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THE IMPACT OF NONI JUICE AND GRAPEFRUIT SEED EXTRACT (CITROSEPT) ON ANAEROBIC INTESTINAL MICROBIOTA

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Abstract: The main aim of the study was to evaluate the impact of *Morinda citrifolia* L. (noni) juice and grapefruit seed extract (Citrosept) on the growth of representatives of human intestinal microbiota. Pharmaceuticals were added to liquid medium at a final concentration of 1.0, 2.5 or 5.0% and their impact on the bacteria number was assessed by measurement of the turbidity after 24 h of culture. Citrosept had the strongest impact. It inhibited the growth of *Bacteroides galacturonicus* even at lowest concentration of 1%. The number of *Ruminococcus gauvreauii* cells was reduced by ~20% only in cultures with 5% Citrosept. Noni juice had diversified impact. In case of *Bacteroides galacturonicus,* the addition of 5% noni juice to medium caused reduction of that bacteria number by ~30%, while *Ruminococcus gauvreauii* growth was slightly stimulated. Neither noni juice nor Citrosept influenced *Bifidobacterium catenulatum* growth. Concluding, dietary supplementation with some pharmaceuticals may exert a negative effect on human health instead of supporting it. The bioactive compound present in consumed pharmaceuticals can modulate the intestinal microbiota.
Key words: antibacterial activity, grapefruit seed, intestinal microbiota, noni

INTRODUCTION

Many natural compounds, including plant polyphenols, have been widely used because of their strong antimicrobial properties against food-borne pathogens, and therefore they can be applied as novel preservatives in the food industry (**Selma et al. 2009**). Although this is important for pathogen elimination, it may have also opposite effect due to elimination of beneficial microflora or negative influence on human body. Recently, the mutagenicity and genotoxicity of different flavonoids and their metabolites has been demonstrated (**Skibola, Smith 2000**). Although it is unlikely that the doses of polyphenols taken together with a balanced diet could negatively influence our health, food supplements and pharmaceuticals that are available without any control contain even 20-fold higher level of polyphenols than in a typical vegetarian diet. The usage of pharmaceuticals rich in polyphenolic antioxidants that are recommended in terms of gram rather than milligram doses could result in exposure to potentially toxic concentrations of those compounds (**Skibola, Smith 2000**). A lot of scientific studies show the inhibitory effect of antioxidants on different species of bacteria, but only few of them refer to the intestinal microbiota (IM). In our previous study we demonstrated that flavonoid aglycones, but not their glycosides, may inhibit growth of some intestinal bacteria (**Duda-Chodak 2012**). The aim of the present study was to evaluate the impact of pharmaceuticals commonly used by people because of their recognized health benefit or medical effects on cultures of bacteria typical for human intestine. We chose two preparations available without a prescription in health food stores or in pharmacies: noni juice and grapefruit seed extract.

MATERIAL & METHODS

The materials for the study were noni juice (juice form *Morinda citrifolia*) and grapefruit seeds extract (Citrosept). Pharmaceuticals (PC) were filtered through the syringe filter (0.45 μm) before analysis. Pure cultures of *Bifidobacterium catenulatum* (DSM 16992), *Bacteroides galacturonicus* (DSM 3978), *Ruminococcus gauvreauii* (DSM 19829) were provided by Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Germany). Bacteria were grown anaerobically at 37°C in special media designed for particular microorganism. TSYE medium (30 g/l Trypticasein Soy Broth, 3 g/l yeast extract, pH 7.0-7.2) was used for *Bacteroides* and *Ruminococcus*. Medium for *Bifidobacterium* contained (per 1 l): peptone from casein, pancreatic digest (10,00 g), yeast extract (5,00 g), beef extract (5,00 g), soy peptone (5,00 g), glucose (10,00 g), K_2HPO_4 (2,00 g), $MgSO_4 \times 7\ H_2O$ (0,20 g), $MnSO_4 \times H_2O$ (0,05 g), Tween 80 (1,00 ml), NaCl (5,00 g), cysteine-HCl x H_2O (0,50 g), resazurin (1 mg), oraz 40 ml salt solution. Composition of salt solution (per 1 l): 0,25 g $CaCl_2 \times 2\ H_2O$, 0,50 g $MgSO_4 \times 7\ H_2O$, 1,00 g K_2HPO_4, 1,00 g KH_2PO_4, 10,00 g $NaHCO_3$, 2,00 g NaCl.

The impact of pharmaceuticals on bacteria was assessed in liquid medium inoculated with tested bacteria (3×10^6/ml) with or without various concentrations (1, 2.5 and 5%) of noni juice or Citrosept. The turbidity of the bacterial cultures after 24 h of incubation at 37°C was assessed nephelometrically by measuring the turbidity in McFarland scale (densitometer DEN-1B, Biosan) and the number of cells was calculated. As controls the number of bacteria in medium without PC (positive control), and turbidity of empty medium without bacteria (double blank) was determined. As the addition of PC to different media changed the medium color and turbidity, blank samples (medium with appropriate concentration of PC without bacteria) were also prepared. The value obtained for the blank was each time subtracted from the value obtained for the sample turbidity, taking into account the kind of medium and the concentration of polyphenol. The obtained results were expressed as % of positive control in order to facilitate the comparison between different bacteria species. All determinations were performed at minimum triplicate, and results were presented as arithmetic mean ± SD.

RESULTS

The result obtained in the study are presented in Fig. 1 and Fig. 2.

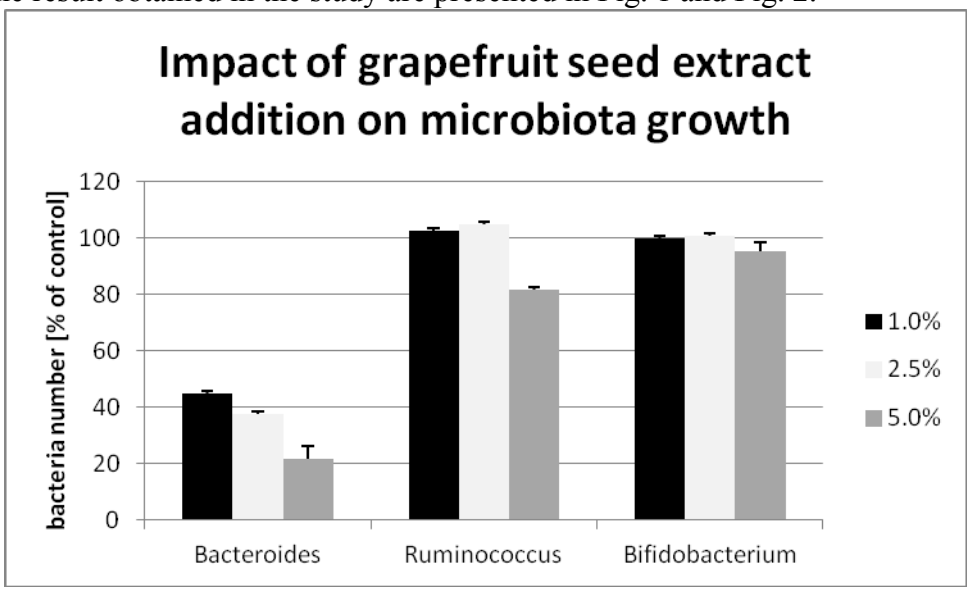

Fig. 1. The impact of grapefruit seed extract (Citrosept) at concentration of 1, 2.5 or 5% on the growth of intestinal microbiota representatives (mean ± SD)

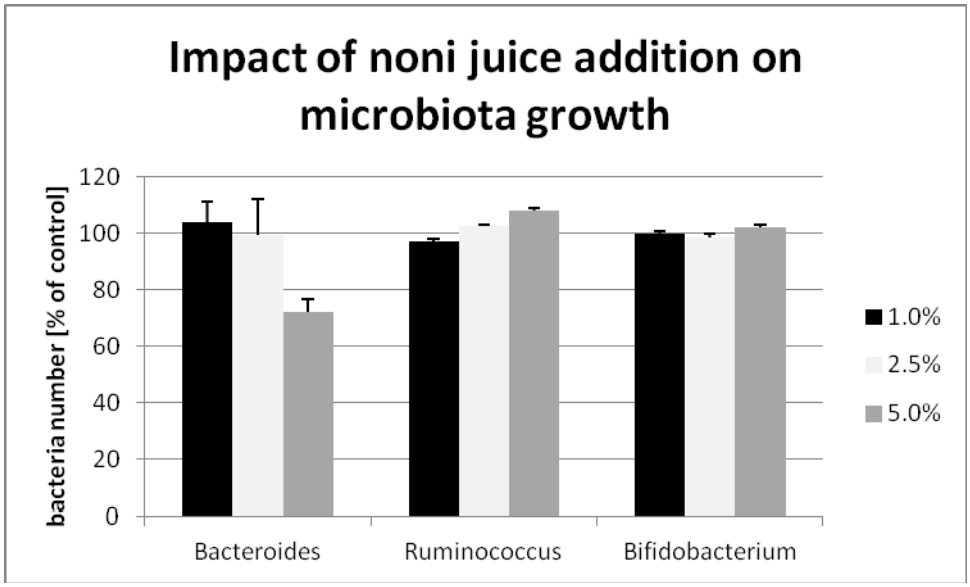

Fig. 2. The impact of noni juice at concentration of 1, 2.5 or 5% on the growth of intestinal microbiota representatives (mean ± SD)

DISCUSSION

A balance between the host and the gut microbiota is crucial for maintaining health and depends on many mechanisms. When this balance is disturbed (dysbiosis), the host–microbe relationship may progress towards a disease state. Many food compounds can influence the intestinal microbiota composition.

When comparing the results of all experiments it can be clearly demonstrated that the influence of pharmaceutical on microbiota depends on the analyzed species. The strongest impact on the microbial growth was demonstrated for Citrosept, which inhibited *Bacteroides galacturonicus* even when present in medium at lowest concentration of 1% (Fig. 1). At higher concentration the grapefruit seed extract reduced also the number of *Ruminococcus gauvreauii* cells by ~20%. Those results are in accordance with earlier studies. Cvetnić and Vladimir-Kneževic (2004) had shown that grapefruit seed extract strongly inhibited the growth of Gram positive bacteria, such as *L. monocytogenes, B. subtilis, S. faecalis, S. aureus,* but had no influence on Gram negative species. The grapefruit seed extract is also a potent fungal growth inhibitor, especially against *Candida tropicalis* and *C. krusei* (Kędzia 2001).

Morinda citrifolia (noni) is a medicinal plant used in folk remedies by Polynesians for over 2,000 years. It was proved that can act as therapeutic and preventive agent, with antioxidant, anti-inflammatory, antithrombotic, anticancer, and analgesic properties (Wang et al. 2002, Pawlus & Kinghorn, 2007, Potterat & Hamburger, 2007). It is also considered to have antimicrobial activity, however scientific research are inconclusive. In our research we proved that *Bacteroides galacturonicus* was sensitive to noni juice; the addition of 5% noni juice to medium caused reduction of that bacteria number by ~30% (Fig. 2).

However, noni juice cannot be considered as a potent antimicrobial agent without detailed experiment, as we had shown that *Ruminococcus gauvreauii* growth was stimulated by noni juice addition. Noni extract had no effect on the growth of *Escherichia coli, Salmonella typhimurium*, *Salmonella typhi, Shigella sonnei and Helicobacter pylori* (Sakunpak&, Panichayupakaranant, 2012). In other research, ethanolic extract of *Morinda citrifolia* at concentration of 10 mg/ml inhibited the growth of *E. coli, B. subtilis* and

S. aureus (Kumar et al. 2010). Neither noni juice nor Citrosept influenced *Bifidobacterium catenulatum*.

Concluding, dietary supplementation with some pharmaceuticals may exert a negative effect on human health instead of supporting it. *Ruminococcus gauvreauii*, *Bacteroides galacturonicus* and *Bifidobacterium catenulatum* are members of the commensal human intestinal microbiota. Its activity is essential for digestion of many food compounds as well as for increasing the bioavailability of polyphenols. This implies that the polyphenols present in consumed pharmaceuticals can modulate the intestinal microbiota and thus affect their own bioavailability.

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