

BACTERIOSTATIC AND ANTIOXIDANT PROPERTIES OF PAULOWNIA LEAF EXTRACTS (*Paulownia* spp.) AS NATURAL PRODUCTS IN CROP PROTECTION

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ABSTRACT

Paulownia leaf extracts were tested for their bacteriostatic and antioxidant properties against six pathogenic bacteria in vegetable and fruit crops. Paulownia leaf extracts were most effective against the *Clavibacter michiganensis* ssp. *michiganensis* and *Xanthomonas hortorum* pv. *carotae*. Paulownia extracts were less effective against *Agrobacterium tumefaciens*, *Pseudomonas syringae* pv. *lachrymans* and *Pseudomonas syringae* pv. *tomato*. Only *Erwinia carotovora* was resistant to the tested plant extracts. The type of extraction solvent significantly impacts the antibacterial activity and the flavonoid and polyphenol contents of plant extracts. Acetone and alcoholic extracts had a higher content of flavonoid and polyphenolic compounds than water extracts, which resulted in their better bacteriostatic properties. The growth inhibition zones of the tested bacteria and the contents of flavonoids and polyphenols were significantly correlated. However, the bacteriostatic properties and antioxidant activity were not significantly correlated.

Keywords: plant extracts, bacterial plant pathogens, agar well diffusion method, flavonoids, polyphenols, paulownia

INTRODUCTION

Paulownia is a deciduous tree native to central and western China [Zhu et al. 1986]. This plant is also cultivated in Southeast Asia, North and Central America, Western and Southern Europe and Australia [Yadav et al. 2013]. The genus *Paulownia* includes nine fast-growing species [Zhu et al. 1986, Yadav et al. 2013]. The most popular species are *Paulownia tomentosa*, *P. fortunei* and *P. elongata* [Yadav et al. 2013, Morote et al. 2023]. Recently, the ‘Oxytree’

(*P. fortunei* × *P. elongata*), ‘Cotevisa 2’ (*P. elongata* × *P. fortunei*) and ‘Shan Tong’ (*P. tomentosa* × *P. fortunei*) hybrids have also been widely cultivated in the world [Sedlar et al. 2020, Kadlec et al. 2021].

Paulownias are adaptable, extremely fast-growing, multipurpose trees [Zhu et al. 1986, Yadav et al. 2013]. They are widely used, including in biomass production [Zuazo et al. 2013, García-Morote et al. 2014], the wood industry [Woods 2008, Barbu et al. 2022], paper

production [Rai et al. 2000, López et al. 2012], phytoremediation and reclamation of degraded soils [Domett et al. 2008, Tzvetkova et al. 2015], as well as the pharmaceutical and cosmetics industries [Schneiderová and Šmetkal 2015, Guo et al. 2023]. Paulownia leaves are also used as green fertilizer and animal feed [Al-Sagheer et al. 2019, Huang et al. 2022]. Moreover, the flowers of paulownia are melliferous [Woods 2008].

Currently, horticulture faces main challenges related to the increase in the production of high-quality food, the reduction of cultivated areas and the reduction of chemical fertilizers and pesticides [Godlewska et al. 2021]. Furthermore, the problem of plant diseases has become increasingly important in recent years. For example, crown gall is a common disease in fruit tree crops like apples, pears, peaches, and cherries [Mańka and Grzywacz 2023]. However, in Poland, plant protection is based mainly on preventive methods. Therefore, plant extracts may prove beneficial due to their potential biological functions. However, there is little information on the effectiveness of paulownia extracts in horticulture. More than 130 physiologically active components have been isolated in paulownia plants, such as flavonoids, lignans, phenolic glycosides, terpenoids, glycerides and phenolic acids [Schneiderová and Šmetkal 2015, Hawrył et al. 2020, Guo et al. 2023, Sławińska et al. 2023]. These compounds have anti-inflammatory, antimicrobial, antioxidant, cytotoxic and anti-cancer properties. Therefore, paulownia leaves, flowers, wood, fruit and bark have high potential in traditional Chinese medicine.

Many studies have reported that plant extracts are effective against microorganisms. Paulownia extracts exhibit antibacterial activity against human pathogens, including *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* [e.g. Popova and Baykov 2013, Ferdosi et al. 2021, İnci et al. 2021, Škovranová et al. 2024]. Paulownia leaf extracts show confirmed bacteriostatic effect against pathogenic bacteria in animal fodder, i.e., *Bacillus cereus*, *Staphylococcus aureus* and *Yersinia enterocolitica* [Džugan et al. 2021]. However, there is still no reliable information about their properties against plant pathogenic bacteria. Since the number of registered chemical plant protection products in the European Union is limited, there has been a growing

interest that seeks bacteriostatic effects in natural and ecological products. Paulownia extracts may have this potential, although existing literature on this subject is limited. Therefore, this study aimed to evaluate the bacteriostatic and antioxidant properties of paulownia leaf extracts against pathogens that cause diseases in vegetable and fruit crops.

MATERIALS AND METHODS

Plant material and extract preparation

The fully expanded leaves of *P. tomentosa* × *P. fortunei* hybrids ('9503 UR', '9501 UR', 'SH 7 UR') and *P. tomentosa* genotypes ('LuP 4/20A', 'WEG 9 PEG') were obtained from trees growing in the field paulownia collection established in Central Poland (Mazovia; 51°43'51.4" N 21°45'54.5" E). The leaves collected from those paulownia clones contained the most secondary metabolites and antioxidants [Džugan et al. 2021]. The paulownia leaves were dried at room temperature, without exposure to sunlight and grounded in a laboratory mill (MMK-06M, MPM, Milanówek, Poland). Two grams of air-dried plant material were then dissolved in 30 mL of solvent, shaken at 160 RPM at room temperature for 24 hours and filtered. The extracts were stored at 4 °C for further analyses. Several solvent types in various proportions were used to extract bioactive compounds from paulownia leaves (Table 1).

Bacteriostatic properties determination

The bacteriostatic properties of paulownia leaf extracts were tested against Gram-positive bacteria *Clavibacter michiganensis* ssp. *michiganensis* and five Gram-negative bacteria: *Agrobacterium tumefaciens*, *Erwinia carotovora*, *Pseudomonas syringae* pv. *lachrymans*, *Pseudomonas syringae* pv. *tomato* and *Xanthomonas hortorum* pv. *carotae*. The *A. tumefaciens* was obtained from the Research Institute of Horticulture in Skierniewice, Poland. Five other bacterial isolates were purchased from the Bank of Pathogens at the Institute of Plant Protection – National Research Institute in Poznań, Poland.

The assessment of the bacteriostatic properties of paulownia extracts was carried out using the agar well diffusion method. Fresh bacterial suspensions were adjusted to 0.5 McFarland standard and were

Table 1. Composition of solvents used to extract active compounds from paulownia leaves

Solvent designation	Solvent composition
W	water (100%)
MetW	methanol/water (50%/50%)
MetWA	methanol/water/acetic acid (50%/49.5%/0.5%)
MetA	methanol/acetic acid (99.5%/0.5%)
EtW	ethanol/water (50%/50%)
EtWA	ethanol/water/acetic acid (50%/49.5%/0.5%)
EtA	ethanol/acetic acid (99.5%/0.5%)
AcWA2	acetone/water/acetic acid (70%/28%/2%)
AcWA05	acetone/water/acetic acid (70%/29.5%/0.5%)
AcWA02	acetone/water/acetic acid (70%/29.8%/0.2%)

evenly spread on Petri dishes. The *A. tumefaciens* was inoculated on the YEB medium (Thermo Fisher Scientific, Waltham, MA, USA). The *C. michiganensis* ssp. *michiganensis* and *X. hortorum* pv. *carotae* were grown on the PDA medium (BioMaxima, Lublin, Poland). Czapek medium (BioMaxima, Lublin, Poland) was used for *P. syringae* pv. *lachrymans* and *P. syringae* pv. *tomato*. The *E. carotovora* was inoculated on the TSA medium (BTL, Łódź, Poland).

Three wells with a diameter of 5 mm were made in the culture medium using a sterile cork borer. Next, 100 μ L of solvent or paulownia extract solution was introduced into the wells. The Petri dishes were sealed with laboratory film and incubated at 28 °C for 48 hours after bacteria inoculation. After this time, the real inhibition zone of bacterial growth was obtained by subtracting the diameter of the zone of inhibition caused by the extract and solvents from the zone of inhibition due to the solvent. In this way, the antibacterial activity of paulownia extracts alone was determined.

Total flavonoids and polyphenolic compound content determination

The total flavonoid content was determined using the spectrophotometric method with aluminum chloride described by Yadav et al. [2013] with slight modifications. The volumes of 0.1 mL of paulownia extracts, 1.6 mL of methyl alcohol, 0.1 mL of 10% $\text{AlCl}_3 \times 6 \text{H}_2\text{O}$, 0.1 mL of 1M CH_3COONa and 2.8 mL of distilled water were added. After 40 minutes of incubation at room temperature, the absorption of the tested solutions was measured at 415 nm. Quercetin dihydrate was used as a standard to make the calibration curve. Once the readings were obtained,

the total flavonoid content was calculated. The results were expressed in milligrams QE per gram of leaf dry mass. Eight independent replicates were completed for each solvent.

The total content of polyphenolic compounds following the Folin-Ciocalteu method was carried out according to Žbik et al. [2023]. The volumes of 0.1 mL of paulownia extract, 6 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent were mixed. After 3 minutes, 1.5 mL of saturated sodium carbonate and 1.9 mL of distilled water were added. The mixture was then incubated at 40 °C for 30 minutes. The absorbance was measured at 765 nm. A calibration curve was prepared based on the gallic acid concentration. The results were expressed in milligrams GAE per gram of leaf dry mass. Eight independent replicates were performed for each solvent.

Antioxidant activity determination by DPPH and FRAP tests

The DPPH was conducted according to Uğuz and Kara [2019]. A solution of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) diluted with methanol was prepared, with the absorbance of approximately 0.9 at 517 nm. Then, 0.2 mL of paulownia extract and 1.8 mL of DPPH solution were mixed. After five minutes of incubation, the absorbance of tested samples was measured again at 517 nm against methanol as a reference. The results were expressed in μ mol Trolox per gram of leaf dry mass. For each solvent, six independent replications were completed.

Antioxidant properties of paulownia extract were additionally determined using the FRAP test according to Bertoncelj et al. [2007]. The FRAP reagent contained 25 mL of 0.3 M acetate buffer (pH 3.6), 2.5 mL

of a 10 mM 2,4,6-tripyridyl-S-triazine (TPTZ) and 2.5 mL of 20 mM FeCl₃. The volumes of 0.2 mL paulownia extract and 1.8 mL of FRAP solution were mixed. After incubation at room temperature for 10 minutes, the absorbance of tested samples was measured at 593 nm. A calibration curve was prepared based on the Trolox solution, and results were expressed in μmol Trolox per gram of leaf dry mass. For each solvent, six independent replications were performed.

Statistical analyses

The obtained results were statistically analyzed using the Statistica 13.3 program software. The collected data were subjected to ANOVA and LSD mean separation tests at $p < 0.05$ significance level, and a correlation analysis among the obtained parameters was performed. Cluster analysis according to Ward's method and Euclidean distance were also performed. The following traits were used for agglomeration: the diameter of the bacterial inhibition zone, total flavonoid and polyphenol contents, and antioxidant activity of the extracts.

RESULTS

Bacteriostatic properties of paulownia leaf extract

Paulownia leaf extracts have a bacteriostatic effect against five bacteria. Plant extracts were most effective

against the *C. michiganensis* ssp. *michiganensis* and *X. hortorum* pv. *carotae* (Table 2). The average diameter of the growth inhibition zone was over 2 mm. The tested plant extracts were less effective for *P. syringae* pv. *lachrymans* and *A. tumefaciens* with a zone of inhibition of almost 2 mm. Small inhibition zones were also found for *P. syringae* pv. *tomato*. Nevertheless, *E. carotovora* was generally resistant to paulownia leaf extracts (Table 2).

The extraction solvent type influenced the antibacterial activity of paulownia leaf extracts (Fig. 1). Water-acetone-acetic extracts were more effective than alcohol and water extracts. The antimicrobial activity was significantly higher in 'AcWA05' and 'AcWA02' extracts against tested bacteria. For *X. hortorum* pv. *carotae*, the maximum inhibition zone was over 6 mm (Figure 2A). For the *C. michiganensis* ssp. *michiganensis*, the bacteriostatic activity was slightly lower, with a zone of inhibition almost 6 mm in diameter (Figures 2B and C). These two acetone extracts also exhibited high bacteriostatic activities against the other tested bacteria. However, the third 'AcWA2' acetone extract had a slightly weaker bacteriostatic effect (Table 2). For *E. carotovora*, the growth inhibition zone for acetone solvents was approximately 1 mm.

High antimicrobial activity was also found for ethanol paulownia leaf extracts ('EtW' and 'EtA'). *P. syringae* pv. *lachrymans* was the most sensitive to these extracts. Maximum zones of inhibition reached 3.5 mm.

Table 2. Diameter of growth inhibition zone of tested bacterial isolates by paulownia leaf extracts

Solvent	Growth inhibition zone diameter (mm)*					
	<i>Agrobacterium tumefaciens</i>	<i>Clavibacter michiganensis</i> ssp. <i>michiganensis</i>	<i>Erwinia carotovora</i>	<i>Pseudomonas syringae</i> pv. <i>lachrymans</i>	<i>Pseudomonas syringae</i> pv. <i>tomato</i>	<i>Xanthomonas hortorum</i> pv. <i>carotae</i>
W	0.4 a	0.4 a	0.3 ab	0.1 a	0.2 a	0.3 a
MetW	0.3 a	0.7 a	0.2 a	0.2 a	0.4 ab	0.3 a
MetWA	1.2 bc	1.5 b	0.5 bc	0.8 b	0.8 bc	0.6 a
MetA	1.4 cd	2.2 cd	0.5 bc	1.4 c	0.9 c	2.3 c
EtW	0.5 ab	0.4 a	0.4 ab	0.3 ab	0.4 ab	0.4 a
EtWA	2.3 e	2.3 cd	0.7 c	3.4 e	1.7 d	2.1 bc
EtA	2.1 e	1.8 bc	0.5 bc	2.4 d	1.1 c	1.6 b
AcWA2	2.1 de	2.5 d	0.5 bc	3.7 ef	2.5 e	3.3 d
AcWA05	4.0 f	5.8 f	1.1 d	4.1 f	3.6 f	6.1 e
AcWA02	4.5 f	5.1 e	1.0 d	3.1 e	3.3 f	5.6 e
Mean	1.9	2.3	0.6	2.0	1.5	2.3

* real growth inhibition zone diameter, i.e., difference between the growth inhibition zone diameter of paulownia leaf extract and the growth inhibition zone diameter of solvent used for extraction (different letters indicate significant differences at $p = 0.05$)

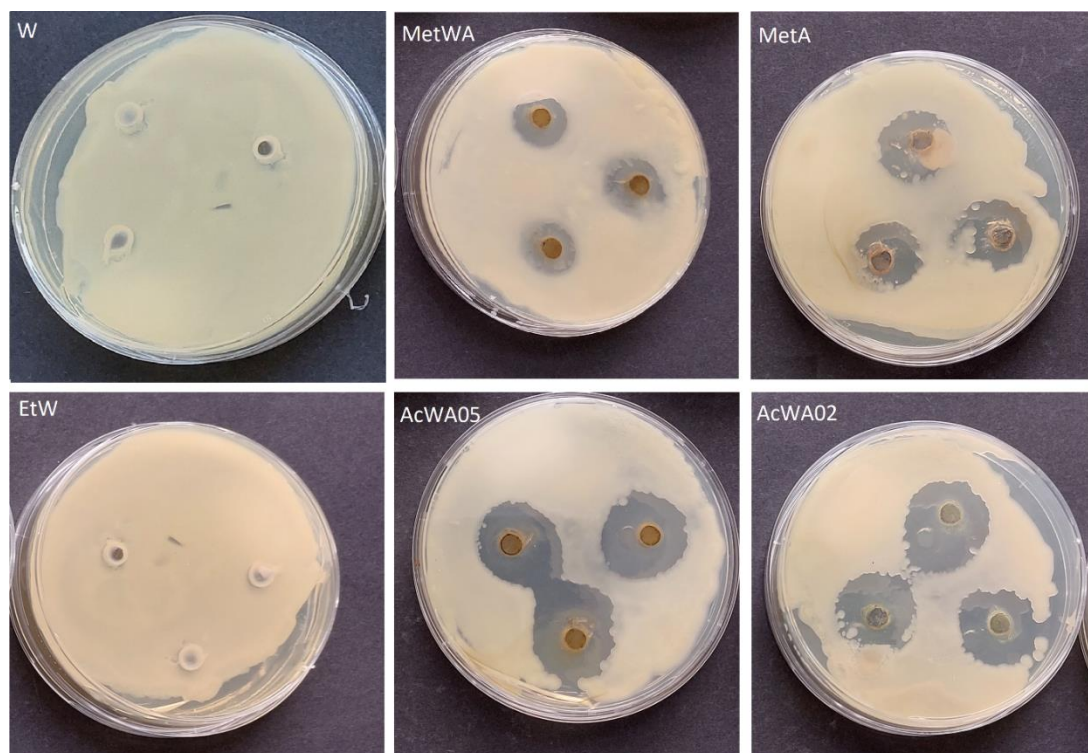


Fig. 1. Effect of paulownia leaf extracts on the growth of *Clavibacter michiganensis* ssp. *michiganensis* depending on extraction solvent type

The ethanol extracts also showed bacteriostatic properties against *A. tumefaciens* and *C. michiganensis* ssp. *michiganensis* with an inhibition zone of approximately 2 mm. The only water-ethanol extract ('EtW') did not inhibit the growth of any of the tested bacteria. The diameter of the growth inhibition zone did not exceed 0.5 mm.

However, the least bacteriostatic properties against tested bacteria were shown by methanol extracts (Figures 2D and E). Nevertheless, *X. hortorum* pv. *carotae* and *C. michiganensis* ssp. *michiganensis* were the most sensitive to 'MetA' extract. The highest inhibition zone diameter of methanol extract was almost 2.5 mm. Water-methanol extract ('MetW') was the least effective for tested bacteria. Nevertheless, the paulownia water extract had the weakest inhibitory effect against all tested bacteria (Table 2). The diameter of the growth inhibition zone did not exceed 0.5 mm.

Total flavonoid and polyphenol contents in paulownia extracts

The total flavonoid content in paulownia leaf extracts depended on the solvent types (Fig. 3). The

content of these compounds ranged from 11 to 56 mg QE·g⁻¹ DM. The highest total flavonoid content was found in the acetone extracts, followed by the ethanol, methanol and water extracts. The total flavonoid content of acetone extracts was over 50 mg QE·g⁻¹ DM. The highest content of these compounds was determined in the 'AcWA02' extract. However, the total flavonoid content of ethanol and methanol extracts ranged from 29 to 46 mg QE·g⁻¹ DM. Among the tested alcohol extracts, the contents of these compounds were higher in 'EtW' and 'MetA' extracts. Meanwhile, the total flavonoid content in water extract was approximately 5 and 3.5 times lower than in acetone and alcohol extracts, respectively.

The total polyphenol content in paulownia leaf extracts was determined using the Folin-Ciocalteu method. The content of these compounds ranged from 30 to 62 mg GAE·g⁻¹ DM. The total polyphenol content of the acetone extracts is higher than other extracts (Fig. 4). The highest total polyphenol content was found in the 'AcWA05' acetone extract. Two other acetone extracts ('AcWA2' and 'AcWA02') were also

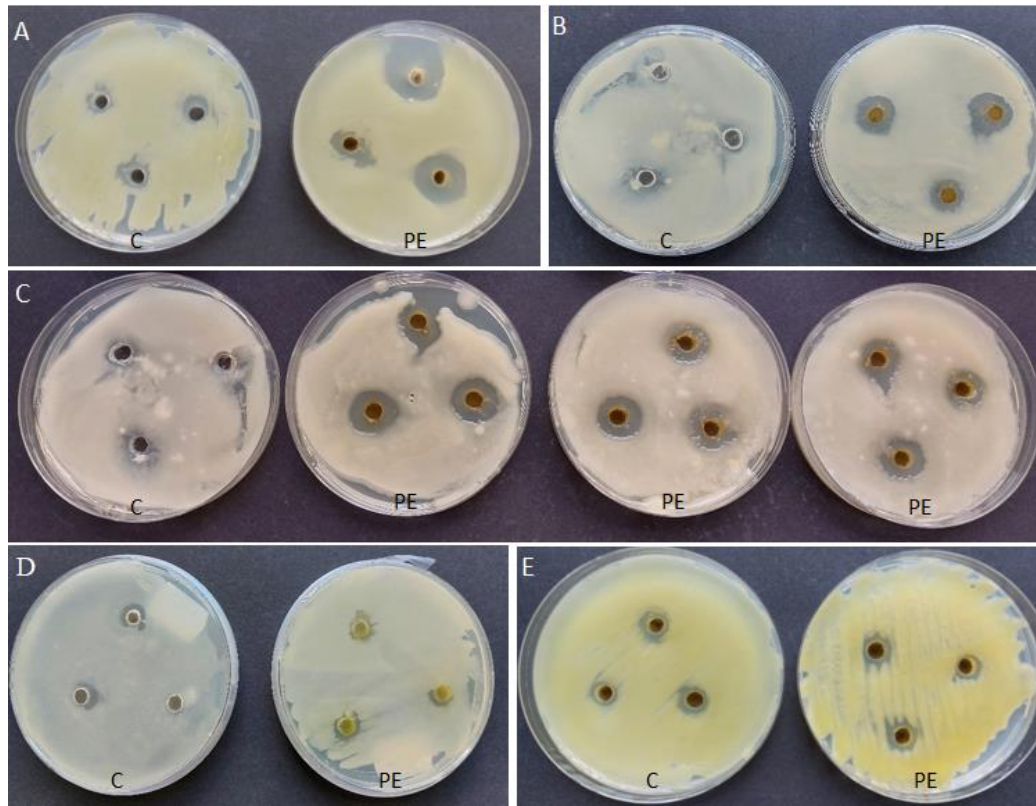


Fig. 2. Growth inhibition zone of bacterial isolates, where C – control and PE – paulownia leaf extracts; *Xanthomonas hortorum* pv. *carotae* ‘AcWA05’ (A), *Clavibacter michiganensis* ssp. *michiganensis* ‘AcWA05’ (B), *C. michiganensis* ssp. *michiganensis* ‘AcWA02’ (C), *Agrobacterium tumefaciens* ‘MetA’ (D) and *X. hortorum* pv. *carotae* ‘MetA’ extract (E)

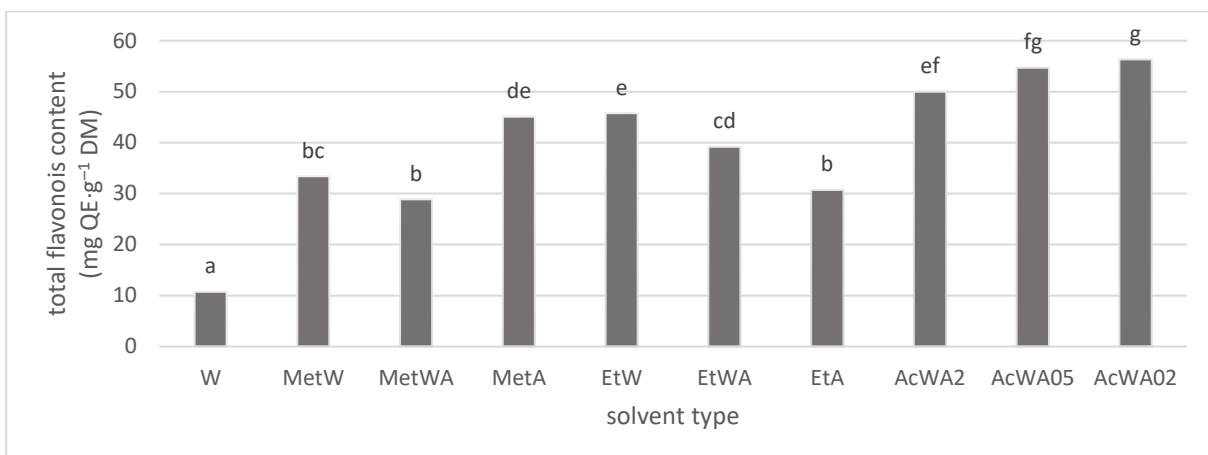


Fig. 3. The total flavonoid content in paulownia leaf extracts depending on the solvent type (different letters indicate significant differences among means at $p = 0.05$)

characterized by a high content of polyphenols. However, the total polyphenol content of the ethanol and methanol extracts was slightly lower (Figure 4), especially for the ‘MetW’ and ‘EtA’ extracts. Nevertheless, the lowest content of polyphenolic compounds was found in the water extract. The total polyphenol content in water extract was up to two times lower than in acetone extracts.

Antioxidant activity of paulownia extracts

The antioxidant activity of paulownia leaf extracts determined by the DPPH method ranged from 128 to

169 $\mu\text{mol Trolox}\cdot\text{g}^{-1}\text{ DM}$. Meanwhile, the antioxidant activity of these extracts measured by the FRAP method ranged from 121 to 167 $\mu\text{mol Trolox}\cdot\text{g}^{-1}\text{ DM}$. The antioxidant activity of the extracts depended on the type of solvent (Figure 5). The alcohol extracts acidified with acetic acid (‘EtA’ and ‘MetA’) were found to have the highest antioxidant activity. High antioxidant activity was also found in the water-methanol-acetic (‘MetWA’) and water-acetone-acetic extracts (‘AcWA2’). However, the lowest antioxidant activity was observed in the ethanol-water extract, which was approximately 25% lower compared with the ‘EtA’ extract.

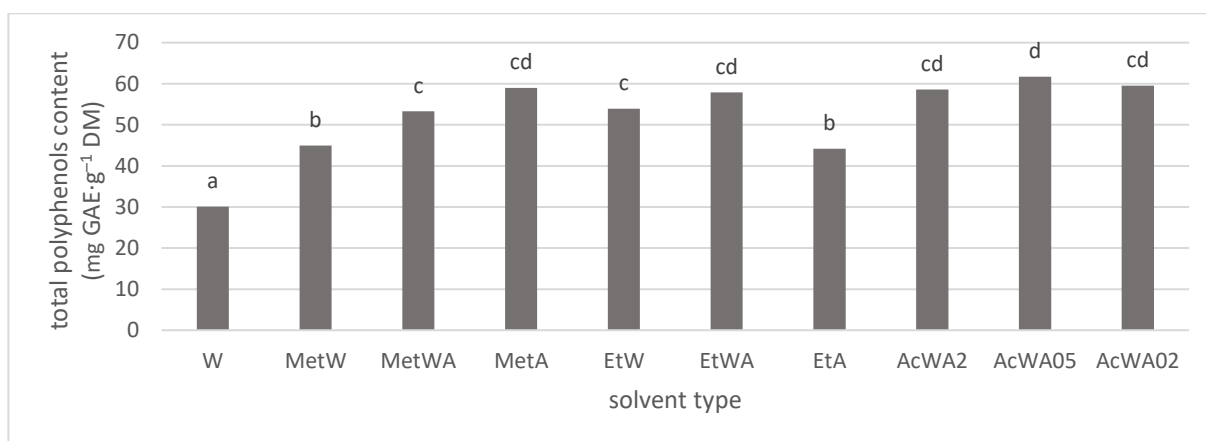


Fig. 4. The total polyphenol contents in paulownia leaf extracts depending on the solvent type (different letters indicate significant differences among means at $p = 0.05$)

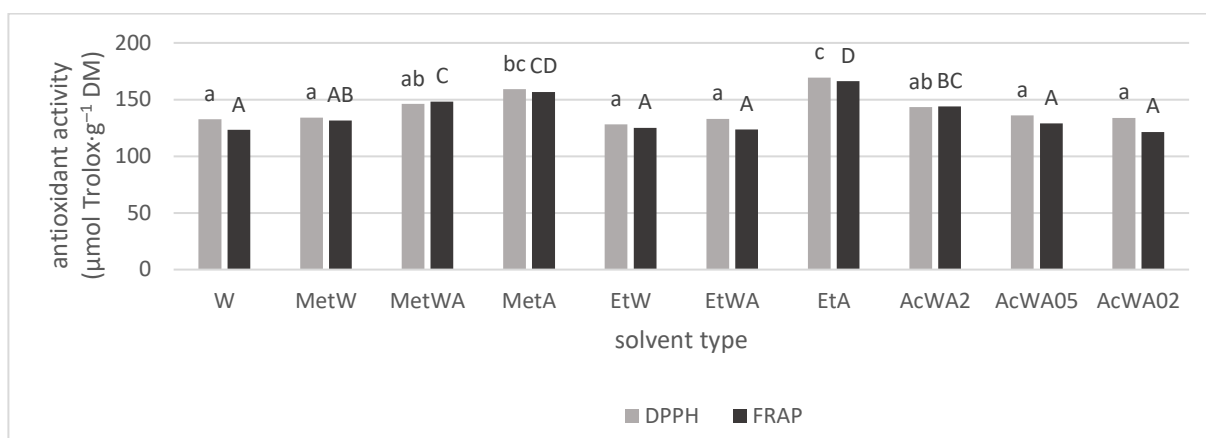


Fig. 5. Antioxidant activity of paulownia leaf extracts (different letters indicate significant differences among means at $p = 0.05$)

Relationships between the analyzed traits

The relationships between the bacterial growth inhibition zone, the total content of flavonoids and polyphenols and the total antioxidant activity of paulownia leaf extracts were determined. The *zone of growth inhibition* of the tested bacteria and the content of flavonoids and polyphenols were significantly positively correlated ($r = 0.61^* - 0.77^*$). However, the bacteriostatic properties of the paulownia extracts and their antioxidant activity were not significantly correlated ($r = -0.22^{ns} - 0.11^{ns}$). A significantly high positive correlation was only found between the DPPH and FRAP methods ($r = 0.96^*$).

A cluster analysis divided the tested bacteria into three groups depending on their sensitivity to paulownia leaf extracts (Figure 6A). The first group consisted of *C. michiganensis* ssp. *michiganensis* (CMM), *X. hortorum* pv. *carotae* (XHC) and *P. syringae* pv. *tomato* (PST). The second group were *A. tumefaciens* (AT) and *E. carotovora* (EC). However, *P. syringae* pv. *lachrymans* (PSL) were separated into the third group.

Ten paulownia leaf extracts were divided into four groups depending on bacteriostatic properties by cluster analysis (Figure 6B). The first group consisted of ‘AcWA02’ and ‘AcWA05’ extracts and was separated from the other samples. These acetone solvents had the highest inhibition zone of bacterial growth.

The second group comprised ‘AcWA2’ and ‘EtWA’ extracts. The ‘MetWA’, ‘MetA’ and ‘EtA’ extracts formed a separate group. The ‘W’, ‘EtW’ and ‘MetW’ extracts were separated into a fourth cluster with the least bacteriostatic activity.

DISCUSSION

Many secondary metabolites were isolated from the paulownia plants [Schneiderová and Šmetkal 2015, Sławińska et al. 2023]. Paulownia leaves, flowers, wood, fruit and bark were used in traditional Chinese medicine. The antibacterial activity of paulownia extracts on human pathogens has been found [Popova and Baykov 2013, Ferdosi et al. 2021, İnci et al. 2021, Škovranová et al. 2024]. These plant extracts were used, among others, against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria. Paulownia leaf extracts demonstrated the bacteriostatic effect against pathogenic bacteria in animal fodder, i.e., *Bacillus cereus*, *Staphylococcus aureus* and *Yersinia enterocolitica* [Džugan et al. 2021]. However, there is still no reliable information about their properties against pathogenic bacteria in plants. Due to the number of registered chemical plant protection products in Poland is limited, there is a growing interest in natural and ecological alternative for their bacteriostatic

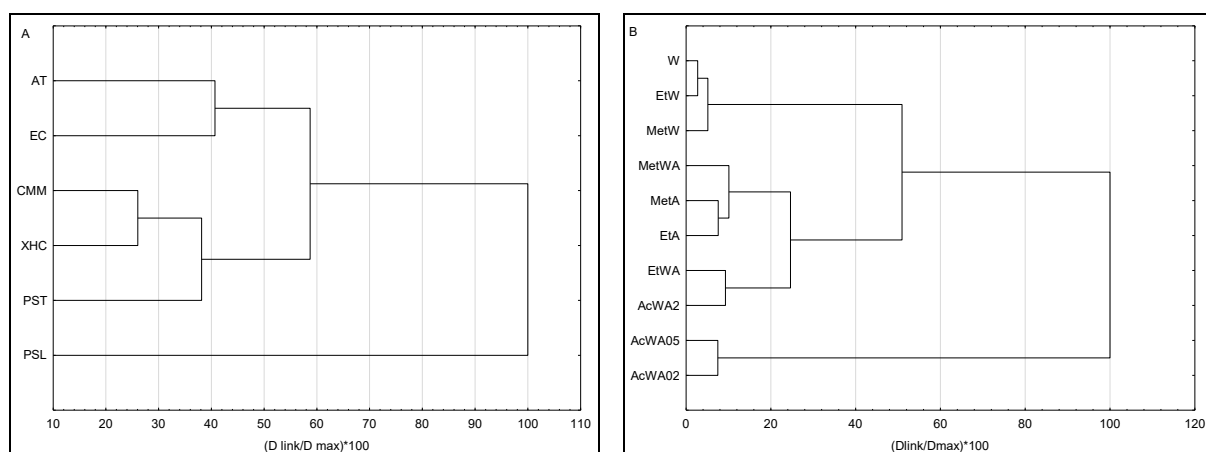


Fig. 6. Dendrogram showing the similarity of paulownia leaf extract effect; *Agrobacterium tumefaciens* (AT), *Clavibacter michiganensis* ssp. *michiganensis* (CMM), *Erwinia carotovora* (EC), *Pseudomonas syringae* pv. *lachrymans* (PSL), *P. syringae* pv. *tomato* (PST) and *Xanthomonas hortorum* pv. *carotae* (XHC)

effects. Moreover, large leaves of paulownia plants are not commonly used in the biomass industry [Jacek and Litwińczuk 2016].

Many studies have reported that plant extracts are more effective against Gram-positive bacteria than Gram-negative bacteria [e.g., Parekh et al. 2005, Lim-suwan et al. 2009, Modarresi-Chahardehi et al. 2012, Koohsari et al. 2015]. Only a few studies have shown that plant extracts have a better inhibitory effect against Gram-negative bacteria [Parekh and Chanda 2007, Popova and Baykov 2013, El Mannoubi 2023]. The first results of the bacteriostatic activity of paulownia plants are promising. In the present study, paulownia leaf extracts were confirmed to have bacteriostatic properties against some pathogenic bacteria causing diseases of vegetable and fruit plants. The paulownia leaf extracts were effective against both Gram-positive (*C. michiganensis* ssp. *michiganensis*) and Gram-negative bacteria (*A. tumefaciens*, *P. syringae* pv. *lachrymans*, *P. syringae* pv. *tomato* and *X. hortorum* pv. *carotae*). Furthermore, paulownia extracts can be used in integrated and organic cultivation.

Paulownia leaf extracts were most effective against the *C. michiganensis* ssp. *michiganensis* and *X. hortorum* pv. *carotae*. These bacteria cause common diseases, such as bacterial canker of tomatoes and bacterial blight of carrots [Scott and Dung 2020, Peritore-Galve et al. 2021]. This is probably the first study of the antimicrobial activity of paulownia leaf extracts against these pathogens. Nevertheless, the Moroccan plant extracts [Talibi et al. 2011], common marigold (*Calendula officinalis* L.) and purple coneflower (*Echinacea purpurea* L.) extracts [Aksoy et al. 2021] were tested against the bacterial canker of tomato. The growth inhibition diameter ranged from 5 to 50 mm. However, the peppermint (*Peganum harmala* L.) and Syrian rue (*Peganum harmala* L.) extracts [Siddique et al. 2020] were less effective. The growth inhibition diameter slightly exceeded 10 mm. On the other hand, Jacobo-Salcedo et al. [2011] and Makhubu et al. [2023] reported that plant extracts were ineffective against *C. michiganensis* ssp. *michiganensis*. However, there is very little information on the effectiveness of plant extracts against *Xanthomonas hortorum* pv. *carotae*.

The paulownia leaf extracts were also effective for *A. tumefaciens*. It is worth underlining that the soil bacteria *Agrobacterium* is polyphagous with a wide host

range [Mańka and Grzywacz 2023]. This is particularly important because of the lack of effective chemical plant protection products on crown gall. Therefore, plant extracts can be useful for plant protection. The mugwort (*Artemisia* L.) and ginger (*Zingiber* Rosc.) extracts [Njagi et al. 2021], as well as garlic (*Allium sativum* L.) extracts [Nabatanzi 2018] were also tested against *A. tumefaciens*. The growth inhibition diameter was approximately 12 mm for these plant extracts. The bacteriostatic effect of the eucalyptus leaf (*Eucalyptus cinerea* L.) extracts against crown gall was also found. The growth inhibition diameter ranged from 0 to 15 mm, depending on the extraction solvent [Kahla et al. 2017]. Okla et al. [2019] also found a bacteriostatic effect of the orange leaf and branch (*Citrus aurantium* L.) extracts on *A. tumefaciens*. The highest diameter of the growth inhibition zone of this bacterium was up to 18 mm. However, branch bark and branch wood extracts were ineffective.

Bacterial soft rot is one of the most common diseases of potatoes [Viswanath et al. 2018]. However, paulownia leaf extracts did not significantly inhibit the growth of *E. carotovora*. Bdiya and Dahiru [2006] found that aqueous leaf and seed extracts of neem (*Azadirachta indica* L.) significantly reduced bacterial soft rot. However, the aqueous leaf extract of river redgum (*Eucalyptus camaldulensis*) was ineffective. In turn, Viswanath et al. [2018] evaluated 13 aqueous plant extracts against soft rot caused by *E. carotovora*. The highest diameter of the inhibition zone was found for jimsonweed (*Datura stramonium* L.) and common fig (*Ficus carica* L.) extracts and was approximately 10 mm. However, the other plant extracts were less effective. Bhardwaj and Laura [2008] tested aqueous extracts of twenty plants against *E. carotovora*. The highest diameter of growth inhibition zone was found for leaf tea (*Camellia sinensis* L.), arabic tree bark (*Acacia arabica* Willd.) and katha bark (*Acacia catechu* Willd.) extracts. However, 13 other plant extracts were ineffective.

In the present study, paulownia leaf extracts demonstrated a bacteriostatic effect against five tested bacteria. Nevertheless, the growth inhibition zones obtained are weaker than the results of the discussed literature. There are no unified methods for plant extract preparation. Various extraction techniques are used to extract bioactive compounds, including hot water ex-

traction, ultrasound or microwave. The bacteriostatic activity also depends on the extraction solvent type, extraction time, sample weight and different plant parts. Plant extracts are prepared from leaves, stems, branches, fruits, flowers and roots. The differences in the bacteriostatic activity of plant extracts may also depend on the plant cultivar, growth conditions, plant part, plant maturity, extraction and storage method [Rashmi and Negi 2022].

The extraction solvent type significantly influenced the antibacterial activity of paulownia extracts. The solvents were ranked by the inhibition zone in the following order: acetone, ethanol, methanol and water. Based on the cluster analysis, 'AcWA02' and 'AcWA05' solvents were separated into a separate group. Acetone extracts obtained from paulownia plants were significantly more effective than water extracts, possibly due to the higher content of flavonoids and polyphenols. Similarly, Basri and Nor [2014] reported that the acetone extracts of tropical fruit trees (*Canarium odontophyllum* Miq.) against *Staphylococcus aureus* had a higher inhibition zone than the methanol extracts. Nevertheless, it is noteworthy that the 'EtWA' and 'EtA' extracts of paulownia leaves also had significantly high inhibition zones, especially against the *P. syringae* pv. *lachrymans*. These two ethanol extracts also exhibited moderate bacteriostatic activity against the *A. tumefaciens* and *C. michiganensis* ssp. *michiganensis*. However, the lowest bacteriostatic activity was found for water ('W') and water-alcohol ('MetW' and 'EtW') extracts. Similarly, Krupiński and Sobiczewski [2001] reported higher bacteriostatic activity of ethanol extracts against *E. amylovora* than water extracts. Likewise, Gniewosz et al. [2012] discovered that the ethanol extracts of common sage (*Salvia officinalis* L.) were more effective than water extracts. In another study, the plant methanol extracts were also more active than the water extracts [Parekh et al. 2005].

Moreover, the flavonoid and polyphenol contents in paulownia leaf extracts and their antioxidant activity depend on extraction solvents. These compounds are the most important secondary metabolites that may be responsible for antibacterial properties [e.g., Safari and Ahmady-Asbchin 2019, Krzepińko et al. 2020]. The antimicrobial activity of flavonoids involves disrupting cell membrane integrity, inhibiting nucleic

acid synthesis and paralyzing the energy metabolism of bacteria [Tagousop et al. 2018, Liga et al. 2023]. A significantly positive correlation was found between the flavonoid content and the diameter of the *growth inhibition* zone of the tested bacteria ($r = 0.65^* - 0.77^*$). Nevertheless, the flavonoid content in the plant extracts varies significantly depending on the solvents used [Gong et al. 2012, Dirar et al. 2019, El Mannoubi 2023], which agrees with the current results. The most effective solvent for flavonoid extraction was acetone. Paulownia acetone extracts contained five times higher total flavonoid content than a water extract. Likewise, Munhoz et al. [2014] reported that water-acetone and water-ethanol extracts contained approximately three times more flavonoids than water extracts. Sasadara and Wirawan [2021] also confirmed a higher total flavonoid content in alcohol and acetone than in water extracts. A high content of these compounds in paulownia water-ethanol extracts was found by Džugan et al. [2021]. Total flavonoid content ranged from 111 to 234 mg QE·g⁻¹ DM, depending on the paulownia clone. However, Na-Young and Ki-Tae found a slightly lower content of total flavonoids in paulownia ethanol extracts [2019]. The content of these compounds was 115 mg CE·g⁻¹ DM. Similarly, Yadav et al. [2013] found that the total flavonoid content in the fresh leaf extracts of paulownia ranged from 103 to 158 µg·mL⁻¹.

Polyphenols damage the cytoplasmic membrane, cell wall and nucleic acid, as well as denature enzymes of microorganisms [Skroza et al. 2019, Krzepińko et al. 2020]. In the present study, the total polyphenol contents and the diameter of the *growth inhibition* zone of the six tested bacteria were significantly positively correlated ($r = 0.61^* - 0.67^*$). The acetone solvent was the most effective in the extraction of polyphenols. The content of polyphenolic compounds was two times higher in acetone than in water extracts. This may partly explain their higher bacteriostatic properties. These results were similar to those obtained by Złotek et al. [2016]. The water-acetone extracts with acetic acid significantly increased the total polyphenol content of basil plants (*Ocimum basilicum* L.). However, the combination of methanol, water and acetic acid was less effective. In studies carried out by Michiels et al. [2012], the acetone/

water/acetic acid system (70/28/2, v/v/v) also proved to be more effective in the extraction of polyphenolic compounds from fruits and vegetables than methanol solvents. Likewise, Abozed et al. [2014] and Dirar et al. [2019] recommended acetone solvents for extracting polyphenols. However, Heś et al. [2012], Tomson et al. [2012], Salih et al. [2021], and Palaiogiannis et al. [2023] reported that alcohol extracts were more effective for the extraction of polyphenols. Nino et al. [2016] also found that methanol extracts of medicinal plants had higher phenolic and flavonoid contents than other solvents used for extraction.

Additionally, flavonoids and polyphenols are perfect sources of antioxidants, which deactivate free radicals and protect cells [Proestos et al. 2013, Krzepińko et al. 2020]. The antioxidant activity of paulownia extracts was determined using the DPPH and FRAP methods. A significantly high positive correlation between the above tests was found ($r = 0.96^*$). The other researchers [Turkmen et al. 2006, Nino et al. 2016, Złotek et al. 2016, Sasadara and Wirawan 2021] reported a positive correlation between the phenolic content and antioxidant activity. However, this relationship was not confirmed in the present study. The diameter of the growth inhibition zone of tested bacteria was also not significantly correlated with the antioxidant activity of paulownia extracts. Nevertheless, Sithisarn et al. [2015] also found no correlation between the total phenolic and flavonoid contents, antibacterial activity, and antioxidant activity of the extracts from *C. orientalis*. Similarly, Adamu et al. [2014] and Ispiryan et al. [2024] found no high correlation between antioxidant activity and antibacterial activity of plant extracts.

The present study found the highest antioxidant activity for ethanol-acetic and methanol-acetic paulownia extracts. It can be assumed that acetic acid improves the antioxidant properties of plant extracts. Żbik et al. [2023] obtained similar results, which also showed that the acidification of methanol solvent enhanced the stability of the extracted antioxidant compounds. Sowndhararajan and Kang [2013] and Nino et al. [2016] reported the highest antioxidant properties in methanol extracts. According to Karthikumar et al. [2007], Sasadara and Wirawan [2021] and Palaiogiannis et al. [2023], the highest antioxidant activity was demonstrated by ethanol extracts.

CONCLUSION

The bacteriostatic properties of paulownia leaf extracts against several Gram-positive and Gram-negative pathogens in the cultivation of vegetable and fruit plants were analyzed. These extracts were most effective against the *C. michiganensis* ssp. *michiganensis* and *X. hortorum* pv. *carotae*. They were slightly less effective against *A. tumefaciens*, *P. syringae* pv. *lachrymans* and *P. syringae* pv. *tomato*. However, only *E. carotovora* was resistant to these plant extracts. It is worth emphasizing that the extraction solvent type influenced the antibacterial activity of plant extracts. Acetone extracts were more effective than alcohol and water extracts. Generally, acetone extracts contained more flavonoids and polyphenolic compounds than other extracts, which probably resulted in their better bacteriostatic properties.

Paulownia leaf extracts could enrich the offer of preparations available on the market and replace chemical plant protection products. Importantly, these preparations can be used in integrated and organic cultivation systems. However, research will continue with paulownia extracts. It is worth assessing the potential bacteriostatic activity of extracts obtained from various plant parts. It is also necessary to expand the research to include pot and field experiments and assessment of phytotoxic effects to use the preparation on a wider scale. A detailed analysis of biologically active compounds that can be extracted from paulownia leaves is also necessary. Nevertheless, the first research results are promising.

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