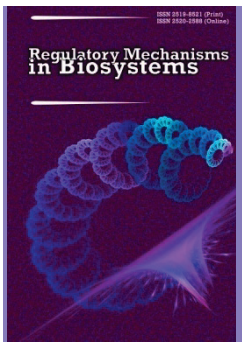

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Antibacterial and anthelmintic activities of *Xanthium strumarium* (Asteraceae) extracts

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Plants of the Asteraceae family are broadly used against microorganisms pathogenic to people and animals, although their potential has not been studied completely so far. In an *in vitro* experiment, we tested ethanolic, ethyl-ether, and dimethyl-sulfoxide extracts from the fruits, leaves, shoots, and roots of *Xanthium strumarium* L. for their effects on 13 species of bacteria and larvae of 3 species of nematodes. Over-8-mm-wide growth-inhibition zones of the colonies around disks saturated with ethanolic extracts from the fruits were observed for 11 bacteria: *Enterobacter cloacae*, *Klebsiella pneumonia* ssp. *rhinoscleromatis*, *K. pneumonia* ssp. *ozaenae*, *K. aerogenes*, *Salmonella enterica*, *Escherichia coli*, *Morganella morganii*, *Enterococcus faecalis*, *E. faecium*, *Staphylococcus aureus*, and *Bacillus subtilis*. Ethanol extract from the leaves inhibited growth of the colonies of 10 bacteria: *E. cloacae*, *K. pneumonia* ssp. *rhinoscleromatis*, *K. pneumonia* ssp. *ozaenae*, *S. enterica*, *E. coli*, *Proteus vulgaris*, *E. faecalis*, *E. faecium*, *S. aureus*, and *B. subtilis*. Ethanol extract from the stem inhibited growth of the colonies of only 6 bacteria: *K. aerogenes*, *E. cloacae*, *P. vulgaris*, *E. faecium*, *S. aureus*, and *B. subtilis*. Ethanol extract from the root inhibited growth of 10 bacteria: *E. cloacae*, *K. pneumonia* ssp. *rhinoscleromatis*, *K. pneumonia* ssp. *ozaenae*, *K. aerogenes*, *S. enterica*, *E. coli*, *Pseudomonas aeruginosa*, *E. faecalis*, *E. faecium*, and *S. aureus*. We analyzed the effects of extracts based on ethyl ether and dimethyl sulfoxide, finding no antiparasitic activity or expressed anthelmintic properties of various extracts from the fruits, leaves, stem, and roots of *X. strumarium* against larvae of the nematodes *Strongyloides papillosus*, *Haemonchus contortus*, and *Muellerius capillaris*.

Keywords: fruits; leaves; stem; root; growth inhibition zone; bacterial colonies; multi-resistant strain; nematodes.

Introduction

Experts of the Global Healthcare Organization note the danger of antibiotic resistance, which is in the top ten of current threats to life of people (www.who.int/health-topics/antimicrobial-resistance). Mortality among the global population in 2019 due to resistance of bacteria to specific antimicrobial drugs accounted for 1.3 M people a year (Huband et al., 2019; Pfaller et al., 2021), and by 2050, the antibiotic tolerance can lead to death of up to 12 M people every year. Microorganisms develop antibiotic resistance more easily than people and animals. Once entering an organism, such bacteria and fungi cause diseases that are hard to treat. Microbial resistance to antibiotics has reached alarming levels all around the world. Tuberculosis, food poisonings and pneumonia are becoming increasingly hard to treat due to reduced antibacterial effects of many commonly used drugs (Bengtsson-Palme et al., 2015; Simonsen, 2018; Zazharskyi et al., 2020a). Metagenome analysis revealed an almost three-fold increase in the number of genes resistant to sulfanilamide and beta-lactams and an 8-fold increase in resistance to trimethoprim. Inhibitory activity towards influenza, adeno- and rhinoviruses was exerted by extracts from the gentian root, primula flower, elder flower, sorrel herb, and verbena herb (Glatthaar-Saalmuller et al., 2011). Phytodrugs also exhibited dose-dependent anti-inflammatory effect, compared with the action of indometacin, preventing formation of exudates and infiltration of the mucous membrane. Plant-based drugs from bark of oak, roots of *Althaea officinalis*, leaves of Persian walnut, herbs of *Taraxacum officinale*, *Achillea millefolium*, *Equisetum*, and flowers of *Matricaria* demonstrated immune-modulating *in vitro* effects, strengthening phagocytosis, activating T-killers, and increasing the cytolitic activity, and also produced in vivo effects by significantly reducing the morbidity with acute respiratory-viral diseases (März et al., 1999).

Hundreds of bacteria species that are pathogenic to people are continuously evolving, adapting to hundreds of pharmaceutical and veterinary antibiotics, and hence there is a dire need of constant development of new antimicrobial drugs to treat infections. In this paper, we continued researching antibacterial dugs based on plant extracts to seek ways to control the spread of antibiotic-polyreistant bacterial strains that are hard to treat (Zazharskyi et al., 2019; Palchykov et al., 2020). Plants produce various secondary metabolites with varying biological activity. We have already found antiparasitic and antimicrobial activities in galenic dugs made of some plants (Boyko & Brygadyrenko, 2016, 2019, 2020, 2021; Palchykov et al., 2020; Zazharskyi et al., 2020b).

Xanthium strumarium L. remains poorly studied for its antimicrobial and anthelmintic activities and can have significant potential in modern medicine and veterinary medicine. Therefore, our objective was identifying the antibacterial and anthelmintic activities of ethanol, ethyl-ether, and dimethyl-sulfoxide extracts of *X. strumarium* L. towards 13 microorganisms and 3 nematodes.

Material and methods

The leaves, roots, fruits, and shoots of *X. strumarium* L. plants were collected in the steppe zone of Ukraine (Boyko & Brygadyrenko, 2016, 2019). We prepared ethanol, ethyl-ether, and dimethyl-sulfoxide extracts from those parts in 1:3 proportion (to 5 g of raw plant material we added 15 mL of extracting agent). To study nematocidal properties of the initial 33.3% extract, we prepared three concentrations of them (10%, 1%, and

0.1%) by adding respective extracting agents. From the filtering paper, we cut disks (6 mm diameter), and soaked them in the extracts and dried them in a laminar box (LAM 2 kl, PORSA, Ukraine).

Antbacterial activity of different parts of *X. strumarium* and its ethanol, ether, and dimethyl-sulfoxide extracts was identified using the disk-diffusion method with epizootic microorganism strains (Table 1). From daily cultures of the mentioned microorganisms, we prepared weighed amounts where the number of bacteria (1.5×10^8 CFU (colony-forming units) according to the turbidity standard) was determined using a DEN-1 densitometer (Biosan, Latvija, 2020).

At the next stage of the research, a weighed amount was evenly inoculated in Petri dishes with Mueller Hinton Agar (Himedia, India, 2023). Onto the surface of medium with inoculant, using a sterile pincette, we put disks (keeping minimal distance of 24 mm between the disks, and 10–15 mm from the margins of Petri dishes), which were soaked in prepared extracts and cultivated in a TCO-80/1 Thermostat (Mediko-Instrumental'-nyi Zavod "Novye Tekhnologii I Marketing", Ukraine, 2015) in an upside-down position, at the temperature of 37 °C for 24 h. As the positive control, we used disks with two standard antibiotics (streptomycin and amoxicillin) in the concentration of 10 μg/disk. Amoxicillin is a semi-synthetic penicillin (beta-lactam antibiotic) that inhibits one or more enzymes in the biosynthetic pathway of bacterial peptidoglycan, which is an integral structural component of cellular wall of bacteria. Streptomycin is an antibiotic that forms over the process of vital activity of bacteria of the *Streptomyces* genus. On top of the inoculations, we put disks (n = 5) saturated with ethanolic, ethyl-ether, and dimethyl-sulfoxide extracts of *X. strumarium* (Table 2). Twenty four hours later, growth of the cultures was measured using the zone scale for reading the sizes of growth-inhibition zones of microorganisms (Antibiotic Zone Scale-C, model PW297, India, 2023) and the software TpsDig2 (F. James Rohlf, Stony Brook, USA). Growth inhibition zone was considered to be an area of complete absence of growth, seen visually (Tendencia, 2004; Zazharskyi et al., 2019, 2020b).

Table 1
Taxonomic composition of the 13 studied microorganisms

Class	Family	Species, strains
Gammaproteobacteria	Enterobacteriaceae	*Enterobacter cloacae* 1
		Enterobacter cloacae 2
		Klebsiella pneumonia ssp. *rhinoscleromatis*
		Klebsiella pneumonia ssp. *ozaenae*
		Klebsiella aerogenes
		Salmonella enterica subsp. *enterica (typhimurium)* UNCSM-014
		Escherichia coli ATCC 25923
		Proteus vulgaris UNCSM–011
	Morganellaceae	*Morganella morganii*
	Pseudomonadaceae	*Pseudomonas aeruginosa*
Bacilli	Enterococcaceae	*Enterococcus faecalis*
		Enterococcus faecium
	Staphylococcaceae	*Staphylococcus aureus UNCSM-017*
	Bacillaceae	*Bacillus subtilis* ATCC 6633

Table 2
The most important data on antibacterial activities of *X. strumarium*

Part of plant used	Most important literature sources about medical properties of plant
Fruit	Nasir & Khan (2012), Cesur et al. (2022), Zarifikhosroshahi & Ergun (2022)
Leaves	Sahu et al. (2010), Devkota & Das (2018)
Shoots	Keya et al. (2018), Ozturk et al. (2021)
Roots	Nibret et al. (2011), Salisbury (2019), Chavan & Kulkarni (2021)

To study nematocidal properties of the extracts, we used larvae of the nematode of the respiratory ways of goats and sheep – *Muellerius capillaris* (Mueller, 1889) (Strongylida, Protostrongylidae) (first stage – L_1), cultivated in advance for 10 days at the temperature of 20 °C; and the digestive-system nematodes *Strongyloides papillosus* Wedl, 1856 (Rhabditida, Strongyloididae) (first–third stages – L_1, L_2, L_3) and *Haemonchus contortus* (Rudolphi, 1803) Cobb, 1898 (Rhabditida, Trichostrongylidae) (third stage – L_3). Using the generally adopted Baermann's method (Zajac & Conboy, 2011), we isolated nematode larvae from feces. The species were identified according to the morphological features of larvae (Van Wyk et al., 2004; Van Wyk & Mayhew, 2013). Afterwards, the fluid with larvae was distributed into test tubes and centrifuged at 1,500 rpm (2 min). The supernatant was removed and the sediment with larvae was evenly distributed into 1.5 mL plastic test tubes, 0.1 mL into each. Then, to this sediment, we added extracts from *X. strumarium* in four-time repetition for each variant. The nematode larvae were exposed to the tested extracts for 24 h at 20 °C. Then, we counted live and dead nematode larvae.

The data in the tables are presented as $x \pm SD$ (mean ± standard deviation). The differences were considered significant at $P < 0.05$. The samples were compared to the previous using analysis of variance (ANOVA) with the Bonferroni's Correction. In case of detecting significant differences between the samples according to ANOVA, we used the Tukey's Honest Significant Difference (HSD) Test.

Results

The greatest antibacterial effect was exerted by ether tincture of *X. strumarium* L. It produced growth-inhibition zones ranging 10.0 (*E. coli*) to 14.0 mm (*E. cloacae* 2) in 11 cases. Alcohol extracts performed only slightly more poorly, in 10 cases, with the growth-inhibition zone of 10.2 (*E. coli*) to 12.8 mm (*K. pneumonia* ssp. *ozaenae*, Table 3). Inhibition of growth of the colonies by dimethy-sulfoxide-based extracts was observed in 8 of 29 cases and measured 10.3 (root tincture on *E. cloacae* 2) to 13.7 mm (action of fruit extract on *K. pneumonia* ssp. *rhinoscleromatis*).

Alcohol extract of the fruits caused antibacterial effects on *E. cloacae* 1, *K. pneumonia* ssp. *ozaenae*, *K. aerogenes*, and *E. coli*, with inhibition zones of 10.4, 10.9, 12.8, and 10.3 mm, respectively. Ether extract of the fruits showed antibacterial activity against *K. pneumonia* ssp. *ozaenae* and *E. coli*, with inhibition zones of 13.0 and 10.2 mm. Fruit extract, based on dimethyl sulfoxide, demonstrated antibacterial effects on *E. cloacae* 1, *K. aerogenes*, and *E. coli*, inhibition zones measuring 12.1, 10.5, and 11.1 mm, respectively. We saw the inhibiting effect of ether extract from the leaves on *E. cloacae* 1, *E. cloacae* 2, *K. pneumonia* ssp. *ozaenae*, *K. aerogenes*, and *S. enterica* (12.7, 10.9, 11.6, 10.0, and 12.0 mm inhibition zones). Alcohol extract from leaves inhibited reproduction of the bacteria *E. cloacae* 1, *E. cloacae* 2, and *E. coli* (10.6, 11.6, and 11.8 mm). Dimethyl-sulfoxide tincture of the leaves inhibited the growth and development of only *E. coli* (11.5 mm).

Stem extracts demonstrated the following inhibiting actions: alcohol extract towards *K. aerogenes* (10.0 mm), ether extract towards *E. cloacae* 2 and *E. coli* (14.0 and 11.1), and dimethyl-sulfoxide extract towards *E. cloacae* 2 (11.0 mm). We saw bacteriostatic effect of alcohol extract from the root on *E. cloacae* 1 and *E. coli* (11.1 and 10.2), ether extract – on *K. pneumonia* ssp. *ozaenae* and *E. coli* (10.1 and 10.5) and dimethyl-sulfoxide extract – on *E. cloacae* 2 and *K. pneumonia* ssp. *rhinoscleromatis* (10.3 and 13.7). The strongest effects on *P. vulgaris* were caused by alcohol extracts from the leaves and stem (9.0 and 8.7 mm). As with inhibitory action against this bacterium, efficacy was seen for only ether extract from the stems (8.9), and dimethyl-sulfoxide extracts from all the parts of the plant (fruits – 9.7, leaves – 8.8, stems – 8.9, and roots – 8.9; Fig. 1). Against *M. morganii*, alcohol and ether extracts from fruits produced growth inhibition zones of 9.0 and 8.5 mm, and ether extracts from the leaves, stems, and roots caused 8.2, 8.5, and 9.6 mm inhibition zones, respectively. At the same time, antibacterial effect of dimethyl-sulfoxide extract was significantly higher: 10.0 (roots) to 10.7 mm (leaves), which

was higher than the control (amoxicillin). Growth of the colonies of *P. aeruginosa* was inhibited by alcoholic tinctures only from the roots (8.9 mm), ether extracts from stem and root (8.4 and 9.0 mm), and dimethyl-sulfoxide extract from three parts of the plants: fruits, stem, and root (9.0, 8.8, and 9.7 mm). This was similar to the control variants of the experiment (amoxicillin, 9.3 mm).

Study of effects of the extracts on the microorganisms of the families Enterococcaceae and Staphylococcaceae revealed inhibition zones of *E. faecalis*, *E. faecium*, and *S. aureus* produced by tinctures of the fruits (10.0, 10.3, and 14.2mm), leaves (15.4, 9.8, and 12.4mm), shoots (7.1, 11.3, and 9.6 mm), and roots (12.0, 10.0, and 11.7 mm). Ethyl-ether extracts exhibited the following effects: from fruits – against *E. faecium* (11.8 mm inhibition zone), from leaves – *E. faecium* and *S. aureus* (11.8 and 12.6 mm inhibition zone), stems – *E. faecalis* (9.5 mm), and from roots – *E. faecium* (10.9 mm).

Dimethyl-sulfoxide extracts displayed noticeable inhibiting effect on growth of the colonies, particularly extracts from the fruits – against *S. aureus* (12.8 mm inhibition zone), leaves – *E. faecium* and *S. aureus* (9.0 and 9.8 mm), stems – *E. faecalis* (9.5 mm), and roots – against *E. faecalis*, *E. faecium*, and *S. aureus* (10.0, 9.0, and 11.6 mm).

Table 3

Antibacterial effect (width of growth-inhibition zone, mm) of ethanol, ethyl-ether, and dimelthyl-sulfoxide extracts from different parts of *X. strumarium* on the families Enterobacteriaceae, Morganellaceae, Pseudomonadaceae, Enterococcaceae, Staphylococcaceae, and Bacillaceae (x ± SD, n = 5)

Variant of the experiment	Control, streptomycin, 10.0 µg	Control, amoxicillin, 10.0 µg	Experiment											
Part of the plant	–	–	fruits	leaves	stems	roots	fruits	leaves	stems	roots	fruits	leaves	stems	roots
Extracting agent	–	–	ethyl alcohol	ethyl alcohol	ethyl alcohol	ethyl alcohol	ethyl ether	ethyl ether	ethyl ether	ethyl ether	dimethyl sulfoxide	dimethyl sulfoxide	dimethyl sulfoxide	dimethyl sulfoxide
Enterobacter cloacae 1	21.1 ± 0.5^{a}	27.6 ± 0.4^{b}	10.4 ± 0.8^{cd}	10.6 ± 0.8^{cd}	8.4 ± 1.7^{d}	11.0 ± 0.9^{cd}	8.8 ± 0.3^{d}	12.7 ± 0.8^{e}	7.3 ± 0.4^{f}	7.1 ± 0.3^{gf}	6.7 ± 0.2^{g}	9.0 ± 0.3^{d}	7.9 ± 0.2^{f}	6.7 ± 0.2^{g}
Enterobacter cloacae 2	17.9 ± 0.2^{a}	24.4 ± 0.6^{b}	9.5 ± 0.5^{c}	11.6 ± 2.0^{d}	8.9 ± 0.7^{c}	9.5 ± 0.5^{c}	9.4 ± 1.0^{c}	10.9 ± 0.7^{cd}	14.0 ± 0.7^{e}	8.5 ± 0.5^{c}	12.1 ± 1.5^{d}	9.1 ± 0.6^{c}	11.0 ± 0.7^{d}	10.3 ± 0.4^{cd}
Klebsiella pneumonia ssp. rhinoscleromatis	19.8 ± 0.8^{a}	24.4 ± 1.0^{b}	8.6 ± 0.4^{c}	8.4 ± 0.9^{c}	7.6 ± 0.4^{cd}	8.0 ± 0.3^{cd}	8.2 ± 0.6^{c}	7.7 ± 0.2^{cd}	9.7 ± 0.8^{ce}	9.3 ± 0.6^{ce}	10.6 ± 1.2^{e}	9.1 ± 0.6^{ce}	8.7 ± 0.2^{c}	13.7 ± 0.9^{f}
Klebsiella pneumonia ssp. ozaenae	22.2 ± 0.8^{a}	24.8 ± 0.4^{b}	12.8 ± 1.0^{e}	7.9 ± 0.5^{d}	7.9 ± 0.6^{d}	8.6 ± 0.7^{d}	13.0 ± 0.8^{e}	11.6 ± 0.7^{e}	7.5 ± 0.3^{d}	10.1 ± 0.5^{d}	7.8 ± 0.2^{d}	8.3 ± 0.4^{d}	8.0 ± 0.6^{d}	8.5 ± 0.3^{d}
Klebsiella aerogenes	15.6 ± 2.0^{a}	19.4 ± 0.7^{b}	10.3 ± 0.9^{c}	9.4 ± 2.4^{c}	10.0 ± 0.3^{c}	9.1 ± 1.1^{c}	8.9 ± 0.6^{c}	10.0 ± 0.6^{c}	8.6 ± 1.1^{c}	9.3± 0.7^{c}	10.5 ± 0.5^{c}	6.4 ± 0.4^{d}	9.6 ± 0.4^{c}	7.6 ± 0.5^{e}
Salmonella enterica	20.7 ± 0.6^{a}	27.0 ± 0.3^{b}	8.0 ± 0.3^{c}	8.0 ± 0.3^{c}	7.6 ± 0.4^{c}	8.1 ± 0.4^{c}	6.6 ± 0.4^{d}	12.0 ± 0.3^{e}	7.8 ± 0.2^{c}	6.7 ± 0.6^{f}	7.8 ± 0.2^{c}	8.7 ± 0.4^{cg}	9.3 ± 0.4^{g}	8.5± 0.3^{cg}
Escherichia coli	19.3 ± 1.6^{a}	20.8 ± 0.5^{a}	11.9 ± 1.2^{b}	11.8 ± 0.5^{b}	7.6 ± 0.6^{c}	10.2 ± 0.5^{b}	10.2 ± 0.7^{b}	8.6 ± 2.0^{bc}	11.1 ± 0.7^{b}	10.5 ± 0.9^{b}	11.1 ± 0.6^{b}	11.5 ± 0.5^{b}	9.7 ± 0.2^{b}	9.4 ± 0.6^{bc}
Proteus vulgaris	16.3 ± 0.8^{a}	17.8 ± 0.5^{b}	7.5 ± 0.5^{c}	9.0 ± 0.3^{d}	8.7 ± 0.4^{d}	6.6 ± 0.6^{c}	7.4 ± 0.4^{c}	6.9 ± 0.6^{c}	8.9 ± 0.4^{d}	7.8 ± 0.5^{cd}	9.7 ± 0.5^{d}	8.8 ± 0.5^{d}	8.9 ± 0.4^{d}	8.9 ± 0.4^{d}
Morganella morganii	16.0 ± 1.1^{a}	9.5 ± 0.7^{b}	9.0 ± 1.2^{b}	6.5 ± 0.3^{c}	7.5 ± 0.5^{d}	7.7 ± 0.5^{d}	8.8 ± 0.5^{b}	8.2 ± 0.5^{bd}	8.5 ± 0.7^{b}	9.6 ± 1.2^{b}	10.1 ± 1.4^{b}	10.7 ± 0.4^{b}	9.8 ± 0.5^{b}	10.0 ± 0.7^{b}
Pseudomonas aeruginosa	14.0 ± 0.6^{a}	9.3 ± 0.4^{b}	7.2 ± 0.2^{c}	7.1 ± 0.4^{c}	7.0 ± 0.4^{c}	8.9 ± 0.4^{b}	7.1 ± 0.2^{c}	7.3 ± 0.4^{c}	8.4 ± 0.7^{bc}	9.0 ± 0.6^{b}	9.0 ± 0.6^{b}	7.1 ± 0.4^{c}	8.8 ± 0.5^{b}	9.7 ± 0.7^{b}
Enterococcus faecalis	8.4 ± 0.4^{a}	8.8 ± 0.4^{a}	10.0 ± 1.6^{ab}	15.4 ± 1.6^{c}	7.4 ± 0.7^{ad}	12.0 ± 1.4^{b}	7.6 ± 0.6^{ad}	7.4 ± 0.6^{d}	9.5 ± 0.7^{ab}	8.3 ± 1.1^{ad}	7.4 ± 0.6^{d}	7.8 ± 0.5^{ad}	10.1 ± 1.6^{ab}	10.0 ± 0.7^{b}
Enterococcus faecium	11.8 ± 1.2^{a}	27.0 ± 0.7^{b}	10.3 ± 0.4^{a}	9.8 ± 0.5^{a}	11.3 ± 0.8^{a}	10.0 ± 0.3^{a}	11.8 ± 0.5^{a}	11.8 ± 1.2^{a}	7.9 ± 0.4^{c}	10.9 ± 1.5^{a}	7.6 ± 0.4^{c}	9.0 ± 0.3^{a}	7.9 ± 0.6^{c}	9.0 ± 0.7^{ac}
Staphylococcus aureus	20.4 ± 0.5^{a}	20.8 ± 0.9^{a}	14.2 ± 1.6^{b}	12.4 ± 0.9^{b}	9.6 ± 0.4^{c}	11.7 ± 0.4^{d}	8.0 ± 0.7^{e}	12.6 ± 1.5^{d}	8.5 ± 1.1^{ce}	7.9 ± 0.7^{ce}	12.8 ± 0.9^{b}	9.8 ± 0.9^{c}	6.5 ± 0.3^{f}	11.6 ± 0.4^{d}
Bacillus subtilis	16.8 ± 0.8^{a}	23.0 ± 0.7^{b}	15.0 ± 0.6^{c}	17.6 ± 0.6^{a}	8.7 ± 0.4^{d}	6.7 ± 0.2^{e}	15.0 ± 0.7^{c}	14.9 ± 0.7^{c}	9.0 ± 0.7^{d}	8.0 ± 0.7^{de}	15.0 ± 0.7^{c}	16.0 ± 0.7^{ac}	7.0 ± 0.7^{e}	8.0 ± 0.7^{de}

Note: different letters in a row indicate samples that are significantly ($P < 0.05$) different from one another according to the Tukey's Honest Significant Difference (HSD) Test.

Powerful antibacterial effect on *B. subtilis* was caused by all the tested extracting agents from the fruits and leaves: ethanolic caused 15.0 and 17.6, ether – 15.0 and 14.9, and dimethyl-sulfoxide extracts – 15.0 and 16.0 mm inhibition zones, respectively. We also found that the inhibition zone of *B. subtilis* produced by alcohol extract from the leaves of *X. strumarium* was larger than in the control with streptomycin ($P < 0.05$). When determining the effects of *X. strumarium* extracts on survival of the larvae of the studied nematodes, we saw no significant nematocidal effects. Over 90% of the larvae survived in 24 h experiment subject to all the tested extracts from roots, stems, leaves, and fruits.

Discussion

The *Xanthium* genus includes annual herbs that often act as weeds. *Xanthium strumarium* has derived from the Greek word xanthos, meaning "yellow", and strumarium, meaning "cushion-like swelling" (Shahed-Al-Mahmud & Lina, 2017). Species of this genus are characterized by a peculiar spiky head. Since Karl Linnaeus described the first two *Xanthium* plants in 1753 (*X. strumarium* L. and *X. spinosum* L.), species of this genus have been given a lot specific epithets. In the last revision of the genus using molecular (Doğan & Kıran, 2017) and phylogenetic analyses, five valid species were described Rojas-Sandoval (2022). *Xanthium strumarium* (common cocklebur). CABI Compendium. http://doi.org/10.1079/cabicompendium.56864). *Xanthium strumarium* is an annual plant that has become a common agricultural and ecological weed. Moreover, it is considered one of the world's most harmful weeds. The plant produces a great number of seeds that easily spread thanks to their abilities to float and move via clothes of people and coating of mammals (Krstić et al., 2021). Once *X. strumarium* emerges in a biotope, it can quickly become a dominating species in the area because of intensive seed production, and also their high germination rate and survivability over a long period. This plant dominates in disturbed areas (wastelands, roadsides, worked over lands), river banks, meadows, beaches, and riverbank thickets. It occurs in scattered populations, but can also form dense populations that displace local vegetation, inhibiting the germination of seeds of other plants, altering the natural processes of regeneration. *Xanthium strumarium* is broadly known as an invasive weed in Serbia, it is one of the most competitive weeds, seriously affecting yields of maize, soybean, rape, and other crops (Ana et al., 2016).

Xanthium strumarium is a monoecious plant with staminate flowers in the upper inflorescences and pistillate flowers in the sheath inflorescences. When it blossoms, the sheath buds become the shoots, each having a terminate staminate inflorescence and sheath pistillate flowers (Salisbury, 2019). Atractyloside is a glycoside of diterpene-kaurane type; toxic, as it inhibits adenine nucleotide translocator (Fig. 1). Atractyloside can cause serious lesions of the liver and kidneys, even after entering through the

human skin. To ensure safety of *X. strumarium*-containing cosmetics, it is necessary to accurately estimate the safe concentration of active agents (Ozturk et al., 2021). Atractyloside concentration in the seeds of *X. strumarium* was 3.04 mg/g in August, 3.50 mg/g in September, and 3.80 mg/g in October. In other parts of the plant (leaves, stems, and roots), atractyloside was not found (Ozturk et al., 2021).

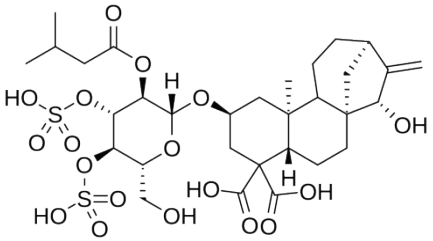

Fig. 1. Formula of carboxyatractyloside (Plumlee, 2004)

Xanthium strumarium is a valuable medicinal plant because of antimicrobial and antioxidant compounds (Azimova & Glushenkova, 2012). It exerted antioxidant and anti-inflammatory properties that could be integrated into anti-aging cosmetics, as well as drugs used for healing various wounds (Shravani et al., 2023). Compounds isolated from the fruits of *X. strumarium* mitigated the harm imposed by UV light, stimulated production of collagen, and enhanced natural wound healing. *Xanthium strumarium* and compounds isolated from it have chances to become a new panacea in cosmetology, being quite promising and effective ingredients for various extracts, serums, and creams. Those drugs have no less powerful potential than hyaluronic and retinoic acids and can enhance their activities in anti-aging drugs (Shravani et al., 2023).

To satisfy the needs of people for vegetal oil, fats isolated from various plants are very important. Cesur et al. (2022) identified contents of oil, composition of fatty acids, and some properties of the seeds of *X. strumarium* subsp. *strumarium*. The seeds were found to contain 24.19% fat of seed mass. Its oil is rich in linoleic (76.97%) and oleic (11.91%) acids. The seeds and seed oil are inedible. Zarifikhosroshahi & Ergun (2022) determined the content of fatty acids of mature and immature oil from whole fruits of *X. strumarium*. Although the main fatty acid in immature samples of the fruits contained oleic acid (50.17%), its amount decreased in oil from samples of mature fruits (25.96%); this compound turned into linoleic acid, which is the main fatty acid of oil from the seeds of *X. strumarium* (Zarifikhosroshahi & Ergun, 2022). Oil of mature fruits contained a greater amount (81.34%) of unsaturated fatty acids (USFA) than oil of immature fruits (56.09%). Those studies revealed that the degree of ripening significantly influenced the concentration of fatty acids in the fruits.

Herbs of *X. strumarium* are traditionally used to treat various diseases, including leukoderma, bites of dangerous insects, epilepsy, salivation, allergic rhinitis, sinusitis, and other (Shravani et al., 2023). Inorganic, biocompatible, and non-toxic, titanium is a compound used in pharmaceutics and biomedicine, and also spheres such as bone-tissue engineering. Shravani et al. (2023) studied nanoparticles of titanium dioxide (TiO NP) synthesized from *X. strumarium*. Their study evaluated cytotoxic properties of extract from the leaves of *Xanthium strumarium* and TiO nanoparticles.

Heavy metals that enter the environment as a result of human activity contaminate the soil and deteriorate its quality. Eren (2018) studied phytoextracting activity of *X. strumarium* plants. The plant could be used to cleanse soil and is utilized in the phytoremediation method to reduce Cu concentration in soil.

Kore et al. (2023) confirmed the antihistamine activity of *X. strumarium* fruit extract (in the dose of 100 mg/kg intraperitoneally) using haloperidol-induced and clonidine-induced catalepsy in laboratory rats.

An important class of natural products with a unique pharmaceutical activity is 8,12-sesquiterpene lactones (STLs). Zheng et al. (2022) reported about a unique germacrene A oxidase (XsGAO) from *X. strumarium*. Unlike the classic enzyme GAO, which catalyzes the three-stage oxidation of germacrene A with formation of germacrene acid A (GAA), XsGAO catalyzes only one-stage transformation of germacrene into germacrene alcohol (Zheng et al., 2022). Sahu et al. (2010) evaluated the pharmaceutical properties of the leaves of *X. strumarium*, finding that alcohol extract exerted a significant anti-inflammatory, anesthetic, and antimicrobial actions.

Devkota & Das (2018) studied antibacterial activity of aqueous and methanol extracts from the leaves of *X. strumarium* in various concentrations (50, 100, 150, 200, and 250 mg/mL) against the bacteria *Klebsiella pneumoniae*, *Proteus mirabilis*, *Escherichia coli*, *Bacillus subtilis*, *Enterococcus faecalis*, and *Staphylococcus aureus*. Phytochemical screening revealed presence of terpenoids, saponins, flavonoids, tanning substances, and alkaloids. Gram-negative bacteria were more tolerant than Gram-positive. The most susceptible bacteria was *S. aureus*, and the most tolerant was *E. coli*. Methanolic extract was more effective than the aqueous one (Devkota & Das, 2018).

Xanthium strumarium deserves further studies because of its valuable pharmaceutical activity (Kozuharova et al., 2019). Extracts from its leaves have a bactericidal potential that may be successfully used in production of antibacterial drugs (Kamboj & Saluja 2010; Nibret et al., 2011; Nasir & Khan, 2012; Keya et al., 2018; Chavan & Kulkarni, 2021).

Antimicrobial action of various parts of *X. strumarium* has been described in the literature in much less detail. According to our results, ethanol, ether, and dimethyl-sulfoxide extracts inhibited growth of colonies of many microorganisms of the families Enterobacteriaceae, Morganellaceae, Pseudomonadaceae, Enterococcaceae, Staphylococcaceae, and Bacillaceae. A somewhat concerning discovery was that amoxicillin produced less than 10 mm inhibition zones of the strains *Morganella morganii*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* (9.5, 9.3, and 8.8 mm), and *Enterococcus faecalis* showed low sensitivity to streptomycin (8.4 mm).

As with antiparasitic properties of this plant, the data are practically absent. There are only reports about effects on malaria-causing *Plasmodium*. According to Sahoo et al. (2020), *X. strumarium* is used by traditional doctors to treat malaria in Northeast India. Those authors conducted a fractioning of extracts from *X. strumarium*, isolating five compounds from the above-ground part and the fruits (stigmasta-5,22-dien-3β-ol (1), xanthinosin (2), stigmasterol-3-O-β-D-glucopyranoside (3), oleic acid (4), and (E)-2, 3-dihydroxypropyl-octadec-9-enoate (5)). Sahoo et al. (2020) found an anti-malaria activity of xanthinosin (2) and stigmasterol-3-O-β-D-glucopyranoside (3), suggesting that this plant or its components should be used against malaria in the future.

Kamaraj et al. (2022) also analyzed the traditional methods of treating parasitic diseases using plants of the Asteraceae family, and reviewed the main chemical classes discovered in those plants and their interaction against parasites. Those authors pointed out flavonoids, terpenoids, and alkaloids which had antiparasitic properties. The authors have extensively presented the data about biologically active compounds that can inhibit the parasitic protozoans (*Plasmodium*, *Leishmania*, *Trypanosoma*, *Entamoeba*, and *Toxoplasma*). They recommend further more detailed research on plants of this family to design drugs with anti-protozoan properties.

Conclusion

This is the first time that antibacterial activity of ethanolic, ethyl, and ether, and dimethyl-sulfoxide extracts from *X. strumarium* were tested against 14 strains of bacteria and 3 nematodes of sheep and goats, the species *S. papillosus*, *H. contortus*, and *M. capillaris*. We found that ethanol extracts from the fruits inhibited 9 strains, from the leaves 8 strains, from the shoots 4 strains, and from the roots 6 strains. Ethyl-ether extracts from the fruits inhibited 5 strains, from the leaves 9 strains, from the shoots 6 strains, and from the roots 5 strains. Dimethyl-sulfoxide extract from the fruits inhibited 9 strains, from the leaves 6 strains, from the shoots 5 strains, and from the roots 8 strains out of the 14 polyresistant bacterial strains examined. We believe that we can recommend ethanol extracts from the fruits and leaves, ether extracts from the leaves, and dimethyl-sulfoxide extracts from the fruits and roots of *X. strumarium* for further studies of combating polyresistant bacterial strains. We found no pronounced nematocidal properties of the extracts of various parts of *X. strumarium* against the nematode larvae *S. papillosus*, *H. contortus*, and *M. capillaris*.

The authors declare no conflict of interests.

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