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Short Communication

Vaccinium myrtillus extract is effective against *Staphylococcus aureus* and does not interfere on the activity of antimicrobial drugs

Gustavo José da Costa[1], Renan Martins dos Santos[2,4], Flávio Júnior Barbosa Figueiredo[3,4], Marcus Vinícius Dias-Souza[4,5*]

1 – University Center of Caratinga - UNEC, Brazil.
2 – Faculty of Pharmacy, Federal University of Minas Gerais, Brazil.
3 – Health and Human Development Faculty, Santo Agostinho Institute, Brazil.
4 – Integrated Pharmacology and Drug Interactions Research Group (GPqFAR), Brazil.
5 – Biological Sciences Institute, Federal University of Minas Gerais, Brazil.
*Corresponding Author: souzamv@ufmg.br

Staphylococcus aureus is a bacterial species that is part of the human microbiota, but virulent strains can cause diseases including endocarditis, bacteremia, septic arthritis, and in the skin, folliculitis[1]. Its growing resistance to antimicrobial drugs is a serious public health concern worldwide, and the lack of new drugs raises the need of exploring new antimicrobials. *Vaccinium myrtillus* (Blueberry) fruits are small barries blue-to-black in color due to the high levels of anthocyanins[2]. Beyond its antioxidant and anticancer effects and benefits for the cardiovascular system, extracts of *V. myrtillus* have been described to present antimicrobial and anti-inflammatory potentials[3,4]. However, the antimicrobial activity of the juice remains poorly investigated. Here we provide evidence on the anti-staphylococcal potential of the methanolic extract of the juice.

A commercially available *V. myrtillus* juice (Juxx, Brazil) was purchased at a local store at Minas Gerais state. The fruit juice was lyophilized and the concentrates obtained were preserved at 4 °C until subsequent use. The 80% methanolic extract was prepared with the dried powder of the juice under magnetical stirring at maximum speed during 24 h. The solution was then centrifuged, liophylized and stored at 4 °C until used. For the assays, a total of 10 *S. aureus* isolates were used in this study. They are from the clinical isolates collection from the Microbiology Laboratory of the Santo Agostinho Institute. All isolates were cultured overnight in BHI broth (Difco) at 35±2 °C for activation, and tested with Gram-positive identification cards for VITEK 2 system (bioMérieux, France) for identity confirmation.

The minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) of the extract were determined by broth microdilution in untreated polystyrene plates as previously described[5], with slight adaptations. For these tests, stock solutions of 4 mg/mL were prepared in DMSO and diluted in sterile warm 0.9% saline to reach final concentrations with the bacterial inoculum (prepared at 0.5 MacFarland scale) ranging from 1 mg/mL to 7.8 µg/mL.

Following, 10 µL of 0.1% resazurine solution was used for staining the wells. MIC was established as the lowest concentration in

which no color modification from blue to pink was observed in all strains. MBC was established as the lowest concentration that yielded no bacterial growth in all strains after 10 µl aliquots of each well were spread-plated in Mueller-Hinton agar (Difco) plates and incubated overnight at 35±2°C. In order to further examine the antimicrobial properties of the extract, we combined it at the MBC concentration with antimicrobial disks against *S. aureus* isolates, using a method previously standardized by our group[6].

Results of the antimicrobial assays are shown in table 1. MIC and MBC values varied within the isolates, ranging from 15.62-62.5 and 125-500 µg/mL, respectively. As MIC and MBC values were very different (MIC < MBC), a bacteriostatic effect can be attributed to the extract. MIC was established at 62.5 µg/mL and the MBC was established at 500 µg/mL. The methanolic extract prepared with *V. myrtillus* juice was more potent than the leaf extract, which was reported to be active against *S. aureus* strains in concentrations ranging from 0.75 to 1.5 mg/mL (MIC values)[4]. This difference might be attributed to the variety and availability of antimicrobial secondary metabolites such as flavonoids in such extracts[3,4].

Table 1 – MIC and MBC results for *V. myrtillus* extract

Isolate number	MIC (µg/mL)	MBC (µg/mL)
S. aureus 1	62.5	250
S. aureus 2	62.5	250
S. aureus 3	62.5	500
S. aureus 4	62.5	500
S. aureus 5	31.25	250
S. aureus 6	15.62	125
S. aureus 7	31.25	250
S. aureus 8	62.5	250
S. aureus 9	15.62	125
S. aureus 10	62.5	500

Final volume in MIC assays: 100 µL.

As the extract presented antimicrobial activity, we investigated if it would present synergistic or antagonistic effects when combined to synthetic antimicrobials of different mechanisms of action. We used levofloxacin, amoxicillin and gentamicin (5, 10 and 10 µg/disks, all from Sensifar, Brazil). The statistical analyses were conducted by ANOVA followed by Tukey test (significance level: $p<0.05$), and no significant interference effect was detected, although some slight variations of 1 to 2 mm were seen.

Table 2: Interference assay of *V. myrtillus* extract on antimicrobial drugs

Isolate	Inhibition zone (mm) of drugs alone or combined to the extract					
	Levo	Levo+E	Amox	Amox+E	Genta	Genta+E
S. aureus 1	28	28	17	17	13	12
S. aureus 2	26	25	18	19	12	11
S. aureus 3	21	22	22	23	18	19
S. aureus 4	25	25	24	25	16	15
S. aureus 5	24	23	19	19	14	14
S. aureus 6	27	26	16	17	15	16
S. aureus 7	22	21	17	17	17	16
S. aureus 8	25	25	19.5	18	18	17
S. aureus 9	27	26	16	16	15	14
S. aureus 10	29	28	23	22	21	20

Levo: levofloxacin; Amox: amoxicillin; Genta: gentamicin; +E: addition of the extract at the MBC. Interference criteria: disks with addition of the extract should present significant ±2 mm difference from disks free of extract.

Our group has been investigating interactions of natural products and antimicrobial drugs in order to obtain synergistic combinations that might be explored for clinical use[6,7]. We believe that this is an important alternative on the research of new antimicrobial solutions for infectious diseases caused by bacteria. We have described relevant combinations against different bacterial species[6,7], and so far, we have confirmed the observations of others regarding the unpredictability of such combinations *in vitro*[7]. We provide evidence here for the first time of the effects of *V. myrtillus* extract on antimicrobial drugs. Additional studies are being planned for testing combinations of this extract with other antimicrobials and other bacterial species.

In summary, we have provided evidence of the antimicrobial effect of the methanolic extract of *V. myrtillus* juice for the first time, as well for its lack of interference when combined to antimicrobial drugs. Further chromatographic studies are necessary for isolating bioactive molecules for *in vivo* investigations, which are necessary for confirming the clinical efficacy of the extract and its safety alone or combined to antimicrobials.

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