Study on the chemical composition of teak wood extracts in different organic solvents

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Teak wood (Tectona grandis Linn. F.) is known for its high natural resistance to attack by microorganisms. For this reason, teak wood is used for restoration works. This paper provides an assessment of the extraction capacity of various organic solvents and the chemical and physical-structural characteristics of extracts of teak wood with an age of 40 years. On the basis of literature data, we selected the solvents with potential synergetic activity in preservation treatments. For this purpose we used the SEM-EDX and GC+MS methods, assisted by computerized processing software, and corroborated the data obtained from these two instrumental techniques.

Keywords: Teak Wood, Composition, Organic Solvents, Extracts, SEM-EDX, GC+MS

Introduction

Wood is an organic material that is degraded by bacteria, fungi, xylophagous insects and termites (Walker et al. 1993). The use of plant extracts has introduced new approaches for protection against fungi and insects (Schultz & Nicholas 2002). They include wood extracts, which are natural products rich in bioactive compounds such as tannins, polyphenols, alcohols, aldehydes, ketones, esters, and oxygenated hydrocarbons, which are toxic for the microorganisms that degrade wood (Yatagai et al. 2002, Okanalwon & Olaoye 2020).

Regarding natural durability, expressed as the degree of physical deterioration and chemical degradation of the structural compounds under the action of biotic and/or abiotic agents, wood is affected differently along the three sections (R-radial, T-tangential, L-longitudinal – Sandu 2008).

The specificity of the wood’s resistance to the attack of fungi and xylophagous insects (anobides, termites, beetles, and marine borers) is contingent on this anisotropy. Also, the presence of an extractable organic compound rises the resistance against fungi (Vasilache et al. 2009, Hayashi et al. 2010).

It is known that wooden species with high natural durability have a wide range of uses and a much greater added value on the market. Among them, teak wood (Tectona grandis Linn F.) is renowned for its high resistance to the attack of many microorganisms (Kirk et al. 2013, Tascioglu et al. 2013, Brocco et al. 2017).

Once it is processed into an object, wood generally deteriorates and/or degrades under the influence of climatic factors and of chemical (pollution), microbiological (fungi, xylophagous insects, rodents, etc.) and radiative agents (fire and very-high temperatures, UV and gamma radiation). The two effects are the result of processes that use different mechanisms (physical-structural destruction or chemical alteration), which are regulated by the presence of one or several components with insect-fungal, hydrophobic or fire-retardant activity that originate from the native wood (natural product) or from the use of dispersed systems for mitigating or stopping the deterioration and/or degradation by means of preservation operations (Iurcovschi et al. 2017, Sandu 2008, Spiridon et al 2017).

Concerning heritage goods, we cannot overlook human impact (the anthropic factor), which through unauthorized preservation or restoration interventions, through manipulation, transport or improper storage or through vandalism, explosions, fires, and floods (accidental or willful), can lead the artefact to the state of precollapse or collapse (end of use life – Sandu 2008).

The natural resistance of wood to exogenous factors and agents depends on the species, age of the tree, origin (area of harvest), time of felling, period and manner of processing and preservation, as well as the quantity and type of the active natural principle against insects and fungi or of the one introduced by the preservation treatments (Bhat et al. 2005, Luta et al. 2006, Thulasi-das & Bhat 2007, Sandu 2008, Sandu et al. 2010, Dungani et al. 2012, Sandu et al. 2015).

As such, teak wood has a high commercial value, as it meets the requirements for its use as a wood with superior natural durability, which does not require preservation interventions (Dungani et al. 2012).

It is known that the wooden species that have low natural durability require preservation treatments when turned into final
products or later during their use life, in order to improve their performance as materials and extend their lifespan. The most common treatment procedures employ – depending on the species, the age of the tree, the age of the timber, the structural-ornamental complexity, and the size and state of the artifact – various ecological systems for preservation based on active principles anti insects and fungi, in the form of solutions of aqueous or alcoholic microdispersions or of other organic solvents. The procedures used for applying the preservative, brushing or spraying, as well as vacuum or pressure technologies, which provide high protection against insecto-fungal attacks (Bolin & Smith 2011, Lebow 2010, Sandu 2008).

According to some authors (Lin et al. 2009, Feradayoni & Hosseinihasemi 2012, Kartal et al. 2015), these active principles can include various chemical substances such as: arsenic salts, chrome salts, copper salts, zinc salts, and boron salts, which have the drawback of being expensive and toxic to the operator, owner and the environment. Moreover, these procedures required periodical re-treatments through time, which makes their use dangerous (Wang et al. 2016).

Currently, a wide range of synthetic (Luta et al. 2006, Sandu et al. 2015) or natural (Vasudeva et al. 2009) substances are used for this purpose, as aqueous or organic solutions, emulsions or nanodispersions, which on account of being applied on the surface (as protection films) or inside the wood require periodic treatments that are hard to carry out once the artefact has been executed.

Given these aspects and the insecto-fungal protective role played by a number of natural extractable compounds from various plants (including flowers and seeds) or trees (oak, acacia, chestnut, mimosa, and teak), their investigation has become a very active area of research. Indeed, there is a large number of studies on the use of substances extracted from wooden species with high natural durability, as eco-friendly preservation agents (Nakayama et al. 2001, Mburu et al. 2007, Syo-funa et al. 2012, Kirker et al. 2013, Tascioglu et al. 2013, Mohammed et al. 2016).

Many studies on teak wood are present in the literature, though most are focused on the base wood components (lignin, cellulose and hemicellulose – Hisamochi et al. 2018). Few authors were concerned with the study of the nature and composition of the extracts from wooden species that are very resistant to insect and fungal attack (Du Silva Leonardo et al. 2015, Brocco et al. 2017). In regards to teak wood, the antimi-
secto-fungal activity of, which the most representative are: 4-tert-butyl-2-fenil-fenol, 2-metilantraquimine, and 2,3-dimetil-1,4,4a,9a-tetrahidro-9,10-antracendione.

The specialized literature on treatments for preserving old processed wood using active principles extracted from teak is limited to hot water and ethyl alcohol extracts, without presenting the chemical composition of the components (Aboaba et al. 2014). In some studies the low natural durability of teak grown in plantation from Panama were investigated (Haupt et al. 2015), and the active wood extracts from different provenances was studied by GC-MS analysis (Xie et al. 2011). Moreover, the effect of some heartwood extractives was studied in order to highlight the impact on symbiotic protozoan communities and termite species (Babar et al. 2017). This paper assesses the capacity to obtain the active principles of the teak wood in extraction processes using various solvents, determining the rate of extraction and the nature of the main components with various potential. Moreover, the purpose is the optimisation of the extraction conditions by using different organic solvents, alone or in mixture, in order to determine the efficacy of the extraction processes (quantity and number of extracted compounds). The experimental data highlighted the difference from the nature of the extracted compounds and their quantity for each studied extraction system.

Materials and methods

We used thin teak wood veneer (Tectona grandis L.f.) with no anatomical defects (knots, gnars, торсades, etc.), with an age of 40 years. The samples were provided by a furniture factory in Iasi, Romania and imported from Thailand. Teak wood veneer weighing 400 g were dried and mechanically divided by chopping, and subsequently ground in a ceramic ball mill, in order to obtain particles below 0.1 mm. For each sample 10 g were weighted, and the powder was dispersed into a series of preset solvents, from which the active components were extracted in the form of organic mixture. Organic solvents of the p.f.a. (pure for analysis) group were used for extraction, specifically: ethyl alcohol (96%), isopropyl alcohol (99.7%), acetone (99%), and acetone-isopropanol (60:40 ratio) co-solvent mixture.

The experiment consisted in 12 Erlenmeyer screw-cap flasks with each of the three solvents and the acetone-isopropanol mixture. In each flask 50.0 mL of solvent was added to 10 g of teak powder. The flask was shaken every hour for 60 sec, for a period of 10 h. The dispersion was subsequently decanted for 14 h and then filtered with a Büchner funnel. One mL of filtrate was taken and its solvent evaporated on a watch glass that was weighted beforehand (m0). The watch glass and the filtrate were weighted (m1), and after the evaporation of the solvent (normal conditions for 24-72 h, depending on solvent) the watch glass with the extract was weighted (m2). The concentration of the organic extract was determined according to the relation (ASTM International 2001 – eqn. 1):

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c(%) = 100 \frac{m_1 - m_0}{m_2 - m_0}
\]

For each solvent the evaluation was done as the arithmetic average of the three values obtained by evaporation.

The extract was further analyzed by means of scanning electron microscopy, coupled with X-ray diffraction (SEM-EDX) and liquid chromatography, coupled with mass spectrometry (GC-MS).

The scanning electron microscopy produced microphotographs showing the morphology of the longitudinal and radial sections of the teak wood used for obtaining the powder and the extract, and the EDX spectra provided the elemental composition in mass percentages of the main chemical components. For this purpose we used a scanning electron microscope (SEM), model VEGA II LSH (Tescan, Czech Republic), coupled with an EDX detector model Quantax QX2 (Bruker/Roentec, Germany). The analysis of the samples was performed at a magnification of 200× to 2500×, with an acceleration tension of 30 kV, and a work pressure lower than 1×10⁻³ Pa. The SEM image was acquisitioned using secondary (SE) and backscattered electrons (BSE).

The analysis of the chemical derivatives in the extracts was performed by GC (Gas Chromatography) technique (model 6890 N, Agilent Technologies Inc., CA, USA) in conjunction with an Agilent 5975 mass spectrometer (Sandu et al. 2003). The chromatographic conditions were differentiated according to the devices involved in the chromatography, as follows: the oven with a temperature 50 °C at the onset, then raised at a rate of 15 °C per minute firstly to 150 °C and then to 320 °C, which was retained for 15 min; the HP-5MS column, measuring 30 mm × 0.25 mm × 0.25 μm, with flow rate of 1.0 mL min⁻¹; the sample injection was splitless (the entire sample) at a temperature of 270 °C. The mass spectrometry conditions were an EI (electronic ionization) source with an electron bombardment energy of 70 eV, an ion source temperature of 230 °C, and transfer line temperature of 280 °C. The compounds were identified by comparing the MS (mass spectrometry) spectra to the National Institute of Standards and Technology library (NIST 2018) with retention time and indexes.

In this experiment, for the three components of the GC technique, the following parameters were used: (i) oven, which started from an initial temperature of 50 °C and reached a maximum temperature of 320 °C in 44.92 min; (ii) syringe for the splitless injection of sample (the entire sample...
is injected once, in order to obtain a higher concentration and a better selection of the component substances) at an initial temperature of 270 °C, a pressure of 7.12 psi, with the flow rate varying for 2 min from 20.0 to 24.1 mL min⁻¹; (iii) column – product separation was achieved by using the carrier gas helium with a flow rate of 1 mL min⁻¹.

Detection was achieved using an acquisition system, with the information processed according to an S975 Agilent database, in scan mode, with the mass unit from 30.0 to 550.0.

Results and discussion
To identify the main organic derivatives from teak wood that account for its great resistance to insect and fungal attack, we initially resorted to a wide range of solvents. After a series of preliminary tests on their extraction efficiency, only three solvents were selected (ethanol, isopropanol and acetone), along with the acetone-isopropanol mixture, which were used in the selective extraction of the organic compounds with insecto-fungicidal activity from teak wood.

Tab. 1 shows that the acetone-isopropanol mix has the greatest extraction yield, followed by acetone, ethyl alcohol and isopropanol, respectively. This result was expected, as the available literature shows that acetone extracts better than ethyl alcohol (Deng 2006), and among alcohols, isopropanol is miscible in water and nonpolar solvents, without evaporation traces left behind, making it the most recommended solvent for extraction protocols (Burlage 1947, Reeve et al. 1979, Yaws 1999, Green & Wittcoff 2003).

SEM-EDX analysis was first conducted on the two sections – radial and longitudinal – of the teak wood from which the powder was obtained, in structurally representative areas, for which the SEM microphotographs were made and incorporated into the EDX spectra (Fig. 1a, Fig. 1b). We continued with the analysis of the films of evaporated extract from the four organic solutions (Fig. 1c-f). The SEM-EDX analysis allowed assessing the texture, morphology and distribution of the anatomo-structural components of the wood fibers in the two sections, alongside the uniformity and morphology of the mixture of products from the evaporated extracts. On the basis of the EDX spectra, Tab. 2 presents the elemental composition of the teak wood in the two sections and for the four organic extracts.

We notice that in the radial section the microphotograph allowed a detailed rendering of the anatomo-structural components, with the parallel orientation of the fibers and of the resinophoric or gumiphoric channels, alongside the parenchymatous reserve cells with thickened and lignified walls, whereas in the longitudinal section only oriented arrangements of cellulose fibers and their defects were visible.

Conversely, the films of organic compounds obtained by evaporating the extracts appear as shiny resins, weakly colored in yellowish-brown, with a uniform arrangement of certain components that solidified differently when the solvent evaporated, in the form of glomerules and microalveoli, some of which were ruptured.

Tab. 2 shows that the elemental composition in gravimetric percentages (%) differs in the two sections for all the elements identified, except for magnesium, which has approximately equal concentrations, and for chlorine, which is present in both sections of the wood sample. In the two sections (R) and (L) there is an inversion of carbon and oxygen, specifically about two times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of silicon and four times diminishing of 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ars/poly saccharides, fatty acids, esters, phenols, terpenes, oils, and tannins had a greater capacity of selective extraction. Ethyl alcohol and acetone extracted a large number of derivatives, but acetone and the acetone-isopropyl alcohol mix had the highest efficiency. The number of organic derivatives identified by GC-MS in the extracts obtained using the four solvent systems decrease as follows: acetone (8 derivatives), ethyl alcohol (8 derivatives), acetone-isopropanol mix (4 derivatives), and isopropanol (2 derivative); this was unexpected, given that the last is considered a solvent with very high emollience and washing power for foulants, and which does not leave evaporation traces, but which increased the efficiency of the extraction in the mixture with acetone.

Of the eight derivatives extracted in acetone (Tab. 3, Fig. 2), four are also found in the ethyl alcohol extract (Tab. 4, Fig. 3), four in the acetone and isopropanol alcohol mix (Tab. 5, Fig. 4), which had the highest extraction efficiency and a single organic derivative, namely 9,10-Anthracenedione, 2-methyl- (Fig. 5) extracted in isopropanol, which can be found in all the extracts.

Tab. 3 presents the organic derivatives extracted from the teak wood in acetone, identified by GC-MS, according to the mass spectra from Fig. 2.

Tab. 4 presents the eight organic compounds from teak wood extract in ethyl alcohol, identified according to the mass spectrum displayed in Fig. 3. Four of the compounds were also found in acetone (Phthalic anhydride, 2H-Naphtho [2,3-b]pyran-5.10-dione, 2,2-dimethyl, 9,10-Anthracenedione, 2-methyl- and 2-(Hydroxymethyl)anthraquinone), but at somewhat lower minutes: 7.761, 14.812, 15.708, 19.769.

Tab. 5 presents the organic derivatives extracted from the teak wood in the acetone-isopropyl alcohol mixture (6/4 vol.),

![Mass spectra of the eight organic derivatives identified in the teak wood extract in acetone. (a): Phthalic anhydride; (b): 1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1αR-(1α,alpha.,4α.beta.,7alpha.,7αa)] ; (c): Naphthalene, 1,2,4α,5,6,8α-hexahydro-4,7-dimethyl-1-(methylalyl)-; (d): Naphthalene, 1,2,4α,5,6,8α-hexahydro-4,7-dimethyl-1-(methylalyl)-, [1α.alpha.,4α.alphα.,8α.alphαa]; (e): 9,10-Anthracenedione, 2-methyl-; (f): 9,10-Anthracenedione, 2-methyl-; (g): 2-(Hydroxymethyl)anthraquinone.](Image)
identified by GC-MS, according to the mass spectra from Fig. 4.

Fig. 5 presents the two derivatives, 2H-Naphtho [2,3-b]pyran-5,10-dione, 2,2-dimethyl- and 9,10-Anthracenedione, 2-methyl- extracted in isopropyl alcohol, identified from the mass spectrum at minutes 14.812 and 18.094, respectively, which were also found in the ethanol alcohol extract, but at different times (17.176 and 18.175).

Of the eight derivatives extracted in acetone, identified according to the mass spectra, four were found in the ethanol alcohol extract (Phthalic anhydride – 2H-Naphtho [2,3-b]pyran-5,10-dione, 2,2-methyl-, 9,10-Anthracenedione, 1-methyl-), but at much higher minutes (9.208, 17.176, 18.175 and 21.740). Only four derivatives (Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(methylethyl)-(1.alpha.,4a.alpha.,8a.alpha)-, alpha.-Cadinol-, 9,10-Anthracenedione, 2-methyl- and 2-(Hydroxymethyl)anthraquinone-), but at much higher minutes (9.208, 17.176, 18.175 and 21.740).

No. Organic derivative Minute Spectrum
1 Phthalic anhydride 9.208 Fig. 3a
2 .alpha.-Cadinol- 10.034 Fig. 3b
3 2H-Naphtho [2,3-b]pyran-5,10-dione, 2,2-methyl- 17.176 Fig. 3c
4 [1,1’-Biphenyl]-2-ol, 5-(1,1-dimethylthyl)- 17.331 Fig. 3d
5 9,10-Anthracenedione, 1-methyl- 17.981 Fig. 3e
6 9,10-Anthracenedione, 2-methyl- 18.175 Fig. 3f
7 2-(Hydroxymethyl)anthraquinone- 21.740 Fig. 3g
8 Butyl(2-chlorocyclohexyl) methyl phthalate 28.817 Fig. 3h

Among the derivatives extracted using the four selected organic solvent systems, most have good insecto-fungicidal activity, with the most active being the anthraquinones (9,10-Anthracenedione, 1-methyl-; 9,10-Anthracenedione, 1-methyl-; 2(Hydroxymethyl)anthraquinone-; 2-Methyl-anthraquinone), naphthalenes (Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(methylethyl)-, and ethyl alcohol two (Butyl(2-chlorocyclohexyl) methyl phthalate and [1,1’-Biphenyl]-2-ol, 5-(1,1-dimethylthyl)-), which are not found in other extracts, but which have a good insecto-fungicidal activity (Luta et al. 2006).
The mass spectra of the four organic derivatives identified in the teak wood extract in the acetone-isopropyl alcohol mixture (6/4 vol.). (a): Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(methylthethyl)-(1 alpha,4a,8a-alpha); (b): alpha-Cadinol; (c): 9,10-Anthracenedione, 2-methyl; (d): 9,10-Anthracenedione, 1-methyl.

Fig. 5 - Mass spectra of the organic derivatives identified in the teak wood extract in isopropyl alcohol. (a): 2H-Naphtho [2,3-b]pyran-5,10-dione, 2,2-dimethyl; (b): 9,10-Anthracenedione, 2-methyl.

Following conclusions can be drawn:
- teak wood is part of the group of exotic species that are very resistant to biotic attacks, containing a large number of bioactive principles with insect-fungal activity, which so far are not sufficiently known;
- for their extraction from fine powder of old teak wood from 40-year-old trees, acetone selectively extracts three derivatives that are not found in other extracts (1H-Cycloprop[e]azulene, decahydro-1,1-dimethyl-4-methylene-, [1aR-(1a,1-dimethyl-4-methylene-2H-Naphtho [2,3-b]pyran-5,10-dione, 2,2-dimethyl-) is also found in the ethyl alcohol extract, and the second (9,10-anthracen, 2-methyl-) is found in all four extracts;
- acetone selectively extracts three derivatives that are not found in other extracts (1H-Cycloprop[e]azulene, decahydro-1,1-dimethyl-4-methylene-, [1aR-(1a,1-dimethyl-4-methylene-2H-Naphtho [2,3-b]pyran-5,10-dione, 2,2-dimethyl-) is also found in the ethyl alcohol extract, and the second (9,10-anthracen, 2-methyl-) is found in all four extracts;
- of the compounds extracted using the four selected organic solvent systems, the most active are: anthraquinones (9,10-Anthracenedione, 1-methyl; 9,10-Anthracenedione, 1-methyl; 2-(Hydroxymethyl)anthraquinone; 2-Methyl-anthraquinone), naphthalenes (naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(methylthethyl)- and alpha-Calacoren), and ethyl alcohol two (butyl (2-chlorocyclohexyl) phthalate methyl and (1,1'-diphenyl)-2-0H, 5-(1,1-dimethylthethyl));
- of the compounds extracted using the four selected organic solvent systems, the most active are: anthraquinones (9,10-Anthracenedione, 1-methyl; 9,10-Anthracenedione, 1-methyl; 2-(Hydroxymethyl)anthraquinone; 2-Methyl-anthraquinone), naphthalenes (naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(methylthethyl))- and alpha-Calacoren), and ethyl alcohol two (butyl (2-chlorocyclohexyl) phthalate methyl and (1,1'-diphenyl)-2-0H, 5-(1,1-dimethylthethyl));
- of the four extracts the most indicated to be used in the preservation treatments of old wooden artifacts, with active biotic attack, is the concentrated extract in ethyl alcohol, which has the lowest toxicity and which extracts the chlorinated compound, the most effective in the bio-icide treatments.

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