FROM BIOMASS OF POPLAR UTILIZATIONS TO BYPRODUCTS

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ABSTRACT: According to recent bioeconomy programs, chemical compounds derived from natural sources will be more available in regions where these compounds can be obtained economically than more expensive synthetic chemicals. In addition, the biorefinery is becoming an important aspect for green chemistry development aimed at ensuring the necessity to achieve the best objectives as favourable as possible from restricted natural resources such as forest biomass. One of the main scope is to generate diversified, innovative and renewable products using on-site bioresources such as biomass of wood and tree residues. Poplar tree species, including all their huge varieties, are largely cultivated in the world as a fast growing bioenergy crop. However the enormous potential of this specie in the other fields is still under-evaluated. The aim of this study was to verify the influence of thermal treatment at 180, 200 and 220 °C on wood extracts obtained via three different extraction techniques: maceration, ultrasound-assisted extraction and accelerated solvent extraction. Results showed the effect of heat treatments and extraction techniques on polyphenol, flavonoid contents and antioxidant activity. This investigation clearly showed the differential effects of temperature and extraction techniques on both antioxidant activity and secondary compounds contents.
Keywords: poplar, wood, biomass, yield, bioeconomy, biochemical.

1 INTRODUCTION

Wood material is widely marketed for numerous applications, from building to packaging. Nowadays, the demand for lignocellulosic biomass from wood, energy crops or agroforestry residues as a renewable source of energy is becoming an important output for the production in the EU. Cultivation of bio-energy crops provides a positive contribution not only in the energy sector but also in other industrial sectors, such as chemical or pharmaceutical. An European directive (2009/28/EC) has established that by 2020 at least 20% of primary energy consumption should come from renewable sources [1]. Thus, in recent years, the cultivation of plants for biomass production as a source of renewable energy has notably increased, including the use of rapidly growing species for short rotation coppice (SRC) such as poplar. On the other hand, in the last century the thermal treatment process is one of alternative treatments for different wood final uses. Usually, during thermal treatment, the most volatile extractives may leave the wood or be degraded [2], with new chemical compounds being obtained. However, still too few are knowledges is available on the potential use of such extractives as chemical value-added products.

According to recent bioeconomy stategy, chemical compounds achieved from natural sources will be more available where these compounds can be obtained economically than more expensive synthetic chemicals. Shimizu et al. [3] highlighted that, in commercial applications, a large proportion of wood is used as timber, while the rest is most often used as a source for energetic scope. Several researchers showed how different biomass sources could provide interesting results to exploit the economic and industrial potential of the biomass refinery. Although the extractives of wood and bark are usually considered minor components, they are believed to play an important role. Recent studies were focused on the characterization of bark extractives [4-5] and the antioxidant properties of the bioactive compounds from Eucalyptus [6-7], Cameroonian woods [8] and other wood species.

Also the biorefinery field is becoming an important aspect for green chemistry development aimed at ensuring the necessity to achieve the best objectives as favorable as possible from restricted natural resources such as biomass. The possible scenarios and the future direction of such transformation system was deeply explained by Fernando et al. [9] since 2006. The importance of biomass as a source for biopesticide active substances was recently highlighted by Villaverde et al. [10].

Poplar (*Populus* spp.), belong to Salicaceae family, grows in Europe from the British Isles to the Mediterranean coast. Poplar tree species, including all their huge clones, are largely cultivated in the world as a fast growing bioenergy crop. The wood is commonly used as fuel, but is also used for furniture, panelling and plywood production. Due to its high cellulose and relatively low lignin content, poplar is suitable for pulp and paper production. However the enormous potential of this species in the field of bio-based chemicals is still underevaluated. The potential commercial opportunities, the limitations and challenges of poplar byproducts as value-added was recently discussed by Devappa et al. [11]. However, biomass of poplar species could have a relevant role in the biorefineries or other different fields by enabling the development of the bioeconomy. In this respect, the various implications of this evidence and the gained attention could play an important future role by increasing the attention toward the poplar plantations as a potential source for use in biorefinery processes.

Ayadi et al. [12] showed that the total phenol and sugar contents of poplar increased after thermal treatment, due to degradation of the wood chemical components, such as hemicelluloses, lignin and extracted compounds. Some studies have aimed to understand other uses for wood components of *Populus* spp., such as the use of extracted components for biomass energy. However, few studies have focused on the extractive yield or have evaluated the wood after thermal treatment [13-14]. Although poplar belongs to a group of woody plants mainly used for bio-energy production, it is possible that useful secondary metabolites could be derived from the wood. Secondary metabolites are chemical compounds produced by several plant tissues (e.g. leaves, bark, roots, buds, wood) that provide different applications, including antioxidant, anticancer, anti-inflammatory, antifungal and other properties [15-16]. Previous studies on the potential of Poplar spp. extracts are particularly related to the nutraceutical properties of wood, bark, leaves and buds [17].

A compendium of analytical information on the extractives is needed to realistically gauge its useful potential. Bibliographic information on the content of many wood species or parts of plants, are available in recent works. To date, however, there is neither a widely accepted common method for analyzing nor a unified scheme for separating main chemical components prior to each analysis.

The focus of this work is to study the effects of thermal treatment on the polyphenolic and flavonoid contents and antioxidant activity of poplar wood extractives. Additionally, GC-MS was used for the identification and quantification of chemical compounds typical of poplar extractives.

2 MATERIAL AND METHODS

2.1 Thermal treatment

Poplar boards were cut from logs and thermally modified at different temperature into a vacuum plant, developed by WDE Maspell srl (Terni, Italy). A dual function machine was used, which can dry wood under vacuum conditions and treat wood at a high temperature (up to 250 °C). The laboratory kiln (4 × 1 m) can hold two layers of boards, which are loaded manually. Boards lie between two metal plates, which contain diathermic hot oil that provides conductive heat transfer to the boards. Pressure in the kiln can be regulated in the range 60-1000 mbar. Vacuum is maintained through a water ring-type pump equipped with a heat exchanger. Under pressure, the plates provide a force on the boards that prevents potential deformation of the wood [18]. Drying and thermal treatment were applied in the same plant. Before treatment, each board was cut in two. One board was thermally modified, while the other was used as a reference. Wood was dried to 0% moisture content under vacuum (185 mbar) at 85 °C for 12 h. From an initial temperature of 30 °C, the temperature was increased 5 °C each hour to 85 °C. Next, the wood was thermally treated at different temperatures.

2.2 Extraction methods

All samples, including untreated controls (Ctrl) and wood treated at 180 °C (TH 180 °C), 200 °C (TH 200 °C) and 220 °C (TH 220 °C), were reduced to a small size with a mill saw. Each sample were prepared and subjected to three different solid/liquid extraction techniques: maceration extraction (ME), ultrasound assisted extraction (UAE) and accelerated solvent extraction (ASE). ME was carried out at room temperature by stirring the sample (10 g) for 1 h in solvent at a sample-to-solvent ratio of 1:12 (w/v). Samples were extracted with a mixture of ethanol: water (70:30 v/v). UAE was performed for 1 h in an ultrasonic bath (Branson 1800), while ASE extraction was carried out with an ASE system (Dionex Corp.). Biomass material (10 g) was introduced in a 22 mL cell and treated three times in an ethanol/water (70:30 v/v) solution at 100 °C. All extracts were filtered through a paper filter and the solvent was removed with a rotary evaporator at 37 °C.

2.3 Determination of antioxidant compounds

Total polyphenol content (TPC) was evaluated by the Folin–Ciocalteu reagent method, total flavonoid content (TFC) was determined by using aluminium chloride ($AlCl_3$) and total tannin content (TTC) was evaluated by protein precipitation, that is bovine serum albumin solution.

2.4 Evaluation of antioxidant activity

Antioxidant activity was measured in terms of hydrogen-donating or radical scavenging ability by using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and expressed as milligrams of Trolox equivalent (TE) per gram of dried sample. Ferric reducing antioxidant power (FRAP) was determinate by reduction of a colourless ferric complex (Fe3+ tripyridyltriazine) to a blue coloured ferrous complex (Fe2+ tripyridyltriazine) was determined at 593 nm. FRAP values were expressed as milligrams of TE per gram of dried extract. Ability of extracts to prevent inhibition of lipid peroxidation was tested by using a β-carotene bleaching assay (BCB). Results were expressed as the percentage of antioxidant activity (AA), measured on the basis of β-carotene bleaching inhibition, calculated as follows:

$$\% AA = [1 - (A\ sample\ T0' – A\ sample\ T180') / (A\ blank\ T0' – A\ blank\ T180')] * 100$$

2.5 Chemical identification

The chemical characterization of wood extractives was performed by Gas Chromatography-Mass Spectrometry (GC-MS). It is an analytical technique suitable for qualitative and quantitative determination of organic compounds in gaseous, liquid or solid samples. The dried wood extractives were solubilized with the methanol (MeOH) and injected manually into the column of GC-MS system at an initial temperature of 80 °C for 3 min. The identification of the compounds was based on computer matching of the mass spectra with the NIST11 library.

3 RESULTS AND DISCUSSION

Different extractive yields of poplar components by using three different extraction techniques were obtained (Fig. 1).

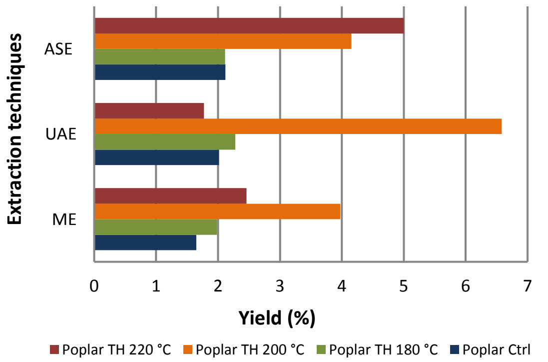


Figure 1: Different yield (%) of extractives obtained from thermally treated and untreated Poplar biomass related to three types of extraction techniques, where: maceration (ME); ultrasound assisted extraction (UAE) and accelerated solvent extraction (ASE).

The thermal treatment process mainly produced polar compounds. An important increase in polar compounds in poplar wood extractives was observed between 200° and 220 °C, particularly by using maceration (ME) or ultrasound assisted extraction (UAE) at 200 °C and using accelerated solvent extraction (ASE) at 220 °C. As it well known, it is important to note that the different extraction yield technique performances could be explained by the different acting mechanisms such as breakup of plant tissue [19] or by a diffusion process as observed by Gironi and Piemonte [20].

Polar extracts, obtained with a mixture of EtOH-H_2O (70:30 v:v), were tested to evaluate the content and antioxidant activity of polyphenols, flavonoids and tannins (Tab. I). TPC was evaluated by using Folin-Ciocalteu reagent, while TFC was quantified spectrophotometrically before and after thermal treatment by using the $AlCl_3$ method.

Table I: Total Polyphenols Content, and Total Flavonoids Content of thermally trated (TH) and untreated (Ctrl) extractives of poplar wood achieved from three different extraction techniques, where: maceration extraction (ME); ultrasound assisted extraction (UAE) and accelerated solvent extraction (ASE).

Treatment	Extraction techniques	TPC (mgGAE/g)	TFC (mgQE/g)
Ctrl	ME	225.20±8.55	215.59±6.84
	UAE	181.50±18.88	129.57±2.28
	ASE	141.90±4.12	133.33±7.60
TH 180 °C	ME	96.68±0.43	22.04±0.76
	UAE	101.87±2.60	23.12±6.84
	ASE	125.51±6.73	14.52±3.80
TH 200 °C	ME	111.96±7.16	220.43±7.60
	UAE	135.76±4.12	204.84±12.93
	ASE	193.66±11.29	373.12±22.81
TH 220 °C	ME	303.18±6.51	344.09±10.64
	UAE	334.85±3.91	401.61±0.76
	ASE	329.59±6.08	563.44±10.64

TPC significantly increased after treatment at 220 °C, with similar contents being obtained by ultrasound assisted extraction (ME) (303.18±6.51 mg GAE/g) and UAE (334.85±3.91 mg GAE/g). The lowest TPC values were observed at 180 °C (96.698±0.43 mg GAE/g for ME, 101.87±2.60 mg GAE/g for UAE, and 125.51±6.73 mg GAE/g for ASE). Table I also shows the highest TFC value (563.44±10.64 mg QE/g) after the 200 °C treatment with accelerated solvent extraction (ASE). This extraction method greatly enhanced the extraction of flavonoids from poplar wood thermally treated at 200 °C and 220 °C. The lowest TFC values were obtained when extraction was performed by ASE technique at 180 °C (14.52±3.80 mg QE/g). In all extracts, tannins in only trace amounts were found.

On the other hand, from untreated and thermo-treated poplar wood extracts evaluation of antioxidant activity was investigated. No single assay can represent total antioxidant capacity; for this reason, three complementary assays were used to evaluate antioxidant activities of extracts. To get a complete picture of the antioxidant capacity, relative antioxidant capacity index (RACI), a hypothetical concept, is used (Fig. 2). This index was calculated comparing the antioxidant activity values obtained from different chemical assays. As shown in the Fig. 2, poplar extractives obtained after thermal treatment at 220 °C showed the highest RACI value. This result confirms that high temperature of thermal treatment increases the antioxidant activity.

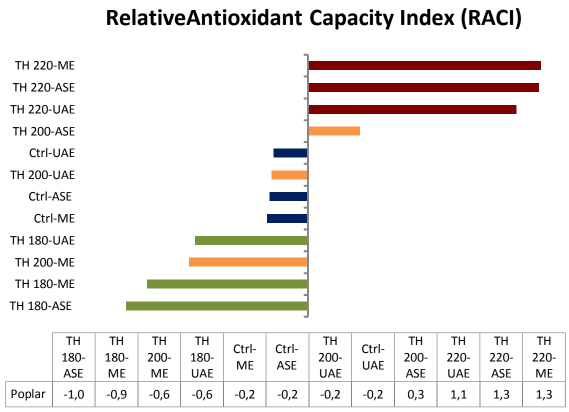


Figure 2: RACI of poplar wood extractives at different temperature of thermal treatment, where: untreated, Ctrl; thermally treated, TH; maceration extraction(ME); ultrasound assisted extraction (UAE) and accelerated solvent extraction (ASE).

Usually, GC-MS is used for the identification and quantification of chemical compounds in several plant extracts [21-22]. Thus, a chemical characterization by GC-MS was done. Table II summarizes the poplar extractives referred to untreated wood (Ctrl) and thermally treated (TH).

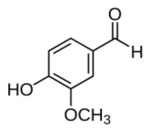
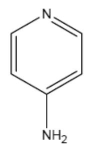
Table II: Principal compounds found in extractives of poplar thermally treated (TH) at different temperature (180, 200 and 220 °C) and untreated (Ctrl).

Compound	Ctrl	TH 180° C	TH 200° C	TH 220° C
Dimethylamine		X		
Phenol	X	X	X	X
Fampridine		X		
Cycloheptasiloxane, tetradecamethyl			X	
Vanillin	X	X		X
1-Dodecanol	X			
Methylparaben				X
Dodecyl acetate	X			
4-hydroxy-3,5-dimethoxy-Benzaldehyde	X		X	
N-(4-Methoxyphenyl)-2-hydroxyimino-acetamide			X	
Phenol, 2,6-dimethoxy-4-(2-propenyl)-			X	X
Cyclononasiloxane, octadecamethyl	X			
Hexadecanoic acid, methyl ester		X		
n-Hexadecanoic acid		X		
Squalene	X			
Benzenamine	X			

The GC-MS analysis showed how some natural compounds appeared such as fampridine (TH 180 °C-UAE) or fatty acid as Hexadecanoic acid, methyl ester (TH 200 °C-ME) and n-Hexadecanoic acid (TH 180 °C-UAE) and some other desappared with increase of thermal treatment such as 1-Dodecanol (Ctrl-UAE), lauryl acetate (Ctrl-UAE), squalene (Ctrl-UAE) or benzeneamine (Ctrl-ASE). However, an abundant presence of phenol was observed in all cases. Apolar compounds and fatty acids with long chain such as Octadecanoic acid or n-Hexadecanoic acid that were found are very important to bring a contribution the production of alternative fuel called biodiesel. Nowadays, most of the biodiesel that is currently made uses soybean, methanol, an alkaline catalyst and waste vegetable oil [23]. This favorable results, could be an useful contribute to think to substitute or consider extractives from wood for the industry of biodiesel. On the other hand poplar cultivation could be successfully not only providing timber for traditional uses, but also natural products with high value added, such as extractives.

Among poplar wood extractives found, the most important compounds identified are reported in Table III.

Table III: Chemical properties and application of vanillin and fampridine found in extractives of poplar wood.

Compounds	Chemical structure	Applications
Vanillin		pharmaceuticals, cosmetics (e.g. parfums)
Fampridine		pharmaceuticals (improve visual function and motor skills in patients with multiple sclerosis)

4 CONCLUSIONS

Populus is not only an abundantly species destined for the biomass cultivation, but is also present along the rivers or riparial zones. Recovery of this material could provide a sustainable and environmentally friendly means for obtaining natural compounds suitable for various industrial applications. Few systematic studies regarding the effect of thermal treatment combined with extraction techniques are reported in literature. Although the potential industrial role of wood extractives could have a relevant application also in the pharmaceutical field, it has not been sufficiently evaluated. Heat treatment modified the poplar extracts and temperatures over 180 °C clearly increased the extractive yield of poplar. The thermo-process produced mainly polar compounds, particularly for poplar wood subjected to temperatures between 200 and 220 °C. Extracts obtained after the 200 °C treatment showed the highest contents of secondary metabolites, such as flavonoids and polyphenols, and the highest antioxidant activity levels. Extracts of poplar wood obtained at the highest temperature reported the highest RACI values. The study can be considered the first report on the reducing antioxidant power (FRAP) and the inhibition of lipid peroxidation (BCB) of extracts derived from thermally treated wood, together with the evidence about the influence of differential thermal-treatment vs extraction techniques. Moreover the GC-MS analysis in all extractives of wood species showed the presence of important natural compounds that could be interesting for different field, from pharmaceutical to petrochemical industry.

Further investigations should be performed on poplar extracts to determine their potential applications as food supplements, nutraceuticals and health promoting natural compounds. However, more studies are needed to understand which compounds are the main responsible of the measured biological activities, through their isolation and identification by observing their potential structural changes in response to thermal treatment. In addition, this research should be consider as significant start to try to encourage the firms towards the innovation and implement the bioeconomy development expanding the functions current of poplar cultivation.

5 REFERENCES

[1] Covarelli, L., Beccari, G., Tosi, L., Fabre, B., Frey, P. (2013). Three-year investigations on leaf rust of poplar cultivated for biomass production in Umbria, Central Italy. Biomass and Bioenergy, 49, 315-322.

[2] Esteves, B., Pereira, H. (2009). Wood modification by heat treatment: A review. BioResources, 4(1), 370-404.

[3] Shimizu, K., Kondo, R., & Sakai, K. (2002). Antioxidant activity of heartwood extracts of Papua New Guinean woods. Journal of Wood Science, 48(5), 446-450.

[4] Hofmann, T., Nebehaj, E., Stefanovits-Bányai, É., Albert, L., 2015. Antioxidant capacity and total phenol content of beech (*Fagus sylvatica* L.) bark extracts. Industrial Crops and Products, 77, 375–381.

[5] Rosdiana, N.A., Dumarçay, S., Gérardin, C., Chapuis, H., Santiago-Medina, F.J., Sari, R.K., Syafii, W., Gelhaye, E., Raharivelomanana, P., Mohammed, R., Gérardin, P., 2017. Characterization of bark extractives of different industrial Indonesian wood species for potential valorization. Industrial Crops and Products, 108, 121–127.

[6] Luís, Â., Neiva, D.M., Pereira, H., Gominho, J., Domingues, F., Duarte, Â.P., 2016. Bioassay-guided fractionation, GC–MS identification and in vitro evaluation of antioxidant and antimicrobial activities of bioactive compounds from *Eucalyptus globulus* stump wood methanolic extract. Industrial Crops and Products, 91, 97–103.

[7] Santos, S.A., Vilela, C., Domingues, R.M., Oliveira, C.S., Villaverde, J.J., Freire, C.S., Neto, C.P., Silvestre, A.J., 2017. Secondary metabolites from *Eucalyptus grandis* wood cultivated in Portugal, Brazil and South Africa. Industrial Crops and Products, 95, 357–364.

[8] Saha, J.B.T., Abia, D., Dumarçay, S., Ndikontar, M.K., Gérardin, P., Noah, J.N., Perrin, D., 2013. Antioxidant activities, total phenolic contents and chemical compositions of extracts from four Cameroonian woods: padouk (Pterocarpus soyauxii Taubb), tali (Erythrophleum suaveolens), moabi (Baillonella toxisperma), and movingui (Distemonanthus benthamianus). Industrial Crops and Products, 41, 71–77.

[9] Fernando, S., Adhikari, S., Chandrapal, C., Murali, N., 2006. Biorefineries: current status, challenges, and future direction. Energy Fuels, 20 (4), 1727–1737.

[10] Villaverde, J.J., Sandín-España, P., Sevilla-Morán, B., López-Goti, C., Alonso-Prados, J.L., 2016. Biopesticides from natural products: current development, legislative framework, and future trends. BioResources, 11 (2), 5618–5640.

[11] Devappa, R.K., Rakshit, S.K., Dekker, R.F., 2015. Forest biorefinery: potential of poplar phytochemicals as value-added co-products. Biotechnology Advances, 33 (6), 681–716.

[12] Ayadi, N., Lejeune, F., Charrier, F., Charrier, B., Merlin, A., 2003. Color stability of heattreated wood during artificial weathering. Holz als Roh-und Werkstoff, 61, 221–226.

[13] Kamdem, D., Pizzi, A., Triboulot, M., 2000. Heat-treated timber: potentially toxic byproducts presence and extent of wood cell wall degradation. Holz als Roh-und Werkstoff, 58, 253–257.

[14] Chaouch, M., Dumarçay, S., Pétrissans, A., Pétrissans, M., Gérardin, P., 2013. Effect of heat treatment intensity on some conferred properties of different European softwood and hardwood species. Wood Science and Technology, 47, 663–673.

[15] Todaro, L., Russo, D., Cetera, P., Milella, L. (2017). Effects of thermo-vacuum treatment on secondary metabolite content and antioxidant activity of poplar (*Populus nigra* L.) wood extracts. Industrial Crops and Products, 109, 384-390.

[16] Cetera, P., D'Auria, M., Mecca, M., Todaro, L. (2019). Gallic acid as main product in the water extractives of *Quercus frainetto* Ten. Natural Product Research, 33(19), 2864-2867.

[17] Palo, R.T., 1984. Distribution of birch (*Betula* spp.), willow (*Salix* spp.), and poplar (*Populus* spp.) secondary metabolites and their potential role as chemical defense against herbivores. Journal of Chemical Ecology, 10, 499–520.

[18] Ferrari, S., Allegretti, O., Cuccui, I., Moretti, N., Marra, M., Todaro, L., 2013. A revaluation of turkey oak wood (*Quercus cerris* L.) through combined steaming and thermo-vacuum treatments. BioResources 8, 5051–5066.

[19] Aspé, E., Fernández, K., 2011. The effect of different extraction techniques on extraction yield, total phenolic, and anti-radical capacity of extracts from *Pinus radiata* Bark. Industrial Crops and Products, 34 (1), 838–844.

[20] Gironi, F., Piemonte, V., 2011. Temperature and solvent effects on polyphenol extraction process from chestnut tree wood. Chemical Engineering Research and Design, 89 (7), 857–862.

[21] Pisoschi, A. M., Negulescu, G. P. (2011). Methods for total antioxidant activity determination: a review. Biochem Anal Biochem, 1(1), 106.

[22] De Falco, B., Fiore, A., Bochicchio, R., Amato, M., Lanzotti, V. (2018). Metabolomic analysis by UAE-GC MS and antioxidant activity of Salvia hispanica

(L.) seeds grown under different irrigation regimes. Industrial Crops and Products, 112, 584-592.

[23] Shu, Q., Gao, J., Nawaz, Z., Liao, Y., Wang, D., & Wang, J. (2010). Synthesis of biodiesel from waste vegetable oil with large amounts of free fatty acids using a carbon-based solid acid catalyst. Applied Energy, 87(8), 2589-2596.

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