Characterization of Guayule Resin as a Natural Wood Preservative: Carrier System and Leaching Rate Analysis

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The environmental and health concerns of traditional preservatives have led to investigations into an effective solution for protecting wood products with preservatives from natural sources. Guayule resin, derived from the *Parthenium argentatum* plant, has been reported to be effective as a wood preservative due to its bioactive properties. However, its high viscosity, limited penetration, and unknown leaching behavior may affect its durability and efficiency. This study investigated guayule resin concentration and solvent carrier efficacy. Yellow poplar and southern pine specimens were treated with guayule resin at four concentrations in three different solvents including acetone, ethyl acetate, and toluene. The leaching test was performed according to the AWPA E11-16 standard. Significant interaction between solvent type and concentrations of guayule resin were found in both species in which a lower mass loss was observed when 5% concentration of guayule combined with toluene as compared to any other guayule concentrations in southern pine. On yellow poplar specimens, 0.5% concentration of guayule in combination with toluene exhibited lower mass loss as compared to any other guayule concentration. In conclusion, toluene was found to be the best performing polar carrier system for guayule resin, considering its functionality and dose-dependence in both species.

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Keywords: Guayule resin; Leaching resistance; Natural wood preservative; Polar solvent; Southern pine and yellow poplar

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INTRODUCTION

Wood is one of the most versatile, renewable, and eco-friendly materials on the planet. It is globally essential for a wide range of structural and non-structural applications. The necessity of wood for construction, furniture, and numerous other applications is due to its low carbon footprint, recyclability, light weight, and aesthetic appeal (Shmulsky and Jones 2019). Although wood has several advantages, its chemical composition (lignin, cellulose, and hemicellulose) makes it highly susceptible to biological degradation, particularly under humid conditions (Broda 2020). Wood decay has been shown to reduce the lifespan and structural integrity of wood products, leading to an increase in replacement costs as well as a decrease in forest resources (Shmulsky and Jones 2019). Thus, effective wood protection strategies are needed to ensure long use life for wood products.

Over the years, various wood protection methods have evolved including wood preservative treatment, using chemicals to prevent wood deterioration, and wood modification involving application of high-temperature treatments to activate chemical compounds found in wood cell walls (Welzbacher and Rapp, 2007; Ahmed *et al.* 2012; Khademibami and Bobadilha 2022). Synthetic preservatives, such as the oil-borne chemicals creosote and pentachlorophenol, as well as water-borne chemicals including chromated copper arsenate, copper zinc arsenate, alkaline copper quaternary, copper azole, and borates, have been effective against termites, fungi, bacteria, marine borers, fire, and weathering (Nakayama *et al.* 2001; Richardson 2002; Groenier and Lebow 2006). However, many of these traditional preservatives have been restricted in use due to their toxic heavy metal content, causing adverse environmental and human health effects (Gerengi *et al.* 2014). Thus, there is a need for environmentally friendly alternatives, derived from natural compounds belonging to both plants and animals (Nakayama *et al.* 2001; Singh and Singh 2012; Broda 2020).

Secondary metabolites including alkaloids, flavonoids, phenolics, and tannins have been shown to play a role in protection of plants against herbivores, microbial diseases, and various forms of abiotic stress (Mazid *et al.* 2011). In addition, plant extracts have shown antifungal activities that can be beneficial in protecting wood products against decay (Bhagat *et al.* 2014; Broda, 2020). Essential oils, tannins, propolis, wood extractives, and chitosan are natural substances that belong to either plant or animal that have demonstrated promising results in protecting different wood species (Tascioglu *et al*. 2013; El-Gamal *et al.* 2016; Broda 2020). However, their practical application is often limited by issues including leaching rate and volatility (Singh and Singh 2012; Cai *et al*. 2019). To address these challenges, the extractives grafting method was developed for treating wood specimens through impregnation with laccase enzyme and the desired extractive (Fernández-Costas *et al.* 2017; Cai *et al.* 2019). However, this approach has been shown to be limited to specific technology and equipment that may not be feasible for industrial application (Cai *et al.* 2019). Thus, development of effective sustainable wood preservatives from natural compounds remains a significant challenge.

Despite these obstacles, plant-based biocides are gaining attention on account of their environmental benefits, with guayule resin emerging as a potential option (Moharreri *et al.* 2017). Guayule (*Parthenium argentatum*) is a drought-resistant, semi-arid shrub plant that is domesticated in the Chihuahuan desert located in northern Mexico with a range extending into the southwestern United States (Mooibroek and Cornish 2000). Previously, the main product of guayule resin was latex rubber; however, its by-products resin and bagasse have become favorable for various commercial applications.

Guayule resin composition varies with factors such as plant age, harvesting techniques, and extraction methods, but it consistently contains a complex mixture of bioactive compounds, including terpenoids, polyphenols, fatty acids, sesquiterpene esters (*e.g.,* guayulins and argentatins), and other organic compounds (Schloman *et al.* 1986; Dehghanizadeh and Brewer 2020). Essential oils extracted from guayule leaves mainly consist of monoterpenes and sesquiterpenes, while its seeds contain oil abundant in fatty acids and proteins (Dehghanizadeh *et al.* 2020). Guayule resin`s possible functions, including biocontrol agents, insecticides, antimicrobials, antifungals, adhesives, coatings, and composite materials, are facilitated by the above-mentioned complex compounds (Nakayama *et al.* 2001; Moharreri *et al.* 2017; Dehghanizadeh and Brewer 2020; Dehghanizadeh *et al.* 2023; Entsminger *et al.* 2023). For instance, Bultman *et al.* (1998)

found that guayule resin provides strong resistance for wood composites against termites. Likewise, pine and birch species treated with guayule resin demonstrated significant protection against fungi such as *Gloeophyllum trabeum* (brown-rot) and *Trametes versicolor* (white-rot), as well as termites such as *Coptotermes formosanus* (Bultman and Schloman 1993; Nakayama *et al.* 2001; Dehghanizadeh *et al.* 2021).

The resin's termiticidal activity is mainly linked to specific terpenoids including argentone, panthenol, and incanilin (Gutiérrez *et al.* 1999). More specifically, argentone has been identified as the most potent antifeedant and toxicant among these compounds (Gutiérrez *et al.* 1999). The mode of action of guayule resin in eliminating termite feeding is linked to the disruption of their nervous and respiratory systems, leading to paralysis and death (Bultman *et al.* 1991). Significant reductions in termite feed intake and an increase in mortality rate were observed in termites subjected to guayule resin (Bultman *et al.* 1991; Gutiérrez *et al.* 1999).

Although the biological activity of wood preservatives against wood decay agents is crucial, their retention and penetration rates are necessary properties to determine their efficacy (Shmulsky and Jones 2019) According to Entsminger *et al.* (2023), guayule is highly viscous, sticky, water-insoluble, and semi-solid at room temperature resulting in difficulty in its application and penetration. Unlike other plant resins, guayule resin also contains low-molecular-weight rubber, which might impact its penetration as well as adherence activities to wood substrates (Thames and Wagner 1991). Guayule resin wood penetration can be facilitated using carrier solvents and/or heating methods (Entsminger *et al.* 2023). Preheating the resin to approximately 70 °C reduces its viscosity, transforming it into a free-flowing liquid suitable for wood preservative applications (Bultman *et al.* 1991).

Guayule resin contains a complex mixture of compounds with varying molecular weights and polarities, which requires careful selection of solvents to ensure optimal resin solubility. It is well-documented that polar solvents are particularly effective in dissolving the resin during solvent extraction (Cornish *et al.* 2013; Pearson *et al.* 2013). Preliminary studies also indicate that polar solvents such as alcohol, esters and ketones can effectively dissolve guayule resin and facilitate its penetration into wood (Dehghanizadeh and Brewer 2020). For example, 5% concentrations of resin in acetone reduced guayule resin viscosity and increased its adhesive properties (Entsminger *et al.* 2023). Furthermore, undiluted resin heated at 70 °C and resin combined with 75%, 50%, and 25%, w/v of acetone have shown promising results as a preservative (Bultman *et al.* 1991). A combination of 10 wt.% of the ethanol with acetone and acetone with chloroform was successfully tested as a bio-insect repellant (Dehghanizadeh 2022). Additionally, the combination of the acetone and pentane with 52% to 97% concentrations of guayule resin resulted in a decrease in termites (*Reticulitermes spp*.) in southern pine specimens (Nakayama *et al.* 2001). Although high concentrations of guayule resin (greater than 10%) have shown promising results as a wood preservative, these concentrations are not yet economically viable for commercial applications.

Acetone has been chosen as a carrier solvent for guayule resin due to its polar property, which allows it to effectively dissolve a wide range of resin constituents, including terpenoids, fatty acids, lipids, and pigments. It has also relatively low boiling points that can contribute to efficiently removing solvent and to recovery of the resin (Cornish *et al.* 2001). Moreover, methanol was used to dissolve 6.4 to 36.1 ppm of the argentatins A and B, which are the components of guayule resin that have been found to inhibit insect activities (Céspedes *et al.* 2001). Cornish *et al.* (2013) suggested that acetone and ethanol (polar solvents) were effective in the extraction of resin from guayule, while hexane, cyclohexane, and chloroform (non-polar solvents) can be used in subsequent extraction for efficient separation and quantification of both resin and rubber fractions without cross-contamination.

Another critical factor to determine the effectiveness of wood preservatives is the leaching rate. A persistent challenge limiting the widespread adoption of biobased preservatives, such as guayule resin, is to ensure active compounds do not leach out of the treated products rapidly under challenging conditions such as high humidity. Strategies such as incorporation of natural preservatives into polymer matrices or hydrophobic additives (Dehghanizadeh *et al.* 2021), enzymatic grafting with laccase (Cai *et al.* 2019), and supercritical CO_2 extraction (Cornish *et al.* 2001) have previously been shown to enhance retention but remain limited by technological and practical constraints (Fernández-Costas *et al.* 2017). Bultman and Schloman (1993) reported the low percentage leaching rate (99% resin retention over 193 days) of guayule resin in pine wood discs under controlled seawater conditions when full-strength resin without solvents was used. Although these results indicate the potential of the resin to be retained in wood over time, it is essential to evaluate its leaching behavior when diluted with various solvents. Furthermore, polar solvents such as acetone, ethanol alone, or in combination have shown promising results in reducing the viscosity of guayule resin as well as increasing penetration into wood specimens. To date, there is no clear understanding of the efficacy of solvents and subsequent concentrations to maximize its flow into wood cells. This creates the need to identify the optimal carrier system that increases the resin's preservative performance and maximizes treated product markets and applications.

In this study, it is hypothesized that determining the most effective solvent and concentration of resin can result in an increase in uptake/flow of guayule resin and decrease in leaching rate in both hardwood and softwood species. Therefore, the main objective of this study was to develop an optimal guayule resin formulation in a polar solvent as a wood preservative. Specifically, the goals were to identify carrier systems that can enhance resin penetration and durability while minimizing environmental impact and maximizing preservative efficacy. A further goal was to assess the leaching potential of guayule resin to ensure effective retention within different wood types for long-term protection.

EXPERIMENTAL

Material Preparation

Yellow poplar (*Liriodendron tulipifera*), as a representative of a hardwood species, and southern pine (*Pinus spp*.), which is a softwood species, were used in this study. Defect-free, kiln-dried sapwood specimens from these two species were prepared at the Department of Sustainable Bioproducts at Mississippi State University. A total of 624 specimens (312 from each species) were prepared to the dimensions of 1.9 cm × 1.9 cm × 1.9 cm (radial × longitudinal × tangential), according to the procedure presented in AWPA Standard E11-16 (2024) for the leaching test. The guayule resin used in this study to prepare the treatment solutions was received from the United States Department of Agriculture-Agriculture Research Service (USDA-ARS). Solvent candidates used in this study were: acetone (≥99.5%, 58.08 g/mol), butanol (≥98.5%, 74.12 g/mol), dimethyl sulfoxide (DMSO) (≥99.5%, 84.17 g/mol), ethanol (≥99.5%, 46.069 g/mol), ethyl acetate (≥99.5%, 88.106 g/mol), isopropanol (≥99.0%, 60.10 g/mol), methanol (99.8%, 32.04 g/mol), and toluene (≥99.5%, 92.14 g/mol). All of the solvents were purchased from Fisher Scientific (Hampton, New Jersey, USA).

Carrier System Selection and Solubility Testing

To choose the best carrier system for guayule resin, nine solvents for initial testing were selected based on the previous findings and hypotheses of this study. These solvents included acetone, butanol, chloroform, dimethyl sulfoxide (DMSO), ethanol, ethyl acetate, isopropanol, methanol, and toluene.

The different characteristics of these nine solvents including flammability, toxicity, biodegradability, odor, solubility of guayule resin in these solvents and environmental impact are shown in the Table 1. These properties were considered according to the environmental and practical suitability of each solvent. Solubility tests were performed by stirring 5% or 10% concentrations of guayule resin in each solvent on a hot plate at 38 to 50 °C for 5 min, after that the mixture was left at room temperature for 24 to 72 h to achieve maximum solubility. Solubility was determined by visual evaluation in four categories, which included 1) completely or highly soluble (no visible particles) which means that the mixture between solvent and guayule resin was complete without any separate phase, 2) soluble which means that the mixture between solvent and guayule resin had little residue. 3) partially soluble (presence of undissolved particles) which means that there were two separate phases, in which the supernatant belonged to the dissolved phase of solvent and guayule resin, and 4) not soluble or insoluble (resin largely undissolved) and the guayule resin and solvents were completely separated. The above-mentioned categories are illustrated in Fig. 1. The other visual evaluations that were considered included the color of solution, amount of residue, and the consistency of the solution.

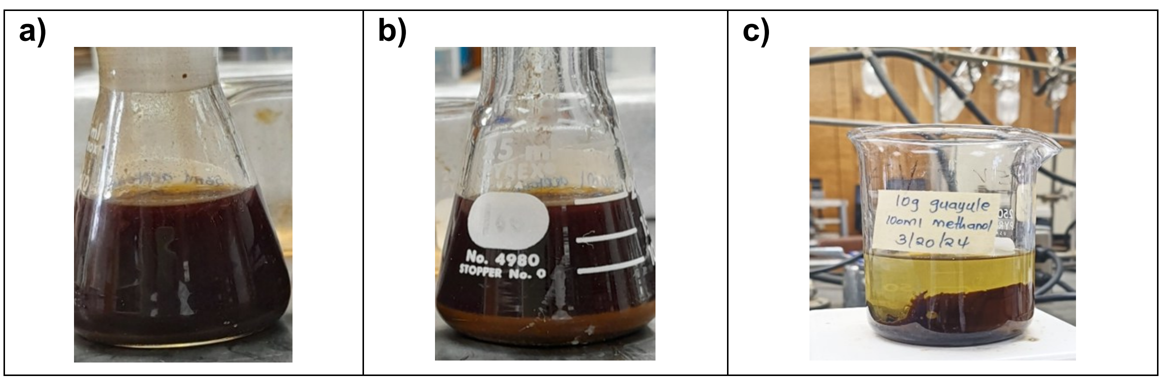

Fig. 1. Demonstration of solubility of guayule resin in different solvents categorized as a) highly soluble, b) partially soluble and c) insoluble

Guayule Resin Treatment Solutions and Treatment Layout

Based on the results from carrier system selection and solubility testing, treatment solutions were prepared with guayule resin in four different concentrations of 0.5%, 1%, 3%, and 5% (w/v) in the pure solvents of acetone, ethyl acetate, and toluene. Guayule resin was weighed according to the required concentration and then mixed with 100 mL of each solvent. For instance, 1 g of guayule resin was dissolved in 100 mL of pure solvent to reach 1% solution. The fully dissolved solution was later transferred into tightly sealed, labeled containers and then stored at room temperature until the wood treatment application.

Table 1. Solvent Characteristics for Initial Testing of Guayule Resin Carrier System

Solvent	Flammability[1] (NFPA 704)	Toxicity[2] (GHS)	Biodegradability[3] (OECD)	Odor[4]	Volatility[5] (Vapor Pressure)	Solubility[6] (Based on the OECD)	References (Application)	Environmental Impact	Environmental Implications	Common Uses
Acetone	3 (High)	Warning (H225)	Readily biodegradable	Strong sweet	Very high (180 mmHg at 20°C)	Soluble	Cornish *et al.* 2001 Nakayama et al. 2001; Spano *et al.* 2018; Entsminger 2024	Biodegradable	Low persistence, but high volatility	Solvent, cleaning, nail polish
Butanol	3 (High)	Danger (H225, H370)	Readily biodegradable	alcoholic-like	low (9.8 mmHg at 20°C)	Soluble	Thames and Kaleem 1991	Low	Low persistence, high toxicity to aquatic life	Laboratory solvent
Chloroform	0 (Minimal)	Danger (H351, H331)	Poor	Pleasant sweet	Moderate (160 mmHg at 20°C)	Soluble	Luo *et al.* 2019 Dehghanizadeh 2022	Environmental hazard	Persistent, toxic, potential groundwater contaminant	Laboratory solvent, anesthetic
Dimethyl Sulfoxide (DMSO)	3 (High)	Warning (H225)	Not readily biodegradable.	Odorless	0.41 mmHg) at 20 °C (68 °F)	Partially soluble	Foster and Thames 1999	Low	Low persistence, low to moderate aquatic toxicity	Pharmaceutical, solvent
Ethanol	3 (High)	Warning (H225)	Readily biodegradable	Strong alcoholic	High (44.6 mmHg at 20°C)	Soluble based on the LR/it is not pure/Partially soluble	Suchat *et al.* 2012; Cornish *et al.* 2013; Spano et al. 2018 Dehghanizadeh, 2022	Biodegradable	Low persistence in the environment, low toxicity	Cleaning, disinfecting, fuel
Ethyl Acetate	3 (High)	Warning (H225)	Readily biodegradable	Sweet fruity	High (97 mmHg at 20°C)	Soluble	Banigan *et al.* 1982; Schloman *et al.*1983 Jara *et al.* 2019	Moderate	Low persistence, low to moderate aquatic toxicity	Solvent, nail polish remover
Isopropanol	2 (low)	Danger	Readily biodegradable	alcoholic-like	low (33 mmHg at 20°C)	Partially soluble	Suchat 2012 Rousset *et al.* 2023	Biodegradable	Aqueous solution has high mobility in soil, low toxicity	Cleaning, disinfecting, solvent
Methanol	3 (High)	Danger (H225, H331, H370)	Readily biodegradable	Mild alcoholic	High (97 mmHg at 20°C)	Insoluble	Dehghanizadeh 2022 Céspedes *et al.* 2001	Environmental hazard	Low persistence, high toxicity to aquatic life	Industrial solvent, fuel
Toluene	3 (High)	Danger (H225, H361)	Poor	Strong aromatic	High (28.4 mmHg at 20°C)	Highly soluble	Dehghanizadeh *et al.* 2020; Wagner and Parma 1988	Environmental hazard	Low persistence, low to moderate aquatic toxicity	Paints, adhesives, solvents

[1] Flammability (National Fire Protection Association's (NFPA 704): [3]*(High):* Indicates serious flammability hazard; can ignite at ambient temperatures. [1]*(Low):* Indicates slight flammability hazard. [0]*(Minimal):* Indicates minimal flammability hazard.
[2] Toxicity (GHS - Globally Harmonized System): *Warning (H225):* Highly flammable liquid and vapor. *Danger (H225, H361):* Highly flammable liquid and vapor, suspected of damaging fertility or the unborn child. *Danger (H225, H304):* Highly flammable liquid and vapor, may be fatal if swallowed and enters airways. *Danger (H351, H332):* Suspected of causing cancer, harmful if inhaled. *Danger (H331):* Toxic if inhaled. *Danger (H370):* Causes damage to organs.
[3] Biodegradability (OECD): *Readily biodegradable:* Easily broken down by microorganisms. *Poor:* Not easily broken down, persistent in the environment.
[4] Odor As provided by fisher Scientific.
[5] Volatility (Vapor Pressure): *High:* Solvent easily evaporates at room temperature. *Very high:* Solvent evaporates very quickly. *Moderate:* Noticeable evaporation rate, but slower than high volatility solvents.
[6] Solubility: *Soluble:* Solvent dissolves guayule resin effectively. *Partially soluble:* Solvent dissolves guayule resin to a lesser extent. *Insoluble:* Solvent doesn't dissolve the guayule resin

Control treatments were water (untreated), acetone, ethyl acetate, or toluene only. The four control solvents were not prepared at different concentrations, and therefore those solvents that contained guayule resin and relevant concentrations were combined to be statistically comparable with control groups. A total of 16 treatments were prepared, including 4 control groups, and the rest of the 12 belonging to three solvents (acetone, ethyl acetate, and toluene) that were combined with guayule resin at four different concentrations (0.5%, 1%, 3%, and 5%). All treatments, including control groups solvent and guayule resin concentration treatment combinations, are listed and described in Table 2.

Table 2. Illustration of Wood Specimens' Treatment Groups

	Treatment Number	Treatment Type	Concentration of Guayule Resin (%)
Main treatments	1	Guayule in acetone	0.5
	2		1
	3		3
	4		5
	5	Guayule in ethyl acetate	0.5
	6		1
	7		3
	8		5
	9	Guayule in toluene	0.5
	10		1
	11		3
	12		5
Control	13	Acetone	No guayule resin (pure solvent)
	14	Ethyl acetate	
	15	Toluene	
	16	Water	

Vacuum Impregnation of the Wood Sample

Wood specimens were oven-dried at 103 °C for 48 h, and subsequently their dry mass and dimensions were recorded with a digital caliper and balance (accuracy: ±0.1 mg, Mettler Toledo ME103TE/00). The dried specimens were then impregnated with the guayule resin solutions under vacuum (>28 mmHg) for 20 min, followed by 15 min atmospheric pressure. The excess solution was wiped off, and the specimens were reweighed and air-dried for 48 h to ensure optimal fixation.

Mass Gain

The amount of the impregnated preservatives retained in treated wood specimens was measured in terms of mass gain using Eq. 1,

$$Mass\ gain = \frac{m_2 - m_1}{m_1} \times 100 \qquad (1)$$

where m_2 is the dry mass(g) after treatment and m_1 is the dry mass (g) before treatment.

Resistance to Leaching

To evaluate the efficacy of guayule resin for long-term protection, the leaching resistance of treated and control specimens was performed according to AWPA E11-16 standards (2024). From each treatment (Table 2), 24 blocks were selected, and then they were divided into four groups that each contained six replicates. Of those four groups, three were assigned for leaching and one was reserved for an unleached test. Each group was immersed in 300 mL of deionized water under vacuum conditions at >28 mmHg for 20 min to ensure thorough saturation. The saturated specimens were then submerged in deionized water for two weeks, with the water being replaced at predetermined intervals. At the end of the period, the blocks were oven-dried at 103 °C for 48 h and reweighed. Mass loss due to leaching was calculated using the following Eq. 2,

$$Mass\ loss = \frac{m_1 - m_2}{m_1} \times 100 \quad (2)$$

where m_1 is the dry mass (g) before leaching and m_2 is the dry mass (g) after leaching. Table 2 shows all treatments together for the leaching test.

This study used gross mass loss rather than component-based mass balance to estimate leaching. This protocol was followed because guayule resin is a chemically complex mixture (terpenoids, polyphenols, fatty acids, sesquiterpene/triterpenoid esters including guayulins and argentatins, and minor rubbery components), comprising >50 constituents that vary with plant material and extraction history. As such, a component-based mass balance approach seemed less viable and had more chance for error in this initial screening process.

Statistical Analysis

Each specimen for either yellow poplar or southern pine served as an experimental unit, and a Completely Randomized Design (CRD) was the experimental design. The number of treatment replications was determined according to the recommendation described by AWPA E11-16 (2024) standard recommendation. The effect of species was separately analyzed and excluded in statistical analysis of the combination of solvent and concertation due to significant differences between hardwood and softwood species on mass loss. All leaching percentage data were analyzed using one-way analysis of variance (ANOVA) to test for the main effect of species or combined solvent and corresponding concentrations. Treatment differences were performed using General linear mixed models (PROC GLIMMIX) of SAS 9.4© (SAS Institute 2013) with significance considered at $P \leq 0.05$. Furthermore, treatment means separations was set to be Fishers protected least significant difference (Steel and Torrie 1980). The following model was used for One-Way ANOVA analysis,

$$Y_i = \mu + T_i + E_i \quad (3)$$

where μ was the population mean, T_i was the effect of solvent and concentration treatment combinations (i = 1 to 16) or the effect of species (i = 1 to 2), and E_i was the residual error.

RESULTS AND DISCUSSION

Carrier System Selection and Solubility Testing

The solubility test to find an ideal carrier system for guayule resin as a natural wood preservative was performed with the selection of nine solvents for guayule resin including acetone, butanol, chloroform, dimethyl sulfoxide (DMSO), ethanol, ethyl acetate, isopropanol, methanol, and toluene. These nine solvents ranged from highly polar (DMSO, ethanol, and methanol) to moderately polar (acetone, butanol, ethyl acetate, and isopropanol) and non-polar (chloroform and toluene). Chloroform was ultimately excluded from further testing due to its health risks (Dehghanizadeh and Brewer 2020). Guayule resin in two different concentrations (5% and 10%) was dissolved in the eight solvents, and 16 solutions were visually compared with each other at different time intervals (24, 48, and

72 h). Different properties such as the solubility of guayule resin in these solvents, color of the solution, presence of residues, as well as consistency of the solution after 72 h were considered. The results of the solubility tests of guayule resin in different solvents are shown in Table 3. The solubility of guayule resin in these solvents was deemed in four categories including highly soluble, soluble, partially soluble, and insoluble.

Homogeneous solutions were observed at both 5% and 10% concentrations of guayule resin with toluene as solvent and guayule resin at concentration of 5% with ethyl acetate as solvent. Residues were not observed in guayule resin solutions with these two solvents. Guayule resin at both concentrations of 5% and 10% with both solvents acetone and butanol were soluble, but with the presence of partial residues on both solutions. Furthermore, guayule resin in both concentration of 5% and 10% in three solvents of dimethyl sulfoxide (DMSO), ethanol, and isopropanol were partially soluble with the presence of a large amount of residues. Additionally, guayule resin at both concentrations was completely insoluble in methanol as solvent, forming a heterogeneous mixture. These solubility differences are linked to the compositions of the guayule resin (Dehghanizadeh and Brewer 2020; Rousset *et al.* 2023).

Table 3. Solubility Characteristics of Guayule Resin in 8 Different Solvents at 5 and 10% Concentrations

Solvent	Concentration (%)	Solubility	Color	Residues	Consistency
Acetone	5%	Soluble	Light brown	Partially present	Heterogenous
	10%	Soluble	Dark brown	Present	Heterogenous
Butanol	5%	Soluble	Dark brown	Partially present	Oily homogenous
	10%	Soluble	Dark brown	Saturated	Oily homogenous
Dimethyl Sulfoxide (DMSO)	5%	Partially soluble	Dark brown	Suspensions on surface	Heterogenous
	10%	Partially soluble	Dark brown	Suspensions on surface	Heterogenous
Ethanol	5%	Partially soluble	Light Brown	Present	Heterogenous
	10%	Partially soluble	Dark brown	Large particles	Heterogenous
Ethyl Acetate	5%	Highly soluble	Light brown	Absent	Homogenous
	10%	Soluble	Light brown	Fine residues	Homogenous
Isopro-panol	5%	Partially Soluble	Dark brown	Present	Heterogenous
	10%	Partially Soluble	Dark brown	large amount of residues	Heterogenous
Methanol	5%	Insoluble	-	Present	Heterogenous
	10%	Insoluble	-	Present	Heterogenous
Toluene	5%	Highly soluble	Dark brown	Absent	Homogenous
	10%	Highly soluble	Dark brown	Absent	Homogenous

The observed solubility trends align with guayule resin's molecular composition and the solvents' physicochemical properties. Guayule resin is rich in polyphenols, sesquiterpene esters, triglycerides, triterpene alcohols (argentatins A–H), free fatty acids, and other lipophilic materials, which interact preferentially with non-polar and moderately polar solvents (Gallego *et al.* 2023; Rousset *et al.* 2023).

The high solubility of guayule resin in toluene as a non-polar solvent ($C_6H_5CH_3$) is linked to hydrophobic constituents of guayule resin such as sesquiterpene esters (guayulins), triterpenoid esters (argentatins), fatty acids, and sterols. These compounds dissolve efficiently in toluene through van der Waals interactions with the solvent's non-polar aromatic structure (Reichardt and Welton 2011). Triterpenoids, particularly argentatins, represent up to 30% of guayule resin and have structures with extensive hydrocarbon frameworks that favor solubility in non-polar environments. Additionally, fatty acids such as linoleic ($C_{18}H_{32}O_2$), palmitic ($C_{16}H_{32}O_2$), and stearic ($C_{18}H_{36}O_2$) acids, which are present in significant amounts in the guayule resin, exhibit high solubility in toluene due to their long hydrocarbon chains and low polarity (Cheng *et al.* 2020). Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) and gas chromatography-mass spectrometry (GC–MS), confirm the presence of hydrocarbons, fatty acids, and oxygenated terpenoids in guayule resin, which dissolve more effectively in aromatic solvents such as toluene (Cheng *et al.* 2020; Dehghanizadeh *et al.* 2020). The FT-ICR MS analysis further supports toluene's efficiency in dissolving guayule resin by detecting a high abundance of C18 to C30 triterpenoids and sesquiterpene esters in toluene-extracted resin samples. These compounds are structurally similar to other plant-derived resins, which dissolve best in non-polar aromatic solvents. The use of toluene in guayule resin extraction aligns with established solvent selection criteria, where the Hildebrand solubility parameter ($\delta \approx 8.9$) of toluene falls within the optimal range (8 to 10) for triterpenoids and other hydrophobic compounds, optimizing resin dissolution (Reichardt and Welton 2011; Cheng *et al.* 2020).

In addition, ethyl acetate's ($C_4H_8O_2$) solubility of guayule resin is also linked to its moderate polarity and ability to dissolve flavonoids and polyphenols (Silva *et al.* 2018; Palaiogianni *et al.* 2023). Its Hildebrand solubility parameter (~8.5 to 9.1) aligns well with the solubility range of triterpenoids and other semi-polar compounds, enabling efficient dilution (Burke 1984). Studies confirm that ethyl acetate is particularly effective for extracting polyphenols and flavonoids, which are among the key constituents of guayule resin (Shikov *et al.* 2022; Palaiogiannis *et al.* 2023). It has been used to isolate flavonoid-rich extracts from various plant sources, indicating its ability to selectively dissolve compounds with hydroxylated and esterified functional groups (Shikov *et al.* 2022). Studies by Latorre *et al.* (2022) revealed that resin dilution in ethyl acetate was notably richer in argentatins C2 and C, indicating its preferential extraction of specific triterpenoid esters. Additionally, ethyl acetate exhibits strong extraction efficiency for phenolic terpenes and oxygenated sesquiterpenes esters, which are structurally similar to guayule resin components guayulin C, and D (Palaiogiannis *et al.* 2023). Furthermore, ethyl acetate's ability to extract essential oils, sterols, and lipid-soluble antioxidants suggests that it effectively isolates valuable bioactive compounds from guayule resin (Amiralian *et al.* 2014; Shikov *et al.* 2022). Moreover, its low toxicity and volatility make it a safer alternative to more hazardous organic solvents, reinforcing its suitability for industrial-scale resin extraction and formulation process (Shikov *et al.* 2022).

Acetone (C_3H_6O) as a moderately polar solvent also demonstrated solubility due to its moderate polarity and ability to dissolve both polar and non-polar resin constituents (Reichardt and Welton 2011). The Modified Soxhlet Extraction method confirms acetone as an effective solvent, as it is the primary solvent used for guayule resin extraction before rubber removal with hexane/pentane (Nurthen *et al.* 1986; Schloman 2005; Cornish *et al.* 2006). Acetone's dipole-dipole interactions with ester linkage (R-CO-OR) and hydroxyl groups (-OH) further justify its efficiency in dissolving guayule resin (Reichardt and Welton 2011). Spano *et al.* (2018) further confirmed that acetone effectively dissolves guayulins A ($C_{24}H_{30}O_2$) and B ($C_{23}H_{30}O_3$), which are abundant in guayule resin and act as metabolic reservoirs for the plant. The high solubility of these sesquiterpene esters in acetone is attributed to their ester linkages and oxygenated functional groups, which facilitate strong interactions with the solvent (Gallego *et al.* 2023). Moreover, Silagy *et al*. (2024) reports that acetone effectively dissolves monoterpenes and oxygenated sesquiterpenes which contribute to the resin's diverse chemical composition.

Butanol (C_4H_9OH) solubility can be linked with previous findings that slightly polar triterpenes dissolve best in solvents with Hildebrand solubility parameters (δ) between 10 and 12 (Burke 1984; Reichardt and Welton 2011; Ramanjaneyulu and Reddy 2019). It also exhibits similar solubility properties as ethyl acetate, as that solvent has been successfully used to extract phenolics and flavonoids, from various plant sources, demonstrating its affinity for oxygenated compounds (Ramanjaneyulu and Reddy 2019; Shikov *et al.* 2022). However, its high boiling point (117.7 °C), strong odor, and relatively high viscosity limits its efficiency in large scale application (Thames and Kaleem 1991; König *et al.* 2018; Jara *et al.* 2019). Furthermore, in plant extraction, butanol is commonly used for fractionation rather than primary extraction, often following ethanol or water extraction to isolate bioactive compounds (Shikov *et al.* 2022).

The highly polar solvents methanol (CH_3OH) and ethanol (C_2H_5OH) performed poorly due to their strong hydrogen bonding and high polarity. These short-chain alcohols are more effective in dissolving small polar compounds rather than large hydrophobic molecules (Patra *et al.* 2006). Methanol, with a solubility parameter of ~14.5 MPa^0.5, forms extensive hydrogen bonds due to its single hydroxyl (-OH) group, reducing its ability to interact with non-polar resin molecules. This leads to phase separation and undissolved residues, particularly with argentatins and guayulins (Rousset *et al.* 2023). Ethanol (C_2H_5OH), with a slightly lower solubility parameter (~12.9 MPa^0.5), exhibited marginally better but still insufficient solubility. Its additional carbon group slightly increases its non-polarity but still allows extensive hydrogen bonding, restricting its compatibility with the hydrophobic resin matrix (Patra *et al.* 2006). DMSO, despite being a strong aprotic solvent, exhibited limited solubility, which may be linked to its lack of compatibility with the resin's lipophilic nature (Lewis 2016). These findings align with previous reports on guayule resin solubility (Rousset *et al.* 2023; Dehghanizadeh *et al.* 2020).

In summary, while guayule resin partially dissolves in some polar solvents, non-polar and moderately polar solvents such as toluene, acetone, and ethyl acetate offer superior solubility due to their compatibility with key resin constituents including triterpenoids, sesquiterpene esters, and fatty acids. The solubility observed in this study highlighted the need for careful selection of a carrier system that aligns with the structural characteristics of the resin's predominant components. Based on the results and with considering high solubility of guayule resin in toluene as non-polar solvent as well as ethyl acetate and acetone as moderate polar solvents, these three solvents were chosen for further treatment process to do leaching test. This study utilized small sapwood specimens to provide a controlled system for comparative evaluation of guayule resin-solvent formulations. While this approach minimizes anatomical variability and diffusion limitations, it does not fully represent penetration challenges associated with larger specimen dimensions, heartwood material, or commercial treatment conditions. Therefore, solvents are discussed here as relative carrier systems under laboratory-scale conditions, and further research is required to assess their effectiveness in less permeable or industrially relevant wood products.

Mass Gain

The means of mass gain percentage results of yellow poplar and southern pine species after treating specimens in guayule resin solutions at four different concentrations (0.5, 1, 3, and 5%) with three different solvents indicated that across all treatments yellow poplar exhibited higher mass gain percentage than southern pine, indicating greater guayule resin uptake. In both species, among the solvents, ethyl acetate and acetone showed the higher mass gain percentage at 5% concentration of guayule resin. In yellow poplar, the mass gain percentage of 5% of guayule resin at ethyl acetate and acetone solvents were 5.02% and 5.34%, respectively, while in southern pine, mass gain percentage of 5% of guayule resin at ethyl acetate and acetone solvents were 4.78% and 3.87%, respectively. In addition, the lowest mass gain percentages were observed in the specimens treated with guayule resin with toluene as solvent in different concentrations of guayule resin in both yellow poplar and southern pine.

In southern pine species, negative mass gain percentages were observed in the treated specimens with 0.5% concentrations of guayule resin in three different solvents (acetone, toluene and ethyl acetate) and 1% concentrations of guayule resin in two different solvents (acetone and toluene). The reason for this negative mass gain might be the chemical reactions involved with preservative and wood species causing degradation or removal of certain wood components. Another reason associated with this negative mass gain could be the evaporation of solvents after the process and the wood could lose mass as the solvent escapes.

The variation in mass gain percentage after treatments between yellow poplar and southern pine could be attributed to differences in their wood anatomy. Yellow poplar, a diffuse-porous hardwood, has a more open cellular structure, allowing for greater solvent penetration and resin absorption. In contrast, southern pine, a softwood, has a denser structure with higher extractive content, which may limit resin diffusion and retention (Hoadley 1990; Radivojevic and Cooper 2010). This is consistent with studies on preservative treatments, where more permeable wood species generally exhibit higher mass gain due to increased fluid penetration (Avram *et al.* 2023).

Higher resin concentrations resulted in greater mass gain percentages, confirming that increasing resin availability enhanced retention. Similar trends were observed in quaternized nano-chitosan treatments, where higher preservative concentrations improved uptake, leading to greater weight percent gain (WPG) (Nowrouzi *et al.* 2015; Khademibami *et al.* 2020). Mass gain percentage or WPG is a key indicator of preservative retention and treatment efficacy, where higher values correlate with increased resin deposition and better bulk impregnation of wood cell walls (Rowell 2012). Acetone and ethyl acetate exhibited the highest mass gain, which is linked to their low viscosity, enabling deeper penetration and improved resin retention (Reichardt and Welton 2011). These results are consistent with Avram *et al.* (2023), who also reported higher treatment retention with ethyl acetate due to its strong adhesion properties. However, while acetone increased resin uptake, its rapid evaporation caused dimensional instability in treated samples, potentially affecting long-term performance (Avram *et al.* 2023).

Resistance to Leaching

The statistical analysis of mass loss percentage (representative for leaching rate) was significantly different (P_{value} <0.0001) between yellow poplar and southern pine species (Fig. 2). These results indicated that the leaching rate was significantly higher in yellow poplar as compared to the southern pine species.

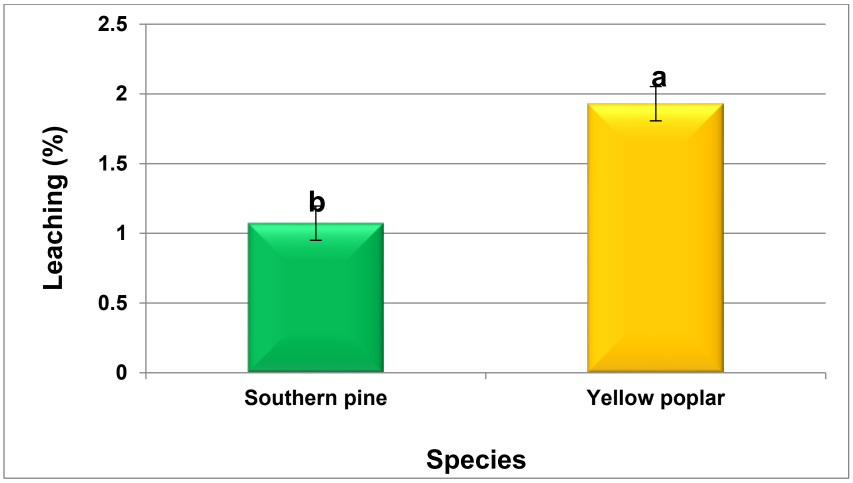

Fig. 2. Means leaching rate percentage between two wood species. a, b Treatment means within the same column within effect without common superscripts are significantly different (P-value ≤ 0.05).

Summary statistics of mass loss percentage (leaching extent) of southern pine and yellow poplar related to all treatments are demonstrated in Tables 4 and 5. Based on these two tables and according to Figs. 3 and 4, there were statistical differences in the leaching rates among all treatments in both species: southern pine and yellow poplar (P_{value} <0.0001). In southern pine, guayule resin at all four concentrations 0.5%, 1%, 3%, to 5% with the combination of acetone exhibited a higher (P_{value} < 0.0001) leaching as compared to any other treatments (Fig. 3). The lower (P_{value} < 0.0001) leaching belonged to 5% concentration of guayule resin in toluene. The other concentrations of guayule (0.5%, 1%, and 3%) combined with toluene and all concentrations of guayule resin with ethyl acetate behaved the same as each other as well as control treatments.

In yellow poplar, any concentrations of guayule resin in ethyl acetate exhibited a higher (P_{value} < 0.0001) leaching extent relative to all other treatments. Moreover, no treatment differences were observed between control groups or among any concentrations of guayule resin in toluene. In addition, the lower leaching rate belonged to toluene alone control group as compared to any treatment containing guayule resin. Additionally, 0.5% concentration of guayule resin in acetone and all concentrations of guayule resin in toluene not only had similar leaching percentage, but also it was lower than 1 to 5% concentration of guayule resin in acetone or all concentrations of guayule resin in ethyl acetate (Fig. 4).

Therefore, in southern pine the best treatment belonged to 5% concentration of guayule resin in toluene which had the lowest leaching. In yellow poplar, the best treatment was 0.5% concentration of guayule resin in toluene.

The leaching behavior observed in this study can be associated with the interactions between wood structure, solvent properties, and the chemical composition of guayule resin. The difference between leaching percentages on yellow poplar and southern pine is largely influenced by the inherent anatomical differences between these two wood species (Radivojevic and Cooper 2010).

Table 4. Summary Statistics of Mass Loss Percentage (Leaching) of Treated Southern Pine Specimens

	Guayule + Acetone				Guayule + Ethyl acetate				Guayule + Toluene				Acetone	Ethyl acetate	Toluene	Water
	Guayule concentration (%)				Guayule concentration (%)				Guayule concentration (%)				No guayule resin (pure solvent)			
	0.5	1	3	5	0.5	1	3	5	0.5	1	3	5				
Mean	1.33	1.42	1.56	1.55	0.99	1.01	0.89	0.87	1.03	0.94	0.89	0.71	1.06	1	1.1	0.79
St Dev	0.14	0.17	0.21	0.19	0.21	0.15	0.18	0.53	0.16	0.17	0.16	0.17	0.08	0.13	0.15	0.11
Min	1.12	0.95	1.22	1.18	0.61	0.73	0.46	0.19	0.70	0.6	0.59	0.4	0.92	0.82	0.91	0.65
Max	1.62	1.73	2.08	1.93	1.35	1.23	1.17	2.51	1.26	1.17	1.15	0.94	1.16	1.19	1.35	0.97

Table 5. Summary Statistics of Mass Loss Percentage (Leaching) of Treated Yellow Poplar Specimens

	Guayule + Acetone				Guayule + Ethyl acetate				Guayule + Toluene				Acetone	Ethyl acetate	Toluene	Water
	Guayule concentration (%)				Guayule concentration (%)				Guayule concentration (%)				No guayule resin (pure solvent)			
	0.5	1	3	5	0.5	1	3	5	0.5	1	3	5				
Mean	1.29	1.63	2.02	2.14	3.35	3.58	4.33	3.79	1.15	1.33	1.4	1.33	0.97	1.07	0.94	0.54
St Dev	0.27	0.28	0.25	0.36	0.53	0.69	0.4	0.59	0.32	0.22	0.34	0.45	0.42	0.08	0.18	0.24
Min	0.62	1.09	1.46	1.01	2.43	1.49	3.37	4.98	0.28	0.89	0.68	0.29	0.059	0.98	0.76	0.34
Max	1.72	2.15	2.47	2.58	4.37	4.26	4.98	4.49	1.67	1.67	1.86	2.12	1.76	1.18	1.19	1.01

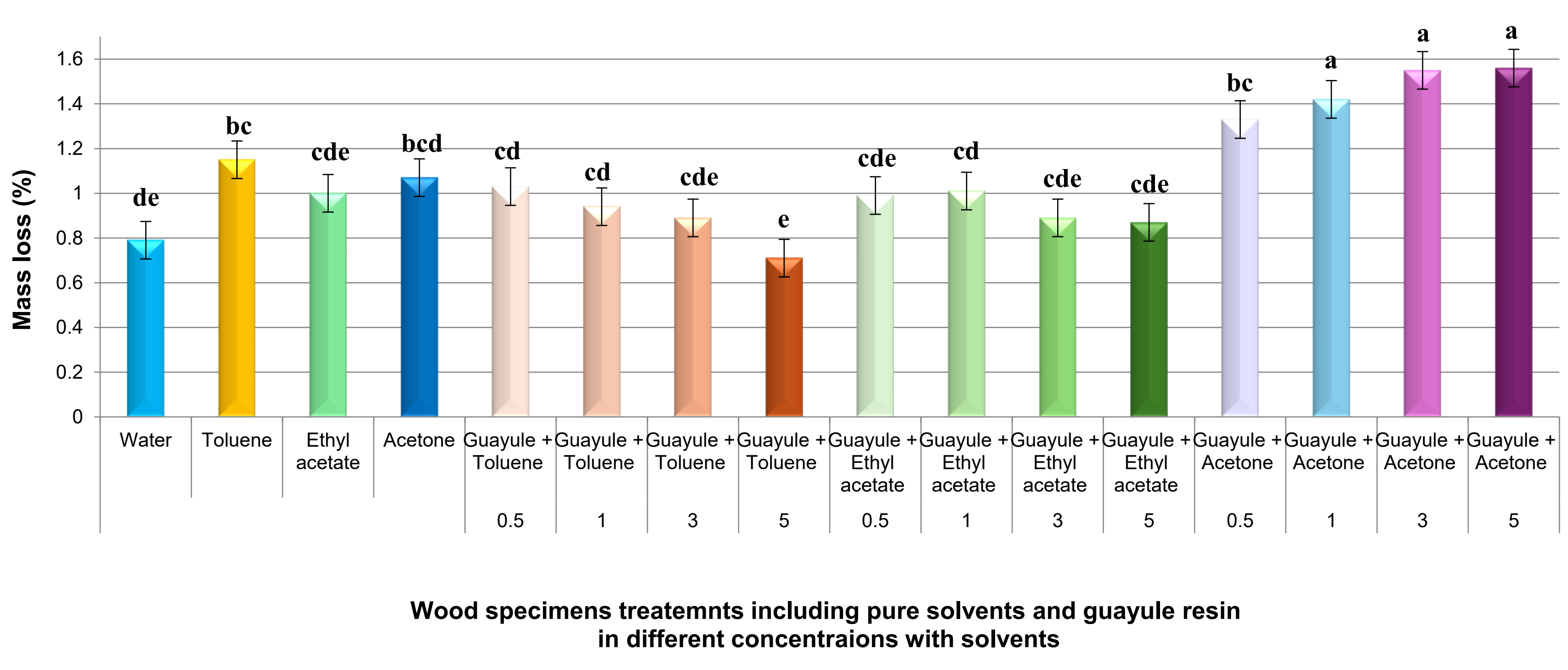

Fig. 3. Means mass loss percentage (leaching) among guayule resin at various concentrations with solvents and different solvents in southern pine. [a-e] Treatment means within the same column within effect without common superscripts are significantly different (P-value ≤ 0.05)

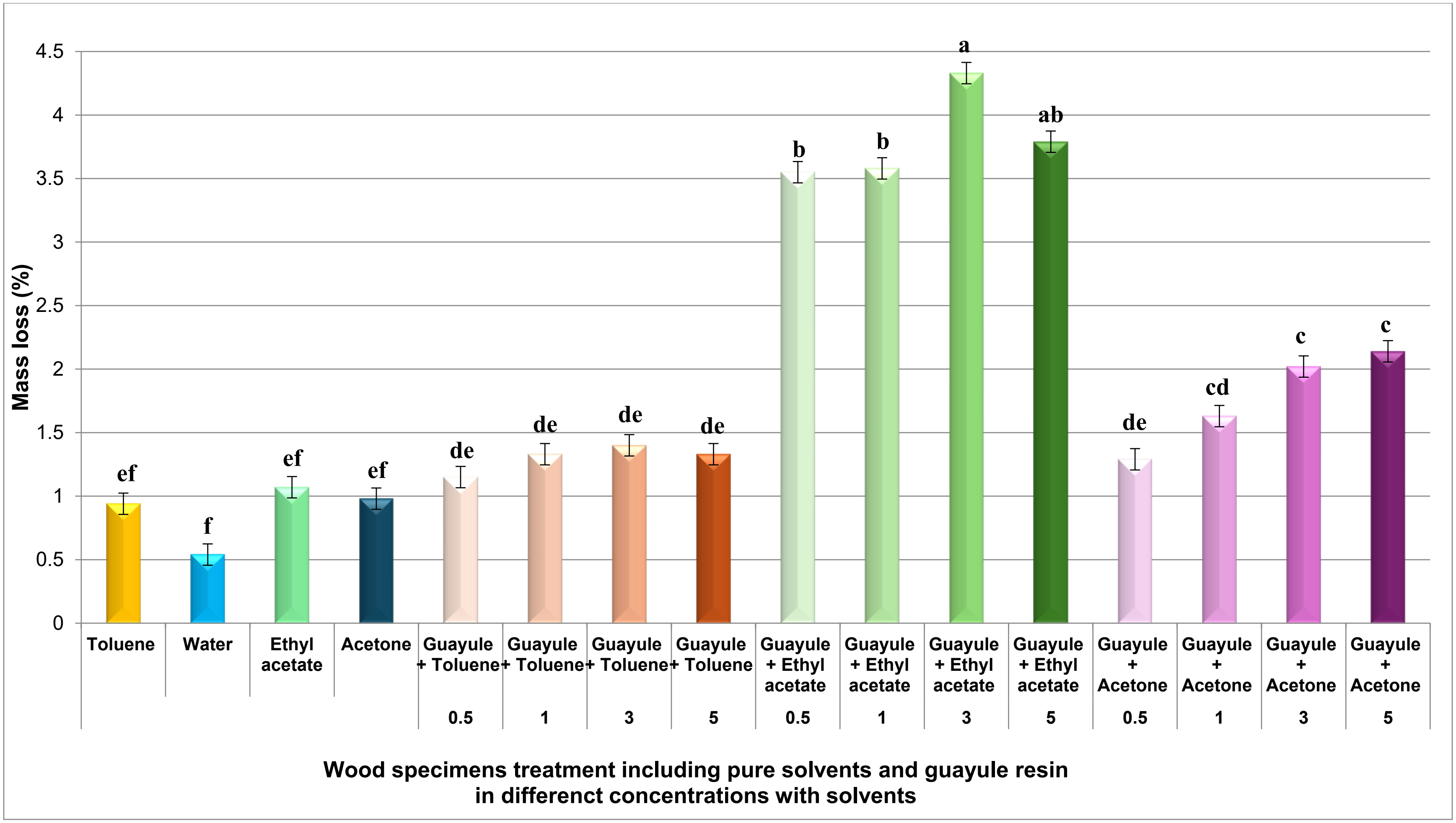

Fig. 4. Means mass loss percentage (leaching) among guayule resin at various concentrations with solvents and different solvents in yellow poplar. $^{a\text{-}e}$ Treatment means within the same column within effect without common superscripts are significantly different (P-value ≤ 0.05).

Yellow poplar, a diffuse-porous hardwood, has a more open cell structure with a higher proportion of vessel elements, which likely facilitates increased solvent penetration and, consequently, higher resin loss during leaching. In contrast, southern pine, a resinous softwood, contains more extractives and a tighter cellular matrix that may contribute to improved retention of the applied resin (Hoadley 1990). These results align with previous studies that have highlighted that hardwoods generally exhibit higher leaching than softwoods due to these structural and chemical differences (Radivojevic and Cooper 2010; Temiz *et al.* 2014). Radivojevic and Cooper (2010) reported that low leaching extent in softwoods due to having more lignin content than hardwoods which plays a crucial role in binding preservatives within the wood matrix. Additionally, the lignin has a greater proportion of free phenolic hydroxyl groups, which serve as primary reactive sites for preservative fixation, enhancing retention and reducing leaching (Radivojevic and Cooper 2010; Temiz *et al.* 2014).

Solvent properties play a crucial role in determining resin retention. Acetone, which exhibited an upward trend in leaching (Figs. 3 and 4), is characterized by its low viscosity, high volatility, and water-miscible nature (NCBI 2025). Acetone has a low boiling point (56.08°C) and a high vapor pressure (231 mm Hg at 25 °C), causing it to evaporate quickly upon exposure to air (Haynes 2016; NCBI 2025). This rapid evaporation reduced the time available for guayule resin to infiltrate the wood matrix, leaving a significant portion of the resin deposited on the surface rather than being absorbed into the cellular structure as observed during experiments. This might possibly account for an increase in leaching as guayule acetone concentration increased. As concentrations increased, more resin (solid content) was present in the solution, which means as acetone evaporated, much of it accumulated on the surface as unfixed materials. This unfixed resin was easily washed away during leaching (Temiz *et al.* 2014). Although acetone is highly volatile and not expected to remain in the wood after treatment, its complete miscibility with water can influence resin distribution during impregnation. Rapid evaporation may result in less effective fixation of guayule resin within the wood matrix, leaving a higher proportion of resin susceptible to mobilization during subsequent leaching. This behavior contrasts with non-polar solvents, which promote stronger hydrophobic interactions and improved resin retention.. In contrast, slightly water-miscible solvents, such as toluene, do not interact as readily with water, leading to lower leaching rates as guayule resin remains more firmly bonded within the wood (Temiz *et al*. 2014; Haynes 2016; NCBI 2025). The lower leaching extent in guayule-toluene-treated wood strong retention of guayule resin within the wood matrix, facilitated by toluene's non-polar nature and wood's permeability characteristics (Reichardt and Welton 2011; Temiz *et al.* 2014).

According to Siau (1970), permeability is the primary factor influencing solvent retention, with wood exhibiting higher permeability to non-polar than polar solvents. Non-polar solvents such as toluene interact weakly with wood's polar components, allowing deeper penetration and more uniform resin distribution. In contrast, polar solvents interact strongly with cellulose, hemicellulose, and lignin, leading to swelling and restricted movement, which reduces deeper penetration (Wang and Schniewind 1985). In deep wood cells the resin forms strong bonds that are not easy to break, meaning that once guayule resin was absorbed into the wood matrix, it remained less prone to being washed away. The high retention after 193 days of sesquiterpene (guayulins), triterpenoid (argentatins), which are highly soluble in toluene further supports, as these components remain well-fixed within the wood (Bultman and Schloman 1993). Moreover, Bultman and Schloman (1993) suggested that the presence of rubber-like compounds stabilizes the resin and reduces leaching. Since toluene, like hexane, is non-polar, it likely retains resin in a similar manner, stabilizing the impregnated compounds within the wood matrix. Additionally, toluene's low water solubility (0.07% at 23°C) reduces the likelihood of guayule resin in toluene treatment dissolution in water, further minimizing leaching (Haynes 2016). These properties collectively ensure that toluene effectively stabilized guayule resin within the wood matrix, making it an ideal carrier solvent for wood treatment.

The concentration of guayule resin within the solvent also appeared to influence leaching resistance as observed in ethyl acetate and toluene (Figure 3). Higher resin concentrations led to better retention, likely due to the increased hydrophobic (solute-solute) interactions preventing resin migration (Reichardt and Welton 2011). However, this was the opposite for acetone, owing to the aforementioned reason, further demonstrating the importance of solvent selection and resin concentration in determining the overall performance of guayule-based wood treatments.

Overall, mass loss due to leaching was below 2%, aligning with previous studies on the leachability of guayule resin-treated wood. Research has demonstrated that guayule resin remains stable under extended exposure due to its high composition of hydrophobic compounds that do not dissolve in water (Bultman and Schloman 1993). Nakayama *et al.* (2001) suggested that the presence of low molecular weight rubber in guayule resin-impregnated wood may contribute to reduced leaching by enhancing resin retention within the wood matrix. Moreover, according to Temiz *et al.* (2014) short-term laboratory leaching tests, such as AWPA E11-16 standards (2024), are known to be more aggressive than natural weathering conditions due to relatively high surface areas exposed to water, leading to higher leaching extents than what would be expected in real-world service environments. Therefore, while these tests provide valuable comparative data, they do not fully represent the long-term leachability of guayule resin-treated wood in practical applications.

CONCLUSIONS

1. The present findings confirm that non-polar and moderately polar solvents, particularly toluene, acetone, and ethyl acetate, provide superior solubility for guayule resin due to their ability to dissolve its predominant constituents, whereas the highly polar solvents methanol and ethanol showed poor solubility, dissolving only a limited range of resin compounds. This highlights the importance of selecting an appropriate carrier system that aligns with the resin's structural composition to optimize resin solubility and overall penetration and retention.
2. Southern pine exhibited superior resin retention, with significantly lower leaching extents than yellow poplar, demonstrating that wood species play a critical role in guayule resin retention. This aligns with the study's objective of assessing leaching potential across different wood types to ensure effective retention.
3. Overall, toluene proved to be the most effective solvent, demonstrating the highest solubility for guayule resin by dissolving it completely at all tested concentrations without residue formation. Additionally, it resulted in the lowest leaching extent, indicating strong resin fixation within the wood matrix. These characteristics make toluene the most suitable carrier system for enhancing resin penetration, retention, and long-term preservative performance.
4. Although toluene was judged to be the best laboratory solvent benchmark, fully

dissolving guayule resin at all tested concentrations and yielding comparatively low percentage mass loss under accelerated leaching for the application of guayule resin as a sustainable and ecofriendly wood preservative, it is recommend follow up solubility tests using biobased oil and lower- toxicity solvents benchmarked against toluene and validated by component-resolved E11-16 leaching.

5. The percentage mass loss due to leaching remained below 2%, confirming guayule resin's strong retention and durability as a wood preservative even when used with solvents. Its stability is attributed to its hydrophobic composition of over 50 compounds, reinforcing its potential for long-term wood protection. These findings highlight guayule resin as a promising biobased preservative with effective leaching resistance, supporting its viability for sustainable wood treatment applications.
6. The findings should be limited to laboratory-scale comparative indicators of guayule resin-solvent performance. Further studies using larger specimens and/or less permeable wood materials, such as those containing heartwood, are needed to evaluate treatment scalability and commercial relevance.

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