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Research Article

PHYTOCHEMICAL ANALYSIS OF *PICEA ABIES* BARK OBTAINED BY ACCELERATED SOLVENTEXTRACTION

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ABSTRACT

Extractive substances from spruce bark (*Picea abies*) were studied after accelerated solvent extraction (ASE). ASE was carried out by using ethanol and the temperatures were 100, 130, 150 and 180°C. The composition of the bark extractives were analysed by gas chromatography/mass spectroscopy (GC/MS). The extractives consisted mainly of fatty acid and monoterpene fractions, especially methyl oleate; methyl isopimarate; 7-oxodehydroabietic acid, methyl ester; methyl lignocerateand methyl behenate and methyl dehydroabietate.

INTRODUCTION

Bark is an attractive renewable raw material composed of all types of silviculture vegetation (Sládková *et al*., 2016). Currently, more than half of the bark is burned and/or landfilled and the remainder is mainly used as a source of energy in saw/pulpmills (Feng *et al*., 2013). Bark is considered as a promising source of biomass which can complement traditional forestry biomass for the production of energy, phytochemicals, and fine chemicals. Valorisation is a key component of an economic lignocellulosic biorefinery (Jablonsky *et al*. 2015a; Surina *et al*. 2015). A simple and clean fractionation of the main components of biomass represents a relevant step in the "clean", renewable carbon economy. Several papers describe extraction by an agent in different conditions (Ghitescu *et al*., 2015; Haz *et al*., 2013; Jablonsky *et al*., 2015b; Kemppainen *et al*., 2012; Krogell *et al*., 2012; Spigno and Faveri 2009). Accelerated solvent extraction (ASE) is an important technique for extracting valuable compounds from lignocellulosic materials. ASE of extractive substances may be influenced by factors such as time of extraction, moisture content, particle size, solid-liquid ratio, type and composition of solvent, temperature and number of extraction cycles. The aim of Co *et al*. (2011) paper was to obtain antioxidants from spruce bark extracts. Several compounds such as stilbene glucosides, astringin (as stilbenoid), piceid and isorhapontin were identified. Kylliainen and Holmbom (2004) studied aqueous extracts from bark of *Picea abies*. In this work, saccharides (glucose, galactose, galacturonic acid), stilbenes, stilbene glycosides, tannins and resin acids were determined. Ajuong and Berkinshaw (2004) confirmed that the ethanol extract from spruce chips contains condensed tannins, flavonoids, and phenolic compounds. Composition of extracts was confirmed in other experiments by using different types of

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extraction (Co *et al*. 2011, Feng *et al*. 2013, Krogell *et al*. 2012). Conventional separation techniques such as solvent extraction and distillation have the drawback of leaving trace amounts of solvents or to cause thermal degradation (Ahluwalia *et al*., 2013). The present work studied the impact of temperature on the yield of extractives and phytochemical analysis of extractives isolated from spruce bark using ASE.

Experimental

Materials

Spruce bark characterisation

Spruce bark was kindly supplied by Bioenergo Ltd. (Ruzomberok, Slovakia). The bark was air-dried, milled, particle sizes 1.0 mm. The spruce bark was extracted using the accelerated solvent extraction method (Sluiter *et al*. 2008), weighed, dried, and analysed to determine the content of lignin, ash, and holocellulose (Table 1). The residual lignin content was determined as Klason lignin (TAPPI T222 1998), and the extractive content was determined according to Sluiter *et al*. (2008). Ash was determined using TAPPI T211 (1998), and holocellulose was quantified with sodium chlorite treatment following the procedure of Wise *et al*. (1946).

Table 1 Composition of Spruce Bark

Spruce Bark Component	Composition (%)
Holocellulose	52.0 ± 0.2
Lignin	26.4 ± 1.3
Ash	3.6 ± 0.4
Extractives	18.0 ± 1.6

Note: Values represent the average of six replicates ± standard deviation

METHODS

Accelerated solvent extraction (ASE)

For the isolation of extractive compounds, the milled samples were extracted with ethanol (96.6 %) in a Soxhlet apparatus for 14 hours. Additional extractions were carried out by accelerated solvent extraction with ethanol. Extractions were performed with the model 200 Accelerated Solvent Extractor, Dionex ASE 350. The extraction pressure (1500 psi) was imposed by the ASE 350 apparatus. Samples, typically 15 g (milled samples), were *placed* into stainless steel extraction chambers. Parameter of static time (time for reaching final temperature) was 5 min for 100°C, 6 min for 130°C, 8 min for 150°C, and 10 min for 180°C. After static time sample was flushed with 50 % volume of used extraction ethanol in stainless steel cell and the extract was collected in a vial. Samples were sequentially extracted three times. Two replicate samples were used for each sample. All extracts were evaporated to dryness.

Derivatization

From the published derivatization techniques alkylation (methylation) with DMF-DMA (N, N-dimethylformamide dimethyl acetal) is shown as the preferred. Pyridine was used as solvent for derivatization. For derivatization of 50 mg dried sample was used a mixture (1:1) of 0.5 ml pyridine and 0.5 mL of DMF-DMA. Derivatization was carried out at 75 °C and the time was 15 min.

GC/MS

The GC/MS analysis was performed on a gas chromatograph (Agilent 7890 GC) coupled with mass detector (Agilent 5975C) which ran electron ionization equipped with a capillary column (HP-5MS, 30 m × 250 µm i.d., 0.25 µm film thickness; Agilent). Helium was used as carrier gas at a rate of 2 mL/min. Chromatograph oven temperature program was 120 °C held for 2 min, then the heating of 10 °C/min to 300 °C. The final temperature was held for 10 min. Recording and evaluation of data was performed by using ChemStation software E 02/01/1177 and identification of compounds by using electronic libraries NIST and Wiley.

Yield of extractives

The yield of extractives (*Y*, %) was determined after each experiment by drying the samples at 105 °C to a constant weight. The results are expressed on the basis of the dry matter before and after extraction as shown in Eq. 1,

$$Y(\%) = 100 \times m_{extr} / m_i \quad (1)$$

where m_i and m_{extr} are the mass (g) of the samples before and after extraction and drying, respectively.

RESULTS AND DISCUSSION

The valorisation of products from forest and silvicultural waste (bark, branches, roots), for example, through the exploitation of the high value and low molecular weight compounds from bark, which are rich in phytosterols, triterpenic acids, lignans and other phenolic compounds which can be obtained before burning or integrated with the valorisation of the individual compounds as well as mixtures of compounds, is a major goal of the bio refinery. A large number of well-known natural bio substances were identified, determined and synthesized; however, current and future studies will focus its attention on the development of green chemistry and new bio refineries. The selection of the most suitable extraction solvent is a critical step in the extraction process. Plant materials are generally extracted with organic solvents such as methanol, propanol and ethanol (Waksmundzka-Hainos *et al*. 2007), of which the latter is often more preferable than methanol, which is dietary toxic. Content of extractive compounds isolated from spruce bark at 100°C was 6.7%, which was similar to the yield obtained at 130°C (6.8%). Extractives content obtained at 130 ° C was 1.1 times lower than the yield obtained at 150 ° C, which had a value of 7.5%. The largest amount of extractives were obtained from the bark of spruce prepared at 180 °C (9.2%), and this value was 1.2 times higher than yield at 150 °C. Except for ASE, the sample was subjected to the spruce bark Soxhlet extraction. Extraction yield in this case was 6.9%, similar to the extraction of ASE data at 150 ° C.

Table 1 Yield of extractives obtained with accelerated solvent extraction and Soxhlet extraction

	***Yield of extractives* (%)**
ASE, 100°C	6.7 ± 0.3
ASE, 130°C	6.8 ± 0.2
ASE, 150°C	7.5 ± 0.1
ASE, 180°C	9.2 ± 0.4
Soxhlet	6.9 ± 0.2

Note: Values represent the average of two replicates ± standard deviation

Table 2 The compounds specified in spruce bark extract using accelerated solvent extraction and Soxhlet extraction.

Class	Methods	ASE				Soxhlet
	Compounds	100°C	130°C	150°C	180°C	
Monoterpene	3,5-Dimethoxytoluene	0.18	-	0.13	-	-
	Methyl 3,4-dimethoxybenzoate	-	-	0.10	0.38	-
	Cyclohexanepropanol, 2,2-dimethyl-6-methylene-	-	-	-	1.22	-
	Methyl pimarate	-	2.51	-	2.37	-
	Methyl isopimarate	7.54	6.60	6.44	6.83	6.43
	Methyl levopimarate	0.68	0.71	0.68	0.71	-
	Methyl dehydroabietate	19.45	18.43	17.16	17.60	19.53
	Isosteviol methyl ester	-	-	1.11	-	-
Diterpene	Cembrene	0.56	0.53	0.46	0.53	0.30
	oplopanone	0.26	0.22	0.38	0.25	-
	Methyl abietate	3.13	3.35	3.16	3.34	2.64
	Sclareol	-	-	0.42	-	-
Sesquiterpene	Longifolene	-	0.21	-	-	-
	Germacrene D	-	-	-	0.38	-
	γ-muurolene	0.16	-	0.15	-	-
	α-muurolene	-	0.16	-	-	-
	δ-cadinene	0.18	0.19	0.17	0.33	-
	γ-cadinene	0.33	0.28	0.36	-	-
	α-cadinol	-	0.14	0.17	-	-
	Cadalene	0.23	0.22	0.32	0.42	-
	β-neoclovene	-	0.19	-	-	-
	Bicyclo[5.2.0]nonane, 4-methylene-2,8,8-trimethyl-2-vinyl-	-	0.43	0.30	-	-
	13-epi-manoyl oxide	0.46	0.38	0.33	0.33	0.33
	Manoyl oxide	-	-	-	-	-
	Alloaromadendrene oxide-(1)	-	-	-	-	0.28
	(-)-Isolongifolol, methyl ether	0.46	0.42	0.46	0.41	-
	5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-yl)-3-methyl-pent-2-enal	0.49	0.50	-	0.42	-
	Stigmastan-3,5-diene	0.46	0.54	0.50	0.80	0.42
	15-Hydroxy-7-oxodehydroabietic acid, methyl ester	-	-	0.55	-	-
	Caparratriene	-	-	-	-	0.51
Fattyacid	Methyl sandaracopimarate	1.89	2.09	1.97	2.05	2.72
	Methyl pimara-8(14),15- dien-18-oate	2.49	-	2.34	-	2.85
	Kauren-18-ol, acetate, (4.beta.)-	0.64	-	0.89	-	1.06
	Methyl 12-methyltetradecanoate	0.23	0.23	0.27	-	0.28
	Methyl 13-methyltetradecanoate	-	-	-	-	0.09
	Methyl 10-methyldodecanoate	-	-	-	-	0.17
	Methyl pentadecanoate	0.09	0.08	0.12	-	-
	Methyl 10,13-dimethyltetradecanoate	0.15	0.14	0.18	-	-
	Methyl palmitoleate	0.27	0.29	0.31	0.31	0.41
	Methyl palmitate	2.77	3.27	3.08	3.02	4.00
	Methyl myristate	0.11	0.12	0.12	0.44	0.14
	Dimethyl azelaate	0.38	0.35	0.42	0.53	0.21
	Methyl 14-methylhexadecanoate	-	-	-	-	2.23
	Methyl 18-methylnonadecanoate	1.62	1.79	1.80	1.75	1.11
	Methyl 15-methylpalmitate	1.82	1.96	1.84	1.92	-
	Dimethyl hexadecanedioate	0.70	0.74	0.86	0.88	-
	Margaric acid methyl ester	0.21	0.24	0.24	0.22	0.28
	Ethyl oleate	-	-	-	-	2.95
	Methyl vaccenate	2.14	-	-	-	-
	Pinolenic acid methyl ester	2.92	2.28	2.61	2.18	4.82
	Methyl oleate	17.18	20.72	18.97	17.07	29.68
	Methyl stearate	1.24	1.30	1.24	1.19	1.22
	Stearyl acetate	-	0.41	0.38	0.37	-
	Butyl 6,9,12-hexadecatrienoate	-	-	-	-	0.34
	Methyl (6E,9E,12E,15E)-6,9,12,15-docosatetraenoate	-	0.80	-	-	-
	Dimethyl octadecanedioate	0.35	0.35	0.40	0.40	-
	Methyl abieta-8,13(15)-dien-18-oate	1.61	1.55	1.77	-	-
	Methyl Behenate	4.03	4.64	4.44	4.08	2.30
	15-Methoxydehydroabietic acid, methyl ester	2.76	2.37	2.39	2.50	1.83
	7-Oxodehydroabietic acid, methyl ester	5.43	4.87	5.26	5.64	3.50
	Dimethyl icosanedioate	-	-	-	2.08	-
	14-Methylpentadec-9-enoic acid methyl ester	-	-	-	0.72	-
	Dimethyl henicosanedioate	-	0.54	-	-	-
	Dimethyl docosanedioate	1.44	1.53	1.51	1.74	-
	Methyl Lignocerate	4.23	5.01	4.72	4.36	2.12
	Methyl 11-methyloctadecanoate	-	-	-	-	1.65
	Methyl 20-methyl-docosanoate	1.05	1.05	1.13	1.46	-

Aromatic hydrocarbon	**9-Hexacosene**	**0.71**	**1.29**	**0.82**	**0.97**	**0.56**
Wax	n-Nonadecanol-1	-	-	-	-	0.31
Lipid	9-Tetradecenal, (Z)-	0.25	0.26	-	0.31	-
Vitamin A	Retinol, acetate	0.68	-	-	-	-
	4-Isopropyl-1,6-dimethyl-1, 2,3,4,4a,7-hexahydro-naphthalene	0.11	0.10	-	-	-
	Cyclododecyne	0.80	-	-	-	-
	3-n-Heptyl-7-methyl-9-(2,6,6-trimethylcyclohex-1-enyl)nona-2,4,6,8-tetraenal	1.89	0.47	0.53	0.63	0.81
	1,5-Cyclodecadiene	-	-	0.85	-	-
	3-Vinyl-1-cyclooctene	-	-	-	0.79	-
	trans-β-Ionone	-	-	-	0.19	-
	UNK	2.52	1.8	4.7	3.18	1.38

The duration of ASE extraction method was 35 min, while the Soxhlet extraction was carried out for 540 minutes, which is 15 times longer than the ASE. From these results we consider the ASE as a more efficient and rapid method in particular such as Soxhlet extraction, due to increased temperature and pressure.

Our results demonstrated that *Picea Abies* bark extracts can be used as an effective source of compounds in the food and pharmaceutical industries. Forty-seven constituents, representing 96.76 % of the extract bark were identified (Table 2) for ASE at 100°C:methyl dehydroabietate (19.45 %), methyl oleate (17.18 %), methyl isopimarate(7.54%), 7-oxodehydroabietic acid, methyl ester (5.43 %), methyl lignocerate (4.23 %)and methyl behenate (4.03 %) were the main constituents of the monoterpene and fatty acid fraction of the bark extract. Fifty constituents, representing 98.2 % of the extract bark were identified (Table 2) for ASE at 130°C: methyl dehydroabietate (18.43 %), methyl oleate (20.72 %), methyl isopimarate(6.60%), 7-oxodehydroabietic acid, methyl ester (4.87 %), methyl lignocerate (5.01 %) and methyl behenate (4.64%). Forty-nine constituents, representing 95.3% of the extract bark were identified (Table 2) for ASE 150°C: α methyl dehydroabietate (17.16 %), methyl oleate (18.97 %), methyl isopimarate(6.64%), 7-oxodehydroabietic acid, methyl ester (5.26 %), methyl lignocerate (4.72 %) and methyl behenate (4.44 %). Forty-three constituents, representing 96.82% of the extract bark were identified for ASE 180°C: methyl dehydroabietate (17.6 %), methyl oleate (17.07 %), methyl isopimarate (6.83%), 7-oxodehydroabietic acid, methyl ester (5.64 %), methyl lignocerate (4.36 %) and methyl behenate (4.08 %). Thirty-four constituents, representing 98.62 % of the extract bark were identified for Soxhlet extraction: methyl dehydroabietate (19.53 %), methyl oleate (29.68 %), methyl isopimarate(6.43%), 7-oxodehydroabietic acid, methyl ester (3.5 %), methyl lignocerate (2.12 %) and methyl behenate (2.3 %).Extract composition is very heterogeneous since it contains fatty acid, monoterpenes, diterpenes, sesquiterpenes. The extract contains more than 55 % fatty acid and 25 % monoterpenes. The ASE at 100 °C the fatty acid contains 53.45 %, at 130 °C 57.17 %, at 150 °C 55.67 %, at 180 °C 55.11 % and 59.33 % for Soxhlet extraction. Monoterpenes represented on the ASE extracts as follows: 27.85 %, 28.25 %, 25.62 %, 29.11 % for Soxhlet 25.96 %, respectively.

The importance of extractive compounds isdue to their multiple applications andbioactivity. These compounds have been reported for several biological activities such as antioxidant, antitumor, cytotoxic, antimitotic (González *et al*., 2010), antibacterial (Gu& Wang, 2010), cytoprotective (Dhayal *et al*., 2008), anti-inflammatory (Hua *et al*., 2006) and so on.

The chain length of fatty acid was between C11 and C25 in this study. The number of identified compounds with a length of fatty acid chains for example C20 is 20%, C19 (14.3%), C18 (11.4%), C18 (11.4%), C16 (8.6%) and C21 (8.6%). Demirbas (2009) reported that fatty acid with chain length between C14-C22 were recognized as the most common fatty acid contained in biodiesel.

The choice of the extraction method had a great influence in the yield and quality of the extracted compounds. A starting point in biorefineries design lies on the complex treatment of input biomass in integrated systems. In some cases commercially applicable procedures for obtaining biologically active substances are available. As examples, obtainment of tanines, stilbenes, polyphenols, astrigin and isohapaporitin can be introduced. It must be frankly admitted that this research field and commercialism of procedures applied in isolating individual compounds with added-value exhibit still some problematic features. There are several open questions on how to do it such as which mode must be used in order to isolate individual compounds or their groups exhibiting biologically significant properties in an economic acceptable level. Extraction and subsequent utilization of compounds with added value is of importance, along with complex processing of biomass also from the viewpoint of other technologies transforming raw materials intobiorefineries to following products and materials. They are just extractives that cause considerable technological problems which are reflected in cost-related aspects. As an example, wood processing and kraft pulping may be used. Here due to variations in input raw materials and imperfect debarking extractants enter the system. It results in excretion of resinous substances in the process of delignification, whitening, or the problems are transferred up to technology of converting fibres into paper.

CONCLUSION

The phytochemical composition of bark *Picea abies* in terms of monoterpenes, sesquiterpene, diterpenes and fatty acid fractions was very complex. On the other hand, fatty acid constituted more than 55% of the analysed extract and another fraction were monoterpenes, which represented more than 25% of the extract. The highest yield of extractives (7.04 %) from a 1 mm fraction was reached at 180°C by ASE. From GC/MS analysis is obvious that the composition of extractives consisted mainly of methyl oleate; methyl isopimarate;7-oxodehydroabietic acid, methyl ester; methyl lignocerate; methyl behenate and methyl dehydroabietate which belongs to the fatty acid, monoterpene class.

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References

Ahluwalia S., Shivhare U.S., Basu S. 2013. Supercritical CO2 extraction of compounds with antioxidant activity from fruits and vegetables waste -a review. Focusing on Modern Food Industry. 2, 42-62.

Ajuong, E. M. A., Birkinshaw, C. 2004. The effects of acetylation on the extractives of Sitka Spruce (Piceasitchensis) and Larch (Larixleptoleptis) wood. *European Journal of Wood and Wood Industries.*

Co, M., Fagerlund, A., Engman, L., Sunnerheim, K., Sjöberg P.J.R., Turner, Ch. 2011. Extraction of antioxidants from spruce (*Piceaabies*) bark using eco-friendly solvents. *Phytochemical Analysis*. 23(1), 1-11.

Dhayal, S., Welters, H., Morgan, N. 2008. Structural requirements for the cytoprotective actions of mono-unsaturated fatty acids in the pancreatic β-cell line, BRIN-BD11. *Br. J. Pharmacol*. 153(8), 1718-1727.

Demirbas, A. 2009.Progress and recent trends in biodiesel fuels.Energy Convers. *Manag*. 50, 14–34.

Feng, S., Cheng, S., Yuan, Z., Leitch, M., Xu, Ch. 2013.Valorization of bark for chemicals and materials: *A review. Renew. Sust. Energ. Rev.* 26, 560-578.

Ghitescu, R.-E., Volf, I., Carausu, C., Bühlmann, A.-M., Gilca, I. A., and Popa, V. I. 2015.Optimization of ultrasound-assisted extraction of polyphenols from spruce wood bark. *UltrasonicsSonochemistry.* 22, 535–541.

González, M.A., Pérez-Guaita, D., Correa-Royero, J., Zapata, B., Agudelo, L., Mesa-Arango, A., Betancur-Galvis, L. 2010.Synthesis and biological evaluation of dehydroabietic acid derivatives. *Eur. J. Med. Chem*. 45 (2), 811-816.

Gu, W., Wang, S. 2010. Synthesis and antimicrobial activities of novel 1H-dibenzo[a,c]carbazoles from dehydroabietic acid. *Eur. J. Med. Chem*. 45(10), 4692-4696.

Haz, A., Jablonský, M., Vrška, M., Straková, M., Sládková, A., Šurina, I. 2013. Extractives from biomass - Source of chemical compounds with added value. Zvolen. Selected processes at the wood processing, X. international symposium. (Extraktívnelátky z biomasy-zdrojchemickýchzlúčenín s pridanouhodnotou. Zvolen). Vybranéprocesyprispracovanídreva – X. medzinárodné symposium (in Slovak).

Hua, K.F., Hsu, H.Y., Su, Y.Ch., Lin, S.S., Chen, Y.M., Chao, K. 2006. Study on the Antiinflammatory Activity of Methanol Extract from Seagrass Zostera japonica. *J. Agric. Food Chem*. 54(2), 306-311.

Jablonsky, M., Skulcova, A., Kamenska, L., Vrska, M., and Vrska, M. 2015a. Deep eutectic solvents: fractionation of wheat straw. *BioResources*.10(4), 8039-8047.

Jablonsky, M., Vernarecova, M., Haz, A., Dubinyova, L., Skulcova, A., Sladkova, A., and Surina, I. 2015b.Extraction of phenolic and lipophilic compounds from spruce (*Piceaabies*) bark using accelerated solvent extraction by ethanol. *Wood Research*. 60(4), 583-590.

Kemppainen, K., Inkinen, J., Uusitalo, J., Nakari-Setälä, T., Siika-aho, M. 2012. Hot water extraction and steam explosion as pretreatments for ethanol production from spruce bark. *Bioresour. Technol*. 117, 131-139.

Krogell, J., Holmbom, B., Pranovich, A., Hemming, J., Willför, S. 2012. Extraction and chemical characterization of Norway spruce inner and outer bark. *Nordic Pulp Paper Res. J.* 27, 6–17.

Kylliäinen, O., Holmbom, B.R. 2004. Chemical composition of components in spruce bark waters. *Pap. Puu.-Pap. Tim*. 86(4), 289-292.

Spigno, G., De Faveri, D., 2009. Microwave-assisted extraction of tea phenols: a phenomenological study. *J. Food Eng*. 93, 210-217.

Sládková, A., Benedeková, M., Stopka, J., Šurina, I., Ház, A., Strižincová, P., Čižová, K., Škulcová, A., Burčová, Z., Kreps, F., Šima, J., & Jablonský, M.2016. Yield of Polyphenolic Substances Extracted From Spruce (Piceaabies) Bark by Microwave-Assisted Extraction. Bioresources 11(4), 9912-9921

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. 2008. Determination of Extractives in Biomass (Technical Report, NREL/TP-510-42619), National Renewable Energy Laboratory, Golden, CO.

Surina, I., Jablonsky, M., Haz, A., Sladkova, A., Briskarova, A., Kacik, F., and Sima, J. 2015. Characterization of non-wood lignin precipitated with sulphuric acid of various concentrations. *BioResources.* 10(1), 1408-1423.

TAPPI T211 om-93 1998. Ash in wood, pulp, paper and paperboard: combustion at 525 °C. TAPPI Press, Atlanta, GA.

TAPPI T222 om-98 1998. Acid-insoluble lignin in wood and pulp. TAPPI Press, Atlanta, GA.

Waksmundzka-Hainos, M.,Oniszczuk, A.,Szewczyk, K.,Wianowska, D. 2007. Effect of sample-preparation methods on the HPLC quantitation of some phenolic acids in plant materials. *Acta Chromatographica.*19, 227-237.

Wise, L. E., Murphy, M., and Daddieco, A. 1946.Chlorite holocellulose, its fractionation and bearing on summative wood analysis and on studies on the hemicelluloses. *Technical Association Papers*. 29(6), 210-218.

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