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Assessment of Blackcurrant Twigs Potential as a Renewable Source of Proanthocyanidins in Latvia

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***Abstract*— Blackcurrant (Ribes Nigrum L.) is one of the most common shrubs in Latvia, in addition to fruits and leaves, twigs cut in spring and autumn are rich in oligomeric polyphenols - proanthocyanidins, which have powerful antioxidant, antimicrobial, anti-inflammatory properties. The yield of proanthocyanidin-containing extracts ranged from 12 to 22%/DM. At the same time, the twigs cut in autumn were richer in proanthocyanidin than in spring. The strong antimicrobial properties of the extracts showed their effectiveness against a wide range of bacteria, which makes the extracts promising for use in medicine and the food industry. All extracts introduced into the lipid-based cosmetic cream showed antioxidant activity comparable to reference samples. This indicates the potential for using these extracts as active ingredients in cosmetics aimed at protecting the skin from the negative effects of free radicals. The results obtained open up new opportunities for developing innovative cosmetic formulations with improved antioxidant properties that help slow down the ageing process of the skin and maintain its health. The use of blackcurrant twigs is a sustainable and cost-effective source of valuable bioactive compounds.**

Keywords—Blackcurrant, extract, polyphenols, proanthocyanidins, twigs.

I. INTRODUCTION

Blackcurrant (Latin: *Ríbes nígrum*), growing in temperate regions of Central and Northern Europe and Northern Asia, is one of the most widespread shrubs, the fruits and leaves of which are rich in biologically active compounds of polyphenolic nature, possessing antimicrobial, antioxidant and health-improving properties. According to FAOSTAT, Poland ranks first among European countries in blackcurrant production [1]. Numerous studies confirm that blackcurrant fruits are a valuable source of vitamin C, P, BB, potassium, γ-linolenic acid and antioxidants such as anthocyanins, and quercetin, and have a positive effect on human health [2]-[4]. Currant extracts are widely used in the food, pharmaceutical and cosmetic industries. The high nutritional value and wide range of health properties make blackcurrant an important component of healthy nutrition and a promising raw material for developing functional foods and drugs. However, in recent years, several works have been published on using other anatomical parts of currants, in addition to the fruits, as a source of biologically active substances. Studies of the potential of leaves, which are plant waste of blackcurrant, have shown that leaves are the main source of phenolic acids or flavonoids [5].

Currently, special attention is paid to the complex and waste-free processing of fruit bushes with maximum extraction of biologically active substances and expansion of the range of preparations from them. Chemical characterization of blackcurrant branch extracts is relevant and will help to create a waste-free processing scheme for blackcurrant cultivation. Our preliminary studies reported

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that blackcurrant twigs contain a complex of polyphenolic compounds, including oligomeric polyphenols – proanthocyanidins [6]. The proanthocyanidins also known as condensed tannins are present in various plants, as a defence against biotic and abiotic stressors. Proanthocyanidins are oligomeric or polymeric products of the flavonoid biosynthetic pathway. The building blocks of proanthocyanidins include catechin and epicatechin. Proanthocyanidin's degree of polymerization can range between 2 and 11 [7].

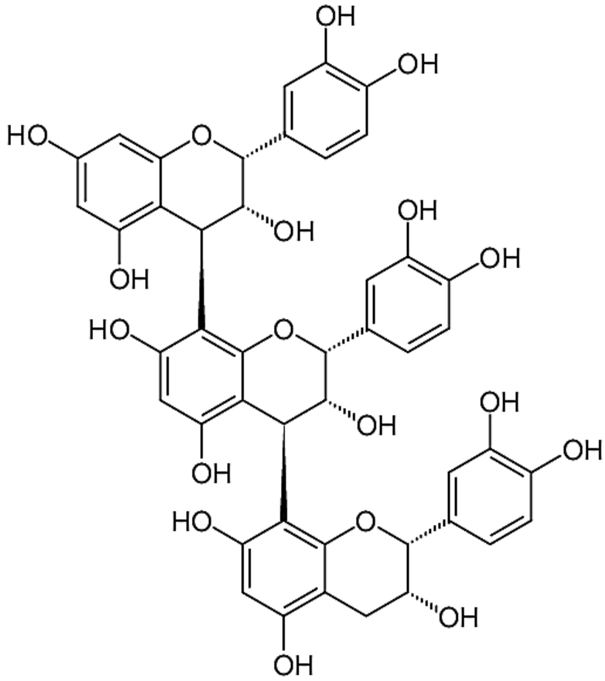

Fig 1. Chemical structure of B-type proanthocyanidins [7].

A review of the literature and our studies shows that oligomeric proanthocyanidins are highly active compounds with a wide spectrum of biological activity, which is determined by their polyphenolic structure [8]. Proanthocyanidins have antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory and antiviral properties [9]. The interest in these substances in the world is extremely high due to their various biological effects and almost complete absence of toxicity.

The purpose of this work was to evaluate the twigs of black currant, collected in the spring and autumn seasons, as a raw material for obtaining proanthocyanidins. Additionally, the study was aimed at testing the proanthocyanidins-rich fraction's antimicrobial activity and antioxidant activity, for assessment of the practical application possibilities.

II. Materials and methods

A. Collection of Blackcurrant Material

The twigs of blackcurrant were collected from the blackcurrant plantation area in Baldone parish, Kekava county of Latvia, in March and October of 2022, 2023 and 2024.

Fig. 2. Blackcurrant plantation area in Baldone parish.

The twigs were dried at room temperature, and ground with a knife mill Retsch SM100 (Retsch, Haan, Germany) and Retch SM200 (Retsch, Haan, Germany). These fractions were stored at −8 °C.

B. Hydrophilic Extract Isolation from Biomass

Extracts from the twigs' biomass of the black- currant were isolated by extraction at 60 °C for 30 and 60 min with distilled water and ethanol-water (1:1, v/v) solution. The extracts after ethanol evaporation were freeze-dried to yield a brown solid. The yield of the extracts is presented as a percentage per dry mass (DM).

C. Determination of Total Phenol Content in the Extract

The total polyphenol (TP) content in all extracts was quantified by the Folin-Ciocalteu method using gallic acid as a reference compound. An aliquot (1 mL) of the extract was transferred into the test tube and addition of 5 mL Folin-Ciocalteu reagent and 4 mL 7% aqueous sodium carbonate solution, the tube was vortexed and placed into a water bath at 30 °C for 1 h. Then absorbance of the mixture was recorded at 760 nm with a UV/VIS spectrometer Lambda 650 (Perkin Elmer, Inc., Waltham, USA) against a blank containing 1 mL of extraction solvent. The content of total phenolics was calculated as a gallic acid equivalent (GAE) from the standard curve and expressed as mg GAE/g extract. All measurements were done in triplicate. The final values were expressed as gallic acid gram equivalent (GAE). The confidence interval (CI) for the results did not exceed 3% at α=0.05).

D. Determination of Proanthocyanidins in the Extract

The content of proanthocyanidins was measured by the butanol-HCl method using procyanidin dimer B2 as a reference compound. An aliquot (1 mL) of the extract was transferred into the test tube and the addition of 6 mL of acid butanol (5% (v/v) concentrated HCl in n-butanol) and 0.2 mL of iron reagent (w/v) ($FeNH_4(SO_4)_2 \cdot 12\ H_2O$ in 2 N HCl), the tube was vortexed and placed into a water bath at 80 °C for 50 minutes. Then absorbance of the mixture was recorded at 550 nm with a UV/VIS spectrometer Lambda 650 (Perkin Elmer, Inc., Waltham, USA) against a blank containing 1 mL of extraction solvent. Each extract was analyzed in triplicate, and assay results were expressed as a percentage per dry extract (DE). The CI for the results did not exceed 3% at α=0.05).

E. Determination of Antimicrobial Activity of Extract

Antimicrobial activity was studied in 96-well plates by the two-fold serial broth microdilution method, which allowed the determination of the minimum inhibitory (MIC) and minimum bactericidal/fungicidal concentrations (MBC/MFC), as described by Andersone [10].

F. Determination of Antioxidant Activity of Extract

The effect of extracts on the oxidative stability of the lipid based system was performed with cosmetic cream (basic compositions without antioxidant additives), and were studied with the Oxipress apparatus (Mikrolab Aarhus) [11]. The oxidative stability was determined under the optimum conditions: 20 g of the reaction mixture (corresponding to 5 g of the lipid phase), oxygen pressure of 0.5 MPa, at 120°C. Ascorbic acid and gallic acid served as a reference antioxidant. The lipid-based systems without antioxidants were used as a control. First, 0.01–0.4 g of antioxidant was mixed in the reaction vessel with the lipid-based substrate (5 g in terms of lipids), and then thoroughly mixed for 30 min under an inert atmosphere. Then the reaction vessel was filled with O2 to 0.5 MPa, and placed into a furnace at 120°C and the changes of O_2 pressure were recorded as a function of time. Protection factor (PF) of samples was calculated: PF = IPx /IPc where IPx and IPc are the induction period of substrate oxidation in the presence of samples and that of the control, respectively.

G. Statistical Analysis

All measurements were conducted in triplicate (n=3). The results are presented as the mean value ± standard deviation (SD). Statistical analyses were performed using Microsoft Excel 2016. Confidence intervals (CI) were calculated for a mean using a Student's T distribution at a significance level α = 0.05. For a yield of extractives: CI≤1.2% at α = 0.05; for total phenolic content in the extract: CI≤3 mg GAE/g at α = 0.05; for proanthocyanidins content in the extract: CI≤1.5% at α = 0.05; for antioxidant activity: CI≤0.3 mg/L at α = 0.05.

III. Results and discussion

A. Chemical Characterization of Blackcurrant Twigs

The yield of the hydrophilic extracts isolated from blackcurrant twigs samples from both seasons varied from 12 to 20%/DM. Higher extract content was isolated using 50% EtOH from twigs samples collected in autumn (Fig. 3).

The proanthocyanidin content in the extracts was quite similar and varied from 12 to 36%/DM (Fig.4). The total polyphenols content in extracts varied from 14 to 38 GSEg/100g DM. The blackcurrant twigs collected in autumn have the highest PACs content in the extract in all years.

Compared to other berry bushes, blackcurrant is poorer than chokeberry and sea buckthorn in terms of proanthocyanidin content. Our previous studies have shown that the proanthocyanidin content in *Hippopae rhamnoides* L. and *Aronia melanocarpa* extracts isolated with 50% EtOH was ~40 and ~70% per DM [12].

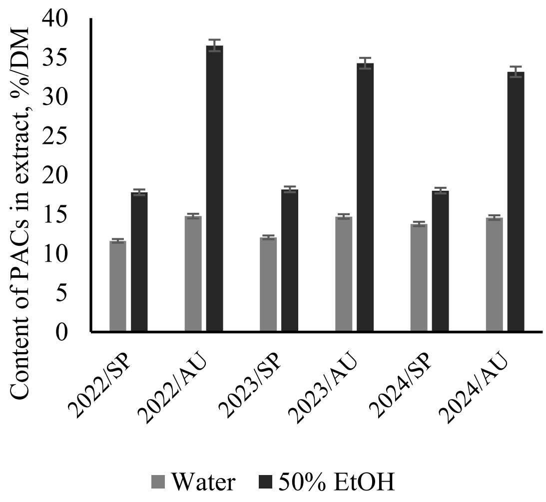

Fig. 3. Yield of extract from blackcurrant twigs biomass.

In this regard, proanthocyanidins will not be isolated from the extract but will be studied in the extract composition. This will allow us to evaluate their potential as a preservative and antimicrobial agent.

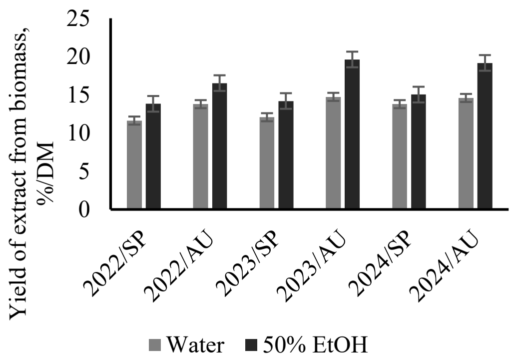

Fig. 4. Characterization extract isolated from blackcurrant twigs by water and 50% EtOH.

B. Antimicrobial Activity of Blackcurrant Extracts

The antimicrobial activity of hydrophilic extracts of blackcurrant extract was studied against four pathogenic bacteria: P. aeruginosa, S. aureus, E. coli, B. cereus. The MIC and MBC of extracts ranged from 0.6 to 5 mg mL^{-1} (Table 1).

The observed antimicrobial activity may be due not only to the presence of proanthocyanidins but also to the presence of other polyphenolic compounds in the extract. The relatively low MIC and MBC values suggest that blackcurrant extracts have potent antimicrobial activity. This is especially important given the growing concern about antibiotic resistance, with traditional antibiotics becoming less effective against certain bacterial strains.

TABLE 1. ANTIMICROBIAL ACTIVITY OF BLACKCURRANT EXTRACTS

Sample	***B. cereus*** **330**	***E. coli*** **332**	***P. aeruginosa*** **331**	***S. aureus*** **(MRSA) 993**
	MIC/MBC, mg/mL			
E/SP/W	1.25/>5.0	5.0/5.0	1.25/1.25	0.31/>5.0
E/SP/50Et	1.25/>5.0	2.5/2.5	0.63/1.25	2.5/>5.0
E/AU/W	1.25/1.25	2.5/>5	1.25/>5	0.63/0.63
E/AU/50Et	0.63/>5	2.5/5	1.25/5	0.63/0.63

E/SP/W - extract isolated by water from blackcurrant twigs collected in spring, 2023
E/SP/50Et - extract isolated by 50% EtOH from black currant twigs collected in spring, 2023
E/AU/W - extract isolated by water from black currant twigs collected in autumn, 2023
E/AU/50Et - extract isolated by 50% EtOH from black currant twigs collected in autumn, 2023

The extracts offer a potential alternative or complementary approach to combat bacterial infections. The potential of blackcurrant extracts as antimicrobial agents requires further study and development. The extracts may be incorporated into new medicinal formulations, wound dressings or other antimicrobial products.

C. Antioxidant Activity of Blackcurrant Extracts

The antioxidant activity of the samples was assesmed by determining the protection factor (PF) for lipid-containing cosmetic cream from oxidation. For this purpose, the Oxipress method was used. The antioxidant activity of the studied samples was expressed as the induction period (IP), for which the antioxidant can delay the oxidation of the substrate. Ascorbic acid and gallic acid were used as references.

TABLE 2. ANTIOXIDANT ACTIVITY OF BLACKCURRANT EXTRACTS

AO concentration, %	**Sample**	**IP, h**	**AP**
1	LC without AO	1.3±0.1	-
	Blackcurrant extract isolated by 50% EtOH	2.3±0.1	1.8±0.1
	Blackcurrant extract isolated by water	1.9±0.1	1.7±0.1
	Ascorbic acid	1.9±0.1	1.5±0.1
	Gallic acid	2.0±0.1	1.5±0.1
2	Blackcurrant extract isolated by 50% EtOH	2.7±0.1	2.1±0.1

The antioxidant activities show that the extracts in focus are promising agents for stabilisation of lipid-based systems.

When comparing the results obtained, at an antioxidant concentration of 1%, the 50% EtOH extract showed the best results.

In turn, the water extract, taking into account the lower proanthocyanidin content (10.2% per dry extract), had a lower protection factor than the 50% EtOH extract. By increasing the antioxidant concentration in the cream suspension to 2%, the protection factor increased only 1.2 times, which means that the cream suspension was saturated with the studied extract, preventing the sample from dissolving further in the given system.

CONCLUSIONS

In this study, blackcurrant twigs collected in spring and autumn and extracted with 50% EtOH solution showed high antioxidant and antimicrobial activity. Blackcurrant lignocellulosic biomass is a valuable source of proanthocyanidin-containing extracts for the creation of antibacterial and antioxidant drugs for health care, food industry and cosmetics.

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