

IN VITRO ANTIMICROBIAL ACTIVITY OF DOGWOOD FRUIT (*Cornus mas* L.) PUREE

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ABSTRACT

The *in vitro* antimicrobial effect of ground dogwood fruit puree (*Cornus mas* L.) was tested using the classic agar-gel diffusion method. Sixteen microbial strains of the species *Esherichia coli*, *Salmonella enterica*, *Staphylococcus aureus* and *Candida albicans* were used, respectively by 1 reference (ATCC) and 3 clinical isolates of each species. A high antimicrobial activity of the investigated plant product was found. The tested dogwood puree showed a well-expressed inhibitory effect against the microbial strains, exceeding that of the broad-spectrum antibiotic Thiamphenicol used as a control. The quantities of the tested microorganisms, added at a final concentration of 2.10^6 cells/ml puree, were reduced by almost half after about 2 hours. After 24 hours, 50% of *E. coli* cells, 42% of *S. enterica* cells, 25% of *S. aureus* cells and 28% of *C. albicans* cells remained viable in the dogwood puree.

Key words: dogwood fruit (*Cornus mas* L.), *Esherichia coli*, *Salmonella enterica*, *Staphylococcus aureus*, *Candida albicans*, antimicrobial activity.

Introduction

Due to the increasing antibiotic resistance of pathogenic bacteria, a current problem of modern microbiology is the search for alternative antimicrobial agents, non-toxic to animals and humans, which effectively suppress the multiplication of pathogenic microorganisms and are suitable as preservatives in the food and cosmetic industries, as well as for prevention and treatment of various infections. One of the possibilities in this regard is the means of plant origin. Dogwood *Cornus mas* (*Cornus mascula* L.) of the *Cornaceae* family, is a widespread plant with edible fruits that have not yet been studied in this aspect. It has been used for medicinal purposes since ancient times. The medicinal properties of *C. mascula* have been described since Hippocrates (Kyriakopoulos and Dinda, 2015; Hosseinpour-Jaghdani *et al.*, 2017).

Common dogwood *C. mas* is a shrub or low tree up to 8 m tall. Its leaves are opposite, 4–10 cm long, ovate or elliptic, pointed, short-hairy and entire. It blooms in February–March. Its flowers are yellow, develop before the leaves unfold and are located axillary, gathered in umbellate or head-shaped inflorescences (Fig. 1). The fruits (dogwoods) are drupe, elongated, up to 15 mm long, with a red fleshy part. It is widespread in Europe and Asia. In our country, it is found throughout the country at an altitude between 0 and 1300 m (Palmarev, 1979; Delipavlov *et al.*, 2011; Stoyanov *et al.*, 2022).



Figure 1: Flowering dogwood.

The plant is used as an ornamental and honey, but its nutritional and medicinal properties are also highly valued in our country. It has a tanning and dyeing effect. The fruits contain about 9% sugar, 3.5% acids (mainly malic), which is why they are used for food in fresh, dried and canned form, as well as in the food industry. The fruits, leaves and bark are used in folk medicine as an astringent, hemostatic and anti-fibrillary agent. Its wood is very hard and is used in the furniture industry (Palmarev, 1979; Delipavlov *et al.*, 2011). Fruits (Fructus Corni) are applied for medicinal purposes. They are collected in autumn when they are fully ripe. Most often they are dried in the sun, spread out individually. The fruits are used in cases of diarrhea, bleeding in the gastrointestinal tract, gout, anemia, etc. Most often, a decoction is prepared, boiling 50 grams of fruit for 15 minutes in 1 liter of water and drinking it instead of water. Jams, purees, compotes and juices are prepared from the fruits, which, in addition to being tasty and nutritious, also have a medicinal effect. Dogwoods can also be successfully used to prevent atherosclerosis, regulate blood sugar levels, lipid profiles and reduce fat accumulation in the liver. In recent years, dogwood has been reported to exhibit antimicrobial, anti-parasitic, anti-inflammatory, antioxidant and anti-cancer effects. It protects the liver, kidneys and cardiovascular system (Hosseinpour-Jaghdani *et al.*, 2017). Aurora *et al.* (2023) reported antioxidant, antimicrobial and cytoprotective effects of dogwood on kidney cells exposed to gentamicin. Other authors also reported antimicrobial activity (Yigit, 2018; Savaş *et al.*, 2020).

Since dogwood puree is one of the widely used options for the storage and use of the fruits, the task of the present work was to determine *in vitro* the degree of inhibitory effect of ground fruits (puree) of common dogwood (*Cornus mas* L.) on the development of strains of *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus* and *Candida albicans*.

Materials and methods

Plant material

The antimicrobial effect of fruit puree from common dogwood (*Cornus mas* L.) in a 500 ml package was tested (Fig. 2). It is intended for use as a food supplement to maintain a normal metabolism and for the normal functioning of the immune system. The composition of the product is presented in Table 1.



Figure 2: Puree from common dogwood (*Cornus mas* L.) used in the tests.

Table 1: Composition of the tested dogwood fruit puree.

Ingredients	Quantity
Dogwood fruit puree (<i>Cornus mas</i>)	99.7 %
Standardized fruit extract of <i>Myrciaria dubia</i> , containing natural vitamin C	0.3 %

The product does not contain alcohol, gluten, colorings, preservatives, sugar or other enhancers. *M. dubia* extract is the only preservative in it.

Controls

Sterile saline solution was used as a negative control. The broad-spectrum antibiotic thiamphenicol (Nikovet - Sofia) was applied as a positive control.

Microorganisms

Pure cultures of 16 microbial strains of four species were used as follows: *Escherichia coli* ATCC 8739 and 3 clinical isolates, *Salmonella enterica* subsp. *enterica* ATCC 1304 and 3 clinical isolates, *Staphylococcus aureus* ATCC 3350 and 3 clinical isolates and *Candida albicans* ATCC 10231 and 3 clinical isolates. The clinical strains used were from the Collection of microbial strains of the Microbiological Laboratory, Faculty of Veterinary Medicine, University of Forestry, Sofia, Bulgaria, which belongs to the Global Registry of Scientific Collections. Suspensions of the strains with a density of 0.5 by McFarland were prepared in a sterile saline solution.

Nutrient media

Meat peptone agar and Muller-Hinton agar (BUL BIO NCIPD - Sofia) were used.

Antimicrobial tests

The antibacterial and antifungal effect of dogwood fruit puree was studied by the classical **agar-gel diffusion** method of Bauer and Kirby (Bauer *et al.*, 1966), designed for fast-growing microorganisms under aerobic conditions, on Mueller Hinton agar with a pH of 7.2-7.4 and a layer thickness of 4 mm. The inoculation of the microbial suspensions on the agar was at a dose of 2.10^6 cells/ml. Dogwood product (undiluted and in 50% and 25% dilutions in sterile saline solution) and controls were administered by instillation of 0.1 ml into 9 mm diameter wells. The control antibiotic Thiamphenicol was tested by instilling into wells of 0.1 ml of a solution in sterile saline at a standard final concentration of 30 µg per well. Cultivation was at 35-37 °C for 18-24 and 72 hours. The reading of the results was carried out by measuring the diameters of the inhibitory zones in millimeters with accuracy to the nearest whole millimeter, including the diameter of the well, on the outside of the bottom of the petri dishes. Complete inhibition of growth was considered as the boundary of the zone. The measured zones of inhibition were interpreted according to the Bauer and Kirby three-level categorization system. The sensitivity of the tested microorganisms to the studied dogwood product was determined as for non-antibiotic agents, namely: resistant (R) – for areas with diameters < 12 mm, intermediate (I) – for areas in within 13 – 16 mm and sensitive (S) - > 17 mm. For thiamphenicol, the corresponding limits are: R < 12 mm, I – 13 – 17 mm and S - > 18 mm (NCCLS).

A suspension test was also applied to examine the duration of survival of the tested microorganisms in dogwood puree. To 9 ml of the puree distributed in test tubes, by 1 ml of suspensions of the studied microbial strains were added at a concentration of 2.10^7 cells/ml, in which a final concentration of 2.10^6 cells/ml in the puree was achieved. After homogenization at Vortex model heidolph; 100 - 2500 r. p. m., at different time intervals (30 min, 60 min, 90 min, 120 min and 24 h) cultures were made on Mueller Hinton agar, which were incubated at 35-37 °C for 18-24 and 72 hours. The number of colonies was then determined and compared with that of untreated controls.

Statistical analysis

The statistical analysis of the results was carried out using the classic Student-Fisher method. Student's t-test for independent samples was reported for 4 results in each group. Significance of differences was determined at levels $P < 0.001$, $P < 0.01$ and $P < 0.05$.

Results and discussion

The summarized results reflecting the effect of dogwood puree on the experimental microbial strains are presented in Table 2. The data show that the growth of all strains was successfully inhibited. The diameters of the inhibitory zones when applying undiluted dogwood puree ranged from 27.00+4.51 to 31.08+4.17 mm. When applying a 50% solution of this puree, the results were also high - from 19.63+3.31 to 23.67+3.52 mm. At high dilution of the puree (25%), its effect decreased, but antimicrobial action was still reported (inhibitory zones 13.25+1.48 - 18.00+2.59 mm).

Table 2: Antimicrobial activity *in vitro* of dogwood puree applied at different concentrations against Gram-positive and Gram-negative microorganisms.

Microorganisms	Diameters of inhibitory zones in mm				
	100%	50%	25%	Th	C (-)
<i>Escherichia coli</i>	30,63±2,46	19,63±3,31	13,25±1,48	17,00±3,18	9,00±0,00
<i>Salmonella enterica</i>	29,42±3,25	22,42±4,25	15,38±4,64	17,75±0,83	9,00±0,00
<i>Staphylococcus aureus</i>	27,00±4,51	22,67±3,68	17,00±2,06	19,25±4,66	9,00±0,00
<i>Candida albicans</i>	31,08±4,17	23,67±3,52	18,00±2,59	21,75±4,14	9,00±0,00

Th – Thiamphenicol; C (-) – negative control

The inhibitory zones at all applied concentrations of the puree for all tested microorganisms were significantly higher than those of the negative control ($P<0.001$).

The tested strains of the fungus *C. albicans* and the Gram-negative bacteria (*E. coli* and *S. enterica*) showed the highest sensitivity to the undiluted puree and that applied at a concentration of 50%. Gram-positive species, however (*S. aureus* and *C. albicans*) showed sensitivity also to the lower tested concentrations of dogwood puree. The inhibitory zones of the undiluted puree were greater than those of the control antibiotic thiamphenicol, but the differences were significant only for the tested Gram-negative bacteria *E. coli* and *S. enterica* ($P<0.001$). The puree at a concentration of 100% exerted a significantly higher antimicrobial effect than that administered at a dilution of 50%, although the differences were significant only for *E. coli* ($P<0.01$). The differences between the diameters of the inhibitory zones of the undiluted puree and those of the 25% tested concentration were much greater ($P<0.001$ for *E. coli* and *C. albicans* and $P<0.05$ for the other microorganisms used).

From the obtained results, it can be seen that dogwood fruits demonstrate high values of an inhibitory effect on the tested pathogenic microorganisms, expressed by clearly defined inhibitory zones and the absence of secondary colonies in them. The performed comparative studies show that tested puree has a better inhibitory effect *in vitro* than the broad-spectrum antibiotic (Table 2).

In all examined strains, there were no colonies in the inhibitory zones, which is evidence of high antimicrobial activity. The sensitivity of all tested strains to dogwood puree at concentrations of 100% and 50% was high. Results were higher at the concentrated product compared to the diluted one (50%). Thiamphenicol, however, showed a significantly lower inhibitory effect on the tested strains compared to dogwood puree. These data, in our opinion, are important, since clinical strains are characterized by high resistance to antimicrobial treatment.

The results of our studies on the antimicrobial activity of dogwood puree are higher than those of Yigit (2018), who reported a 25 mm zones of inhibition when testing a methanolic and aqueous dogwood fruit extract against *S. aureus*. According to their data, only methanol extracts of the fruits have antifungal activity against *Candida* sp. Aurora *et al.* (2023) found an inhibitory effect of dogwood on *Pseudomonas aeruginosa*. Kyriakopoulos and Dinda (2015) also found significant antimicrobial activity against *S. aureus* and *P. aeruginosa* of products obtained from fresh fruits of *C. mas* L. They also considered that the identification of new agents with potent antimicrobial activity against these bacterial species is of medical importance to overcome the problem of universal antibiotic resistance. Negreanu-Pirjol *et al.* (2017) reported that liquid extracts of some wild fruits, including *C. mas* L., exhibit antibacterial activity against *E. coli* and *S. aureus* as well as antifungal activity against *C. albicans* comparable to that of classical antibiotics. According to their results, the most pronounced antibacterial and antifungal activity had been recorded with

growth inhibition diameters of microbial strains between 7 – 13 mm for *C. mas* L. extract in 50% v/v ethanol. The inhibitory zones that we measured in our studies on dogwood puree were significantly larger.

The results obtained by the suspension method to monitor the survival of the different tested microorganisms in dogwood puree are presented in Figure 3.

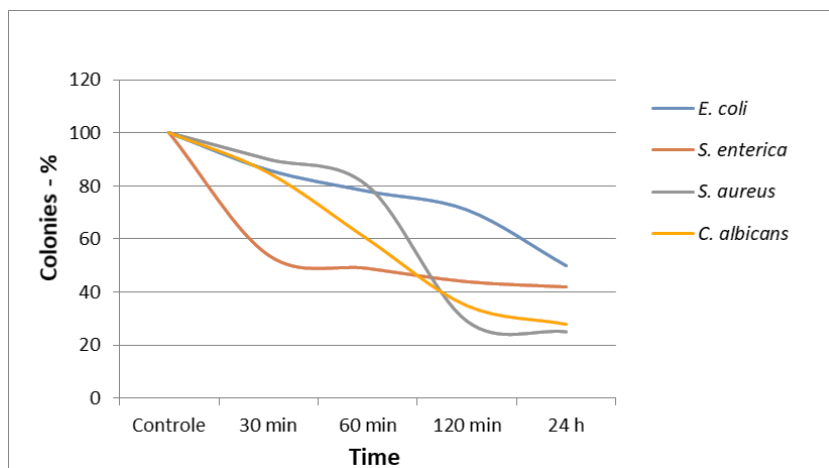


Figure 3: Survival periods of the tested microorganisms added in dogwood puree at a final concentration of $2 \cdot 10^6$ cells/ml.

As can be seen from the graphs, the amounts of the tested microorganisms, added at a final concentration of $2 \cdot 10^6$ cells/ml in the puree, decreased significantly, but did not die completely. After about 2 hours, the number of most of the microorganisms was reduced by almost half, but even after 24 hours, 50% of *E. coli* and 42% of *S. enterica* cells remained viable in the dogwood puree. Gram-positive microorganisms, however, were reduced to a greater extent, with 75% of *S. aureus* and 72% of *C. albicans* cells killed within 24 hours in the puree. We hypothesize that if the microorganisms are added in smaller amounts in the puree, they will die in a shorter time. These results also show that no microorganisms develop in dogwood puree, and their numbers progressively decrease even when added to it in large quantities, so it does not need preservatives during storage. These results are consistent with the data of Effenberger-Szmechtyk *et al.* (2021) who found that dogwood leaf extracts reduced bacterial viability after 24 - 48 hours of exposure. In addition to dogwood fruits, extracts from its leaves also have antibacterial and antioxidant effects. Effenberger-Szmechtyk *et al.* (2020) investigated the chemical composition and antioxidant capacity of *C. mas* leaf extracts and their antimicrobial activity against typical spoilage and pathogenic bacteria found in meat and meat products. A high total phenolic content and the presence of hydroxycinnamic acid derivatives, as well as flavonols, ellagitannins and iridoids, were found. The authors reported action of the extracts as bacteriostatic agents against Gram-negative bacteria (*E. coli*, *S. enterica*, *Moraxella osloensis*, *Pseudomonas fragi*, *Acinetobacter baumannii*, *Enterobacter aerogenes*) and Gram-positive bacteria (*S. aureus*, *Enterococcus faecium*, *Brochothrix thermosphacta*, *Lactobacillus sakei*, *Listeria monocytogenes*), reducing the growth rate and prolonging the lag phase. Effenberger-Szmechtyk *et al.* (2021) investigated the *in vitro* antibacterial mechanisms of leaf extracts of some plants, including *C. mas*, against pathogenic and spoilage bacteria. They

reported that the extracts reduced bacterial viability after 24 h and 48 h of incubation. Acting as pro-oxidants, the extracts induce intracellular generation of ROS (reactive oxygen species) in bacterial cells, with *C. mas* having the strongest influence in this regard. Leaf extracts increased the release of intracellular UV-absorbing components, suggesting a decrease in membrane integrity. They also increase the permeability of the outer membrane of Gram-negative bacteria. Morphological changes occur in the bacteria, including aggregate formation, irregular shapes, wrinkled cell surfaces, cell wall pores, and cell shriveling. In addition, leaf extracts inhibit DNA synthesis in *E. coli* cells by suppressing DNA gyrase activity. We assume that the mechanisms of antimicrobial action of the dogwood puree we studied are analogous.

The results of our research showed a significant activity of the tested plant product against some of the most common pathogens *E. coli*, *S. enterica*, *S. aureus* and *C. albicans* and are an indication of a significant antibacterial activity comparable to and higher than that of some of the most widely used broad-spectrum antibacterial agents, which can be the subject of further research. Savaş *et al.* (2020) consider that the total phenolic content of dogwood fruits provides their antimicrobial and antioxidant activity. According to them, due to its antimicrobial effect, these fruits in the form of marmalade can be used to increase the shelf life of various foods. Our results also show that dogwood puree does not allow growth of microorganisms therein and does not need preservatives. This puree can and should be administered internally as a concomitant medicine in the fight against bacterial infections of the digestive system caused by *E. coli*, *S. enterica*, *S. aureus*, as well as deep mycoses of the digestive tract with the causative agent *C. albicans*. According to Negreanu-Pirjol *et al.* (2017) the rich content of polyphenols, carotenoids, vitamins and minerals is associated with increased antioxidant and antimicrobial activity, which brings important health benefits by helping to prevent many diseases caused by free radicals from the action of oxidative stress. Liquid extracts of wild fruits with high antimicrobial activity would represent possible new nutritional supplements used in various metabolic dysfunctions related to oxidative stress in dermatocosmetics, homeopathic medicines or aromatherapy, with fewer side effects often associated with synthetic antimicrobials (Negreanu-Pirjol *et al.*, 2017).

Conclusion

The studied dogwood puree showed *in vitro* highly pronounced inhibitory effect against clinical and control reference strains of *Escherichia coli*, *Salmonella enterica*, *Staphylococcus aureus* and *Candida albicans*, even at 50% dilution, significantly higher than the effect of the broad-spectrum antibiotic Thiamphenicol.

The amounts of the tested microorganisms, added at a final concentration of 2.10^6 cells/ml puree, were reduced by almost half after about 2 hours. After 24 hours, 50% of *E. coli* and 42% of *S. enterica* cells remained viable in the dogwood puree. Gram-positive microorganisms were reduced to a greater extent, with 75% of *S. aureus* and 72% of *C. albicans* cells dying within 24 hours in the dogwood puree.

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