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ANTIMICROBIAL POTENTIAL OF BARK EXTRACTS OF THE GENUS *SORBUS* PLANTS

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The spread of antibiotic resistance of pathogenic microorganisms poses a serious threat to human health and actualizes the search for new antimicrobial agents of natural origin. Plants of the genus Sorbus L. are known in ethnopharmacology, including their antimicrobial ability. The aim of the work was to establish the spectrum of antibacterial and antifungal activity of bark extracts of Sorbus species and natural hybrids. Ethanol extracts of plant bark, obtained by the method of cold maceration, were tested at a concentration of 30 μg/μL. The antimicrobial activity of the extracts was studied by the disc-diffusion method and evaluated by the size of the inhibition zone of the colony's growth of microorganisms in comparison with reference antibiotics. It was found that the activity of bark extracts of S. aucuparia and S. torminalis was the greatest against Pseudomonas aeruginosa B907 strain (respectively, 63.5% and 53.8% of the ofloxacin level). The greatest inhibition of the growth of Staphylococcus aureus B904 was achieved by the bark extracts of S. hybrida (66.2%) and S. latifolia (60.8%). The growth of the ofloxacin-resistant Proteus mirabilis clinical strain was most inhibited by the bark extracts of S. domestica (inhibition zone diameter 13.5 mm) and S. hybrida (11.9 mm). Against ofloxacin-resistant clinical strain St. epidermidis the most effective were the bark extracts of S. torminalis (inhibition zone 13.8 mm) and S. latifolia (13.5 mm). Clinical fungal strains were resistant to fluconazole but sensitive to bark extracts, most notably Candida albicans to S. domestica (inhibition zone 15.9 mm) and S. hybrida (14.2 mm) extracts, and C. krusei strain to S. aucuparia extract (14.1 mm). Thus, bark extracts of Sorbus plant showed remarkable activity against both Gram-negative and Gram-positive collection bacterial cultures, as well as against clinical antibiotic-resistant bacterial and fungal strains. The obtained results confirm the high antimicrobial potential of Sorbus plant bark extracts and the possibility of their use for the creation of effective antimicrobial agents.

Key words: antibiotic-resistant microorganisms, Sorbus plant extract, antimicrobial activity.

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АНТИМІКРОБНИЙ ПОТЕНЦІАЛ ЕКСТРАКТІВ КОРИ РОСЛИН РОДУ *SORBUS*

Н. О. Хромих, Т. В. Скляр, О. В. Ляшенко

Поширення антибіотикорезистентності патогенних мікроорганізмів створює серйозну загрозу для охорони здоров'я людини та актуалізує пошук нових антимікробних агентів природного походження. Рослини роду Sorbus L. відомі в етнофармакології, у тому числі протимікробною здатністю. Метою роботи було встановлення спектру антибактеріальної та протигрибкової активності екстрактів кори видів і природних гібридів Sorbus. Етанолові екстракти кори рослин, отримані методом холодної мацерації, тестували в концентрації 30 мкг/мкл. Антимікробну активність екстрактів вивчали диск-дифузним методом і оцінювали за розміром зони інгібування росту колоній мікроорганізмів у порівнянні з референтними антибіотиками. Встановлено, що активність екстрактів кори S. aucuparia і S. torminalis проти штаму Pseudomonas aeruginosa B907 була найбільшою (відповідно, 63,5% і 53,8% від рівня офлоксацину). Найбільшого інгібування росту Staphylococcus aureus B904 досягли екстракти кори S. hybrida (66,2%) і S. latifolia (60,8%). Ріст клінічного штаму Proteus mirabilis, стійкого до офлоксацину, найбільше пригнічували екстракти кори S. domestica (діаметр зони інгібування 13,5 мм) та S. hybrida (11,9 мм). Проти стійкого до офлоксацину клінічного штаму St. epidermidis найбільш ефективними були екстракти кори S. torminalis (діаметр зони 13,8 мм) і S. latifolia (13,5 мм). Клінічні грибкові штами були стійкими до флуконазолу, але чутливими до рослинних екстрактів, найбільше штам Candida albicans до екстракту S. domestica (діаметр зони 15,9 мм) і S. hybrida (14,2 мм) та штам C. krusei до екстракту S. aucuparia (14,1 мм). Отже, екстракти кори рослин Sorbus виявили активність як проти грам-негативних, так і грам-позитивних колекційних бактеріальних культур, а також проти клінічних стійких до антибіотиків бактеріальних і грибкових штамів. Отримані результати підтверджують високий антимікробний потенціал екстрактів кори рослин Sorbus і потенційну можливість їх застосування для створення ефективних протимікробних засобів.

Ключові слова: *антибіотикорезистентні мікроорганізми, екстракти рослин Sorbus, антимікробна активність.*

Introduction

The resistance of pathogenic microorganisms to many, most, or all antibiotics available for clinical use, which has become a global problem in recent decades, is a consequence of the excessive and often inappropriate use of drugs (Cook & Wright, 2022). The continuous spread of pathogenic microbial strains resistance to traditional medicines poses a serious threat to human health and food safety, which necessitates the search for new effective antimicrobial agents, including substances of natural origin (Walesch et al., 2023). In this sense, plants are recognized as a promising source of antimicrobial substances with a huge therapeutic potential, since phytocompounds are completely different from traditional antibiotics in terms of chemical structure, mechanisms of action and target sites, which reduces the risk of resistance (Wiart et al., 2023). The most common mechanisms of antimicrobial action of phytocompounds established today include disruption of the functionality of microorganism's cell membranes, inhibition of efflux pumps and inhibition of microbial enzymes (Khameneh et al., 2019). In recent years, the antibacterial and antifungal activity of many mixtures (extracts, essential oils) and individual plant compounds against drug- resistant pathogens has been documented (Kokoska et al., 2019). In addition, the ability of plant extracts and isolated phytocompounds to additive or synergistic interaction with traditional antibiotics was revealed (Walesch et al., 2023), which can contribute to the preservation or enhancement of the activity of antimicrobial drugs.

Plants of the genus *Sorbus* L. (Rosaceae) are widespread in temperate regions, in particular, in Ukraine *S. aucuparia*, *S. domestica* and *S. torminalis* grow naturally, and the species *S. aria* and some hybrids were introduced (Fedoronchuk, 2017). *Sorbus* plants are well known in the ethnopharmacology of Europe and the world (Kültür, 2007; Sołtys et al., 2020), and the plant fruits are included in the pharmacopoeia as multivitamin components (Arvinte et al., 2023). Preparations from *Sorbus* plants were used in folk medicine as remedies for bacterial and viral diseases (Sarv et al., 2020). The bark of *S. decora* and *S. americana* plants was used by aboriginal peoples of Canada to treat diabetes (Bailie et al., 2016). A decoction of *S. aucuparia* bark was used in

Estonia for the treatment of cancer diseases (Sak et al., 2014), and in Lithuania as a means for washing wounds (Pranskuniene et al., 2019).

Scientific studies of the antimicrobial activity of the genus *Sorbus* L. plants are currently fragmentary and insufficient for general conclusions. According to Boncler et al. (2017), fruit extracts of *S. aucuparia* showed high activity only against Gram-negative bacteria, while Bobinaitė et al. (2020) reported the antimicrobial activity of rowanberry pomace extracts, especially against Gram-positive bacteria. Leaf extracts of *S. aucuparia* and *S. caucasica* var yaltirikii had a strong activity against Gram-negative bacteria (Turumtay et al., 2017). *S. umbellata* leaf extracts showed high activity against both Gram-positive (inhibition zone of the *St. aureus* reached 19.3 mm) and Gram-negative bacteria (Kavak & Akdeniz, 2019). The antimicrobial activity of the bark of *Sorbus* plants remains understudied at present. The aim of the work was to find out the spectrum and level of antimicrobial activity of bark ethanol extracts of the genus *Sorbus L.* plants and to evaluate the prospects of this natural resource.

Material and methods

Selection of plant material. We studied the bark of the genus *Sorbus* L. plants from the collection of Botanical Garden of Oles Honchar Dnipro National University: natural for Ukraine species *S. domestica* L., *S. aucuparia* L., and *S. torminalis* (L.) Crantz and introduced *S. aria* (L.) Crantz., *S. latifolia* (Lam.) Pers., and *S. hybrida* L. Bark samples were collected from one-year plant shoots, dried and crushed, and extracts were prepared by maceration in 70% ethanol (1:10, w/v) for 24 hours at room temperature. The filtered extracts were dried using a rotary evaporator and stored at 4 °C, before preparing extracts with a concentration of 30 µg/µl for analysis.

Antimicrobial activity assay. The antibacterial and antifungal activity of *Sorbus* bark extracts was studied by the disk diffusion method (EUCAST..., 2015). For this, Petri dishes containing Mueller-Hinton agar were seeded with 10^9 cfu (colony forming units) suspension of test microbial cultures. Sterile paper discs (6 mm diameter) were impregnated with 10 µL of bark extracts and placed on the agar surface, after which the plates were incubated at 37 °C for 24 h. Both collection (*Pseudomonas aeruginosa* B907, *Escherichia coli* B906, *Klebsiella pneumoniae* B920, *Staphylococcus aureus* B209 and *St. aureus* B904) and clinical bacterial strains (*Proteus mirabilis* and two *St. epidermidis* strains from different sources), and fungal clinical *Candida albicans* and *C. krusei* strains were tested in the study. The antibiotics ofloxacin (5.0 µg per disc) against bacteria and fluconazole (25.0 µg per disc) against fungi served as a positive control. Antimicrobial activity of the bark ethanol extracts was expressed as the inhibition zone diameter (mm) around the discs along with disc diameter.

Statistical processing. The studies were performed at least in triplicate, the results were processed using the Microsoft Excel XP 2007 software package and are presented as mean ± standard deviation (x ± SD). Differences in mean values, assessed by the Tukey test in the Statgraphics Centurion XV Version 15.1.02 program package, were considered statistically significant at $P < 0.05$.

Results

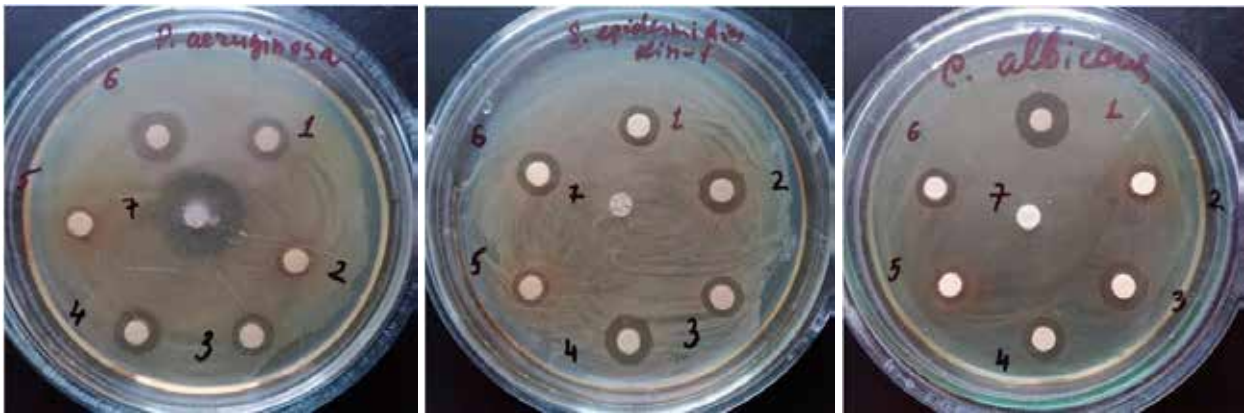
Ethanol bark extracts of Sorbus plants at concentration 30 µg/µl showed antimicrobial activity against both Gram-negative and Gram-positive bacteria, as well as against fungal strains (Fig. 1).

The level of antibacterial activity of bark extracts against collection Gram-negative strains varied within 30.3–63.5% of the effectiveness of ofloxacin (Table 1).

The bark extract of *S. domestica* most actively inhibited the colony growth of *K. pneumoniae* and *E. coli* (48.9% and 55.6% of the ofloxacin effectiveness, respectively), as well as the ofloxacin-insensitive clinical strain *P. mirabilis*. The activity of *S. aucuparia* of bark extract was the highest against *P. aeruginosa* (63.5% of the ofloxacin effectiveness). The activity of *S. torminalis* bark extract was most notable against *P. aeruginosa* and *K. pneumoniae* (53.8% and 48.6% of the ofloxacin effectiveness). Bark extracts of other *Sorbus* species had less pronounced activity against Gram-negative bacteria.

The activity of bark extracts of *Sorbus* plants against Gram-positive collection strains varied within 36.5 – 66.2% of the effectiveness of ofloxacin (Table 2).

The greatest inhibition of the growth of colonies of *St. aureus* B904 achieved the bark extracts of *S. hybrida* (66.2% of the ofloxacin effectiveness) and *S. latifolia* (60.8%). The bark extract of *S. aucuparia* showed the highest activity against *St. aureus* B209 (52.8% of the ofloxacin effectiveness). The growth of insensitive to ofloxacin clinical strains of *St. epidermidis*-1 and *St. epidermidis*-2 were



a b c

Fig. 1. Spectrum of antimicrobial activity of the bark extracts of *Sorbus* L. plants:
1 – *S. domestica*, 2 – *S. latifolia*, 3 – *S. hybrida*, 4 – *S. torminalis*, 5 – *S. aria*,
6 – *S. aucuparia*, 7 – antibiotic;
a – *P. aeruginosa* B907, b – *St. epidermidis*-1, c – *C. albicans*.

Table 1

The activity of *Sorbus* bark extracts against Gram-negative bacteria (n = 3, x ± SD)

Plant species	**Inhibition zone diameter (мм)**			
	***P. mirabilis* (clinical)**	***P. aeruginosa* B907**	***K. pneumoniae* B920**	***E. coli* B906**
S. domestica	13,54 ± 0,34^{d}	11,89 ± 0,04^{b}	13,92 ± 0,42^{c}	13,54 ± 0,20^{b}
S. latifolia	9,82 ± 0,12^{a}	9,16 ± 0,19^{a}	11,02 ± 0,33^{b}	13,18 ± 0,12^{b}
S. hybrida	11,90 ± 0,09^{c}	11,48 ± 0,28^{b}	13,20 ± 0,32^{c}	13,27 ± 0,18^{b}
S. torminalis	10,84 ± 0,16^{b}	12,71± 0,62^{b}	13,85 ± 0,16^{c}	11,86 ± 0,15^{a}
S. aria	10,38 ± 0,34^{ab}	9,65 ± 0,34^{a}	8,63 ± 0,03^{a}	11,10 ± 0,31^{a}
S. aucuparia	9,99 ± 0,15^{a}	15,01 ± 0,86^{c}	9,14 ± 0,12^{a}	11,12 ± 0,54^{a}
Ofloxacin	NA	23,63 ± 0,63^{d}	28,49 ± 0,76^{d}	24,33 ± 0,73^{c}

Note. Different letters in a column indicate statistically significant differences in mean values according to the Tukey test (P < 0.05). NA – no activity.

Table 2

The activity of *Sorbus* bark extracts against Gram-positive bacteria (n = 3, x ± SD)

Plant species	**Inhibition zone diameter (мм)**			
	***St. aureus* B904**	***St. aureus* B209**	***St. epidermidis* (clinical-1)**	***St. epidermidis* (clinical-2)**
S. domestica	11,02 ± 0,17^{a}	11,15 ± 0,34^{bc}	11,72 ± 0,16^{b}	9,33 ± 0,18^{a}
S. latifolia	12,82 ± 0,12^{ab}	11,01 ± 0,26^{bc}	13,47 ± 0,61^{d}	11,36 ± 0,41^{c}
S. hybrida	13,97 ± 0,69^{b}	11,64 ± 0,26^{cd}	11,95 ± 0,35^{bc}	11,69 ± 0,37^{c}
S. torminalis	11,71 ± 0,20^{a}	8,54 ± 0,09^{a}	13,83 ± 0,06^{d}	12,15 ± 0,20^{c}
S. aria	11,64 ± 0,38^{a}	10,53 ± 0,39^{b}	10,37 ± 0,37^{a}	13,97 ± 0,16^{d}
S. aucuparia	11,58 ± 0,10^{a}	12,35 ± 0,35^{d}	12,55 ± 0,30^{cd}	10,36 ± 0,42^{b}
Ofloxacin	21,10 ± 1,65^{c}	23,41 ± 0,45^{e}	NA	NA

Note. Different letters in a column indicate statistically significant differences in mean values according to the Tukey test (P < 0.05). NA – no activity.

most effectively inhibited, respectively, by bark extracts of *S. torminalis*, *S. latifolia*, *S. aucuparia* plants, and *S. aria*, *S. torminalis* plants.

The inhibitory activity of *Sorbus* bark extracts against *Candida* clinical strains was detected in the absence of the antifungal effect of fluconazole (Table 3).

Table 3

The activity of *Sorbus* bark extracts against clinical fungal strains (n = 3, x ± SD)

Plant species	**Inhibition zone diameter (мм)**	
	C. albicans	***C. krusei***
S. domestica	15,85 ± 0,27^{e}	11,46 ± 0,07^{ab}
S. latifolia	11,33 ± 0,40^{b}	12,30 ± 0,40^{b}
S. hybrida	14,18 ± 0,12^{d}	11,77 ± 0,23^{ab}
S. torminalis	12,22 ± 0,23^{c}	11,36 ± 0,18^{ab}
S. aria	9,74 ± 0,32^{a}	10,81 ± 0,33^{a}
S. aucuparia	11,74 ± 0,18^{bc}	14,15 ± 0,78^{c}
Fluconazole	NA	NA

Note. Different letters in a column indicate statistically significant differences in mean values according to the Tukey test (P < 0.05). NA – no activity.

Bark extracts of *S. domestica* and *S. hybrida* plants showed the greatest ability to inhibit the growth of *C. albicans* colonies, while bark extracts of *S. aucuparia* and *S. latifolia* plants had the greatest efficacy against *C. krusei*.

Discussion

The study results testify to a wide spectrum of antimicrobial activity of bark extracts of the *Sorbus* plants in general and to species-specific features of plant extracts interaction with certain pathogenic microorganisms. For example, *S. aucuparia* bark extract had the highest activity against *P. aeruginosa*, *St. aureus* B209 and *C. krusei*, however, low activity against other Gram-negative bacteria and moderate activity against Gram-positive bacteria and *C. albicans*. Variation in the *S. aucuparia* bark extracts activity is consistent with data on the diversity of antibacterial activity of fruit (Boncler et al., 2017) and leaf (Turumtay et al., 2017) extracts of this species.

Bark extract of *S. domestica* had the highest activity against *E. coli*, *K. pneumoniae* and clinical strains of *P. mirabilis* and *C. albicans*, but moderate to low activity against Gram-positive bacteria (especially against *St. epidermidis*-2). Activity of *S. torminalis* bark extract was moderate against all Gram-negative bacteria and clinical fungal strains and low against collection Gram-positive bacteria (especially *St. aureus* B209), but high against both *St. epidermidis* clinical strains. The bark extract of *S. aria* showed the highest activity against *St. epidermidis*-2, while moderate to low activity against other Gram-positive bacteria, all Gram-negative bacteria and *Candida* strains. The peculiarity of the antimicrobial effect of bark extracts of natural hybrids *S. latifolia* and *S. hybrida* was moderate or high activity against almost all microbial strains. The *S. latifolia* bark extract had relatively low activity only against *P. aeruginosa* and *P. mirabilis*, but high activity against Gram-positive bacteria and *C. krusei*. The activity of the *S. hybrida* bark extract was high both against Gram-negative and Gram-positive bacteria, and against fungal strains (especially *C. albicans*).

A similar variation in antimicrobial activity was established (Camadan et al., 2023) for leaf extracts of *S. subfusca*, *S. umbellata* and *S. persica*, which were inactive against Gram-positive bacteria (*B. subtilis*, *S. aureus* and *S. pyogenes*), while effective against Gram-negative bacteria *E. coli* and *P. aeruginosa*. The antifungal activity of *Sorbus* bark extracts against insensitive to fluconazole clinical *Candida* strains are is consistent with data on ability of *S. pohuashanensis* leaf extracts to inhibit the growth of the fungus *Alternaria tenuissi* and other plant pathogens (Song et al., 2021).

The diverse antibacterial activity may indicate that the *Sorbus* bark extracts overcome the resistance of Gram-negative bacteria to plant compounds and antibiotics, which arise due to hydrophilic and negatively charged lipopolysaccharide shield around bacterial cells (Wiart et al., 2023). It is likely that *Sorbus* bark extracts could also block the efflux pumps that function in the cell walls of Gram-positive and Gram-negative bacteria and yeasts and expel antimicrobial substances to the outside.

Conclusions

Bark ethanol extracts of the genus *Sorbus* L. plants demonstrated species-specific activity against collection and clinical strains of Gram-negative and Gram-positive bacteria and *Candida* fungi. Extracts of *S. hybrida*, *S. domestica* and *S. aucuparia* plants had the widest spectrum of high antibacterial and antifungal activity.

Effective inhibition of the growth of clinical bacterial (*P. mirabilis* and two different strains of *St. epidermidis*) and fungal strains (*C. albicans* and *C. krusei*) by plant extracts was recorded in the absence of activity of the reference antibiotics ofloxacin and fluconazole.

The obtained results testify to the significant antibacterial and antifungal potential of *Sorbus* plant bark extracts and the possibility of their use for the creation of effective antimicrobial agents. Further research is needed to establish optimal methods of extraction, dosing and to test the effectiveness of the extracts in vivo.

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