Industrial Crops and Products 61 (2014) 517–525

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Characterization of Tunisian Aleppo pine tannins for a potential use in wood adhesive formulation

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ARTICLE INFO

Article history:
Received 4 April 2014
Received in revised form 10 July 2014
Accepted 17 July 2014
Available online 24 August 2014

Keywords:
Aleppo pine tannin
Wood adhesives
NMR
FTIR
TMA
TGA

ABSTRACT

The phenolic composition of Tunisian Aleppo pine barks was studied. Colorimetric assays, Fourier transformed infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) were used to examine Aleppo pine tannins. Analyzes showed that Aleppo pine barks are rich in condensed tannins, especially in procyanidin and prodelphinidin tannins. The reactivity to formaldehyde test (Stiasny number) showed the possible use of Aleppo pine tannins in wood adhesive formulation. Thermomechanical analysis (TMA) and strength analysis of Aleppo pine tannin/hexamin based resin showed important bonding properties.

1. Introduction

Aleppo pine (*Pinus halepensis Mill.*) is a conifer that belongs to the Pinaceae family. It is a rustic species which characterizes the semi-arid Mediterranean bioclimatic zone (Chakroun, 1986). It is found naturally around the Mediterranean basin, except in Egypt. Its most important mass exists in North Africa (Nahal, 1962).

The use of Aleppo pine barks for tanning has been known for a long time in the Middle East, Europe and North Africa. In Provence, pine barks are removed carefully during tree harvesting, reduced into a powder and mixed with *Quercus coccifera* tannins to hides tanning. They also were used to produce a hot solution in which fishermen dipped their nets every 15 days to ensure their conservation (Nahal, 1962). Tunisia is one of the native lands of Aleppo pine with a total area of 361221 ha. It extends on the dorsal mountains in the South of Medjerda and the Western massif of Zaghouan until the steppic limits of the center (Ayari, 2012; Ministry of Agriculture, 2010). The exploitation of Tunisian Aleppo pine forests consists mainly of wood production, resin tapping and seeds production (for culinary uses) (Chakroun, 1986). The exploitation of Aleppo pine barks as raw material for wood adhesives production could be envisaged. Recently, there have been growing interests on tannin-based resins, which is contributed to tannins structure, suggesting that they can be used as substitutes for phenol in phenol-formaldehyde resins known for their toxicity (Pizzi, 2006). Tannins can be divided into two categories: hydrolysable tannins and condensed tannins. Hydrolyzed tannins have low reactivity toward formaldehyde due to lack of macromolecular structures and also low nucleophilicity, which limits its usage in worldwide adhesive production. Condensed tannins contain complex chemical structures of polyphenolic compounds, which have higher reactivity toward formaldehyde than phenol (Hoong et al., 2011).

Actually, the most common sources of tannins are mimosa (*Acacia mearnsii*) bark, quebracho (*Schinopsis balansae* and *Schinopsis lorentzii*) wood, pine bark (Long, 1991; Pizzi, 1977) and eucalyptus species bark (Fechtal and Riedl, 1993; Vázquez et al., 2009).

This study aims to evaluate, for the first time, the potential use of Tunisian Aleppo pine barks tannins in wood adhesive formulation. For this purpose, polyphenolics, hydrolysable tannins and condensed tannins of Tunisian Aleppo pine barks were analyzed using

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http://dx.doi.org/10.1016/j.indcrop.2014.07.035
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colorimetric, spectrometric and thermal methods. Aleppo pine tannins extraction was optimized. Then, Aleppo pine tannin-based adhesive was characterized using mechanical and thermomechanical tests.

2. Materials and methods

2.1. Materials

Aleppo pine barks were purchased from a market and ground using a grinder (Retsh SK1 rotating knife) with a screen of 1 mm diameter. The resulting powder was kept in darkness at room temperature to conduct the following analyses described in Section 2.2.

2.2. Methods

To estimate the polyphenolics, the condensed tannins and the hydrolyzable tannins contents in Aleppo pine barks, we exploited conventional and standard methods commonly used in the study and the determination of plants polyphenolic composition (Bate-Smith, 1977; Scalbert et al., 1987). It comes to colorimetric methods developed as below.

2.2.1. Extraction of polyphenols for total polyphenols and condensed tannins assay

0.5 g of bark powder was extracted three times with10 mL of 80% methanol solution using a homogenizer at room temperature (3 h × 2 h). The supernatant was collected after each extraction by filtering through Whatman paper no.1. The methanol was evaporated under reduced pressure and then two drops of HCl (6N) were added. The resulting solution was extracted with diethyl ether (3 mL × 5 mL). After drying over sodium sulfate, the diethyl ether was removed and the residue was dissolved in 5 mL of methanol. The volume of the aqueous extract was adjusted to 10 mL (Scalbert et al., 1987).

The experiment was carried out in triplicate.

2.2.2. Determination of total polyphenolic content

Total polyphenolic contents of the ether and aqueous extract were determined according to the method of Scalbert et al. (1987). Briefly, 0.5 mL of a 200-fold diluted aqueous extract (or 0.5 mL of a 20-fold diluted ether extract) was mixed with 2.5 mL of 10-fold diluted Folin–Ciocalteu's phenol reagent and incubated for 1 min, before 2 mL of 7.5% Na_2CO_3 was added. The mixture was allowed to stand for 5 min at 50 °C in a water bath and then transferred into cold water. The absorbance versus prepared blank was monitored at 760 nm. A gallic acid aqueous solution (80 μg/mL) was used for calibration. The final results were expressed as mg gallic acid equivalent (GAE) per g of dry weight (DW). We note that the aqueous extract was diluted with water while ether extract was diluted with absolute methanol (Scalbert et al., 1987).

The experiment was carried out in triplicate. The result was expressed as means ± SD.

2.2.3. Determination of condensed tannins

2.2.3.1. Catechin equivalent content. Determination of condensed tannins by the vanillin assay was based on the procedure reported by Broadhurst and Jones (1978). A 0.5 mL of aqueous extract, contained in a test tube covered with aluminum foil, was mixed with 3 mL of 4% vanillin–methanol solution and then with 1.5 mL of hydrochloric acid. The mixture was allowed to stand for 15 min at 20 °C in the dark. The absorbance of the mixture was measured at 500 nm. A catechin aqueous solution (30 mg/L) was used for calibration. The final results were expressed as mg catechin equivalent (CE) per g of dry weight (DW).

The experiment was carried out in triplicate. The result was expressed as means ± SD.

2.2.3.2. Cyanidin equivalent content. A 0.5 mL of aqueous extract was added to 5 mL of an acid solution of ferrous sulfate (77 mg of $FeSO_4{\cdot}7H_2O$ in 500 mL of 2:3 (HCl $d = 1.18$/n-BuOH)). The tubes were loosely covered and placed in a water bath at 95 °C for 15 min. The absorbance was read at 530 nm and results were expressed as cyanidin g equivalent (Cya) per g of dry weight (DW) (Scalbert et al., 1987).

The condensed tannins content was calculated using the formula given below (Eq. (1)):

$$[CT] = \frac{A \times V \times FD \times V' \times M}{\varepsilon \times \upsilon \times m} \quad (1)$$

where $[CT]$ is the condensed tannin content (mg Cya/g DW); A is the absorbance of the sample; V is the volume of the reaction medium; V' is the volume of the aqueous extract recovered after extraction with diethyl ether; υ is 0.5 mL; D_f is the dilution factor; M is the molecular weight of cyanidin; m is the weight of the dry matter and ε is the molar extinction coefficient (34,700 M^{-1} cm^{-1}).

The experiment was carried out in triplicate. The result was expressed as means ± SD.

2.2.4. Determination of hydrolyzable tannins content

Hydrolyzable tannins were determined by the method of Bossu et al. (2006) with slight modifications. 0.25 g of the ground sample was extracted with50 mL of methanol (80%) using a homogenizer at room temperature for 6 h. After centrifugation (10 min, 2000 rpm), the final extract was diluted three times with water, before determination. 5 mL of KIO_3 aqueous solution (2.5%, w/v) was heated for 7 min at 30 °C, and then 1 mL of the diluted sample was added. After an additional 2 min of tempering at 30 °C, the absorbance was measured at 550 nm. A calibration curve was obtained using tannic acid solution (5000 mg/L) prepared by solubilization of 0.25 g of tannic acid in 50 mL of methanol (80%). The analytical standard solutions of tannic acid were prepared by aqueous dilution. Results were expressed as mg tannic acid equivalent (TAE) per g of dry weight (DW).

The experiment was carried out in triplicate. The result was expressed as means ± SD.

2.2.5. Extraction of tannins for FTIR, NMR and TGA analysis

Tannin extraction from Aleppo pine barks was carried out in water containing 2% of sodium bisulfite and 0.5% of sodium bicarbonate, with a sample-to-water ratio equal to 1/5. The bark powder was immersed in water under continuous magnetic stirring for 6 h at 70 °C. The tannins extract so obtained, was filtered and dried in an oven at 50 °C to yield tannins.

2.2.6. FTIR spectra analysis

Infrared analysis was performed using a Perkin Elmer Spectrum One equipped with a ATR-FTIR module. A few milligrams of milled product were deposited on the crystal (Diamond/ZnSe). The sample was crushed using the module for 10 scans and the scanning wavelength of infrared was 4000–400 cm^{-1} at a resolution of 4 cm^{-1}.

2.2.7. NMR analysis

NMR analysis was carried out on the ^{13}C carbon atom, in liquid phase. The spectra were recorded on a Brüker avance 400 MHz spectrometer. The chemical shifts were calculated relative to TMS (tetramethylsilan). The spectra appear at 100.6 MHz. The number of scans and the acquisition time were 1.36 s and 12,000, respectively.

Tannin extract was dissolved in DMSO-d6 (deuterated dimethyl sulfoxid).