

Antibacterial characteristics of black currant fruit extracts

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Abstract

Background: Our group previously reported from high bactericidal potential of berry juices, especially from blackcurrant fruits. The present study observed different fractions from blackcurrant fruits (all-fruit juice: AFJ, squeezed juice: SJ, non-dialyzable juice material: NDFM, alcoholic fraction from beery skins: AFBS, aqueous fraction from beery skins: AQFBS) in their efficiency of suppressing various oral gram-positive and gram-negative bacteria.

Methods: AFJ, SJ, NDFM, AFBS and AQFBS were prepared from freshly harvested blackcurrant fruits and incubated in direct contact (60s) to planktonic cultures of *Fusobacterium nucleatum* (F.n.), *Agregatibacter actinomycetemcomitans*(A.a.), *Porphyromonas gingivalis*(P.g.), *Streptococcus gordonii*(S.g.), *Streptococcus mutans* (S.m.), *Streptococcus sobrinus* (S.s), *Actinomyces naeslundii* (A.n). and *Enterococcus faecalis* (E.f.).

Results: AFJ was most efficient, causing total suppression of F.n., P.g. and S.g. and induced major reduction in A.a., S.g. and A.n.. Bactericidal efficiency was as follows: AFJ>SJ>EFBS>AQFBS>NDFM. Gram-negative bacteria were more susceptible to blackcurrant extracts compared to gram-positive species. AFJ was efficient as 0.2% CHX in suppressing F. n., P. g. and S. g..

Conclusions: All-fruit juice from blackcurrant shows strong bactericidal activity on F.n., A.a., P.g., S.g. and A.n.. Further research should aim on investigating the function of blackcurrant fruit extracts in controlling oral biofilm formation.

1 Background

Some plant-derived foods are particularly rich in polyphenols, which belong to the group of secondary plant metabolites and can be classified into flavonoids and non-flavonoids [1]. Those metabolic compounds contribute to aroma, color, astringency, and protection of plants against predators and environmental threats. Interestingly, some of these molecules also have pronounced effects on the human body and are therefore considered important phytochemicals [2–5].

In this regard, our group recently showed that especially all-fruit juices derived from various berry fruits have strong antibacterial properties. In particular, juices from blackcurrant (*Ribes Nigrum*) were most efficient in reducing different oral bacteria associated with periodontitis, caries and root canal infection [6].

Fruits are among the most preferred food materials with various effects on individual health [7–12]. Because of their high nutritional content in vitamins and minerals, berry fruits in particular are important components of a healthy diet. Due to their richness in antioxidants, berry fruits also show pronounced anti-inflammatory effects on several oxidative stress related diseases [13–15].

Besides their antioxidant activity, berry polyphenols also show strong antibacterial and anti-fouling characteristics, which makes them attractive candidates for controlling food spoilage and microbial

biofilm formation [16, 17].

In this regard, different authors have found that especially blackcurrant fruits are rich in flavonols (kaempferol, quercetin, and myricetin), their glycosides (kaempferol-3-O-glycoside, quercetin-3-O-glycoside, and quercetin-3-O-rutinoside), anthocyanins, and tannins. Blackcurrant anthocyanins (delphinidin-3-O-rutinoside and cyanidin-3-O-rutinoside) which are considered significant antimicrobial metabolites, constituting between 60% and 85% of the total phenolic content [18–20]. Due to their richness in bioactive natural compounds, blackcurrant fruits in particular enables distinct health promoting effects [20–22].

In times of increasing microbial drug resistance, new and efficient alternative treatment methods are highly needed. As recently shown by our group, especially blackcurrant all-fruit juices are highly bactericidal towards many different oral pathogenic bacteria [6]. But, information about the activity of different fruit fractions that can be obtained from blackcurrant fruits are still missing. The present study therefore focuses on investigating the antibacterial activity of blackcurrant fruits fractions on different gram-positive and gram-negative oral bacteria. Besides the bactericidal effect from all-fruit juices, the efficiency of alcoholic and aqueous extracts from berry skins, squeezed fruit juices and from non-dialyzable fruit material were investigated as well. It was hypothesized that among the single extracts there are no significant differences in regard to their bactericidal activity (H0).

2 Methods

This study used blackcurrants (*Ribes nigrum*) grown regionally in the federal state of Thuringia, Germany. After harvesting the fruits were washed and immediately deep frozen. Deep frozen fruits (-20°C) were transferred to the laboratory for further processing. Test solutions were prepared from the berry fruits by five different protocols and antibacterial activity investigated by means of a direct contact test.

2.1 All-fruit juice (AFJ)

Black currant berries were processed using a conventional blender. The resulting mixture was centrifuged at 4800 rpm for 10 minutes, supernatant collected and centrifuged again at 13.000 rpm for 5 minutes in order to remove all solid components. The resulting juice was then sterilized at 70°C for 30 minutes and stored in 1.5 ml Eppendorf tubes at -20°C.

2.2 Squeezed Berry Juice (SJ)

By carefully squeezing the berries through a sieve, solids and juice were separated. The resulting juice was centrifuged at 13.000 rpm for 5 minutes, and the collected supernatant sterilized at 70°C for 30 minutes. Aliquots were then stored in 1.5 ml Eppendorf tubes at -20°C.

2.3 Non-dialyzable fruit material (NDFM)

Squeezed juice was transferred to a dialysis tube (pore size MWC = 12.00–14.00 MW) and dialyzed for 7 days at 4°C in distilled water with constant stirring and daily change of water. The process was terminated when no visible discoloration of the distilled water was observed anymore. The content of the dialysis tube was then transferred to a sterile container, lyophilized, and stored at room temperature until use. For experimental purpose, the lyophilisate was then dissolved in PBS, according to the initially applied juice volume.

2.4 Alcoholic fraction from beery skins (AFBS)

Berry skins were mixed with ethanol in a ratio of 1:3 and placed on a tumbling table at room temperature for 30 min. The aliquots were centrifuged at 4°C, 1500 rpm for 5 min. The supernatants were collected and transferred to an open, sterile reaction vessel and placed alternately in an incubator at 37°C followed by an evaporation step at room temperature. The cycle was terminated when all ethanol was completely evaporated from the solution. The resulting residues were then dissolved in DMSO and aliquots deep frozen at -20°C. For experimental purpose, the DMSO samples were dissolved in PBS up to the initial volume of the applied berry skins.

2.5 Aqueous fraction from beery skins (AQFBS)

Berry skins were mixed with aqua dest. in a ratio of 1:3 and incubated on a tumbling table at room temperature for 30 min. Subsequently, the solution was centrifuged by 1500 rpm at 4°C. The supernatants were lyophilized and afterwards dissolved in PBS. The PBS volume corresponded to the mass of the applied berry skins. The test solution was then sterilized at 70°C and stored at -20°C.

2.6 Bacterial Species

The following bacterial species were applied: *Fusobacterium nucleatum* (F.n., ATCC 20482), *Aggregatibacter actinomycetemcomitans* (A.a., ATCC 8324), *Porphyromonas gingivalis* (P.g., ATCC 20709), *Enterococcus faecalis* (E.f., ATCC 20376), *Streptococcus mutans* (S.m., ATCC 20523), *Streptococcus sobrinus* (S.s. ATCC 20742), *Streptococcus gordonii* (S.g., ATCC 6777), *Actinomyces naeslundii* (A.n., ATCC 17233).

S. m., S. g., and S. s. were grown in tryptose soy medium. F. n., P. g., A. a., A. n. and E. f. were cultured in Schaedler's medium supplemented with vitamin K (10 mg/L). All bacterial species were incubated under anaerobic standard conditions (10% CO₂, 10% H₂, 80% N₂) at 37°C for 16 to 20 h. Subsequently bacteria were pelleted by centrifugation at 4000 rpm for 5 minutes, washed twice with PBS and re-suspended in PBS to an optical density of OD_{546nm} = 0.5 (corresponds to 10⁶ CFU/ml).

2.7 Direct contact test

Ten µl of the respective bacterial suspensions was gently mixed with 90 µl of each test solution (AFJ, SJ, NDFM, AFBS, AQFBS) and incubated for 60s at 37°C. Subsequently, 900 µl of PBS was added and bacteria pelleted by centrifugation at 4000 rpm for 5 min. The bacterial pellets were dissolved in 200 µl PBS and further diluted up to 10⁻⁵. Twenty µl of each original solution and from the dilution series were

plated onto 6% Schaedler blood agar or TS agar plates in accordance to the strain specific culture conditions, followed by anaerobic incubation for 3–5 days at 37°C. Afterwards, colony-forming units (CFU/ml) were determined. For positive control 0.2% CHX and for negative control PBS was applied. For each test solution n = 8 samples were prepared. Experiments were run in duplicate.

2.8 Statistical analysis

Significant differences were analyzed using the non-parametric Mann-Whitney-U-Test.

The level of significance was set to an alpha of 5%. For statistical analysis, the software SPSS version 29.0 was used.

3 Results

In direct contact to planktonic cells, antibacterial efficiency was dependent upon the bacterial species and type of blackcurrant extract applied (Figs. 1 and 2). In detail, from all tested solutions AFJ presented the highest antibacterial activity, followed by EFBS > SJ > AQFBS > NDFM. In general, gram-negative bacteria were more susceptible compared to gram-positive species.

As shown by the results, incubation with AFJ caused total suppression of *F. nucleatum*, *P. gingivalis* (Fig. 1, dark blue arrows) and *S. gordonii* (Fig. 2, dark blue arrow). High inhibitory effects of AFJ were also observed on *A. actinomycetemcomitans* (Fig. 1) and *A. naeslundii* (Fig. 2). On all other tested bacteria, AFJ presented only minor suppressive effects.

In Fig. 2, the results for all tested gram-positive species are shown. While *E. faecalis* was still reduced by 1.8 log in CFU/ml ($p < 0.05$, Table 2), no significant antibacterial activity was observed by AFJ on *S. mutans* and *S. sobrinus*.

SJ and AFBS also showed efficient antibacterial behavior. Incubation with SJ caused total suppression of *F. nucleatum* and *P. gingivalis* (light green arrows, Fig. 1), while *P. gingivalis* was also completely reduced by AFBS (Fig. 1, dark green arrow). Furthermore, AFBS induced major reduction in CFU/ml among *F. nucleatum*, *A. actinomycetemcomitans*, *E. faecalis*, *S. gordonii* and *A. naeslundii* (Figs. 1 and 2).

Same as with AFJ, both, SJ and AFBS also failed to significantly inhibit the growth of *S. mutans* and *S. sobrinus* (Fig. 2, Table 2).

When compared to the negative control (PBS), aqueous extracts obtained from berry skins (AQFBS) induced minor, but still significant suppressive effects on *F. nucleatum*, *A. actinomycetemcomitans*, *P. gingivalis*, *S. gordonii* and *A. naeslundii*, too. There was no significant inhibitory effect of AQFBS on *E. faecalis*, *S. mutans* and *S. sobrinus* (Fig. 2, Table 2).

From all tested blackcurrant fractions, NDFM showed the lowest antibacterial activity. Significant suppressive effects were only detected on *A. naeslundii* (Fig. 2, Table 2). In contrary, treatment with 0.2%

CHX (positive control) caused total suppression of all planktonic strains, except *A. actinomycetemcomitans* (red column, Fig. 1). In the case of *F. nucleatum*, *P. gingivalis* and *S. gordonii*, blackcurrant AFJ showed identical suppressive activity as 0.2% CHX, resulting in total suppression of these species (Figs. 1 and 2, dark blue arrows).

Table 1
Bacterial reduction in log CFU/ml gramnegative oral bacteria

gramnegative bacteria						
	F. nucleatum		P. gingivalis		A. actinomycetemcomitans	
AFJ	-5.54	p < 0.001	-6.12	p < 0.001	-3.78	p < 0.001
SJ	-5.54	p < 0.001	-6.12	p < 0.001	-3.91	p < 0.001
NDFM	-0.05	p = 0.226	-0.01	p = 0.563	-0,02	p = 0.671
AFBS	-4.33	p < 0.001	-6.12	p < 0.001	-2.58	p < 0.001
AQFBS	-1.38	p < 0.001	-2.88	p < 0.001	-2.11	p < 0.001
CHX	-5.54	p < 0.001	-6.12	p < 0.001	-5.48	p < 0.001

Table 1: Bacterial reduction in log CFU/ml compared to the control (PBS) among gramnegativ oral bacteria. Significance was determined by Mann-Whitney *U*-Test (p < 0.05) to the control (PBS). (AFJ: all-fruit juice; SJ: squeezed juice; NDFM: non-dialyzable fruit material, AFBS: alcoholic fraction from berry skins; AFBS: aqueous fraction from beery skins; CHX: chlorhexidine 0.2%; PBS: phosphate buffered saline)

Table 2
Bacterial reduction in log CFU/ml grampositive oral bacteria

grampositive bacteria										
	<i>E. faecalis</i>		<i>A. naeslundii</i>		<i>S. mutans</i>		<i>S. gordonii</i>		<i>S. sobrinus</i>	
AFJ	-1.77	p < 0.001	-4.21	p < 0.001	-0.03	p = 0.153	-5.24	p < 0.001	-0.20	p = 0.001
SJ	-0.16	p = 0.141	-2.27	p < 0.001	-0.03	p = 0.057	-2.97	p < 0.001	-0.01	p = 0.596
NDFM	-0.30	p = 0.053	-1.31	p < 0.001	0.00	p = 0.752	-0.01	p = 0.634	0.00	p = 0.634
AFBS	-3.84	p < 0.001	-3.98	p < 0.001	-0.30	p = 0.111	-3.98	p < 0.001	0.00	p = 0.832
AQFBS	-0.12	p = 0.528	-1.54	p < 0.001	-0.05	p = 0.417	-0.34	p = 0.832	-0.02	p = 0.064
CHX	-5.95	p < 0.053	-6.11	p < 0.001	-5.28	p < 0.001	-5.24	p < 0.001	-5.18	p < 0.057

Table 2: Bacterial reduction in log CFU/ml compared to the control (PBS) among grampositive oral bacteria. Significance was determined by Mann-Whitney *U*-Test ($p < 0.05$) to the control (PBS). (AFJ: all-fruit juice; SJ: squeezed juice; NDFM: non-dialyzable fruit material, AFBS: alcoholic fraction from berry skins; AFBS: aqueous fraction from beery skins; CHX: chlorhexidine 0.2%; PBS: phosphate buffered saline)

4 Discussion

In the present investigation, different fractions from blackcurrant fruits were tested in their efficiency of suppressing oral pathogenic bacteria that are associated with periodontitis, caries and root canal infection.

Five different types of fruit aliquots were prepared and tested in direct contact to the bacteria. As shown by the results, the antibacterial efficiency differed strongly among the single fruit fractions and was also dependent upon the respective bacterial species. It was found that all-fruit juice (AFJ) was most efficient, followed by the antibacterial activity of alcoholic extractions from berry skins (EFBS) and squeezed juice (SJ). Aliquots from aqueous fractions of berry skins (AFBS) and non-dialyzable fruit material (NDFM) showed only minor effects. Therefore, H0 has to be neglected.

As shown by the results, direct incubation with AFJ for 60 s caused total elimination of *F. nucleatum*, *P. gingivalis*, and *S. gordonii*. In general, gram-negative bacteria were more susceptible compared to the examined gram-positive species. The high antibacterial capacity, especially of AFJ and SJ on *F. nucleatum* and *P. gingivalis*, is in line with already published data of our own research group [6].

Besides blackcurrant, it was verified that especially blueberry extracts show strong antibacterial activity against *F. nucleatum* [23]. This property may result from the ability of blueberry polyphenols to chelate iron. Moreover, it was found that blueberry extracts at concentrations of 62.5 µg/ml are capable of inhibiting *F. nucleatum* biofilm formation by $87.5 \pm 2.3\%$ [23]. If blackcurrant extracts also inhibit biofilm formation of *F. nucleatum*, this still needs to be discovered.

In the present study, *P. gingivalis* was also highly susceptible to blackcurrant juice. It was found that AFJ, SJ and also EFBS suppressed this species completely. This is in line with investigations reporting about the efficiency of proanthocyanidins in suppressing *P. gingivalis* [24]. It was proven that especially b-type proanthocyanidins, such as procyanidin B2 and epigallocatechin, can react with gingipains and hemagglutinin, thus influencing the adhesion ability and virulence of *P. gingivalis* [25, 26]. In this regard, blackcurrants are widely recognized for containing high levels of polyphenols, specifically anthocyanidins and proanthocyanidins which contribute to the sufficient antibacterial activity on *P. gingivalis* [21].

Sufficient antibacterial characteristics of blackcurrant AFJ were also observed in the present study on *A. actinomycetemcomitans* and *A. naeslundii*. While there were still some bactericidal effects on *E. faecalis* by EFBS, none of the prepared juice fractions showed any remarkable inhibitory activity on the gram-positive species *S. mutans* and *S. sobrinus*.

In the present study, *S. gordonii* was the only gram-positive species that was suppressed completely. Application of blackcurrant AFJ caused total eradication of this species. This is in line with former observations of our group which confirmed the strong suppressive activity of blackcurrant juice (concentration 90%, incubation time 60 s) on *S. gordonii* [6].

As also observed in the present investigation, other authors, too, reported from the insufficiency of blackcurrant fruit extracts in suppressing gram-positive *S. mutans* [27]. It was observed that blackcurrant juice in concentrations < 20% is not capable of reducing bacterial growth of this species. Detailed analysis has verified that ferulic acid and chlorogenic acid in higher juice concentrations seem to be the main substances in blackcurrant juice extracts that have a suppressive influence on *S. mutans* [28].

In general, oral streptococci are known to be early colonizers of the enamel and are also able to co-aggregate with other pioneer species, such as some *Actinomyces* species. In a different study, the effect of blackcurrant juice in inhibiting the formation of bacterial aggregates was observed. It was found that blackcurrant juice is able to prevent and also to reverse bacterial co-aggregation between *S. mutans* and *A. naeslundii* [29]. These effects were attributed to the inhibitory effects of polyphenols that are present among blackcurrant fruit juices in concentrations between 0.6 and 1.4 mg/g [29]. Further it was found that proanthocyanidins such as (epi)catechin or (epi)gallocatechin, can bind to pellicle proteins as well as to α -amylase and thus compete with pioneer species for enamel adhesion [30]. In this context, it was also analyzed that the presence of cranberry polyphenols, especially flavonols, can impair the activity of bacterial glucosyl- and fructosyltransferases, causing a loss in bacterial enamel adhesion and biofilm formation capability [31].

In the present investigation, however, incubation with AFJ and SQ caused only minor effects on *E. faecalis*. From all fruit fractions prepared, EFBS still showed the strongest antibacterial activity. Direct contact to EFBS caused a decrease in *E. faecalis* by 3.8 log CFU/ml. Up to now, there are no further studies available that report about a bactericidal effect of blackcurrant extracts on *E. faecalis*. Apparently, the bacteria reacts differently to plant phenols. While extracts from wild rose, chokeberry and elderberry had no antibacterial effects, fractions from cranberry, Japanese quince and sea buckthorn caused strong inhibition [32].

In the present investigation blackcurrant juice also showed significant bactericidal effects on gram-negative *A. actinomycetemcomitans*, with AFJ and SJ being most efficient. This is in line with results of former investigations of our group. It was found that blackcurrant all-fruit juice has a similar antibacterial efficiency on this species as compared to CHX [6].

Similar to *P. gingivalis*, it was shown that proanthocyanidins have a sufficient antibacterial effect on *A. actinomycetemcomitans*. Treatment of *A. actinomycetemcomitans* biofilms with blueberry proanthocyanidins resulted in bacterial viability loss caused by cell membrane damage [33].

5 Conclusions

In the present study, it was verified that blackcurrant fruit juices, especially AFJ have a strong antibacterial effect on certain gram-negative and gram-positive oral pathogenic bacteria. Strong antibacterial activity was also observed from alcoholic fractions obtained from the berry skins, which are essentially rich in polyphenols. Further studies are necessary that observe the activity of berry phenols in their capability of controlling oral plaque bacteria. Incorporation of natural phenols into drug carrier systems might enable even stronger effects on mature biofilms. This will be a subject of further examinations of our study group in future.

Abbreviations

A.a.	<i>Agregatibacter actinomycetemcomitans</i>
A.n.	<i>Actinomyces naeslundii</i>
AFBS	Alcoholic fraction from beery skins
AFJ	All-fruit juice
AQFBS	Aqueous fraction from beery skins
CFU	Colony forming units
CHX	Chlorhexidine
DMSO	Dimethyl sulfoxide
E.f.	<i>Enterococcus faecalis</i>
F.n.	<i>Fusobacterium nucleatum</i>
MW	Molecular Weight
MWC	Molecular Weight Cut-Off
NDFM	Non-dialyzable juice material
P.g.	<i>Porphyromonas gingivalis</i>
PBS	Phosphate buffered saline
S.g.	<i>Streptococcus gordonii</i>
S.m.	<i>Streptococcus mutans</i>
S.s.	<i>Streptococcus sobrinus</i>
SJ	Squeezed juice

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: Data can be obtained on request from the corresponding author

Competing Interests: None

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Authors' contributions: BS supervised, coordinated and reviewed the manuscript. SK wrote and drafted the submission. Data collection and experiments were carried out by FL and AG. Data curation was performed by AG. MR and MH reviewed the manuscript.

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Figures

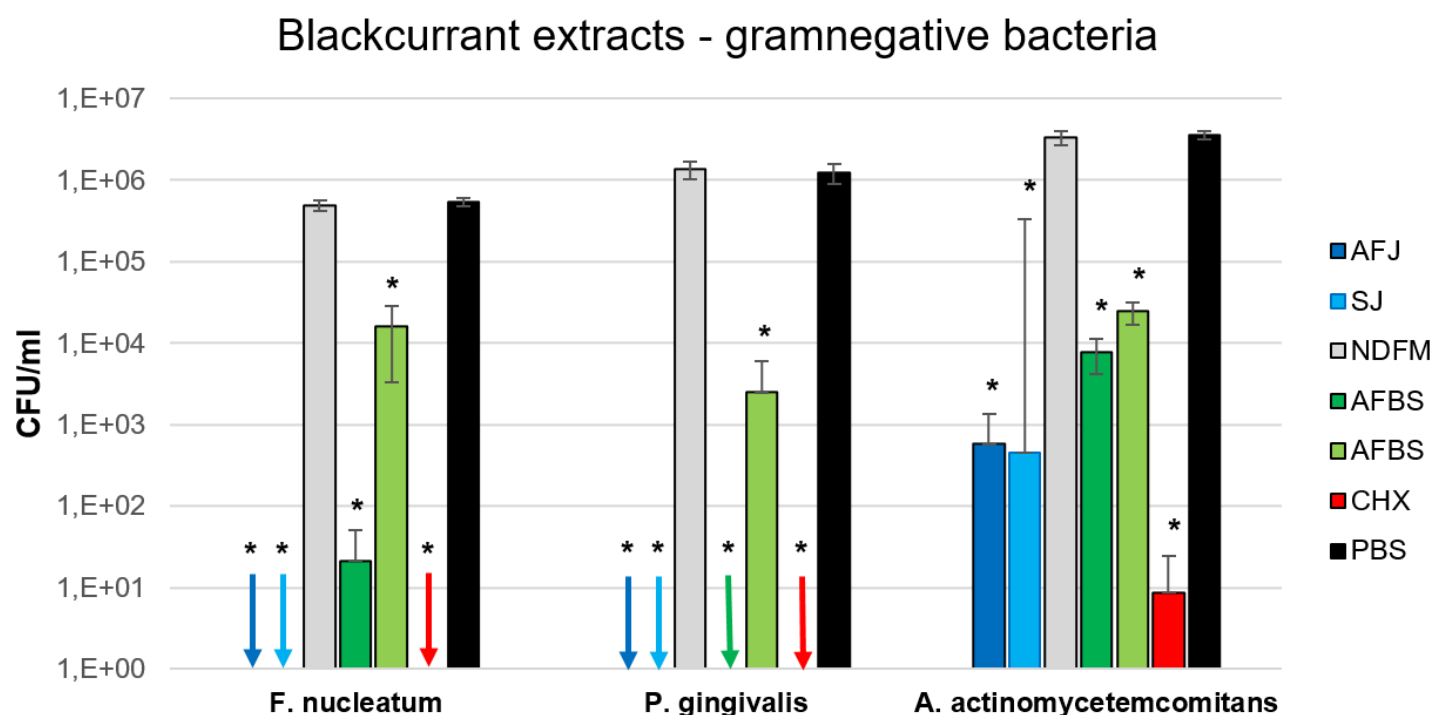


Figure 1

Antibacterial effect of different blackcurrant extracts on gramnegative oral bacteria. Significant difference to the control (PBS) is marked by star ($p<0.05$). Arrows represent total suppression. (AFJ: all-fruit juice; SJ: squeezed juice; NDFM: non-dialyzable fruit material, AFBS: alcoholic fraction from berry skins; AFBS: aqueous fraction from beery skins; CHX: chlorhexidine 0.2%; PBS: phosphate buffered saline)

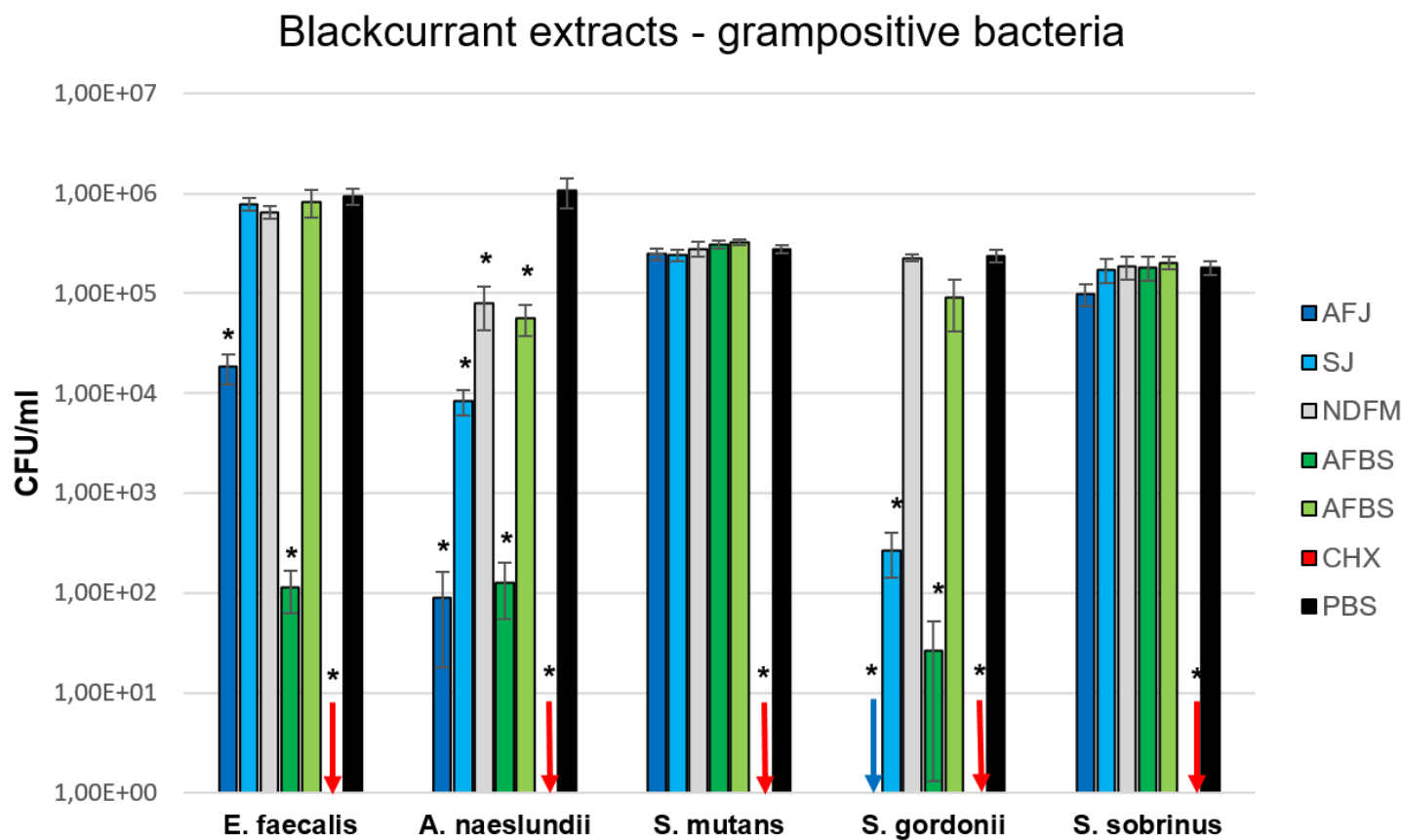


Figure 2

Antibacterial effect of different blackcurrant extracts on grampositive oral bacteria. Significant difference to the control (PBS) is marked by star ($p<0.05$). Arrows represent total suppression. (AFJ: all-fruit juice; SJ: squeezed juice; NDFM: non-dialyzable fruit material, AFBS: alcoholic fraction from berry skins; AFBS: aqueous fraction from beery skins; CHX: chlorhexidine 0.2%; PBS: phosphate buffered saline)