Yield of Stirred Cold Maceration and Extraction of Milled European Black Alder Wood and Bark using Different Solvents

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Wood extractives, especially polyphenols, have great influence on the xylem colour of many wood species, which affects the success of hydrothermal wood processing, such as wood drying. One such wood species is European black alder (*Alnus glutinosa* (L.) Gaertn.), which is prone to forming intense and uneven reddish-orange or reddish-brown discolourations immediately upon harvesting and processing. However, there is lack of published scientific data on the wood and bark extractives of black alder, as well as the most suitable solvents to extract them. In this work, total soluble extractives, phenols, and flavonoids have been quantified in the wood and bark of black alder. Furthermore, the influence of four different polar organic solvents and deionized water on extractives removal yields has been measured. It was found that the bark has much higher levels of extractives as compared to the wood. Furthermore, it has been found that the highest extractive yields were obtained by using methanol as the extraction solvent for all observed compound group classifications.

Keywords: Alnus glutinosa; European black alder; Wood; Bark; Extractives; Phenols; Flavonoids; Polar organic solvents; Water; Extraction; Maceration

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INTRODUCTION

Wood and bark extractives include a wide range of organic compounds, which can be extracted from the biomass matrix using a liquid such as an organic solvent, water, or co-solvent mixtures; such extractions can be conducted in succession with different solvents or co-solvent mixtures (Fengel and Wegener 2003). Wood and bark extractives consist of substances such as waxes, fats, fatty acids, alcohols, steroids, higher carbon compounds, terpenes, lignans, stilbenes, flavonoids, tannins and other aromatics (Boddy 1992; Fengel and Wegener 2003). Extractives have an extensive range of functions in the living tree during the plant's development and defense (Taiz and Zeiger 2006). On the other hand, extractives are of great importance in wood technology; they can affect the successful implantation of hydrothermal wood processing (*e.g.*, wood drying), which is necessary in the production of finished wood products. It has been suggested that extractives, especially polyphenols among others, participate in the colour changes of

wood during tree growth (Vek *et al.* 2014, 2015) or wood processing (Burtin *et al.* 1998; Kreber *et al.* 1998; Pervan *et al*. 2006; Esteves *et al.* 2008; Straže *et al.* 2008; Sandoval-Torres *et al.* 2010; Chen *et al.* 2014; Tolvaj *et al.* 2016). Sundqvist and Morén (2002) concluded that the degradation products of wood polymers and extractives participate in wood colour formation during hydrothermal processing. When wood matrices are exposed to different temperatures, they have relatively good stability up to 100 °C, the chemical changes at temperatures below 100 °C mainly occur due to extractives (Fengel and Wegener 2003; Navi and Sandberg 2012).

Another industrial problem related to wood extractives occurs during particleboard production. Some extractives present in wood can cause particleboard manufacturing issues with resin consumption, resin curing rate, poor water resistance, and board pressing blowout (Moslemi 1974; Maloney 1977). Wood extractives often cause problems during pulping and papermaking; they contribute to the formation of pitch deposits on process equipment, and thus they affect the quality of the product (Baeza and Freer 2001). Regarding bark, the forest products industries mostly use bark as an energy source. However, it has been demonstrated that bark can also be used as a bioactive chemicals resource (Pietarinen *et al.* 2006; Fang *et al.* 2013).

European black alder (*Alnus glutinosa* (L.) Gaertn.) is a species with great potential in the wood processing industry; however, it is rarely used for the production of finished, high-value products in Europe. One of the potential reasons for this is the tendency of black alder wood to develop intense and uneven reddish-orange or reddish-brown wood discolouration immediately after tree felling and during wood processing (Klarić *et al.* 2012). Discolouration of freshly cut alder wood has been described as an oxidative chemical reaction, a chemical reaction of accessory organic compounds assisted by enzymes when oxygen penetrates wood tissue (Bauch 1984). Black alder wood discolouration also occurs in living trees, which is a response by the xylem either to a mechanical wound or an infection; this response is reflected by orange deposits at the reaction zones (Oven and Torelli 2003).

Various *Alnus* species have been investigated frequently to examine the potential biological activities and structures of individual extractible compounds (Roze *et al.* 2011; Sati *et al.* 2011; Telysheva *et al.* 2011). However, information about the influence of extractives in black alder on technological processes is sparse and fragmentary (Bauch 1984). Hence, the first step in order to understand the black alder wood discolouration and the possibilities of bark use, is to determine the extractives content and to investigate the influence of different solvents on extractives yields. The aim of this research was to: (1) investigate the solid-liquid extraction of macerated black alder wood and bark by using various polar organic solvents and water; and (2) identify and quantify extractives, phenolic, and flavonoid compounds obtained from the wood and bark.

EXPERIMENTAL

Chemicals and Reagents

All chemicals in this research were used without purification: methanol (HPLC Grade, J. T. Baker, Avantor Performance Materials Inc., Pennsylvania, U.S.A.), 96% ethanol (Kemika d.d., Zagreb, Croatia), acetonitrile (Scharlab, S.L., Barcelona, Spain), acetone (Gram-Mol d.o.o., Zagreb, Croatia), gallic acid monohydrate (Scharlab, S.L., Barcelona, Spain), sodium carbonate anhydrous (LACH-NER, s.r.o., Brno, Czech

Republic), quercetin hydrate (Sigma-Aldrich, Chemie GmbH, Taufkirchen, Germany), aluminum chloride hexahydrate (Sigma-Aldrich, Chemie GmbH, Taufkirchen, Germany), and Folin-Ciocalteu reagent (Sigma-Aldrich, Chemie GmbH, Taufkirchen, Germany). Deionized ultrapure water (ASTM D1193–06 (2011), Type I) was freshly prepared by using a Siemens UltraClear TWF system (Siemens AG, Munich, Germany). Prior to use, all organic solvents, deionized water, and solutions were tempered in a MEMMERT WNE10 water bath (Memmert GmbH + Co. KG, Schwabach, Germany) at 20 °C. Volumetric glass flasks (class "A") were used for volume determinations, solutions preparations, and stock solutions dilutions.

Sampling and Preparation of Material

A representative European black alder tree without any visible defects was harvested in mid-July 2014 from a 30-year-old forest governed by Hrvatske šume Ltd. within the management unit of "Đurđevačke nizinske šume" (Đurđevac, Croatia), department "98", and section "b". The mean trunk diameter at breast height (1.3 m) was approximately 30 cm. The tree was without central xylem discolouration. The height of 1.5 m from the ground was marked on the trunk. A cross-section segment (*i.e.*, disk) of five centimeters in thickness was cut just above the felling mark. The disc was immediately placed into dark cold storage in expanded polystyrene containers. From the disc, bark (inner and outer bark included) was collected; in addition, a radial wood element from bark to bark without the pith was sawn from the disk and cut into 1 cm^3 cubes. Bark and wood samples were frozen with dry ice. Samples were then milled and homogenized with the addition of dry ice pellets in a RETSCH SM300 mill (Retsch GmbH, Haan, Germany) at 1500 rpms. The mill was equipped with 6-disc rotor with reversible cutting tips of tungsten carbide for milling without heavy-metal contamination. Bottom sieve for milling had 1.00 mm trapezoid holes was used. Milled samples were frozen in dry ice and stored in a freezer at approximately -30 °C until further analysis. Before the extraction process, samples were lyophilized using a CHRIST alpha 1-2 LD freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). Moisture content of the samples after lyophilisation was determined gravimetrically according to EN 13183-1:2002/AC:2003 (2003) standard on six samples of wood and six samples of bark. The mass of the samples was determined with a KERN ABT 220-4M balance with reproducibility to the nearest 0.1 mg (Kern & Sohn GmbH, Balingen, Germany).

Extraction Procedure

Extraction by cold maceration was conducted at room temperature (20 ± 1 °C) using an IKA RO 10 magnetic stirrer (IKA®-Werke GmbH & Co. KG, Staufe, Germany) operating at 600 rpms using 6 x 30 mm PTFE coated magnets. Methanol (MeOH), ethanol (EtOH), acetonitrile (ACN), acetone (ACTN), and deionized ultrapure water (dH_2O) were used individually for extraction. Two separate extractions for each solvent was conducted in parallel on wood and bark samples. Two grams of lyophilized sample was extracted in 200 mL of solvent at sample-to-solvent ratio of 1:100 (w/v). Extractions were performed in 300 mL Erlenmeyer flasks equipped with a ground glass stopper. Extractions of 6 hours were conducted by the procedures of Albert *et al.* (2003), and Vek *et al.* (2014). Afterwards, the extracts were filtered through Munktell 388 quantitative ashless filter paper (Ahlstrom Munktell). The filtered extracts were stored in closed amber glass jars that were placed into a refrigerator.

Estimation of Total Soluble Extractives (TSEs)

Total soluble extractive (TSE) content was measured gravimetrically. From each extract, 10 mL was pipetted into a 22 mL glass test tube and oven dried at 103 ± 2 °C until constant mass was reached. This was replicated six times for each solvent extraction sample. Thus, for each solvent, twelve TSE measurements were performed for wood and twelve TSE measurements for bark. TSEs were expressed as milligrams of TSE per gram of dry mass of wood or bark ($mg \cdot g_{dm}^{-1}$).

Estimation of Total Soluble Phenols (TSPs)

Total soluble phenol (TSP) content was measured by means of the UV-Vis spectrophotometry method of Folin-Ciocalteu that was modified according to Singleton and Rossi (1965), Scalbert *et al.* (1989), and Vek *et al.* (2013a), with some additional minor modifications. Prior to analysis, extracts were tempered in a water bath at 20 °C. Solvent extract aliquots of 125 µL for bark and of 500 µL for wood were taken, and the solvent evaporated using a vacuum desiccator at room temperature (20 ± 1 °C), which was shielded from sunlight. Dry extract residues were then dissolved in 500 µL of MeOH and vigorously shaken for 10 seconds on an IKA digital vortex 4 shaker at 1000 rpms. To this extractives solution (500 µL) 2.5 mL of a 10-fold-diluted Folin-Ciocalteu reagent (aqueous solution) was added; after 90 seconds had passed, 2.0 mL of an aqueous 7.5% (w/v) sodium carbonate solution was added. The mixture was vigorously shaken for 5 seconds on the vortex shaker (1000 rpms). This solution was incubated for two hours at room temperature (20 ± 1 °C). An UV-vis calibration curve was generated using dilutions from an aqueous gallic acid stock solution (0.5 $g \cdot L^{-1}$) at eight concentration levels (0.300, 0.250, 0.200, 0.150, 0.100, 0.050, 0.025, and 0 $g \cdot L^{-1}$), which were likewise treated with Folin-Ciocalteu reagent. Absorbances of solutions were measured at a 765 nm wavelength on a Shimadzu UVmini 1240 single beam UV-Vis spectrophotometer (Shimadzu Europa GmbH, Duisburg, Germany). TSPs were expressed as gallic acid equivalents (GAE) in milligrams of TSP per gram of dry mass of wood/bark ($mg \cdot g_{dm}^{-1}$).

Estimation of Total Soluble Flavonoids (TSFs)

Total soluble flavonoid (TSF) levels in the extracts were measured using the $AlCl_3$ spectrophotometric method (Brighente *et al.* 2007; Diouf *et al.* 2009; Vek *et al.* 2013b) with some minor modifications. Prior to analysis, extracts were tempered in a water bath at 20 °C. Solvent extraction aliquots of 1 mL for bark and of 8 mL for wood were taken and the solvent was evaporated using a vacuum desiccator at room temperature (20 ± 1 °C), which was shielded from sunlight. Dry extract residues were then dissolved in 2 mL of MeOH and vigorously shaken for 10 seconds using vortex shaker at 1000 rpms. Reagent solutions of aluminum chloride hexahydrate (2% w/v) and quercetin (0.250 $g \cdot L^{-1}$) were prepared using MeOH as the solvent. To 2 mL of the prepared MeOH extract solution, 2 mL of the 2% (w/v) aluminum chloride hexahydrate solution was added. The mixture was then vigorously shaken for 5 seconds on a vortex shaker at 1000 rpms. The solution was incubated for one hour at room temperature (20 ± 1 °C). Calibration curves were generated using dilutions of the quercetin stock solution at ten different concentration levels (0.1000, 0.0800, 0.0600, 0.0400, 0.0200, 0.0100, 0.0050, 0.0025, 0.0010, and 0 $g \cdot L^{-1}$). Absorbances of solutions were measured at a 415 nm wavelength on a Shimadzu UVmini 1240 single beam UV-Vis spectrophotometer. TSFs were expressed as quercetin equivalent (QE) in milligrams of TSF per gram of dry mass of wood/bark ($mg \cdot g_{dm}^{-1}$).

Statistical Analysis

Statistical analyses were performed using STATISTICA 12 (Dell, Texas, U.S.A.) and Microsoft Excel (Microsoft EMEA, Issy-les-Moulineaux, France). The Welch's ANOVA and Games-Howell *post hoc* tests were performed at the 0.05 significance level.

RESULTS AND DISCUSSION

The results showed that the trend of TSE yields with different solvents at isothermal conditions was $TSE_{MeOH} > TSE_{EtOH} > TSE_{dH2O} > TSE_{ACTN} > TSE_{ACN}$ for both wood and bark. There was a statistically significant difference among the solvents' TSE yields as determined by the Welch's ANOVA for bark ($F(4, 27.461) = 3767.487$, $p < 0.001$), and for wood ($F(4, 27.326) = 639.253$, $p < 0.001$). Games-Howell *post hoc* tests showed that there were statistically significant differences (for all, $p < 0.001$) among all the solvents' TSE yields for both wood and bark. Furthermore, much higher TSE contents were obtained from the bark than from the wood.

Fengel and Wegener (2003) reported that black alder wood contains 3.8% of extractives, which were obtained with an ethanol-benzene co-solvent system; Roze *et al.* (2011) reported extractive levels of black alder bark obtained from Soxhlet extraction (SOX), fluidized bed extraction (FBE) and accelerated solvent extraction (ASE) (Table 1). These aforementioned values are not fully comparable with the research results reported in this study, but are quite similar to Table 2.

Table 1. Black Alder Bark Extractives (% on oven dry bark) (Roze *et al.* (2011))

Method	Hexane	Ethyl acetate	Ethanol
SOX	2.5	13.9	11.8
FBE	2.4	12.8	12.0
ASE	2.4	12.3	15.0

In contrast to the TSE yields, the results of the TSPs were somewhat different for wood and bark (Table 2). At isothermal conditions, amount of TSPs for bark obtained with different solvents was $TSP_{MeOH} > TSP_{EtOH} > TSP_{ACTN} > TSP_{dH2O} > TSP_{ACN}$; the trend for wood was $TSP_{MeOH} > TSP_{EtOH} > TSP_{dH2O} > TSP_{ACTN} > TSP_{ACN}$. Regardless of the different solubility trends, much higher TSP contents are obtained from bark than wood. There were statistically significant differences among the solvents' TSP yields as determined by the Welch's ANOVA for bark ($F(4, 27.111) = 10575.543$, $p < 0.001$), and for wood ($F(4, 27.165) = 41393.292$, $p < 0.001$). Games-Howell *post hoc* tests confirmed the statistically significant differences (for all, $p < 0.001$) among all the solvents' TSP yields for both wood and bark.

As for the TSF yields, the extracting efficiency ranking of different solvents at isothermal conditions for bark was $TSF_{MeOH} > TSF_{EtOH} > TSF_{dH2O} > TSF_{ACTN} > TSF_{ACN}$, and for wood was $TSF_{MeOH} > TSF_{EtOH} = TSF_{dH2O} > TSF_{ACTN} > TSF_{ACN}$. There were statistically significant differences among the solvents' TSF yields as determined by Welch's ANOVA for bark ($F(4, 26.937) = 8448.543$, $p < 0.001$), and for wood ($F(4, 26.558) = 3375.887$, $p < 0.001$). Games-Howell *post hoc* tests showed that there were statistically significant differences (for all, $p < 0.001$) among all the solvents' TSF yields for bark, while for wood there were no statistically significant differences in TSF yields

with EtOH and dH_2O (p = 0.914). Furthermore, much higher TSF yields were obtained from bark versus wood.

Table 2. Average Content of Total Soluble Extractives (TSEs), Total Soluble Phenols (TSPs), Total Soluble Flavonoids (TSFs) Expressed as $mg \cdot g_{dm}^{-1}$

Solvent	*N*	Mean ± SD	95 CI	Median	IQR	Min	Max
TSE – wood							
MeOH	12	28.19 ± 1.53	27.220 - 29.163	29.03	2.50	26.02	30.03
EtOH	12	21.77 ± 0.97	21.155 - 22.383	22.02	1.50	20.02	23.02
ACN	12	5.59 ± 1.17	4.848 - 6.329	6.01	2.00	4.00	7.01
ACTN	12	8.67 ± 1.07	7.992 - 9.357	9.01	1.50	7.01	10.01
dH_2O	12	18.60 ± 1.38	17.723 - 19.476	18.02	1.50	17.01	21.02
TSE – bark							
MeOH	12	117.04 ± 2.09	115.711 - 118.367	117.04	4.00	114.04	120.04
EtOH	12	80.69 ± 1.83	79.533 - 81.854	81.03	3.00	78.03	83.03
ACN	12	20.34 ± 2.31	18.872 - 21.808	20.51	4.00	17.01	23.01
ACTN	12	38.68 ± 1.97	37.428 - 39.931	38.01	3.00	36.01	42.01
dH_2O	12	43.51 ± 2.02	42.229 - 44.800	44.01	2.50	39.01	46.02
TSP – wood							
MeOH	12	12.01 ± 0.07	11.959 - 12.054	12.01	0.11	11.89	12.15
EtOH	12	8.40 ± 0.06	8.356 - 8.437	8.39	0.08	8.30	8.50
ACN	12	2.19 ± 0.05	2.155 - 2.221	2.18	0.09	2.13	2.28
ACTN	12	3.78 ± 0.08	3.728 - 3.828	3.75	0.14	3.70	3.91
dH_2O	12	7.31 ± 0.04	7.285 - 7.341	7.32	0.08	7.23	7.36
TSP – bark							
MeOH	12	55.34 ± 0.72	54.882 - 55.798	55.62	1.06	53.87	56.12
EtOH	12	38.35 ± 0.42	38.091 - 38.619	38.32	0.27	37.78	39.42
ACN	12	8.56 ± 0.88	7.997 - 9.115	8.23	0.40	8.04	11.16
ACTN	12	18.23 ± 0.46	17.936 - 18.516	18.25	0.84	17.59	18.73
dH_2O	12	15.39 ± 0.44	15.107 - 15.672	15.34	0.82	14.74	15.92
TSF – wood							
MeOH	12	0.24 ± 0.01	0.232 - 0.242	0.24	0.01	0.23	0.25
EtOH	12	0.10 ± 0.00	0.098 - 0.100	0.10	0.00	0.10	0.10
ACN	12	0.03 ± 0.00	0.027 - 0.030	0.03	0.00	0.03	0.03
ACTN	12	0.04 ±0.00	0.043 - 0.046	0.04	0.00	0.04	0.05
dH_2O	12	0.10 ± 0.01	0.090 - 0.103	0.09	0.01	0.08	0.12
TSF – bark							
MeOH	12	3.40 ± 0.09	3.341 - 3.453	3.39	0.16	3.30	3.53
EtOH	12	1.66 ± 0.02	1.647 - 1.673	1.66	0.04	1.63	1.69
ACN	12	0.21 ± 0.03	0.188 - 0.223	0.21	0.01	0.12	0.22
ACTN	12	0.56 ± 0.03	0.546 - 0.579	0.56	0.04	0.51	0.59
dH_2O	12	0.82 ± 0.02	0.808 - 0.832	0.82	0.02	0.78	0.85

Note: *N* – number of measurements; *SD* – standard deviation; *95 CI* – 95%confidence interval of the mean; *IQR* – interquartile range; *MIN* – minimum value; *MAX* – maximum value.

It is evident (Table 2) that the standard deviations of all of the extraction results were relatively small. In this research, the higher contents of TSE, TSP, and TSF were obtained from the bark rather than from the wood, regardless of the type of solvent used. This was expected, since it is well known that bark contains higher amounts of extractives than wood within the same tree, where also living bark (living cells present) contains more extractives than dead bark (rhytidome- no living cells present). Bark's complex chemical composition is due to its main biological functions: metabolites

transport from the leaves to the rest of the tree, as well as protection and defense of the xylem and cambium from environmental influences, pathogens, insects, *etc.* In this research, MeOH produced the highest yields of TSEs, TSPs, and TSFs, from both wood and bark. MeOH is commonly used as a solvent for the extraction of hydrophilic extractives from plant material (Vermerris and Nicholson 2008; Vek and Oven 2011). The choice of solvent depends on the properties and polarity of the targeted solute molecule (*i.e.*, the general principle "like dissolves like"). Polar solvents dissolve more polar solute molecules (*i.e.*, hydrophilic compounds), while nonpolar solvents dissolve less polar solute molecules (*i.e.*, lipophilic compounds). Polyphenols are more soluble in polar solvents, *e.g.*, methanol, ethanol, acetone, and ethyl acetate (Dai and Mumper 2010; Horvath 2006), or in alcohol-water mixtures, or in co-solvent mixtures, which often produce better results than a pure solvent alone (Horvath 2006). Vek *et al.* (2013b) used 70% methanol (*aq*) as a co-solvent mixture for the extraction of total phenols, flavonoids and proanthocyanidins from European beech (*Fagus sylvatica* (L.)). Tham and Liew (2014) examined the optimum extraction temperature and the methanol-water co-solvent compositions for maximizing total phenols and total flavonoids yield from the heartwood and bark of *Acacia auriculiformis*. The authors concluded that higher methanol levels (75% MeOH) produced the highest extracted yields of total phenols and total flavonoids at 75 °C. However, in our research, it was anticipated that deionized water would produce somewhat higher yields. Presumably, if higher extraction temperatures were used, the viscosities and the surface tensions of the solvents would decrease, which would help the solvents to penetrate the samples' matrices, thus improving the extraction rates (Dai and Mumper 2010). Gironi and Piemonte (2011) reported that higher polyphenol yields from chestnut wood were produced by water as the solvent if high extraction temperature was used. Furthermore, when the authors mixed water with ethanol (20% and 40% EtOH) during isothermal extraction conditions (60 °C), they obtained higher yields with a 40% EtOH (*aq*) solution. The extraction time and temperature have a great influence on the recoveries of phenolic compounds from the plant matrix, but in the same time, while solubilization can be improved, the analytes degradation by oxidation and hydrolysis can be accelerated, and *vice versa* (Robards 2003; Vermerris and Nicholson 2008). Additional care should be undertaken if particular compound of interest is being investigated with an analytical method of greater sensitivity, *e.g.*, HPLC analysis. An increase in the extraction temperature can promote both solubility and mass transfer rate; however, longer extraction times and higher extraction temperatures increase the chances of oxidation of polyphenols, which decrease the yield of phenolics in the extracts (Dai and Mumper 2010). As for the choice of the extraction method in this research, the maceration with stirring of relatively short duration (6 h) and at lower extraction temperatures (room temperature) were employed. Furthermore, special care was taken during sample preparation, manipulation and analysis with the intent to minimize solute degradation processes (*i.e.*, oxidation, photodegradation, and thermal degradation).

Regarding wood hydrothermal processing, extractives and especially phenols are well known wood colourants having notable influence on the natural colour, as well as on wood discolouration formation during processing. Discolouration of wood during hydrothermal processing is very complex phenomenon, not only because extractives themselves affect the formation of discolouration, but degradation products can participate in discolouration formation as well. Consequently, more detailed chemical characterization of black alder wood extractives should be conducted in order to determine their relation to the wood discolouration during hydrothermal processing.

Particularly, more research should investigate the within-tree-distribution of involved chemical compounds, as well as the influence of hydrothermal processes parameters on discolouration formation.

CONCLUSIONS

1. In this research, a comparative study of the TSEs, TSPs, and TSFs levels of European black alder wood and bark is presented. The bark of black alder has much higher levels of TSEs, TSPs, and TSFs than wood. This was confirmed with all extraction solvents employed (*i.e.*, MeOH, EtOH, ACN, ACTN, and dH_2O) at the same extraction conditions.
2. Of all the solvents employed, MeOH produced the highest yields of TSEs, TSPs, and TSFs, from both wood and bark. MeOH is a preferable organic solvent for extraction of black alder extractives *i.e.,* phenols and flavonoids. In the case when a specific chemical compound is investigated, additional preliminary research should be conducted in order to determine the most suitable solvent.
3. It was established that dH_2O produces higher yields of TSPs from wood than ACTN; however, in case of bark, this trend is reversed. This phenomenon is unexpected and requires more detailed further studies.
4. During this research it has been visually observed that the use of dry ice during black alder sample collection, storage, and preparation prior to lyophilization inhibits wood and bark samples reddish-orange discolouration.
5. Stirred maceration extraction of wood and bark has been shown to be a simple, fast, convenient, time and money saving extraction method, which can be easily conducted without the need for expensive apparatus or extensive knowledge. Additionally, it can be conducted at low temperatures which prevents analytes degradation.

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