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# *Fruit Shrubs' Twigs as a Source of Valuable Oligomeric Polyphenolic Compounds with Antibacterial and Antifungal Potential*

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***Abstract.*** **To obtain a good harvest, regular pruning of fruit trees and bushes is necessary, which results in the accumulation of piles of cut twigs. These twigs are underutilized and form a large number of agricultural waste. Finding a use for this lignocellulosic biomass is necessary for the sustainable use of resources, as well as for creating additional income for berry growers and rural workers.**

**The purpose of the research was to evaluate the potential of branches of various fruit trees and shrubs as a source of valuable oligomeric polyphenolic compounds – proanthocyanidins, which have a wide range of biologically active properties, including antioxidant, antibacterial, anti-inflammatory, anticancer, etc. Hydrophilic extracts of twigs of sea buckthorn (*Hippopae rhamnoides* L.), black chokeberry (*Aronia melanocarpa*), black currant (*Ribes nigrum* L.), red currant (*Ribes rubrum*), gooseberry (*Grossulariaceae*), quince (*Cydonia oblonga*), raspberry (*Rubus* L.), and grape (*Vitis vinifera*) were studied for the first time. The main process for isolating proanthocyanidins from the twigs is the extraction by ethanol-water solutions. The amount of extractive substances in the branches containing proanthocyanidins varied from 6 to 28% per DM. The highest content of proanthocyanidins was found in black chokeberry, quince, and sea buckthorn.**

**The proanthocyanidins isolation from hydrophilic extracts was carried out by Sephadex LH-20. The antimicrobial activity of dominant hydrophilic extracts and purified oligomeric proanthocyanidins was studied against eleven pathogenic bacteria and fungus: *Pseudomonas syringae* pv. *syringae*, *Erwinia rhapontici*, *Fusarium culmorum*, *Verticillium dahlia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Candida albicans* and *Cutibacterium acnes*.**

**The MIC and MBC/MFC of extracts ranged from 2 to 6 mg/mL. The antimicrobial activity of purified proanthocyanidins was 10 times higher than that of the extracts.**

***Keywords: sea buckthorn, aronia, black currant, red currant, gooseberry, quince, raspberry, grape, antimicrobial activity***

## INTRODUCTION

The effective use of local renewable resources by industry is one of the important factors for the growth of the national economy, underlined in the Latvian Bioeconomy Strategy 2030 [1]. The total estimated orchard area in the European Union is around 11 mln hectares [2]. In Latvia, the cultivation of fruit trees is traditionally an important part of the rural economy, with a total orchard area of 10.4 thousand ha in 2022 [3]. Red currant, blackcurrant, sea buckthorn, quince, black chokeberry, raspberry, and gooseberry are traditionally popular species for growing in Latvia, and altogether they form 48% of the total orchard area in Latvia. All these species demand several types of pruning annually or even twice per year, and pruning and harvesting measures lignocellulosic waste forms approximately 15-20% of the total fruits. This plant material started to attract attention of the scientists in the last years, but it is very poorly studied so far. Our latest studies confirmed the potential of

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the sea buckthorn lignocellulosic biomass as an anti-microbial and anti-inflammation agent [4]. The studies for the sea buckthorn berries showed that they contain almost 200 biologically active substances, including vitamins, essential amino acids, low molecular and high molecular polyphenols (proanthocyanidins), trace elements, etc. [5], [6]. Several studies showed the multiple bioactivities, including antimicrobial, antiviral, and even anti-cancer activity of the sea buckthorn and aronia fruits [6], [7], and leaves [8], [9] [10]. Grape fruits are considered a major source of polyphenolic compounds; fruits, juice and by-products of grape processing, such as pomace and grape seeds, have proven anti-oxidant properties [11], [12], anti-microbial effect [13], polyphenols from grapes reduce the incidence of cardiovascular diseases, improve neuronal function, and have antimicrobial properties with no toxicity effect [14].

There is much less information available for the other fruit shrubs berries. It was shown that polyphenols-rich blackcurrant extract had beneficial effects on preventing atherosclerosis of diabetic patients [15], anti-inflammatory, antioxidant and antimicrobial effects [16]. Polyphenols-containing extracts from black currant, red currant and gooseberry fruits and extracts from black currant processing residues showed high antioxidant activity [17], [18], [19]. Quince fruit extract showed anti-obesity effect [20]; research showed that the concentration of procyanidins/flavan-3-ols has the biggest contribution to the quince fruits antioxidant activity [21]. Red raspberry fruit extracts and polyphenols could reverse the metabolically associated pathophysiologies [22].

Many described bioactivities are associated with the presence of polyphenolic compounds in berries [23].

Considering the high biological activity of the berries and leaves, as well as anti-microbial and anti-inflammatory activity confirmed in our previous research for sea buckthorn twigs, this research aimed to evaluate the potential of a range of the fruit trees/shrubs twigs formed as waste in a result of fruit-shrubs pruning as a source of valuable oligomeric polyphenolic compounds – proanthocyanidins and to evaluate and compare their antibacterial and antifungal potential.

## MATERIALS AND METHODS

### *Plant Material*

Fruit trees' twigs were collected in autumn of 2023 from Baldone parish, Kekava county of Latvia (DD: 56.77306/24.30162). The twigs were dried at room temperature and ground in a mill (Cutting Mill SM100, Retsch, Haan, Germany) until the particle size of 1–4 mm. The samples were stored at −8 °C.

### *Twigs Extraction*

Twigs extraction was performed sequentially by hexane and maceration with ethanol (EtOH)-distilled water solutions (96% EtOH, 50% EtOH) and by distilled water, at 60 °C for 60 min. The extracts were freeze-dried using lyophilization equipment Heto Power Dry HS3000 (Thermo Fisher Scientific, Waltham, MA, USA) to yield a dry weight (DW) extract. The yield of the extracts is given as a percentage based on DW. The extracts were stored at −8 °C.

### *Proanthocyanidins Separation*

The purification of proanthocyanidins from the extracts was done as described by Andersone et al. [4], using a cross-linked dextran-based resin Sephadex LH-20 packed in solvent-resistant column. 96% EtOH (v/v) was used for elution of low-molecular-weight phenolics, and 70% (v/v) acetone/water solutions were used as elution solvent for proanthocyanidins. Solvents remainders were evaporated using a rotary evaporator (Heidolph Instruments, Schwabach, Germany), and purified proanthocyanidins were freeze-dried using lyophilization equipment Heto Power Dry HS3000 (Thermo Fisher Scientific, USA) and stored at 4 ºC.

### *Determination of Proanthocyanidins Content*

Determination of the content of proanthocyanidins was performed by oxidative depolymerization to anthocyanidins in acid butanol (butanol–HCl method) as described in Andersone et al. [24].

### *Determination of the Antimicrobial Activity*

Antimicrobial activity was performed for autumn and spring biomass, 50% and 96% extracts and purified poanthocyanidins, against bacteria strains *Pseudomonas syringae* pv. Syringae MSCL 894, *Erwinia rhapontici* MSCL 651, *Fusarium culmorum* MSCL 1628, *Verticillium dahliae* MSCL 863, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Candida albicans* as described in [25].

## RESULTS AND DISCUSSION

### *The Yield of Lipophilic Extracts*

The yield of lipophilic extracts obtained by hexane from all fruit shrubs' twigs biomass was quite close and varied from 0.7 to 1.5% per DM. The yield of hydrophilic extracts from the same biomasses obtained using ethanol-water solution and distilled water differed statistically significantly and ranged from 9.3 to 18.2% per DM. With 50% EtOH, all extracts yield increased significantly, suggesting that the extractives are more soluble in the ethanol-water solution. The yield of hydrophilic extracts on dry matter (DM) obtained with 50% EtOH from the entire biomass under study were: *Cydonia oblonga* 18.22% > *Aronia melanocarpa* 17.76% > *Ribes rubrum* 16.03% and *Hippopae rhamnoides* L. 15.54% > *Rubus* L. 14.13% > *Vitis vinifera* 12.98% > *Grossulariaceae* 10.69% (Table 1).

The twigs biomass of *Cydonia oblonga* and *Aronia melanocarpa* differs among the studied fruit shrub species not only by the highest total yield of hydrophilic extracts (18.22% and 17.76% per DM) but also by the high content of oligomeric proanthocyanidins (62.7% and 74.01% per DM). The PACs content in *Hippopae rhamnoides* L. and *Ribes nigrum* L. extracts isolated by 50% EtOH was 36.2 and 33.9% per DM. *Grossulariaceae, Vitis vinifera*, and *Rubus* L. had the lowest content of proanthocyanidins in hydrophilic extract composition (Table 2).

TABLE 1 THE YIELD OF LIPOPHILIC AND HYDROPHILIC EXTRACTS FROM FRUIT TREES BIOMASS, %/DM

| **Biomass** | **Yield of lipophilic extract from biomass, % per DM (solvent-hexane)** | **Yield of hydrophilic extract from biomass, % per DM (solvent-50% EtOH)** | **Yield of hydrophilic extract from biomass, % per DM (solvent-water)** |
|---|---|---|---|
| *Hippophae rhamnoides L.* | 1.46±0.04 | 15.54 ±0.05 | 14.42±0.02 |
| *Aronia melanocarpa* | 0.97±0.01 | 17.76±0.02 | 13.99±0.04 |
| *Ribes nigrum L.* | 1.12±0.02 | 15.08±0.03 | 15.03±0.04 |
| *Ribes rubrum* | 0.88±0.05 | 16.03±0.03 | 15.41±0.03 |
| *Grossulariaceae* | 1.16±0.03 | 10.69±0.03 | 6.27±0.04 |
| *Cydonia oblonga* | 1.02±0.03 | 18.22±0.03 | 14.96±0.05 |
| *Rubus L.* | 0.66±0.04 | 14.13±0.03 | 14.76±0.05 |
| *Vitis vinifera* | 1.12±0.04 | 12.98±0.03 | 10.11±0.04 |

TABLE 2 PACS CONTENT IN EXTRACTS OF FRUIT SHRUBS TWIGS THE MINIMUM INHIBITORY (MIC) AND BACTERICIDAL CONCENTRATIONS (MBC) OF EXTRACTS

| **Biomass** | **PACs content in water extract, % per DM** | **PACs content in 50% EtOH extract, % per DM** |
|---|---|---|
| *Hippopae rhamnoides L.* | 12.1±0.1 | 36.2±0.2 |
| *Aronia melanocarpa* | 33.4±0.2 | 74.01±0.3 |
| *Ribes nigrum L.* | 12.9±0.1 | 33.9±0.2 |
| *Ribes rubrum* | 5.3±0.2 | 14.8±0.2 |
| *Grossulariaceae* | 2.1±0.1 | 4.6±0.2 |
| *Cydonia oblonga* | 35.7±0.1 | 62.7±0.2 |
| *Rubus L.* | 1.9±0.1 | 2.2 ±0.1 |
| *Vitis vinifera* | 8.2±0.1 | 11.0±0.1 |

The antimicrobial activity of hydrophilic extracts of *Aronia melanocarpa*, *Cydonia oblonga*, and purified PACs from A*ronia melanocarpa* extract was studied against 11 pathogenic bacteria and fungus: *P. syringae, E. rhapontici, F. culmorum, V. dahlia, P. aeruginosa, S. aureus, E. coli, B. cereus, and C. albicans. S. pyogenes, C. acnes*. The MIC and MBC/MF of extracts ranged from 0.8 to 6.2 mg $mL^{-1}$. The antimicrobial activity of purified proanthocyanidins was to 10 times higher than of the extracts (Table 3).

TABLE 3 THE MINIMUM INHIBITORY (MIC) AND BACTERICIDAL/FUNGUS CONCENTRATIONS (MBC/MFC) OF EXTRACTS AND PACS, MG $ML^{-1}$

| **Samples** | ***E. coli*** | | ***P. aeruginosa*** | | ***S. aureus*** | | ***B. cereus*** | |
|---|---|---|---|---|---|---|---|---|
| | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** | **MIC** | **MBC** |
| PACs | 0.78 | 0.78 | 0.20 | 0.20 | 0.10 | 0.39 | 0.10 | 0.10 |
| *Aronia melanocarpa* extract isolated by 50% EtOH | 0.78 | 0.78 | 0.78 | 0.78 | 0.39 | 0.78 | 0.78 | >6.25 |
| *Cydonia oblonga* extract isolated by 50% EtOH | 0.78 | 0.78 | 0.78 | 0.78 | 0.39 | 0.78 | 0.78 | 6.25 |
| | ***P. syringae*** | | ***E. rhapontici*** | | ***F. culmorum*** | | ***V. dahliae*** | |
| PACs | 0.39 | 0.78 | 1.56 | 1.56 | 3.13 | 12.5 | 3.13 | 6.25 |
| *Aronia melanocarpa* extract isolated by 50% EtOH | 1.56 | 3.13 | 1.56 | 1.56 | 6.25 | 12.5 | 3.13 | 6.25 |
| *Cydonia oblonga* extract isolated by 50% EtOH | 1.56 | 1.56 | 1.56 | 1.56 | 6.25 | 12.5 | 3.13 | 6.25 |
| | ***C. albicans*** | | ***S. pyogenes*** | | ***C. acnes*** | | | |
| PACs | 0.31 | >1.25 | 0.04 | 0.04 | 2.50 | 2.50 | | |
| *Aronia melanocarpa* extract isolated by 50% EtOH | 6.25 | >12.5 | 0.39 | 0.39 | 1.56 | 1.56 | | |
| *Cydonia oblonga* extract isolated by 50% EtOH | 3.13 | >6.25 | 0.05 | 0.05 | 1.56 | 1.56 | | |

## CONCLUSIONS

The results showed that the most suitable solvent for obtaining proanthocyanidins-rich hydrophilic extracts was ethanol-water solution. The biggest yield of hydrophilic extracts obtained with 50% EtOH was for *Cydonia oblonga* (18.22%/DM) and *Aronia melanocarpa* (17.76% per DM), and they also had the highest content of oligomeric proanthocyanidins ( 62.7% and 74.01% per DM).

The antimicrobial activity of purified proanthocyanidins was up to 10 times higher than that of the extracts. The bactericidal and fungicidal properties of the fruit trees' lignocellulosic biomass proanthocyanidins and extracts under study allow us to consider them for the creation of antibacterial preparations. Yearly changes in the chemical composition of fruit shrubs' twigs and in the biological activity of isolated extracts and proanthocyanidins will be further studied.

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