plantarum (Caco-2)			L. acidophilus (Caco-2)			L. rhamnosus (Caco-2)			L. gasseri (Caco-2)			L. reuteri (Caco-2)			L. fermentum (Caco-2)		
		256	128	64	256	128	64	256	128	64	256	128	64	256	128	64	256	128	64
L.plantarum (Caco-2)	256																	***	
	128																		
	64																		
L. acidophilus (Caco-2)	256																**	*	
	128																	**	
	64																		**
L. rhamnosus (Caco-2)	256			**															
	128			**															
	64																		
L. gasseri (Caco-2)	256																		
	128																		
	64																		
L. reuteri (Caco-2)	256																		
	128																		
	64																		
L. fermentum (Caco-2)	256	***	**	****		*	***						*	*		***			
	128		***	****			***						*		*	***			
	64			***												*			

Asterisks show significance of the mean values of three inter-day measurements compared to the control **** (p<0,0001), *** (p<0,001), **(p<0.01), *(p<0.05)