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Addressing the phytochemical prospection of thermally treated *Eucalyptus grandis* wood

Abordagem da prospecção fitoquímica da madeira de *Eucalipto grandis* tratada termicamente

Abordar la prospección fitoquímica de madera de *Eucalyptus grandis* tratada térmicamente

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Abstract

This work aimed to investigate phytochemical prospection in treated and untreated wood of *Eucalyptus grandis* to understand the dynamics of extractives in relation to heat treatment. Samples of *E. grandis* wood were collected and grouped into external and internal regions. Half of the samples from each region were submitted to heat treatment at 190 ºC. From the treated and untreated samples, phytochemical tests were performed to detect classes of metabolites present in the *E. grandis* raw wood, hydrophilic extract and lipophilic extract. Phytochemical analysis detected the presence of alkaloids, phenolic compounds and triterpenoids in all hydrophilic extracts of the studied species. Presence of flavanonols, flavanones and saponins was detected only in the internal region of the wood. Tannins, leucoanthocyanidins, triterpenoids and saponins were influenced by heat treatment. The classes of flavonoids, xanthones and alkaloids are the most resistant to heat treatment. The phytochemical analysis made it possible to identify a new class of extractives that emerged after heat treatment, flavones.

Keywords: Heat treatment; Wood extractives; Lipophilic extract.

Resumo

Este trabalho teve como objetivo investigar a prospecção fitoquímica na madeira tratada e não tratada termicamente de *Eucalyptus grandis* visando compreender a dinâmica dos extrativos em relação ao tratamento térmico. Para isso, foram feitas amostras da madeira de *E. grandis*, sendo essas agrupadas em região externa e interna. Metade das amostras de cada região foi submetida ao tratamento térmico a 190 ºC. A partir das amostras tratada e não tratada, realizaram-se os testes fitoquímicos para detecção de classes de metabólitos presentes na madeira bruta de *E. grandis*, extrato hidrofílico e extrato lipofílico. A análise fitoquímica detectou a presença de alcalóides, compostos fenólicos e triterpenóides em todos os extratos

hidrofílicos da espécie estudada. Apenas na região mais interna da madeira verificou-se a presença de flavanonóis, flavanonas e saponinas. Taninos, leucoantocianidinas, triterpenóides e saponinas, tiveram influência do tratamento térmico. As classes dos flavonóides, xantonas e alcaloides são as mais resistentes ao tratamento térmico. A análise fitoquímica permitiu identificar uma nova classe de extrativos que surgiu após o tratamento térmico, as flavonas.
Palavras-chave: Tratamento térmico; Extrativos da madeira; Extrato lipofílico.

Resumen

Este trabajo tenia como objetivo investigar la prospección fitoquímica en madera tratada y no tratada térmicamente de *Eucalyptus grandis* con el fin de comprender la dinámica de los extractivos en relación al tratamiento térmico. Para eso, se elaboraron muestras de madera de *E. grandis*, las cuales se agruparon en regiones externas e internas. La mitad de las muestras de cada región fueron sometidas a tratamiento térmico a 190ºC. A partir de las muestras tratadas y no tratadas, se realizaron las pruebas fitoquímicas para detectar clases de metabolitos presentes en la madera de *E. grandis* con la madera en bruto, extracto hidrofílico y extracto lipofílico. El análisis fitoquímico detectó la presencia de alcaloides, compuestos fenólicos y triterpenoides en todos los extractos hidrofílicos de las especies estudiadas. Solo en la región más interna de la madera se encontró la presencia de flavanonoles, flavanonas y saponinas. Los taninos, leucoantocianidinas, triterpenoides y saponinas fueron influenciados por el tratamiento térmico. Las clases de flavonoides, xantonas y alcaloides son las más resistentes al tratamiento térmico. El análisis fitoquímico permitió identificar una nueva clase de extractivos que aparecieron tras el tratamiento térmico, las flavonas.
Palabras clave: Tratamiento térmico; Extractos de madera; Extracto lipofílico.

1. Introduction

Wood is a basic, versatile and renewable resource widely used in various applications, and remains indispensable to everyday life and human culture due to its aesthetic aspect and characteristic properties. Despite its great benefits, this lignocellulosic material has undesirable properties, such as dimensional instability, hygroscopicity and susceptibility to biological degradation, thus limiting its use in several applications (Hoseinzadeh et al., 2019; Chien et al., 2018; Borges & Quirino, 2004). To solve these problems, chemical approaches have been used to increase dimensional and thermal stability, and biological resistance in recent years (Yang et al., 2014; Hung et al., 2010; Evans, 2009; Wu et al., 2004). However, chemical modification is costly and time consuming, requiring cumbersome processing. Therefore, heat treatment, a physical modification, has attracted attention in the academic and industrial spheres due to its economic and sustainable aspect.

Heat treatment is said to be an effective process to improve physical and aesthetic properties, besides being considered a method of wood preservation. It consists of applying high temperatures ranging from 160 to 250 °C, with no use of toxic chemical agent, thus being an environmentally acceptable and technically more attractive treatment (Sandberg et al., 2017; Zhu et al., 2014; Poncsak, 2011). Some studies have shown that high temperature treatment reduces the moisture and dimensional shrinkage of wood (Poncsak et al., 2009; Esteves et al., 2008). On the other hand, thermally treated wood suffers changes in chemical compositions, i.e., there are changes in structural components such as cellulose, hemicellulose, lignin and, the non-structural ones, such as natural extractives from wood cell wall (Zanuncio et al., 2014).

The extractives are non-structural components of wood, specifically concentrated in the core, and commonly produced by the tree as defensive compounds against environmental stresses, besides having great influence on wood properties, being quite affected by the action of heat (Klock et al., 2005; Taylor et al., 2012; Singh et al., 2012; Kirker et al., 2013). Most of the original wood extractives disappear with the heat treatment, especially the more volatile compounds, while new extractives appear as structural polymer degradation products. In this perspective, a better understanding of the differences between extracts during heat treatment is necessary to enable their use in different fields, such as pharmaceutical or cosmetic (Esteves et al., 2008).

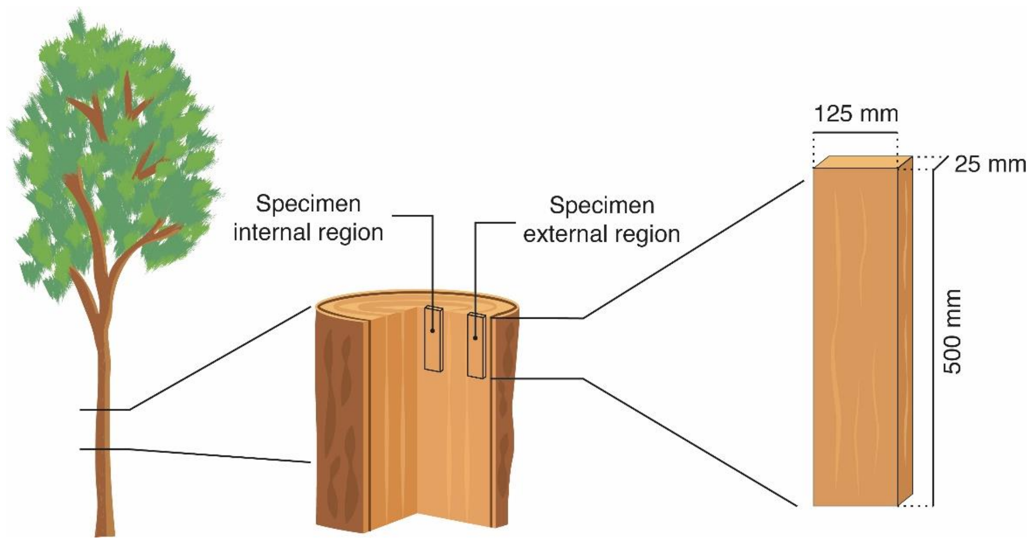
Phytochemical studies are important mainly when not all chemical studies of certain plant species are available, aiming to know the chemical classes and evaluate their presence in them, identifying groups of relevant secondary metabolites (Carvalho, 2009; Simões et al., 2001). In addition, phytochemical analysis in plants confirms the presence of alkaloids, steroids, flavonoids, coumarin, saponins, glycosides and phenols (Gröcer et al., 1998). The studies about wood extractives have been stimulated in the discovery and characterization of new chemical structures, taxonomic classification of species, tree growth processes, obtaining of new products and by-products of commercial value and the determination of problems related to some uses of wood (Santos et al., 2017; Barbosa et al., 2005; Klock et al., 2005). Therefore, the detailed analysis of the chemical composition, and particularly of the less abundant and studied components present in non-modified and thermally modified wood, is a relevant issue to be studied. Thus, the present work was aimed at investigating the phytochemical prospection of the thermally modified *Eucalyptus grandis* wood, in order to understand the dynamics of the extractives in relation to the heat action.

2. Material and Methods

2.1 Sampling and preparation of material

The wood in this study came from six trees of *Eucalyptus grandis* Hil Ex Maiden with 23 years of age. These trees were provided by the company Quinvale, located in Barra do Piraí, Rio de Janeiro State, Brazil, whose geographical coordinates are 22°43'23" latitude (S) and 44°08'08" longitude (W) and at an average altitude of 446 meters. The woods were processed, generating boards of 2.5 x 12.5 x 50.0 cm, and only those considered free of defects were selected, totaling 104 samples (Figure 1).

Figure 1 - Sample collection scheme.



Source: The authors.

In order to evaluate the action of heat on the wood and its influence on the chemical composition, the wood samples were divided into external and internal regions, the former closer to the bark and the latter closer to the pith. For the thermal treatment, a program of 6 hours and 30 minutes program with four stages was adopted: (1) temperature increase up to 100 ºC with 2 hour duration; (2) temperature increase from 100 to 190 ºC with 30 minute duration; (3) thermal treatment at 190 ºC for 3 hours; and (4) temperature decrease up to 60 ºC for 30 minutes.

2.2 Preparation of organic extracts

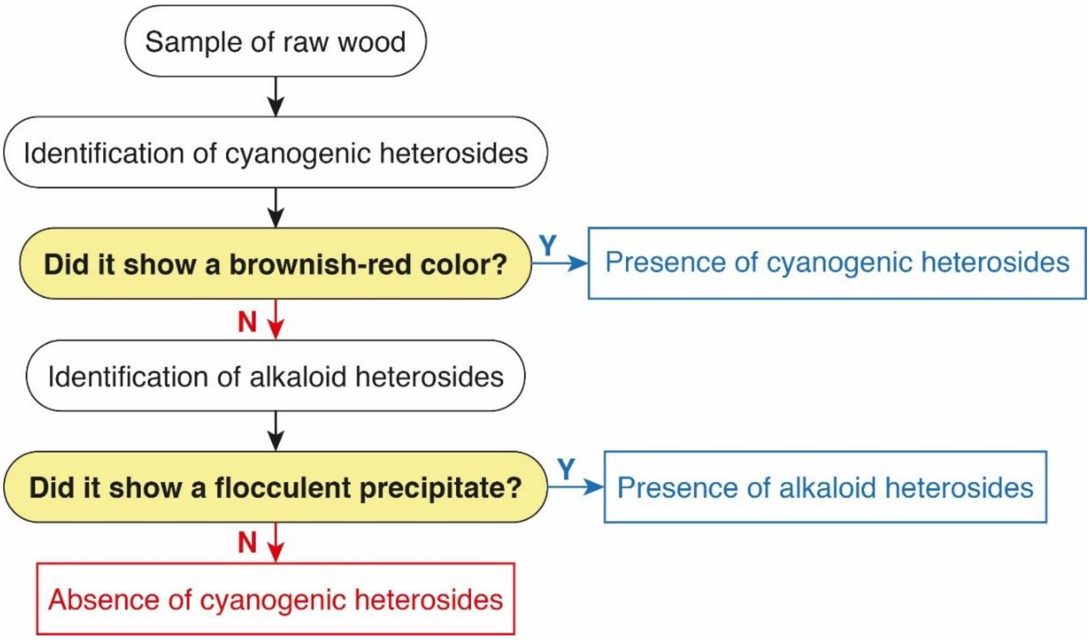
The woods of *E. grandis* were processed in a Willey-type knife mill. To obtain a greater homogenization of the particles, the fraction sieved through the 40 mesh and 60 mesh was used, according to the procedures pointed out by the standard TAPPI T204 cm-97 (TAPPI, 1997). To obtain the organic extracts of the material, it was conditioned to a paper filter cartridge of 15.0g of the wood samples treated and not thermally treated. Then, the material was submitted to the extraction cycle using cyclohexane, ethyl acetate and methanol solvents for 12 hours in a Soxhlet apparatus, using 400 mL of each solvent (Abreu et al., 2006). To obtain the organic extract, the content from the extraction was submitted to separation of each solvent through the application of rotavapor, responsible for the evaporation of the solvent, to obtain a concentrated solution of wood extractives. The concentrates were transferred to a container until reaching the complete evaporation of the solvent at room temperature.

2.3 Phytochemical evaluation

Phytochemical tests for the identification of non-structural components present in woods in this study were performed based on methodologies proposed by Costa (1995), Matos (1997) and Rodrigues et al., (2010). The analyses were carried out from raw wood, hydrophilic extract (methanol) and lipophilic extract (cyclohexane). All tests were performed in duplicate.

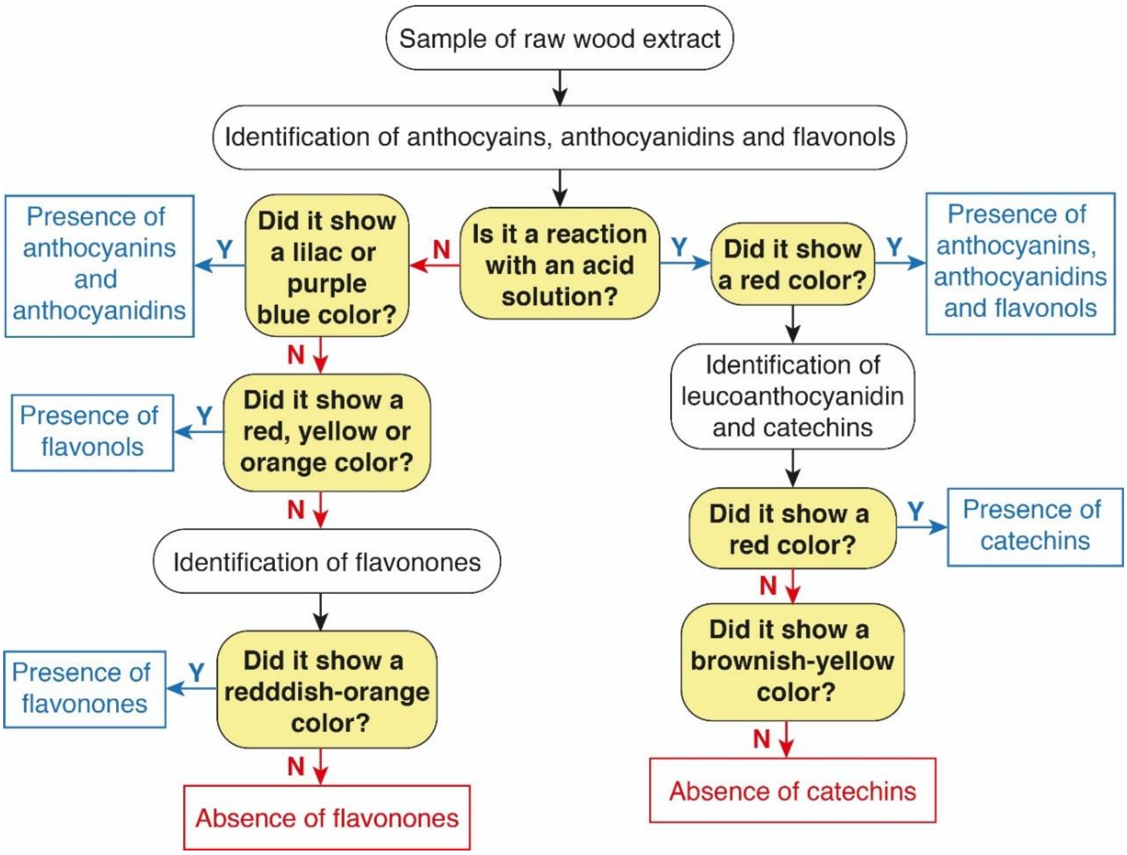
From raw wood, prospection tests were performed for the detection of cyanogenic and alkaloid heterosides (Reaction with Dragendorff's and Mayer's reagents) (Figure 2). The hydrophilic extract was submitted to tests for phenols and tannins (reaction with ferric chloride), anthocyanins, anthocyanidins and flavonoids (pH variation test, with sodium hydroxide and hydrochloric acid) (Table 1), leucoanthocyanidins, catechins and flavones (pH variation and heating test, with sodium hydroxide and hydrochloric acid) (Table 2), flavonols, flavanones and xanthones (Shinoda test), steroids and triterpenoids (Lierbemann - Burchard test), saponins (foam test), resins (extract turbidity test) and alkaloids (reaction with Dragendorff' and Mayer' reagents) (Figure 3).

Figure 2 - Flowchart representing prospection for raw wood.



Source: The authors.

Figure 3 - Flowchart representing prospection for wood extracts.



Source: The authors.

Table 1 - Reactions for identification of anthocyanins, anthocyanidins and flavonols.

Constituents	pH 3	pH 8.5	pH 11
Anthocyanins and anthocyanidins	Red	Lilac	Blue Purple
Flavones, Flavonols and Xanthones	-	-	Yellow
Chalcones and aurones	Red	-	Reddish-orange
Flavanonols	-	-	Reddish-orange

Source: The authors.

Table 2 - Reactions for identification of leucoanthocyanidins, catechins and flavones.

Constituents	pH 3	pH 11
Leucoanthocyanidins	Red	-
Catechins	Brown-yellowish	-
Flavanones	-	Reddish-orange

Source: The authors.

The lipophilic extract was submitted to tests for alkaloids, phenolic constituents, steroids and triterpenoids, using the reactions mentioned above, and anthraquinones (reaction with NH4OH) were also evaluated.

3. Results and Discussion

3.1 Phytochemical prospection of non-thermally treated *E. grandis* wood

Cyanogenic heterosides were not detected in extractives of the studied species (Figure 4). The presence of alkaloids can be observed in the internal and external regions of untreated wood of *E. grandis* and in hydrophilic extract (Figure 4). In studies carried out with other Eucalyptus species, this chemical component was not verified (Lobo, 2014; Döll-Boscardin et al., 2010; Malinowski, 2010). Alkaloids perform functions related to the defense of plants and, in addition, these metabolites are of great pharmacological interest (Croteau et al., 2000).

Among the phenolic compounds, the presence of tannins in hydrophilic extract was identified in the internal and external regions of *E. grandis* wood (Figure 4). Tannins represent the fourth most abundant group of secondary metabolites after cellulose, hemicellulose and lignins and are characterized as defensive molecules of the plant, having as main advantage their phenolic structure and antioxidant action (Sharma, 2019;

Shirmohammadli et al., 2018). Mamani et al. (2012) revealed that tannins act defensively and confer resistance against pathogens in different plant species. Tuominen (2013) revealed that the plant allocates a significant amount of tannins to parts that are more susceptible to attack by natural enemies.

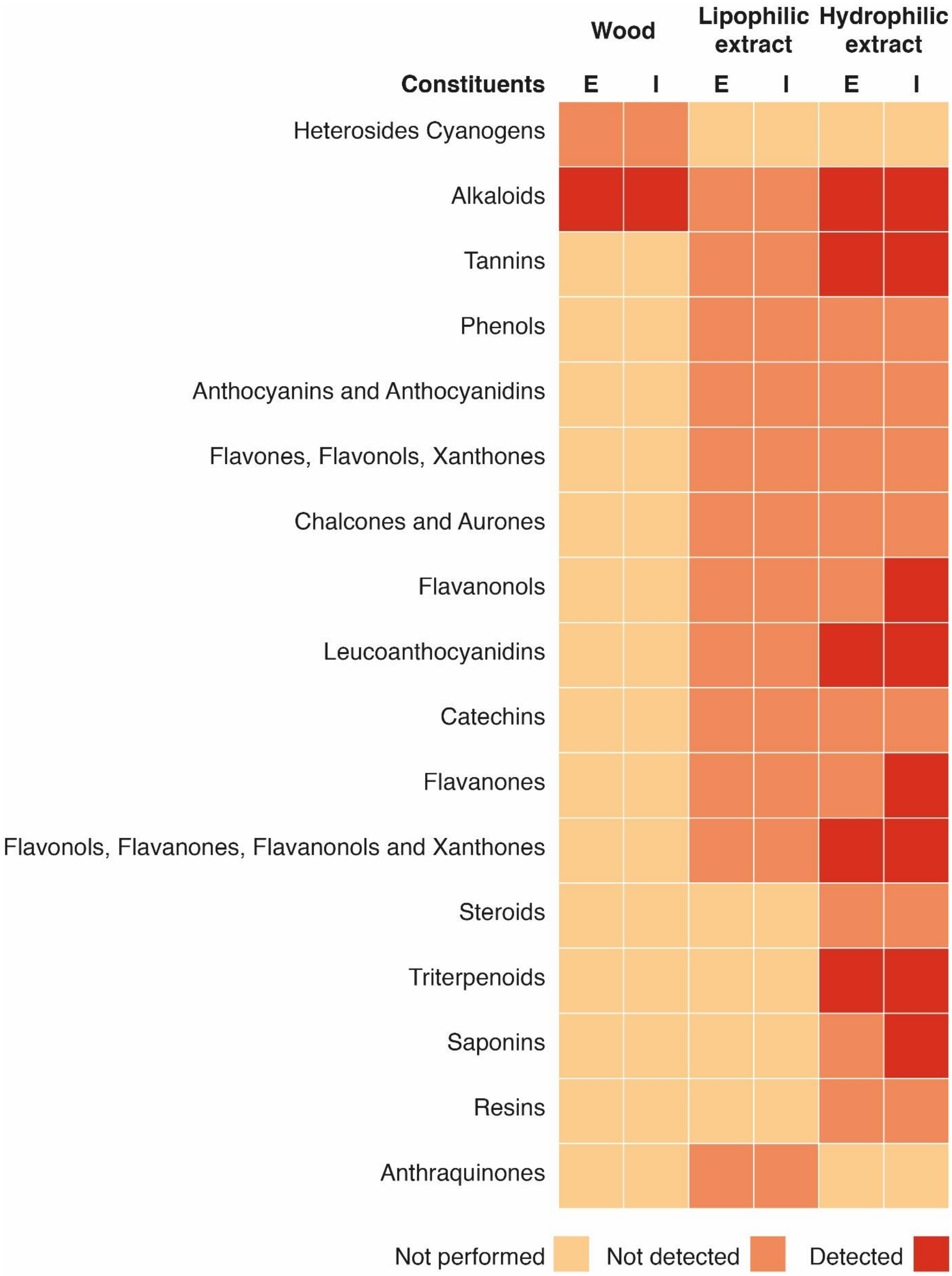
In the test performed on hydrophilic extract of *E. grandis* wood, flavonoids, xanthones, triterpenes and saponins were detected. Among the flavonoids, the presence of leucoanthocyanidins, flavonols, flavanones and flavanonols was detected (Figure 4). Flavonoids are the largest groups of phenolic compounds and include antioxidant, antibacterial and free radical elimination properties (Carvalho et al., 2004; Dai & Mumper, 2010). Thus, it is clarified that the material has the potential to stand out in the health area, as flavonoids have a beneficial effect on health, thanks to its anti-inflammatory, anticancer and antiplatelet activities (Thilakarathna et al., 2013; Yadavalli et al., 2018).

The results presented in this work are in line with those of other phytochemical works for *Eucalyptus* species. A study conducted by Antonio (2011) with *Eucalyptus badjensis* showed the presence of flavonoids and leucoanthocyanidins, indicating that the concentration of these phenolic compounds is very significant. Lobo (2014) found in phytochemical tests with *Eucalyptus elata* the presence of important compounds of the secondary metabolism such as the group of flavonoids, leucoanthocyanidins and steroids and terpenes. In both regions, the presence of triterpenoids was detected. Araújo (2005), when performing a phytochemical study with leaves of *Terminalia brasiliensis*, verified the presence of these secondary metabolites. It is important to emphasize that plants of the genus *Terminalia* are widely known as a rich source of secondary metabolites, such as pentacyclic and glycosylated triterpenoids (Katerere et al., 2003).

Another study found in the literature corroborates this. Döll-Boscardin et al. (2010), when performing phytochemical prospection in extracts of *Eucalyptus benthamii*, verified the presence of triterpenoids. These constituents have antimicrobial and antitumoral action; however, there are reports that some of them may be toxic to the human body (Robbers et al., 1997). It can be verified in the region closest to the core the presence of flavanonols, flavanones and saponins. Flavanones were found only in the internal region of the wood, near the heartwood (Figure 4). In general, the heartwood has a higher concentration of extractives when compared to the sapwood, as its formation leads to the accumulation of extractives, making it more resistant, containing phenolic substances. Moreover, they directly influence the protection against xylophagous agents, wood color and durability (Kampe et al., 2013; Kirker et al., 2013; Nascimento et al., 2013; Kuroda et al., 2014). Studies with *E.*

camaldulensis showed biologically active metabolites such as flavonoids, saponins and hydrolysable tannins (Gambato et al., 2014). Saponins are determined by their amphiphilic behavior and ability to form complexes with steroids, proteins and membrane phospholipids (Simões et al., 2004).

Figure 4 - Phytochemical analysis of non-thermally treated *E. grandis* wood. Where: I = internal region; E = external region; D = detected; ND = not detected; NP = not performed.



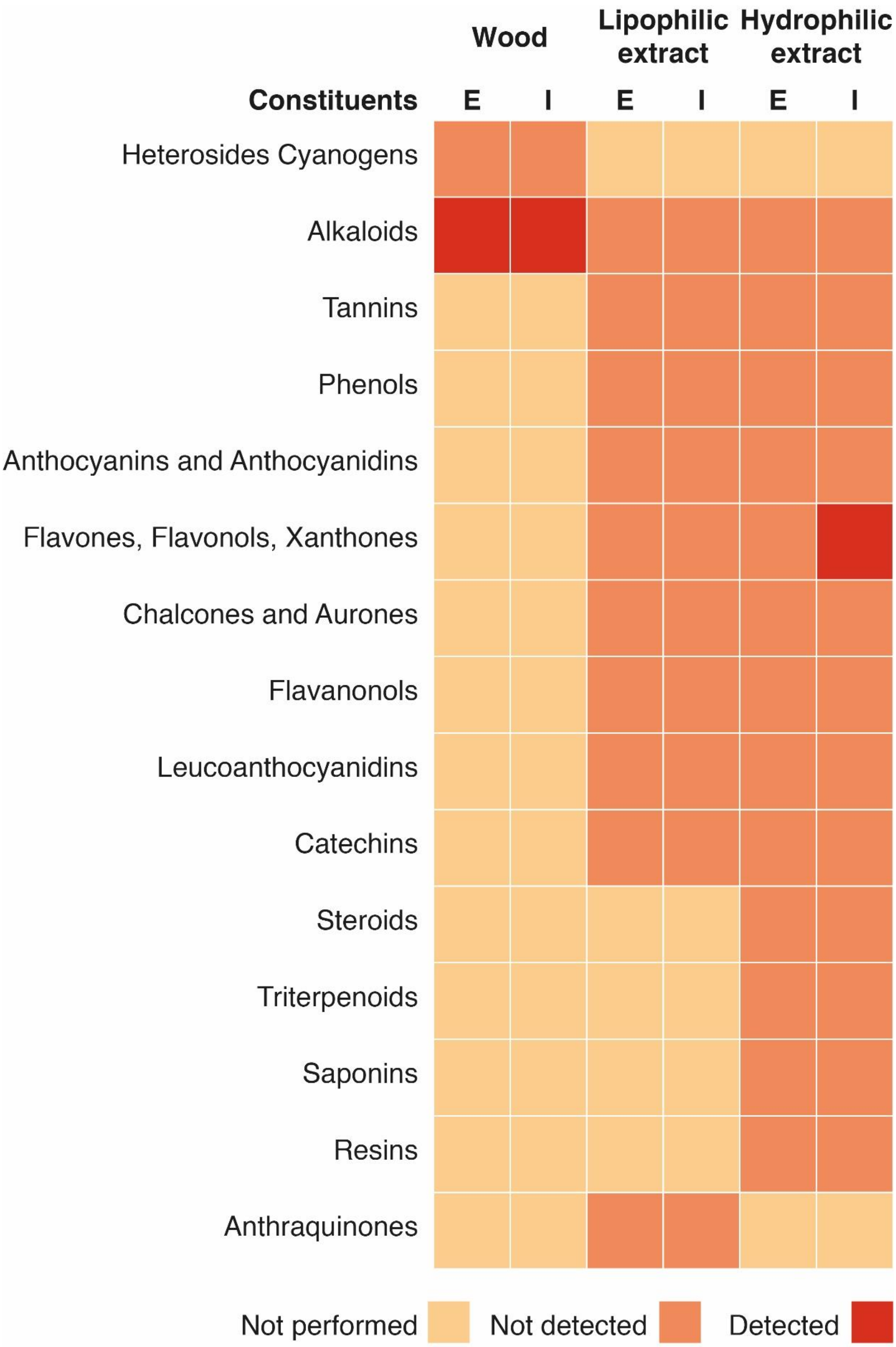
Source: The authors.

3.2 Phytochemical prospection of thermally modified *E. grandis* wood

When performing the phytochemical analysis with the thermally modified wood, the presence of alkaloids was observed in the heartwood and sapwood regions (Figure 5). The result is obtained after the appearance of flocculent precipitates after the addition of Dragendorff's reagent. With the thermal treatment, in both extracts (lipophilic and hydrophilic), most of the extractives disappeared from the wood (Figure 5). These findings are in accordance with those of Nuopponen et al. (2003), who reported that fats and waxes are the first compounds to disappear from wood after thermal treatment, followed by fatty acids and resins. Some authors, such as Esteves; Graça and Pereira (2008); Pierre et al. (2011), and Silva (2012), Zanuncio et al. (2014), show that with the increased rigor of the heat treatment, extractives decrease at temperatures above 170 °C, mainly at 240 °C. Hakkou et al. (2006), when conducting a study with thermally treated *Fagus sylvatica* (beech) wood, observed that the alterations in the contents of extractives began to be significant in the thermally treated wood above 200 °C, this behavior is due to the beginning of the decomposition of hemicellulose (Alén et al., 2002; Hakkou et al., 2006), that is, the quantity of extractives reaches a peak around 240 °C and decreases with the increase in temperature, which leads to their volatilization.

After the heat treatment, the presence of specific classes of extractives (flavones, flavonols and xanthones) in the internal region of *E. grandis* wood can be verified (Figure 5). These chemical compounds can come from the degradation of other classes of extractives, allowing their appearance after the wood is subjected to the action of heat. Among these components, flavonols and xanthones are more resistant to heat treatment. Sung et al. (2019), in a recent study, provided information about thermally treated citrus fruit peel, and their results showed that heat treatment contributed to the significant release of phenolic and flavonoid compounds present in the peel. According to Kabera et al. (2014), flavonoids are widely distributed in plants and various functions are associated with these secondary metabolites, for example, the coloring of flowers, producing the yellow, red or blue pigmentation of petals, and serving as an attraction for pollinating animals. Negi et al. (2013) highlights the importance of the pharmacological properties of xanthones and flavonoids, emphasizing the similarity of their structures and chromatographic behavior in plants. In addition, this class may have a certain connection with the coloring of the wood because, after the heat treatment, it undergoes a change in its color pattern, becoming dark.

Figure 5 - Phytochemical analysis of treated Eucalyptus grandis wood. Where: I = internal region; E = external region; D = detected; ND = not detected; NP = not performed.



Source: The authors.

4. Conclusions

The phytochemical investigation detected the presence of alkaloids, phenolic and triterpenoid compounds in the hydrophilic extract of the untreated *E. grandis* wood. Saponins were detected only in the hydrophilic extract of the internal region.

The extractives of *E. grandis* wood were influenced by the heat treatment. Almost all the extractives were volatilized, with alkaloids and flavonoids being more resistant classes and, due to the chemical reactions, a new class of extractives (flavones) was found in the thermally treated wood. Further studies are recommended to be carried out to carry out a more detailed characterization of the metabolites that resist heat treatment and understanding the action of heat on these compounds.

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