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EXPLORING THE POTENTIAL OF LIGNIN AND EXTRACTIVE FRACTIONS FROM TOBACCO STEM FOR COSMETIC APPLICATION

Tales G. Eckel[1], Marcel B. Santana Jr[1], Felipe Á. Gama[1], Regilsom L. Defante[1], Luiz Carlos de Oliveira Jr[1], Débora Trichez[1], Acácio A. F. Zielinski[1], Jaciane L. Ienczak[1].

[1] *Chemical and Food Engineering Department (EQA), Federal University of Santa Catarina (UFSC), Florianópolis-SC, Brazil.*
** Corresponding author's email address: talesge@gmail.com*

ABSTRACT

This study aims to assess the potential of lignin fractions and extracts derived from tobacco stems as bioactives in the cosmetics industry. The efficiency of an 80% v/v hydroethanolic solution (ethanol/water) was investigated for extracting the stem extracts, along with the optimal extraction time at 60°C. Additionally, a fatty acid profile analysis was conducted on the extracts (apolar fraction). The solid residue remaining after extract extraction underwent an alkaline-glycerol organosolv treatment, resulting in a soluble lignin fraction that was precipitated and recovered. Both the extracts and lignin were evaluated for their antioxidant capacity (IC_{50} of 1.13 and 0.41 mg/mL, respectively) and sun protection factor (SPF ranging from 7.97 to 8.14 for extracts and from 14.85 to 21.55 for lignin). These results demonstrate high potential for future applications in the cosmetics industry.

Keywords: Tobacco. Lignin. Extractives. Cosmetics. Organosolv.

1. INTRODUCTION

The production and use of tobacco have profound impacts on society that extend beyond health concerns. According to the World Health Organization (WHO), tobacco-related deaths are projected to exceed 8 million annually by 2030[1]. Furthermore, research such as that by Novotny TE[2], published in the WHO Bulletin, highlights the extensive environmental impact of tobacco production. This includes land concentration, chemical pollution of water sources, soil nutrient depletion, substantial solid waste generation during manufacturing and post-use, and significant emissions of polluting gases[3]. Research on tobacco residues has revealed substantial potential for bioproduct production [3,4]. Composed of cellulose, hemicellulose, lignin, and extractives, tobacco residues can be efficiently repurposed, serving as an excellent biomass source for biorefineries focused on biofuels, biocosmetics, and other bioproducts[3,5,6]. The extractive fraction from tobacco stems contains a diversity of valuable compounds such as fatty acids, essential oils, solanesol, nicotine, and phenolic compounds, each with distinct characteristics and applicability across multiple industries[7]. Additionally, studies indicate that tobacco stem lignin possesses significant bioactive potential, particularly useful in cosmetic applications[8,9]. Recent research has focused on utilizing polysaccharides like cellulose and hemicellulose for prebiotic production and bioconversion processes [3,4]. However, the extractive fraction and lignin, comprising approximately 40% of the biomass, have not been widely explored in valorization studies. Thus, the aim of this study is to evaluate the bioactive potential of extractives and lignin fractions extracted from tobacco stems, investigating their antioxidant capacity and sun protection properties.

2. MATERIAL & METHODS

Samples of tobacco stems, donated by a Brazilian agro-industry, underwent a drying process in an oven at 45°C and were subsequently reduced in size to achieve particle sizes in the range of 40-50 mesh, as described by [3]. The biomass was then chemically characterized in terms of cellulose, starch, hemicellulose, lignin, ash, and polar and apolar extractives, following NREL methodology[10]. The apolar extractive fraction was analyzed to determine the fatty acid profile, as established by [11]. The biomass was evaluated to determine the optimal conditions for extractive removal, using a hydroethanolic solution composed of 4 parts ethanol to 1 part water [12]. The objective of this stage was to investigate the effectiveness of the hydroethanolic solution in extracting the tobacco stem extractives, analyzing the time required to reach saturation in removing the antioxidant compounds present in this extractive fraction. The experiment was conducted in an orbital shaker at 250 rpm and 60°C for 5 hours. Samples were collected every 30 minutes during the first 2 hours and every 60 minutes thereafter, totaling 5 hours[12]. Saturation of the medium was determined by antioxidant capacity using the DPPH method, with results expressed in mg Trolox/g sample.

The optimal time for removal of antioxidant compounds from the tobacco stem extractive fraction was maximized in a jacketed reactor. Following this stage, the liquid stream, enriched in tobacco stem extractives, underwent rotary evaporation and lyophilization for evaluation of its antioxidant capacity (IC50-DPPH)[13] and sun protection factor (SPF)[14]. Simultaneously, the solid stream was dried in a circulating air oven at 60°C to constant weight and subsequently chemically characterized in terms of cellulose, hemicellulose, lignin, and extractives for mass balance purposes[10]. The solid stream underwent an alkaline-glycerol organosolv pretreatment for lignin extraction, employing conditions of 120°C for 1 hour in an autoclave, with a solid loading of 15% (w/v), 3% NaOH (w/v), and 50% glycerol (v/v)[15]. Following this step, the suspension containing the pretreated solid and black liquor was diluted with distilled water in a 1:8 ratio (v/v). The suspension was filtered to separate the black liquor [4], which was reserved for subsequent lignin recovery by precipitation in acidic medium. The pH of the black liquor was adjusted to between 1-2 using a 10 M sulfuric acid solution 16,17, followed by centrifugation at 5000g. The precipitated lignin was then washed with distilled water to neutrality and subjected to lyophilization for subsequent analysis of antioxidant capacity (IC_{50}-DPPH) and sun protection factor (SPF).

The sun protection factor was determined through an in vitro method that evaluates the relative UVB protection capacity of the extracts, based on the absorption spectrum of the sample at wavelengths from 290 to 320 nm[14]. Antioxidant capacity was assessed in microplates using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) reagent to measure the samples' ability to scavenge free radicals, following the methodology of [13] ,with some adaptations. Analyses were conducted in triplicate.

3. RESULTS & DISCUSSION

The chemical analysis of tobacco stem revealed the following contents: cellulose 31.16 ± 0.65%, starch 5.60 ± 0.01%, hemicellulose 14.05 ± 0.21% (composed of 11.80% xylan, 0.97% arabinose, and 1.28% acetate), lignin 16.96 ± 0.39%, extractives 24,43 ± 1,42%, and ash 6,34 ± 0,09%. These results are consistent with previous studies on tobacco residues[3,4] regarding cellulose, hemicellulose, and lignin contents. The high content of extractives also aligns with findings by [4] and [5], which indicated that tobacco stems can contain between 30-50% of the total biomass weight in extractives. These results highlight a significant concentration of extractives and lignin, which together constitute nearly 40% of the total biomass structure of tobacco stem. The extractive fraction includes a lipid fraction (apolar), accounting for approximately 2.25% of the biomass. The lipid profile of this fraction is depicted in figure 1-B, revealing that the predominant fatty acid is palmitic acid (40.60%), followed by oleic acid (26.71%), linoleic acid (20.37%), stearic acid (11.69%), and margaric acid (0.62%). This diverse lipid profile indicates a composition with 52.91% saturated fatty acids and 47.08% unsaturated fatty acids.

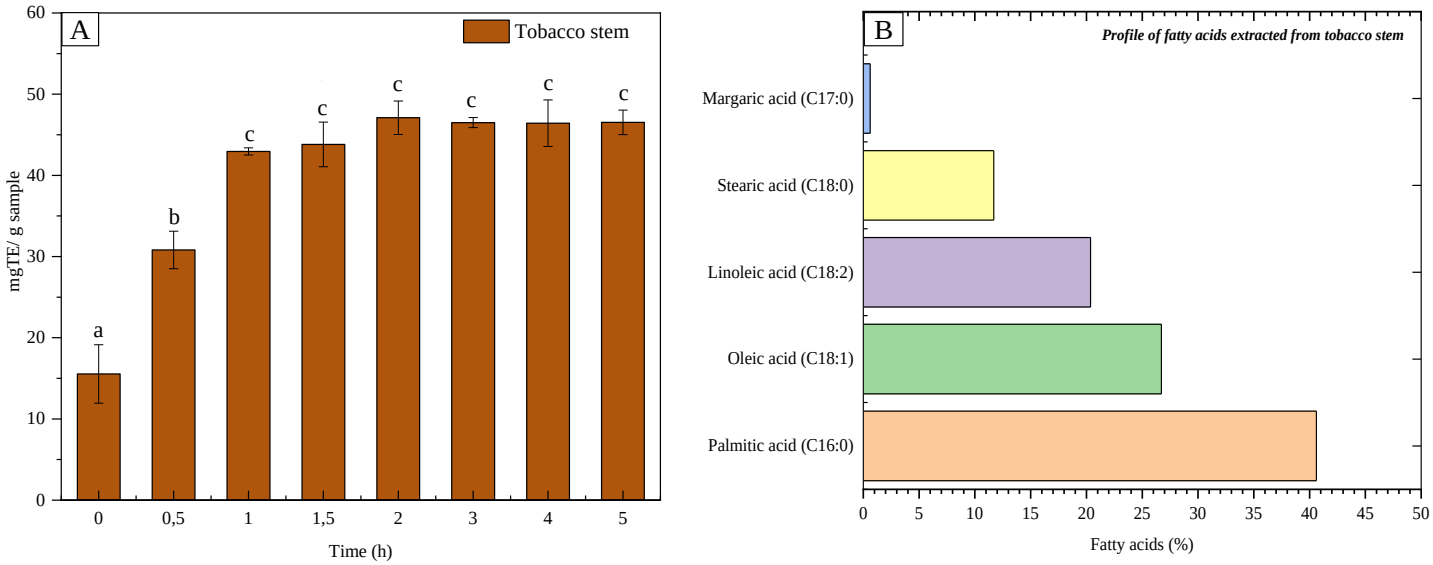

Figure 1. (A) Kinetics of release of antioxidant compounds from the extractive fraction of tobacco stem; (B) Profile of fatty acids present in the non-polar extracts of tobacco stem.

In Figure 1-A, it is observed that the antioxidant capacity of the reaction medium increases as the exposure time between the solvent and biomass is prolonged. Continuous release of active components from tobacco stem shows significant differences until the 1-hour extraction period, reaching equilibrium. This phenomenon, illustrated in Figure 1-A, is based on mass transfer according to diffusion laws. Tobacco stem extractives, rich in phenolic compounds with antioxidant capacity, are transferred to the solvent as exposure time increases, resulting in a gradual increase in antioxidant capacity as observed. Likely, after 1 hour of extraction, the solvent reaches saturation, ceasing the increase in antioxidant capacity of the medium. Approximately 68% of the extractive fraction was removed by the end of the process.

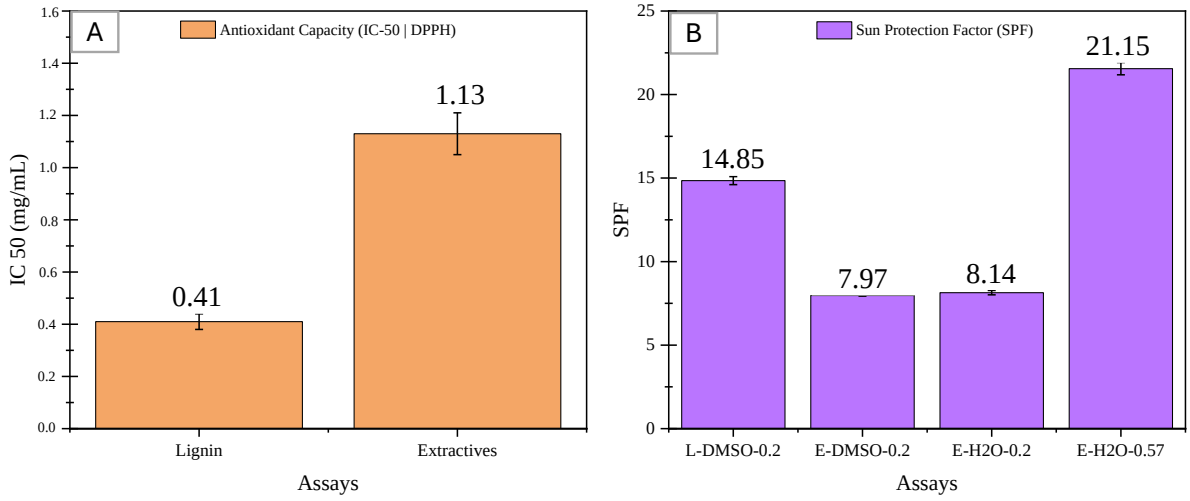

Figure 2. (A) Antioxidant capacity of lignin and solubilized extractives in DMSO solution, analyzed in terms of IC50. (B) Sun protection factor of lignin fraction at a concentration of 0.2 mg/mL solubilized in DMSO (L-DMSO-0.2); extractives at a concentration of 0.2 mg/mL solubilized in DMSO (E-DMSO-0.2), in water (E-H20-0.2), and at a concentration of 0.57 mg/mL solubilized in water (E-H20-0.57).

The radical scavenging capacity was determined by IC50, representing the lowest concentration required to inhibit free radicals by 50%. A lower IC50 value indicates a more efficient inhibitor. As shown in Figure 2-A, all extracts exhibited high antioxidant capacity, especially lignin, with values around 0.41 mg/mL, while extractives were around 1.13 mg/mL. Previous studies on tobacco cultivar extractives revealed an IC50 of 0.65 mg/mL, attributed to high concentrations of flavonoids[6]. Furthermore, tobacco stem lignin was also investigated for antioxidant potential, showing activity superior to commercial antioxidants like butylated hydroxytoluene (BHT)[8,9], with values ranging from 0.09 mg/mL to 0.58 mg/mL depending on the lignin extraction method[8]. These results are consistent with those obtained in this study.

The sun protection factor (SPF) of tobacco stem lignin and extractives was evaluated as shown in Figure 2-B. The results indicate that at a concentration of 0.2 mg/mL, SPF values of 14.85 and 7.97 were obtained for lignin and extractives in DMSO, respectively. There was no significant difference when the extractives were solubilized in water compared to DMSO. Furthermore, increasing the concentration of extractives to 0.57 mg/mL substantially increased SPF, reaching values of 21.55. These results highlight that both lignin and extractives, even at low concentrations, exhibit high sun protection potential compared to literature standards[8,9]. They possess numerous phenolic groups capable of absorbing in the UV non-visible range, making them promise for applications in cosmetic products such as sunscreens[8,9].

4. CONCLUSION

The extractive fraction exhibits a diverse composition of fatty acids, including palmitic acid (40.60%), oleic acid (26.71%), linoleic acid (20.37%), stearic acid (11.69%), and margaric acid (0.62%). These compounds are suitable for application in cosmetic products such as moisturizing lotions. Moreover, the extraction of these antioxidants proved feasible, with an extraction time close to one hour and a removal yield of 68% of the extractives. The results demonstrate a high antioxidant capacity of both lignin and extractives, with IC50 values of 0.41 and 1.13 mg/mL, respectively. This makes them suitable as additives in creams, lotions, and other dermatological products aimed at delaying skin aging. However, future toxicity analyses are necessary if implemented. Other significant data obtained include the Sun Protection Factor (SPF), showing a high potential for protection: the lignin fraction achieved an SPF of 14.85 and the extractive fraction up to 21.55 SPF.

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