The Effect of Gemmoderivatives on Probiotic and Pathogenic Microorganisms

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Abstract

Gemmotherapy is a biotherapeutical treatment method that uses extracts from fresh meristematic tissues obtained from different parts of the plants like buds, young twigs, seeds, seedlings, bark of young branches or rootlets. The gemmoderivatives composition is complex, containing a higher amount of active ingredients that can no longer be found in the adult plant. Therefore, the object of the study was to investigate the effects of commercial meristematic extracts from buds of *Ginkgo biloba*, young branches of blueberry (*Vaccinium myrtillus*), cranberry (*Vaccinium vitis-idaea*) and heather (*Calluna vulgaris*) on bacteria from 2 probiotics (*Linex Forte*, *Eubiotic*) and pathogenic *Staphylococcus aureus* and *Escherichia coli* and on two fungal strains isolates from dogs with mycotic infections. The antimicrobial effect was investigated using disc diffusion method and the inhibition zones were compared with that of different standards like ampicillin for antibacterial activity and nystatin for antifungal activity. Also, the microbial growth in culture media supplemented with tested gemmoderivatives were spectrophotometric analysed. The heather gemmoderivatives showed prominent antifungal activity while *E. coli* and *S. aureus* growth was inhibited mainly by *Ginkgo biloba* extract. The study revealed that gemmoderivatives have different inhibition patterns on probiotic strains and blueberry meristematic extract slightly increased the growth of probiotic strains. This preliminary research is the first report on the effect of gemmoderivatives on commercial probiotics.

Keywords: gemmoderivatives, probiotic, pathogenic bacteria and fungi.

1. Introduction

The gemmotherapy/meristemotherapy is a new phytotherapy branch that uses extracts from fresh meristematic tissues obtained from different parts of the plants like buds, young twigs, seeds, seedlings, bark of young branches or rootlets. Pol Henry is considered the "founding father" of meristemotherapy as the results of their initial studies were published, in 1959, in "Archives Homeopatique de Normandie" [1]. However, ancient Indian medicine (Ayurveda) used plant buds extracts for therapeutic purposes, as well as many medieval botanists were interested in bud-preparations [1].

It has been proved that gemmoderivatives have therapeutic power as their composition is complex and contain a higher amount of active ingredients that can no longer be found in the adult plant [2, 3]. Secondary metabolites, which are in high demand for their therapeutic values, can be found in higher amount in callus and regenerated plant compared to adult plants. Some of these metabolites have showed antibacterial and antifungal activity [4- 7]. Callus cultures from *Hellchrysum pedunculatum* (*Asteraceae*), a plant from South Africa well-known in folklore to have medicinal value, inhibited the growth of the Gram-positive bacteria, *Bacillus cereus, B. pumilus, B. subtilis* and *Staphylococcus aureus* as well as the Gram-negative bacterium, *Serratia marcescens* [4].

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Another study proved the increased inhibitory activities of callus extracts compared to natural adult plant extracts from *Premna serratifolia* L., an important medicinal shrub [8]. Similar results were obtained with steam and leaf callus extracts from *Solanum trilobatum* L. on *Escherichia coli* and *Staphylococcus aureus* [9].

Therefore, the aim of this research was to investigate the effects of commercial meristematic extracts from buds of *Ginkgo biloba*, young branches of blueberry (*Vaccinium myrtillus*), cranberry (*Vaccinium vitis-idaea*) and heather (*Calluna vulgaris*) on bacteria from 2 probiotics (*Linex, Eubiotic*), pathogenic strains *Staphylococcus aureus* and *Escherichia coli* and on two fungal strains isolates from dogs with mycotic infections.

2. Materials and methods

Strains and media: The *Staphylococcus aureus* and *Escherichia coli* strains from *Applied Microbiology Laboratory*, Faculty of Biotechnology (UASVM Bucharest) was grown on Mannitol Salt Agar MSA (*Accumedia*) and MacConkey Agar (*Accumedia*) media, respectively. Probiotics (*Linex Forte, Eubiotic*) were grown on Man, Rogosa and Sharpe MRS agar (*Oxoid*) medium, while the fungal strains isolates from dogs with mycotic infections on Sabouraud medium from Uranotest (*Urano Vet SL*) for *Dermatophytes* isolation. The turbidity (OD_{600}) of fungal and bacterial suspensions used as inoculum in all experiments was 0.41±0.57, measured with UV-1800 spectrophotometer (*CromTech)*.

Probiotics: Linex Forte is a supplement from *Sandoz AG* company and *Eubiotic* is manufactured by *Labormed Pharma*.

Gemmoderivatives: Commercial meristematic extracts from buds of *Ginkgo biloba*, young branches of blueberry (*Vaccinium myrtillus*), cranberry (*Vaccinium vitis-idaea*) and heather (*Calluna vulgaris*) are manufactured in Romania by *PlantExtrakt* company, using ecological plants. They are hydroglyceroalcoholic solutions of macerated fresh plant extracts in the first decimal dilution for therapeutic purposes.

The ethanol content of *Vaccinium* sp. extracts is 18% vol., and 33% vol for *Gingko* and heather, respectively. The pH values of meristematic extracts varied between 6.68 to 6.78.

Disc diffusion assay: Paper discs soaked in different meristematic extracts were placed on the surface of agar medium inoculated with bacterial or fungal strains [10]. Also, standard discs with ampicillin (SAM, 20 µg) and nystatin (100U) were used as positive control. Discs soaked in ethanol (18-33% vol.) were used as negative control. Plates inoculated with *S. aureus*, *E. coli* and strains from 2 probiotic (*Linex, Eubiotic*) were incubated at 37^0C, while fungi strains at 25^0C. The diameter of inhibition zones (Ɵ) were measured at 48 hours for bacteria and 7 days for fungi. In this study, the measurement of the inhibition zones is taken including the 5 mm diameter of the disc.

The antimicrobial activity was expressed as the ratio of the inhibition zone produced by the plant extract and the inhibition zone caused by the standard discs with antibiotics/antifungal. Testing was carried out for each strain in duplicates and the average results were reported.

Growth in liquid media supplemented with gemmoderivatives: The pathogenic and probiotic bacterial strains from *Linex* and *Eubiotic* were growth for 24 hours in 10 ml of Nutrient broth NB (*Acumix*) media supplemented with daily recommended dosage (2 ml) of meristematic extract of blueberry, cranberry, heather or *Ginkgo.*

Data analysis: The data from 3 experiments with 2 replicates were analyzed by ANOVA ($p<0.05$) and the average values and standard deviation (SD) were reported

3. Results and discussion

The extensive use of antimicrobials in therapy has reduced the incidence of infection diseases but has also led to the appearance of drug-resistant microbes. Therefore, it has been proposed to use plant extracts in combination with fewer amounts of traditional antibiotics to inhibit the appearance of antibiotic-resistant bacteria and to reduce the inhibitory effect of antibiotics on gut microbes [11].

Firstly, *in vitro* antimicrobial effects of commercial meristematic extracts from buds of *Ginkgo biloba*, young branches of blueberry, cranberry and heather were assessed using the disc diffusion method. The results showed that the antibacterial and antifungal activities of each meristematic extract are different. Thus, the heather (*Calluna vulgaris*) extract has the highest antifungal activity on 2 strains (P1, P5) isolates from dogs with mycotic infections. The

diameter of the zone of inhibition (mm) was 12±0.7 for fungal strain P1 identified as *Microsporum canis* and 10±0.9 for strain P5. The inhibition zone produced by nystatin standard (100U) on both fungal strains was 24±0.66mm. The antifungal activities, expressed as the ratio of the inhibition zone produced by different plant extracts and nystatin standard, vary between 0.1 to 0.5. The cranberry (*V. vitis-idaea*) meristematic extract showed the lowest antifungal activity.

The highest antibacterial activity on Gram-negative bacterium *Escherichia coli* was obtained with the extract from buds of *Ginkgo biloba.* The results obtained with disc diffusion method were consistent with those obtained after 48 hours growth on medium supplemented with daily recommended dosage of tested extract (Figure 1). All extracts inhibited the *E. coli* strain but only *Ginkgo* hydroglyceroalcoholic extracts had antibacterial activity of 0.7, compared with ampicillin (SAM20) standard.

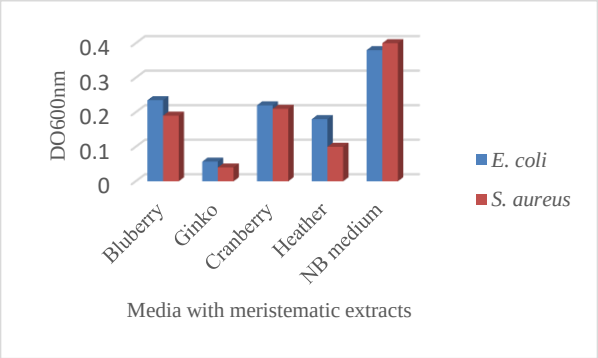

Figure 1. Antimicrobial activity of meristematic extracts of of *Ginkgo biloba*, *Vaccinium myrtillus*, *Vaccinium vitis-idaea* and *Calluna vulgaris* on *E. coli* and *S. aureus*

Also, in case of Gram-positive bacterium *Staphylococcus aureus*, the highest antibacterial effect was obtained with *Ginkgo* extracts (Figure 1). The results obtained with disc diffusion method proved that this extract had antibacterial activity of 0.85 on *S. aureus* using the same standard SAM20. Growth of *E. coli* and *S. aureus* strains in liquid media supplemented with berry gemmoderivative decreased to almost half of the bacterial growth in NB medium without extracts (38.15 – 52.5%).

These results are consistent with other studies which also showed that plant extracts inhibit Gram-positive bacteria more than Gram-negative ones [4-7]. Thus, Dilika et al. noticed that using agar dilution method callus extract from *Hellchrysum pedunculatum* inhibited all the Gram-positive bacteria tested as well as the Gram-negative bacteria *S. marcescens. E cloacae* and *M. kristinae* while *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* showed resistance [4].

The investigation showed different inhibition patterns for probiotic strains. It was noticed that blueberry (*Vaccinium myrtillus*) meristematic extract slightly increased the growth of strains from both probiotics, *Linex Forte* and *Eubiotic* (Figure 2, Figure 3).

Linex Forte dietary supplement containing at least $1.2x10^7$ CFU of lactic acid bacteria from species: *Lactobacillus acidophilus* LA-5 and *Bifidobacterium animals* subsp. *lactis* (BB-12) and it is used to treat gastrointestinal tract diseases in adults and children. Data outlined in Figure 2 showed that blueberry extract slightly increased the growth of lactic bacteria from Linex, while heather and *Ginko* extracts have antibacterial activity. Also, results based on disc diffusion method proved that blueberry and cranberry meristematic extract (*Vaccinium* sp.) do not produced inhibition zones on bacteria from *Linex Forte* and *Eubiotic*.

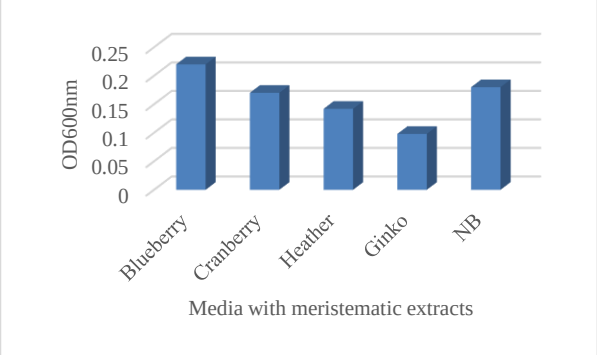

Figure 2. The effect of meristematic extracts of *Ginkgo biloba*, blueberry (*Vaccinium myrtillus*), cranberry (*Vaccinium vitis-idaea*) and heather (*Calluna vulgaris*) on lactic acid bacteria *from Linex Forte*

Eubiotic contains $1.4x\ 10^9$ CFU, with a different composition compared to *Linex*: *Lactobacilus acidophilus LA-5, Bifidobacterium animals subsp. lactis* and *Bifidobacterium BB-12*. Also, the blueberry (*Vaccinium myrtillus*) meristematic extract slightly increased the growth of lactic bacteria strains from *Eubiotic* (Figure 3). This preliminary research is the first report on the effect of gemmoderivatives on commercial probiotics.

Similar results were obtained by Biswas et al., 2012 who have proved that organic compounds derived from blueberries (*Vaccinium corymbosum*)

impacted the growth and viability of *S. typhimurium*, *E. coli* O157:H7, and *C. jejuni* strains without affecting the growth of probiotic species such as *Bifidobacteria bifidum* and *Lactobacillus bulgaris* [12]. Moreover, they have showed that blackberry juice inhibited *L. monocytogenes*, *S. typhimurium*, *C. jejuni*, and *E. coli* O157:H7 but had no inhibitory effect on *Lactobacillus casei*, *L. rhamnosus*, and *Lactobacillus plantarum* [12].

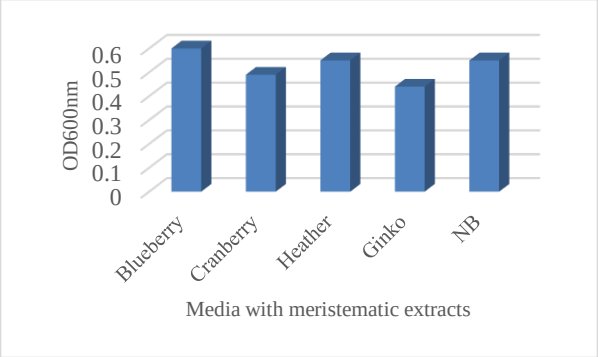

Figure 3. The effect of meristematic extracts of *Ginkgo biloba*, blueberry (*Vaccinium myrtillus*), cranberry (*Vaccinium vitis-idaea*) and heather (*Calluna vulgaris*) on lactic acid bacteria *from Eubiotic*

4. Conclusions

In conclusion, the research showed that commercial meristematic extracts from buds of *Ginkgo biloba*, young branches of blueberry (*Vaccinium myrtillus*), cranberry (*Vaccinium vitis-idaea*) and heather (*Calluna vulgaris*) have different effects on bacteria from probiotics (*Linex Forte*, *Eubiotic*) and pathogenic *Staphylococcus aureus* and *Escherichia coli*, as well as on two *Dermatophytes* fungal strains isolates from dogs with mycotic infections.

The highest antibacterial activity on *E. coli* and *S. aureus* was obtained with the extract from *Ginkgo biloba*, while heather (*Calluna vulgaris*) extract have antifungal activity. Different results were obtained during testing on lactic acid strains from probiotics when the blueberry (*Vaccinium myrtillus*) meristematic extract slightly increased their growth.

This preliminary research offers consumers supplementary information about the health benefits of some commercial meristematic extracts.

Acknowledgements

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