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Red sanders bark extracts as effective bio-protective agents against fungal and termite degradation of plantation timbers

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Red sanders (*Pterocarpus santalinus*), an endemic species of Southern India, is highly valued for its heartwood, yet its bark is frequently discarded as waste. The sustainable utilization of underutilized bark offers a promising route to develop bio-based wood preservatives. This study investigates the bio-protective efficacy of *Pterocarpus santalinus* bark extracts against fungal and termite degradation in plantation timbers. Gravimetric analysis revealed markedly higher yields for aqueous extracts (26.22%) compared to acetone (2.59%) and methanol (1.05%) extracts. Three wood species: rubberwood (*Hevea brasiliensis*: HB), mango wood (*Mangifera indica*: MI), and melia wood (*Melia dubia*: MD) were pressure-impregnated with 3% and 8% extract concentrations for 1 h and 2 h. Retention values in different wood species ranged from 0.94 to 8.81 kg m^{-3}, while weight percent gain reached 17.88%, especially in lower-density MD. Acetone extracts conferred the strongest antifungal protection, reducing brown-rot (*Oligoporus placentus*) mass loss from 46% (control HB) to 11% (HB at 8%), and white-rot (*Trametes hirsuta*) mass loss from 38% to 11%. A similar phenomenon was seen in MI and MD wood. Termite damage ratings declined from 5.0 (complete failure) in untreated HB and MI to 1.7 and 2.8 post-treatment, and to 0.2 in MD. Leaching resistance improved with higher concentrations and longer impregnation times, while FTIR spectra confirmed the preservation of lignin and hemicellulose associated peaks after fungal tests. SEM confirmed that the extracts form protective barriers into the wood, inhibiting microbial degradation and termite infestation.

Sustainability spotlight

This study presents a sustainable solution for wood preservation by value addition of the discarded bark of *Pterocarpus santalinus* (red sanders), an endemic species of Southern India. Traditionally considered waste, the bark is repurposed into a bio-based extract exhibiting strong antifungal activity against both brown and white rot fungi, effectively protecting plantation timbers like rubberwood, mango wood, and Malabar neem wood. Beyond durability, the extract imparts a rich aesthetic resembling red sanders heartwood. Leaching studies confirm the extract's stability and long-term efficacy, while SEM and FTIR analyses reveal its role in forming protective barriers within wood structures. This multifunctional approach offers an eco-friendly alternative to synthetic preservatives, reducing environmental impact and promoting circular use of lignocellulosic biomass. By converting forestry waste into a high-value functional additive, this work contributes to sustainable materials development in the wood industry and supports broader goals of resource efficiency, waste reduction, and climate-resilient practices.

1. Introduction

Red sanders or *Pterocarpus santalinus* Linn is an endemic timber species found mainly in Chittoor and Kaddapa districts of Andhra Pradesh and in some parts of Tamil Nadu (India).[1] Red sanders has dark red heartwood due to the presence of santalin compound, which is used for dyeing, polishing and varnishing in different industries.[2] The timber is highly valued, and it has high demand in the domestic and international markets.[2] Reports on trading of the red sanders suggested that between 2001 and 2007 (only from Andhra Pradesh), there had been illegal trading of 3067 tonnes of red sanders wood at the rate of $6870 to $9160 (USD) per tonne in 2002.[1] Recognizing the high value of this tree's wood, extensive plantations by farmers and industries have been established in Andhra Pradesh. Each tree has a bark thickness of 1.4 cm to 1.8 cm on average. Thus, given the volume of trade of red sanders wood, it is evident that the amount of bark waste generated is quite high.

Although the wood is utilized for high-end furniture manufacturing, cosmetics and pharmaceuticals industries, the bark remains unutilized. There are several studies that have confirmed the presence of several groups of chemical

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compounds like terpenoids, phenolic compounds, flavonoids, alkaloids, tannins, glycosides and saponins.[1,3–8] Kumar *et al.*,[9] reported the anti-UV efficiency of the red sanders bark extract as coatings over the wood. The researchers also discussed a closed-cycle system where the tree bark can be utilized as fuel, charcoal or in the preparation of adhesives from the extracted lignin and in the fabrication of wood-plastic composites to make the molded products stronger.[10] This opened new arenas for work with the extracts; one of them being bio-preservatives.

Fungal attack on wood significantly degrades the mechanical strength of wood and causes severe economic losses.[11] To enhance the fungal resistance of wood, certain preservatives like borates and phenates have already been used in industries. The wood treated with traditional preservative containing toxic chemicals might be responsible for antifungal activity in wood but at the same time when they leach out, they have environmental and human health impacts.[12,13] Plant based preservatives can be a solution to these especially with extractives from naturally durable species which can be isolated and utilized to increase the durability of non-durable wood species.[14,15] The antifungal activity of the crude stem bark extract of *Senna alata* Linn. against dermatophytes was found to be satisfactory at a concentration of 10.00 mg mL^{-1} and 5.00 mg mL^{-1}.[16] Rubberwood and mango wood are highly susceptible to termites, fungal decay, borer and powder-post beetle attacks.[17] The impregnation of plant extracts into several wood species has demonstrated superior antifungal efficacy compared to conventional treatments, as evidenced by Turkish oriental beech and Scots pine woods treated with indigo dye extracts and exposed to *Rhodonia placenta* and *Trametes versicolor* fungi.[18] Wood preservatives from mimosa and quebracho bark,[18] neem oil (*Azadirachta indica*),[19] tannin mixed with Chromated Copper Borate (CCB)[20] have been used as preservatives in indoor applications. *Acacia crassicarpa* and *Acacia mearnsii* tree bark extracts were found to affect the termite feeding habits negatively.[21] Other studies have reported that plant extracts from *Sextonia rubra* wood,[22] *Cinnamomum cassia* bark,[23] and *Piper sarmentosum*[24] can increase durability against fungus in treated woods. Resistance to leaching provides better durability and anti-swelling efficiency to the preserved woods.[25] Fungal decay resistance and termite resistance were reported in *Acacia mangium* bark extract treated rubberwood against brown-rot fungi (*Gloeophyllum striatum*) and white-rot fungi (*Trametes versicolor*).[26,27]

The bark of Red Sanders, though underutilized compared to its heartwood, holds promising potential for medicinal and phytochemical applications.[28] Red sanders is protected under Schedule IV of the Wildlife (Protection) Act, 1972 (as amended in 2022[29]), and listed in Appendix II of CITES, red sanders trade is strictly regulated and permitted only with proper authorization (TRAFFIC & WWF-India, 2023[30]). India's recent removal from the CITES Review of Significant Trade highlights improved compliance, enabling legitimate trade from plantation sources. Nevertheless, bark collection from wild populations remains restricted under the Indian Forest Act, 1972,[31] highlighting the need for sustainable, non-invasive, and legally compliant sourcing. Future utilization should therefore rely on plantation-derived material consistent with national conservation and international trade perspectives.

The utilization of red sanders bark extracts to enhance the durability of rubberwood (*Hevea brasiliensis*), mango wood (*Mangifera indica*) and Malabar neem wood (*Melia dubia*) (all belonging to Class III according to IS 401)[32] in terms of anti-fungal activity and anti-termite properties was explored in this study. Therefore, to address a significant challenge posed by plantation-grown wood species, particularly those with a higher proportion of sapwood and lower natural durability, wood preservation using plant extracts offers an eco-friendly solution. Additionally, since red sanders wood is regarded as auspicious in many cultures and believed to bring good luck and other positive benefits,[2,33] plantation-grown, non-durable wood infused with red sanders bark extract may also possess significant aesthetic appeal.

2. Materials and methodology

2.1. Materials

Wood specimens of rubberwood [*Hevea brassilensis* (HB)], mango [*Mangifera indica* (MI)] and Malabar neem or melia [*Melia dubia* (MD)] were purchased from MS Fabwood Pvt. Ltd, Bengaluru and MS National Timbers, Bengaluru, India. Defect free wood specimens having dimensions of 20 mm × 20 mm × 20 mm were prepared and dried in a hot air oven at 105 ± 2 °C and conditioned in a closed chamber maintained at 25 ± 5 °C temperature and 65 ± 3% relative humidity (RH) prior to impregnation. To obtain smooth surfaces, wood samples were sanded using 80, followed by 120 and finally 220 grit sandpapers.

The bark of *Pterocarpus santalinus* used as a raw material was obtained from the Sri Venkateshwara Wildlife Sanctuary (WLS), Tirupati Biotechnology Research Centre, Tirupati, and Tirupathi-Tirumala Forest belt, Andhra Pradesh, India. Acetone and methanol (LR Grade) and malt agar powder were obtained from MS SD Fine Chemicals, Bengaluru, India. The water used in the experiments was distilled water with pH = 6.9 and was prepared in laboratory.

2.2. Preparation of bark extracts

Small chips of air-dried red sanders bark were made, ground in a pulverizer and sieved between 40 and 60 mesh. The bark powder was then extracted using acetone, methanol, and water. The solid/liquid ratio was kept constant at 1/10 (w/v). For the water extract, the bark powder was soaked in hot water and then boiled at 90 °C for 2 h.[9] The bark's water extract was filtered through a muslin cloth and then concentrated by keeping over a hot plate. The extract was finally dried in an oven at 60 °C. The solvent extraction was carried out in a Soxhlet apparatus at the rate of 5–6 siphons per hour. The temperature for Soxhlet using methanol was kept at 55 ± 5 °C and for acetone at 45 ± 5 °C and it was run until the siphon ran clear. The extracts were then concentrated and finally dried using a rota-vapor (Buchi R3). The solvents used were recovered during concentration of the extracts and subsequently reused for further extractions. The extractive yield (solid extractive content) was computed based

on the initial amount of bark powder on an oven-dry basis using eqn (1).

$$\text{Extractive content percentage(EC\%)} = \left(\frac{\text{weight of the extract}}{\text{weight of total plant material}}\right) \times 100 \quad (1)$$

2.3. Impregnation of bark extracts

The conditioned wood samples (HB, MI and MD) were impregnated with red sanders (RS) bark extract. The concentration of the treating solution was fixed at 3% (A) and 8% (B) (w/v). The treatment solutions were methanol extract, acetone extract, and water extract of *P. santalinus* bark. The wood samples were impregnated in accordance with IS 401.[32] Vacuum was applied at 550–560 mm Hg for 15 minutes followed by pressure impregnation at 110–120 pounds per sq. inch (psi). The pressure duration was varied for 1 hour (T1) and 2 hours (T2) to assess the increase in retention of the samples. Finally, a vacuum of 500–530 mm Hg was applied for 5 min to remove the excess solutions. The impregnated samples were finally conditioned at 25 ± 2 °C and 65 ± 3% relative humidity (RH). The samples were then oven dried at 105 ± 2 °C till constant weight was achieved. The retention (kg m^{-3}) of bark extracts in wood was calculated as follows:

$$R = \frac{(W2 - W1) \times C}{V} \times 10 \text{ kg m}^{-3} \quad (2)$$

The weight percent gain (WPG) of wood was determined as follows:

$$\text{WPG}(\%) = \frac{(W2 - W1)}{W1} \times 100 \quad (3)$$

In eqn (1) and (2), $W1$ and $W2$ is the oven-dry weight of wood (kg) before and after treatment, respectively, C is the concentration of red sanders bark extract solution (%) and V is the volume of the wood samples taken before impregnation (m^3) and unit conversions were done before calculating retention. Both retention and WPG were calculated and reported since WPG gives us the relative increase in mass while the retention value expresses the actual amount of preservatives present per unit volume.

2.4. Colour measurements

The change in colour of the impregnated wood, *i.e.*, lightness (L^* coordinate) and colour hue (a^* and b^* coordinates), was measured using a spectrocolorimeter (LabScan XE, xenon light source, 10° standard observer, D65 standard illuminant). The values were reported as a mean value of four colour readings on each wood sample.

2.5. Resistance of red sanders bark extract treated wood against wood decay fungi

Fungal decay resistance of rubberwood, mango and Melia wood impregnated with red sanders extracts was tested against white-rot fungi *Trametes hirsuta* and brown-rot fungi *Oligoporous placentus* and was examined in accordance with IS 4873 (Part 1)[34] with slight modifications. Agar block culture bottles were prepared with maltose-agar media in the ratio of 1 : 4 (agar: malt extract). Agar bottles were autoclaved for sterilization. Subsequently, small pieces of previously grown fungi on Petri plates were transferred into the bottles under a sterile environment to avoid contamination with other fungus. The inoculated bottles were stored at a temperature of 25 ± 2 °C and relative humidity of 70 ± 4% till mycelium had reached the outer edge of the glass bottles. The samples were weighed prior to exposure to fungal decay. The treated samples and control samples were then autoclaved and sterilized before the test commenced. Six samples of each treatment were exposed to each fungus for a period of 12 weeks at a temperature of 25 ± 2 °C and relative humidity of 70 ± 4%. Glass rims were placed beneath each treated block, to avoid direct contact with agar and fungal matter. After the expiration of the requisite period, *i.e.*, 12 weeks, the samples were again sterilized and cleaned by gently removing any fungal matter that was attached to wood samples. The samples were thereafter oven dried at 105 ± 2 °C. The efficacy of treated wood samples with RS bark extracts was assessed by the percentage of mass loss (ML %) after fungal tests and were calculated as follows:

$$\text{ML}(\%) = \frac{W1 - W3}{W1} \times 100 \quad (4)$$

In the equation, $W1$ is the oven dry weight of the wood sample before any treatment (g) while $W3$ is oven dry weight of the wood sample after fungal exposure (g).

2.6. Resistance of red sanders bark extract treated wood against termites

The termite resistance activity of red sanders bark was analyzed according to IS 4833[35] with slight modifications. Defect free wood samples of 150 mm (length) × 40 mm (width) × 7 mm (thickness) were prepared and treated with red sanders bark extracts (*i.e.*, water, methanol and acetone extracts) at a concentration of 8% (w/v). The samples were impregnated in the same method as followed for impregnating block samples. 10 replicates were taken for each species and each treatment. The samples were conditioned and then introduced into the termite plot. Regular inspection was carried out in the field and numerical ratings based on visual inspection were given.

The grading criterion according to IS 4833[35] suggests a numerical rating of 0 to 5 based on the area of the sample affected by the termites with 0 given to unaffected samples and 5 given to samples 70% or more affected by the termites. The other ratings for 5% to 20%, 20% to 30%, 30% to 40% and 40% to 70% are given as 1 to 4, respectively.

2.6.1. FTIR spectral analysis. The chemical changes undergone in untreated and treated wood samples after exposure to fungi were examined by using FTIR spectroscopy. FTIR spectra were captured using a FTIR Frontier spectrometer (PerkinElmer) with an attenuated total reflectance mode at a resolution of 4 cm^{-1} and 32 numbers of scans. The range for measuring the spectra was 4000–650 cm^{-1}. However, in the

results, only the spectra from 2000 to 650 cm^{-1} were reported as major changes were seen in that area. All the samples were held against the crystal and absorbance was recorded.

2.6.2. SEM analysis. Wood impregnated with red sanders extracts was observed under a scanning electron microscope (Zeiss Ultra 55) at 2.0 kV to 5.0 kV to examine the changes in the microstructure of the untreated wood samples exposed to wood rotting fungus and the microstructure of the treated wood samples with red sanders bark extracts exposed to wood rotting fungus. Sample preparation was done by placing a small piece of wood on the carbon coated grid followed by gold coating of the sample prior to imaging.

2.7. Leaching test

Leaching test was performed based on standard method EN 84[36] for both treated and untreated samples. Six wood blocks of different treatments were kept in a beaker and balanced with weights and placed on an orbital shaker. Sufficient water (150 mL) was added to the samples. Water was replaced after 24, 48, 72, 96 hours and thereafter on alternate days up to 2 weeks. The mass loss % was calculated thereafter as follows:

$$\mathrm{ML}(\%) = \frac{W2 - W4}{W2} \times 100 \quad (5)$$

In the equation, $W2$ is the oven dry weight of the wood sample after impregnation (g) while $W4$ is the oven dry weight of the wood sample after leaching (g).

2.8. Statistical analysis

To compare the efficacy of treated wood with different RS bark extracts, analysis of variance (ANOVA) was performed. One way ANOVA was done to test whether the treatments differ significantly or not. Differences were considered significant at $p \leq 0.05$. The null and alternative hypotheses were articulated as follows: the treatment means are identical, indicating no significant differences between treatments (null hypothesis), and the treatment means are distinct, indicating substantial differences between treatments (alternative hypothesis). Post-hoc ANOVA analysis was done using Duncan *t*-test to group the treatments.

3. Results and discussion

3.1. Bark extracts

A detailed study on the chemical composition of the red sanders bark extracts was reported by the authors in their previous paper.[9] The main compounds present and reported in *P. santalinus* bark are santalin Y, santalin A and santalin B. The other compounds found in the bark are isoflavonoids, terpenoids, and related phenolic compounds, like β-sitosterol, lupeol, (−) epicatechin[37] and lignans. The extractive content percentage of the bark with different solvents was estimated gravimetrically according to the method explained in ref. 9 and it was found that water extract yielded the maximum with around 26.22%, followed by acetone extract at 2.59% and methanol at approximately 1.05%. The authors obtained total phenolic content (mg of GAE/gm of extract) on the different extracts and found that water extract had 111.20 while methanol and acetone extracts had 131.45 and 126.20, respectively. This higher phenolic concentrations in the solvent extracts also resulted in higher antifungal properties. The LCMS/MS analysis showed that acetone extracts have a number of extra compounds present in them, namely santalin A and homopterocarpin which adds on to the antifungal properties of the acetone extracts as compared to the methanol extracts.[9] This section has not been elaborated further to keep the focus of the study on antifungal properties.

3.2. Impregnation of bark extracts into wood

The impregnation of wood with red sanders bark's extract was done according to IS 401 (ref. 32) and the penetration of red sanders bark extracts into HB, MI and MD wood is reported in terms of retention and WPG (Table 1). Solvent choice influenced both the chemical constituents of the applied extracts and also the impregnation behaviour. Acetone and methanol extracts are enriched in terpenoids such as lupeol and other antifungal terpenes and phenolics while the water extraction favoured glycosides and phenolics.[9] Moreover, the anatomical variations of the wood species and the physical properties of the impregnating medium like surface tension, contact angle and viscosity control capillary uptake and penetration kinetics.[38] Perré *et al.*[38] studied the penetration behaviour of the oil and water in poplar and spruce species using X-ray tomography. The authors saw that in the case of poplar, oil exhibited penetration through the vessel network with a small contact angle, while no direct penetration of water was observed in vessels. The authors concluded that the large contact angle for water blocks the capillary rise between vessel cells. However, in the case of spruce, the water exhibited penetration through latewood and subsequently into the growth rings, but this transversal migration is quasi-absent for oil.

This was also observed in our cases where, the retention of acetone and methanol extracts was higher (in most cases) compared to water extracts. Solvents with lower surface tension such as acetone and methanol penetrate lumina and fine pores faster and deeper than water (Lucas–Washburn-type behaviour), which increases bulk retention and access to anatomical pathways. In terms of reduced leaching, water forms hydrogen bonds and swells polysaccharide domains (favoring small polar solutes diffusing into cell walls), whereas organic solvents plasticize the matrix differently and keep mid-polarity compounds solubilized and mobile long enough to adsorb to lignin or deposit at lumen–wall interfaces.[39] Furthermore, fast-evaporating solvents (acetone and methanol) tend to freeze solutes near and in the lumens, while slower drying can allow more time for diffusion into the cell wall. This combined with favourable solute-polymer affinities (Hansen solubility parameter), explains why longer impregnation time and organic solvents reduced leaching and improved efficacy in our tests.[40]

Rubberwood has a density of 540 to 660 kg m^{-3} (air dry),[41] mango wood has a density of 540 to 660 kg m^{-3} (air dry) and melia dubia wood at 371 to 551 kg m^{-3}.[42] The retention of the extracts was in the range from 2.64 to 7.57 kg m^{-3}, 0.94 to 8.81

Table 1 Retention (kg m^{-3}) and WPG (%) of *P. santalinus* bark extract treated rubberwood, mango and melia wood[a,b]

	Retention (kg m^{-3})			Weight percentage gain (%) air dry		
Code	Water extract	Acetone extract	Methanol extract	Water extract	Acetone extract	Methanol extract
HB-AT1	3.06 ± 0.13^{a}	2.64 ± 0.19^{a}	2.78 ± 0.15^{a}	15.16 ± 0.4^{d}	13.12 ± 0.76^{d}	13.51 ± 0.81^{d}
HB-AT2	3.21 ± 0.11^{b}	3.38 ± 0.16^{b}	3.06 ± 0.12^{b}	15.29 ± 0.3^{e}	16.77 ± 0.38^{e}	14.84 ± 0.48^{e}
HB-BT1	6.91 ± 0.55^{c}	6.09 ± 0.68^{c}	5.84 ± 0.53^{c}	13.64 ± 0.61^{e}	12.43 ± 1.3^{e}	12.23 ± 1.13^{e}
HB-BT2	7.57 ± 0.36^{c}	7.36 ± 0.73^{c}	7.03 ± 0.81^{c}	15.25 ± 0.54^{e}	15.01 ± 1.14^{e}	15.18 ± 1.56^{e}
MI-AT1	0.94 ± 0.06^{a}	1.22 ± 0.09^{a}	1.02 ± 0.12^{a}	4.63 ± 0.12^{d}	6.31 ± 0.5^{d}	5.04 ± 0.51^{d}
MI-AT2	1.99 ± 0.14^{b}	2.14 ± 0.1^{b}	2.23 ± 0.15^{b}	10.89 ± 0.52^{e}	11.01 ± 0.32^{e}	11.74 ± 0.43^{e}
MI-BT1	7.16 ± 0.69^{c}	7.10 ± 0.47^{c}	5.78 ± 0.61^{c}	14.31 ± 0.62^{e}	16.23 ± 1.23^{e}	11.76 ± 1.04^{e}
MI-BT2	7.26 ± 0.75^{c}	8.81 ± 1.21^{c}	7.47 ± 0.70^{c}	14.59 ± 1.32^{e}	17.88 ± 2.17^{e}	15.43 ± 1.48^{e}
MD-AT1	1.26 ± 0.04^{a}	1.16 ± 0.08^{a}	1.18 ± 0.07^{a}	6.61 ± 0.19^{d}	6.77 ± 0.35^{d}	6.57 ± 0.26^{d}
MD-AT2	1.96 ± 0.15^{b}	2.14 ± 0.5^{b}	2.21 ± 0.69^{b}	11.52 ± 0.34^{e}	11.41 ± 4.67^{e}	12.28 ± 4.07^{e}
MD-BT1	6.61 ± 0.38^{c}	6.34 ± 1.34^{c}	6.59 ± 0.43^{c}	15.60 ± 1.4^{e}	17.02 ± 0.37^{e}	17.31 ± 0.89^{e}
MD-BT2	5.87 ± 0.53^{c}	6.14 ± 0.38^{c}	6.22 ± 0.49^{e}	14.55 ± 1.6^{e}	16.14 ± 0.40^{e}	16.84 ± 0.47^{e}

[a] Here, A, B, T1, and T2 refers to 3% concentration of extracts, 8% concentration of extracts, 1-hour pressure treatment and 2-hour press treatment respectively. Thus, AT1 means samples treated with 3% extract concentration for 1 hour and so on. [b] Similar alphabets denote non-significant changes in the groups.

kg m^{-3} and 1.16 to 6.61 kg m^{-3} in the case of HB, MI and MD wood respectively. Both the retention and the WPG were in direct proportion to the concentration of the bark extracts used to treat the wood samples. There were statistical differences in treatment method used, with samples pressure treated with the 8% concentration and for 2 hours had the highest WPG%. Moreover, the weight percentage gains were in the range from 12.23 to 16.77%, 4.63 to 17.88% and 6.57 to 17.31% in the case of HB, MI and MD wood, respectively. Low density woods have higher weight percentage gain and a higher concentration of bark extract gave higher retention in wood than lower concentration.[27] Moreover, caking of extracts (layering of extracts on top of the wood surface) was observed when impregnated with a higher concentration of extracts. This layer was wiped off before WPG was calculated and further testing was done. This surface crust formation was also observed when *Acacia mangium* bark extracts were impregnated in rubberwood at higher concentrations.[27] The impregnation of tannin rich solution into wood results in a decrease in penetration due to the high viscosity of the solutions.[14] Moreover, the vessels and lumens get fully saturated with extracts and that results in caking of the solutions. The average WPG was the highest for HB and the lowest for MD wood and this can be attributed to the higher density of the wood. Lower density of wood has greater lumen space and thus, more quantity of extracts can travel into the wood and contribute to the higher WPG.

The color changes after impregnation were represented in terms of L^* (lightness), a^* (redness), and b^* (yellowness). A change in visual appearance of the wood surface was observed on impregnation of extracts into the wood. Wood specimens when impregnated with lower concentration (3%) of the bark extracts had a lightish red shade compared to dark red shades when impregnated with a higher concentration (8%). The colour changes that occurred on the wood surface after impregnating wood samples with 8% concentration of bark extract are represented in Fig. 1. Maximum darkening was observed when wood was treated with water extract and redness was observed when treated with acetone and methanolic extracts. The hues obtained can be explored to utilize the extracts as both bio-preservatives and bio-formulated coatings. Thus, it can be complementary to the previous study[9] in improving the aesthetics of the plantation grown timber. However, mere coatings can be removed away from the surface but impregnating wood with the extracts addresses the issue by creating a two-fold increase in protection; it not only improves the antifungal properties but also provides protection against UV rays. The fixation of extractives and reduction of mass loss after leaching discussed below support the above statement.

3.3. Antifungal properties of the red sanders bark extract treated woods

The red sanders bark extracts were found to be more effective against brown rot fungi *O. placentus* than white rot fungi *T. hirsuta.* This antifungal activity of the red sanders bark extract may be attributed to the presence of higher amounts of phenolic groups in the extract.[9] Kumar *et al.*,[9] studied the percentage growth inhibition of acetone and methanol extracts against brown-rot and white-rot fungi *via* the food poisoning method. It was observed that an increase in dosage to 2% (w/v) increased the fungal growth inhibition percentage against white rot up to 77.92% and 75.97% for methanol and acetone extracts of RS bark, respectively. This antifungal activity of the RS bark extract may be attributed to the presence of higher phenolic contents in the extract. Lupeol is known to be present in *P. santalinus* bark along with an array of other compounds, which contributes to its *in vitro* antifungal activity. Lupeol was found in acetone and methanol extracts in the (+) ve ion mode and (−) ve ion mode, of LCMS and has been detailed by the authors in a previous study.[9]

The percentage of mass loss of acetone extract treated rubberwood was approximately 23% when treated with 3% extract, while it reduced significantly to 11% for both *T. hirsuta* and *O. placentus* when treated with 8% extract. The increase in extract content impregnated into wood as represented by the retention

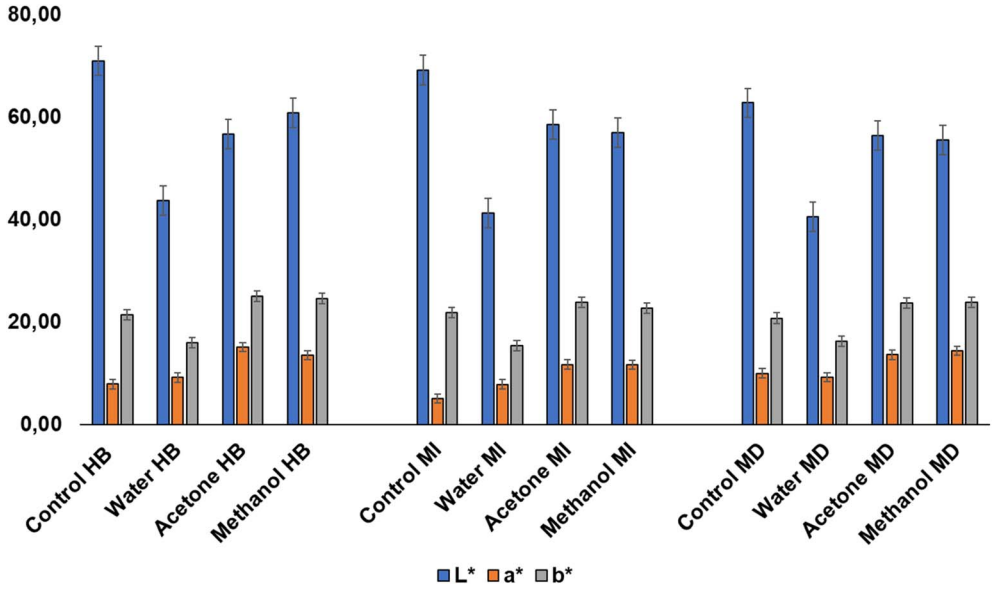

Fig. 1 Changes in colour parameters after impregnation of red sanders bark extract.

(kg m^{-3}) is found to be synergistic to the increase in antifungal activity. The decay percentage of control rubberwood samples was on average (37.86 ± 0.25) % and (46.48 ± 0.62) % for *T. hirsuta* and *O. placentus*, respectively. Table 2 shows a table with treatments significantly differing from the control samples in terms of mass loss %. The mass loss % of treated woods was more in water extract than in the acetone or methanol extract. The acetone extract performed better than other extracts across all the three different species. This may be because most active biochemicals were present in the methanol extract and acetone extracts.[9] There may be concerns regarding the use of solvents for impregnation and the potential impact of residual solvents on biocidal activity. However, since methanol and acetone are highly volatile, the impregnated samples were oven-dried to a constant weight, ensuring complete solvent removal and eliminating any risk of solvent interference with the test results. MD had the least amount of degradation in terms of mass loss; (13.28 ± 2.89) % and (18.21 ± 2.65) % in the case of *T. hirsuta*, respectively. After treatment with 8% extractive, it was found that the mass loss decreased below 1% for *O. placentus* and around 2% for *T. hirsuta*. It may be concluded that the red sanders bark extract along with the extractives found in the MD wood performed synergistically. The extract treated MI wood was also found to be effective. A mass loss of around 7% to 6% was observed against both the tested fungi while the untreated samples had a mass loss of (33.63 ± 1.84) % and (32.91 ± 3.27) % against *T. hirsuta* and *O. placentus*, respectively.

Table 2 Mass loss% of red sanders bark extract treated wood samples against wood rotting fungi[a,b]

	Mass loss (%) against *T. hirsuta*			Mass loss (%) against *O. placentus*		
Code	Water extract	Acetone extract	Methanol extract	Water extract	Acetone extract	Methanol extract
HB-AT1	31.33 ± 4.10^{b}	25.89 ± 1.72^{b}	30.69 ± 3.48^{b}	30.91 ± 1.58^{b}	24.19 ± 2.93^{b}	29.63 ± 2.62^{b}
HB-AT2	28.98 ± 0.72^{b}	21.63 ± 2.30^{b}	25.89 ± 1.08^{b}	28.28 ± 1.42^{b}	21.13 ± 1.43^{b}	24.52 ± 1.12^{b}
HB-BT1	17.99 ± 1.44^{a}	11.92 ± 1.39^{a}	13.90 ± 0.57^{a}	17.17 ± 3.92^{a}	11.87 ± 1.12^{a}	13.80 ± 1.55^{a}
HB-BT2	15.24 ± 1.57^{a}	10.97 ± 1.84^{a}	11.11 ± 1.33^{a}	13.82 ± 1.88^{a}	11.21 ± 0.55^{a}	10.74 ± 3.18^{a}
HB control	37.86 ± 0.25^{c}			46.48 ± 0.62^{c}		
MI-AT1	28.76 ± 1.37^{e}	20.47 ± 0.71^{e}	25.71 ± 1.45^{e}	24.67 ± 1.53^{e}	19.89 ± 1.11^{e}	23.88 ± 2.12^{e}
MI-AT2	23.51 ± 1.88^{e}	18.28 ± 1.50^{e}	22.27 ± 1.05^{e}	21.69 ± 0.79^{e}	17.65 ± 3.75^{e}	20.27 ± 2.72^{e}
MI-BT1	8.44 ± 5.96^{d}	7.81 ± 3.99^{d}	8.01 ± 1.89^{d}	7.89 ± 1.26^{d}	6.07 ± 2.64^{d}	7.53 ± 1.70^{d}
MI-BT2	7.76 ± 0.89^{d}	6.78 ± 2.27^{d}	7.63 ± 1.36^{d}	6.91 ± 1.26^{d}	5.98 ± 1.69^{d}	6.75 ± 2.85^{d}
MI control	33.63 ± 1.89^{f}			32.91 ± 3.27^{f}		
MD-AT1	12.77 ± 2.72^{h}	10.84 ± 1.24^{h}	11.31 ± 0.40^{h}	11.88 ± 1.04^{h}	6.14 ± 0.76^{h}	11.41 ± 2.37^{h}
MD-AT2	11.89 ± 0.83^{h}	10.36 ± 0.57^{h}	11.09 ± 0.24^{h}	10.96 ± 0.15^{h}	5.50 ± 1.59^{h}	10.85 ± 3.32^{h}
MD-BT1	2.84 ± 0.23^{g}	1.89 ± 0.63^{g}	2.65 ± 0.43^{g}	2.39 ± 0.86^{g}	1.49 ± 1.04^{g}	2.32 ± 0.92^{g}
MD-BT2	2.49 ± 0.66^{g}	1.44 ± 0.35^{g}	1.51 ± 0.23^{g}	1.21 ± 0.23^{g}	0.65 ± 0.92^{g}	0.89 ± 0.41^{g}
MD control	13.28 ± 2.89^{i}			18.21 ± 2.65^{i}		

[a] Here, A, B, T1, and T2 refers to 3% concentration of extracts, 8% concentration of extracts, 1-hour pressure treatment and 2-hour press treatment respectively. Thus, AT1 means samples treated with 3% extract concentration for 1 hour and so on. [b] Similar alphabets denote non-significant changes in the groups.

These findings are consistent with the work of Yingprasert *et al.*,[27] who observed that *Acacia mangium* bark extracts reduced fungal mass loss from 28.26% and 26.36% in untreated samples which significantly reduced to 9.23% and 5.18% against *T. versicolor* and *G. striatum*, respectively, when the concentration of bark extracts was increased up to 20% (w/v). Similarly, the present results align with those of ref. 26 which reported that *Acacia mangium* bark extracts were more effective against brown-rot fungi than white-rot fungi. In the present study, the extracts of *P. santalinus* bark showed greater inhibition against *O. placentus* (brown rot) than *T. hirsuta* (white rot), suggesting that phenolic compounds in the bark are more effective in disrupting the oxidative degradation mechanisms of brown-rot fungi. Our findings were comparable to these findings specially in the case of HB, where the control samples had a mass loss of 46.84% which was reduced to 10.74% with methanol extracts against brown rot fungi and from 37.86% to 11.11% against white rot fungi; and in MD specimens treated similarly showed ≤2% mass loss and ≤8% mass loss in MI samples. A close study of Table 2 shows us that there was significant change observed when the samples were treated with extracts. The statistical analysis was done to determine the changes within treatment cycles against white rot and brown rot fungi separately in individual wood species. The groupings were thereby done based on these aspects.

The antifungal efficacy observed here also compares favorably with the activity of other bark extracts reported in the literature. For instance, Alfredsen *et al.*,[43] found that *Acer platanoides* bark extract showed high inhibition against a range of fungi, while Kofujita *et al.*,[44,45] reported the potent antifungal activity of diterpene quinones from *Cryptomeria japonica*. The presence of lupeol in red sanders bark extracts as reported by Kumar *et al.*,[9] likely contributes to the antifungal activity observed in the present work. Nisar *et al.*,[46] found that lupeol was found to have significant fungicidal activity when tested against an array of fungi like *Aspergillus niger*, *Candida albacan*, *Cantharellus flavus*, *Fusarium solani*, *Microsporum canis* and *Candida glabrata*.

When compared to synthetic preservatives, the antifungal performance of *P. santalinus* bark extracts approaches that of commercial wood preservatives formulations such as chromated copper arsenate (CCA), alkaline copper quaternary (ACQ) and copper azole (CuAz), which typically limit mass loss to below 3–5% at ground-contact retentions under laboratory conditions.[47] The results were also comparable to zinc and copper-based nano-compounds (with and without acrylic emulsions) where nanozinc borate combined with acrylic emulsion imparted very high resistance to *T. versicolor*, with only 1.8% mass loss.[48] The authors discussed that the addition of acrylic emulsion significantly improved the leach resistance of nanozinc oxide and nanozinc borate, reducing leaching percentages to 9% and 8%, respectively, compared to higher percentages without the emulsion.

The termite testing was performed according to IS 4833[35] and the visual rating was given to the samples. The average degradation of all samples in each treatment was noted every month. The result of the experiment is represented in Table 3 and Fig. 2 shows the average visual rating of each sample after a period of 6 months. It was found that HB and MI were completely destroyed after a period of 6 months while melia being a durable wood had a degradation of around 5% to 20% after 6 months. The treated MD on an average had degradation less than 5% while treated mango had an average degradation of 30% when treated with red sanders bark extract. The readings were almost similar for all different extracts. HB suffered slightly more degradation than MI but the overall degradation was within the 40% limit. The untreated HB exposed to termites underwent complete destruction within four months of exposure. The untreated MI samples also perished completely after the six-month testing period. These findings suggest that red sanders bark possesses potential for use in protecting wood against subterranean termites, and its efficacy is likely to be enhanced in less humid environments, as termite activity is strongly influenced by surrounding environmental factors. Duncan *T*-test shows significant differences after termite tests of fungal groups, and they were grouped in different classes.

The anti-termite results obtained aligns with previous studies on bark-derived bio-preservatives. Bark extracts from *Lawsonia inermis*,[49] *Acacia mollissima* and *Schinopsis lorentzii*,[18] and *Acacia crassicarpa* and *Acacia mearnsii*[50] all demonstrated substantial termite mortality within three to four weeks, similar to the present findings where *P. santalinus* extracts caused significant anti-termite activity. These results confirm that bark-derived polyphenolic compounds act as both feeding deterrents and toxic agents against termites. Harun and Labosky,[51] investigated the anti-termite and antifungal activities of bark extracts from red pine (*Pinus resinosa*), white pine (*Pinus strobus*), shagbark hickory (*Carya ovata*), red oak (*Quercus rubra*), and red maple (*Acer rubrum*). Acetone : hexane : water (A : H : W) bark extracts were used to investigate these parameters. While the antifungal activities of *C. ovata*, *Q. rubra*, and *P. strobus* were found to be satisfactory against *Lenzites trabea*, complete termite mortality to near complete termite mortality was observed when cellulose pads were treated with extractives of *C. ovata*,*Q. rubra*, and *P. strobus*. Although the red sanders extractives do not yet achieve identical performance levels, their

Table 3 Visual rating of untreated wood and treated woods after impregnation against termite[a]

Wood species	Water extract	Acetone extract	Methanol extract	Control
Rubberwood (HB)	2.6[a]	2.8[a]	3.0[a]	5.0[c]
Mango wood (MI)	2.0[a]	1.7[a]	1.7[a]	5.0[c]
Melia wood (MD)	0.2[b]	0.2[b]	0.2[b]	1.0[b]

[a] Similar alphabets denote non-significant changes in the groups.

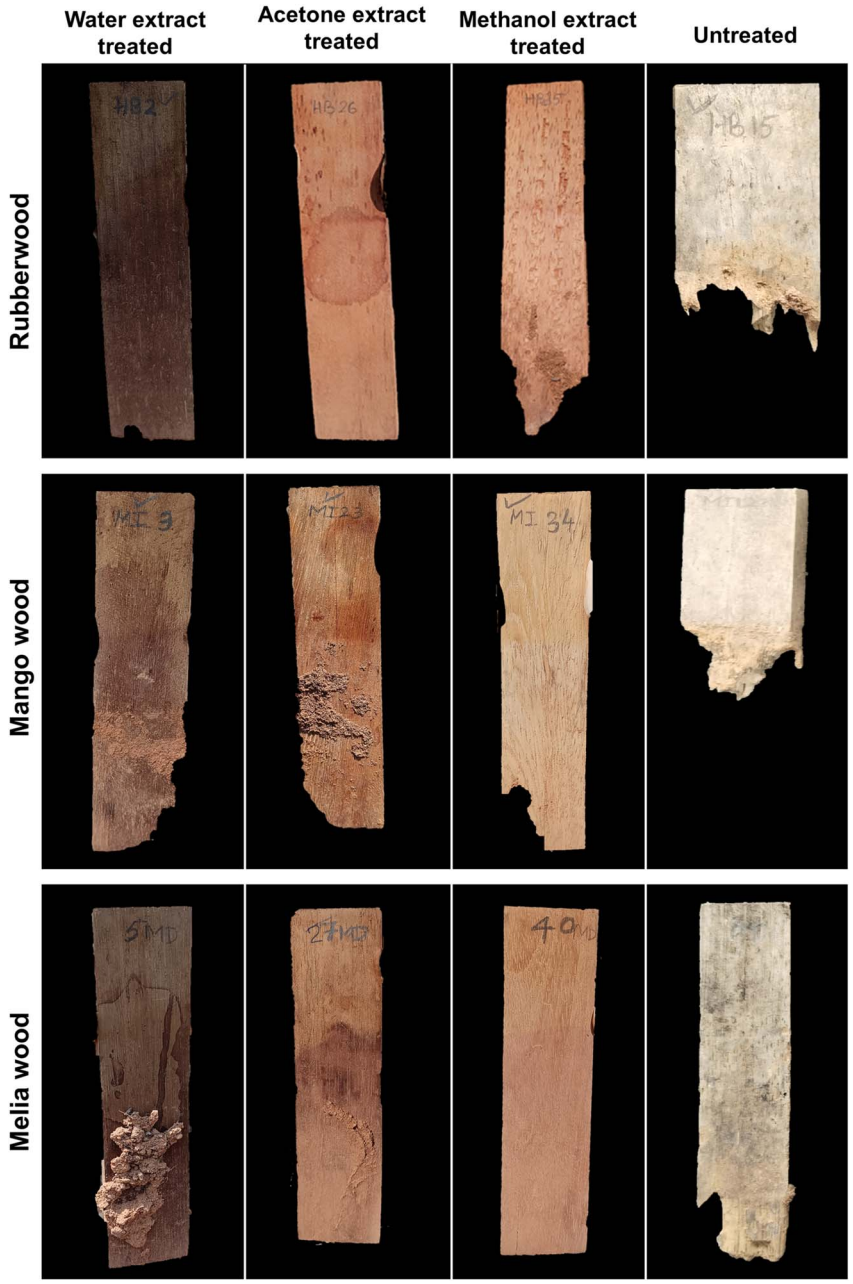

Fig. 2 Degradation against termites in rubberwood, mango wood and melia wood in treated and untreated control wood samples.

environmental safety and renewability make them attractive alternatives, particularly for indoor and non-ground-contact applications.

3.4. Leaching of bio-preservatives from treated woods

The leaching of bio-preservatives is one of the major constraints in conditions where wood is used for outdoor purpose. Fig. 3 represents the mass loss % in treated and untreated HB, MI and MD wood after leaching test for each treatment. It was observed that water extract leached the most, followed by methanol extract and least in the case of acetone extracts. The mass loss after leaching is the combined effect of the loss of treating extract and the secondary metabolites already present in the wood. It was observed that with increasing concentration of the extracts, the leaching reduced. This may be attributed to the fact that increasing concentration of the extracts resulted in bulking in the vessels and thereby reduced leaching.[27] It was also observed that in all treated samples leaching was reduced when the samples were treated for 2 hours. This suggests that increasing treatment duration results in better fixation of the bio-preservatives within the cells and thus reduces leaching. The mass loss observed in the untreated samples (control samples) was due to the loss of the secondary metabolites already present in the samples. HB showed maximum leaching with decrease in mass by almost 4.80% in the case of water extract treated wood (8% for 2 hours). The least leaching is seen

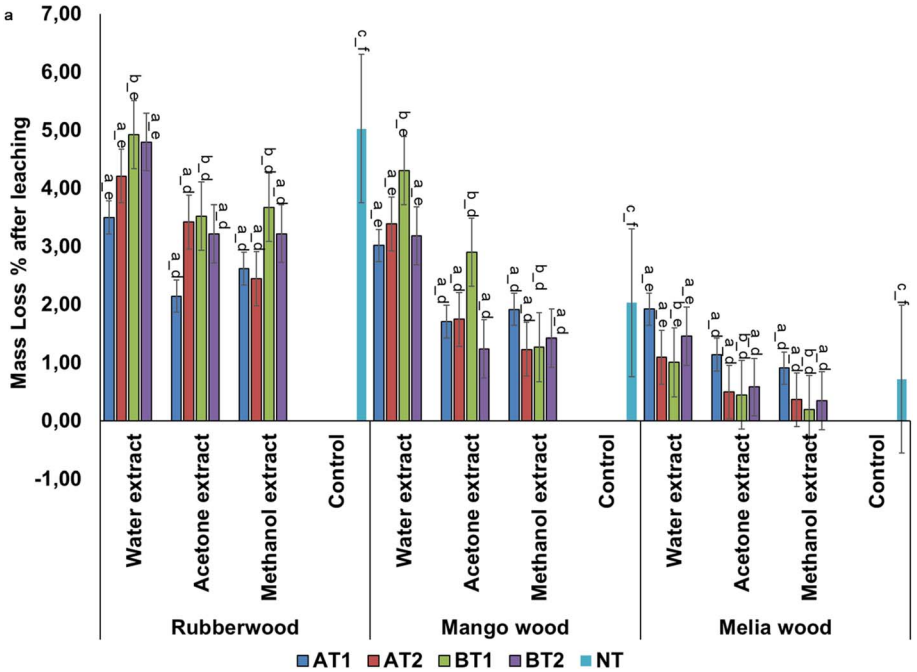

Fig. 3 Mass loss % in treated and untreated wood after leaching test where, A, B, T1, and T2 refer to 3% concentration of extracts, 8% concentration of extracts, 1-hour pressure treatment and 2-hour press treatment, respectively (Note: similar alphabets denote non-significant changes in the groups).

in acetone extract treated woods. While comparing amongst wood, it was observed that MD wood showed lower leaching.

Duncan *T*-test was performed on the samples after the leaching test for grouping or subset formations based on the mass loss % after the leaching test. It was observed that control samples behaved significantly different from the treated samples in both the cases. Therefore, it can be said that there had been significant effect of the treatments and the extracts against leaching. However, among extracts, methanol and acetone extracts behaved almost similarly to each other in offering protection against leaching while the water extracts behaved completely different to it.

Kumar *et al.*,[9] reported the presence of β-amyrone, β-sitosterol, lupeol, epi-lupeol, lupenone, acetyloleonic acid, and a new lupenediol named lup-(20) 29-en-2α,3α-diol along with santalin A, santalin B and santalin Y. Phytochemical analysis of the active fraction from the bark of *P. santalinus* has indicated the presence of flavonoids, glycosides, tannins and phenols.[52] There has been no concrete evidence of what the exact reaction mechanism behind the fixation of the bio-preservatives to wood is, but studies related to metal-tannin have suggested the formation of chelation leading to coordination complexes.[53] Phenolics (including tannins) probably oxidize to quinone-like species which then undergo further coupling reactions or Michael-type additions with nucleophiles (–OH) present in hemicelluloses/lignin or with proteins giving rise to covalent (or strongly stabilized) attachments to the wood substrate.[53] Another possible reaction mechanism can be the *in situ* polymerisation or cross-linking that converts water-soluble phenolics into high molecular weight insoluble networks inside the cell wall or lumen. The samples treated for 2 hours had sufficient duration to undergo this change and thus exhibit lower mass loss after the leaching test.

3.5. FT-IR spectral analysis

The chemical changes observed in the wood after degradation by *T. hirsuta* and *O. placentus* were analyzed by changes in the FT-IR spectra. Significant damage is caused by wood degrading fungi which results in the structural failure of wood. The fungi enter wood through pits, vessels or by penetrating the cell walls. White-rot and brown-rot fungi attack various elements of the wood cell wall in different ways. The main peaks discussed and analysed in the study were ~1729 cm^{-1} (unconjugated C=O present in the hemicelluloses), ~1505 cm^{-1} (aromatic skeletal vibration in lignin), ~1461 cm^{-1} (aromatic C–H deformation in lignin and carbohydrates), ~1370 cm^{-1} (C–H deformation in cellulose and hemicelluloses), ~1242 cm^{-1} (syringyl ring plus C–O stretch in lignin and xylan), ~1155 cm^{-1} (C–O–C vibration in cellulose and hemicelluloses), and ~898 cm^{-1} (C–H deformation in cellulose).[9,54–60] The major spectral changes after fungal degradation were found at all the major concerned peaks as shown in Fig. 4. To summarize, the peaks at 1505 cm^{-1}, 1461 cm^{-1} and 1242 cm^{-1} are associated with lignin while 1370 cm^{-1}, 1155 cm^{-1} and 898 cm^{-1} are associated with cellulose. Weight gain after impregnation and mass loss after fungal degradation experiments (SI data) showed grouping of the fungal degraded samples of the untreated and treated samples.

The spectra for HB, MI and MD samples treated with water, acetone and methanol extracts exposed to *T. hirsuta* are shown in Fig. 4. From the spectra it can be seen that the ~1729 cm^{-1}

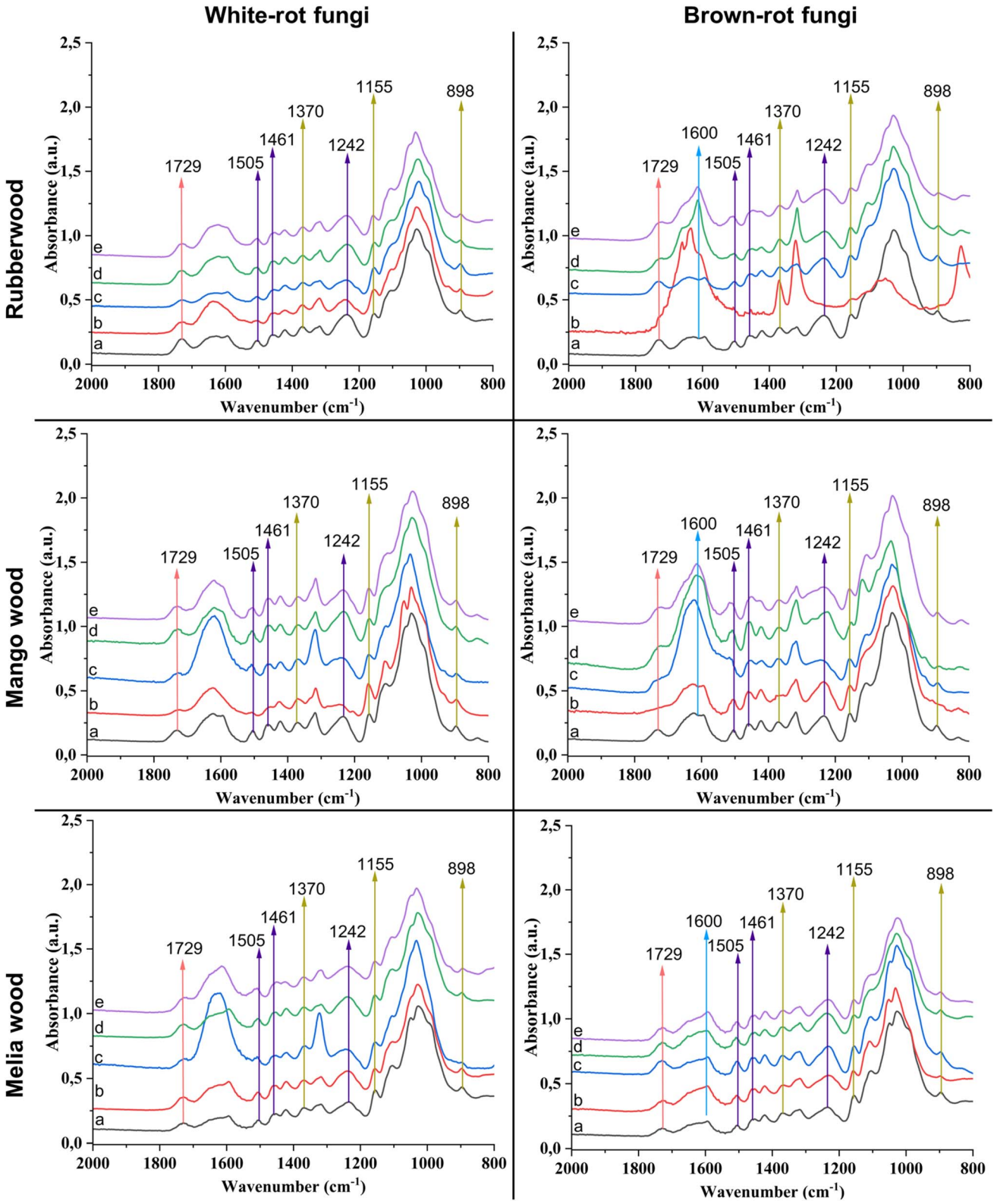

Fig. 4 FTIR spectra of (a) untreated unexposed wood, (b) untreated exposed wood, (c) 8% water extract treated exposed wood, (d) 8% acetone extract treated exposed wood, and (e) 8% methanol extract treated exposed wood against white-rot fungi and brown-rot fungi.

peak undergoes changes in the intensity in the untreated exposed wood while there is minor to almost no change in the intensity of the treated wood. This can be attributed to the degradation in the xylan composition of the untreated wood.[57,58] The almost no change in the same band suggests no degradation in the treated woods. One of the major characteristics of

the white rotting fungi is that it starts degrading the lignin of the wood and thereafter the other components of the cell wall structure and structural carbohydrates.[58] One of the major changes observed in the chemical composition of the wood degraded by white rotting fungi is the elimination or partial removal of the peaks associated with the lignin. Similar findings were observed in the cases of untreated exposed HB and MI and decreased intensity in MD. The peaks of ~1505 cm^{-1}, ~1461 cm^{-1} and ~1242 cm^{-1} were completely removed in mango and rubberwood and partial decrease in intensity of the MD. The spectra for HB, MI and MD samples treated with water, acetone and methanol extracts exposed to *O. placentus* are shown in Fig. 4. The rate of enhancement of lignin proportion is much higher in brown rot decayed wood than in the white rot decayed wood.[58] MD is in general durable wood and thus no major change is observed in the FTIR spectra of the untreated exposed wood except a significant decrease in intensity of the 898 cm^{-1} which corresponds to deformation in cellulose. However, in case of mango and HB major changes are observed. The peaks associated with unconjugated C=O present in the hemicelluloses (*i.e.* ~1729 cm^{-1}) are almost vanished. HB untreated exposed wood showed maximum mass loss percentage and to compliment the findings, the absorbance peaks of the untreated exposed wood showed elimination of peaks at ~898 cm^{-1} and ~1155^{-1}. This was complemented with significant increase in intensity of the ~1600 cm^{-1} peak that is associated with lignin.[58,59] This peak was less pronounced in the treated samples. The results thus obtained from the FT-IR spectra were in accordance with the mass loss percentage observed and thereby it can be concluded that the red sanders bark extracts can be used to prolong the service life of the wood and wooden handicrafts.

3.6. SEM analysis

The treated HB, MI and MD exposed to decaying fungi were also analysed by SEM to visualise the morphological changes in the

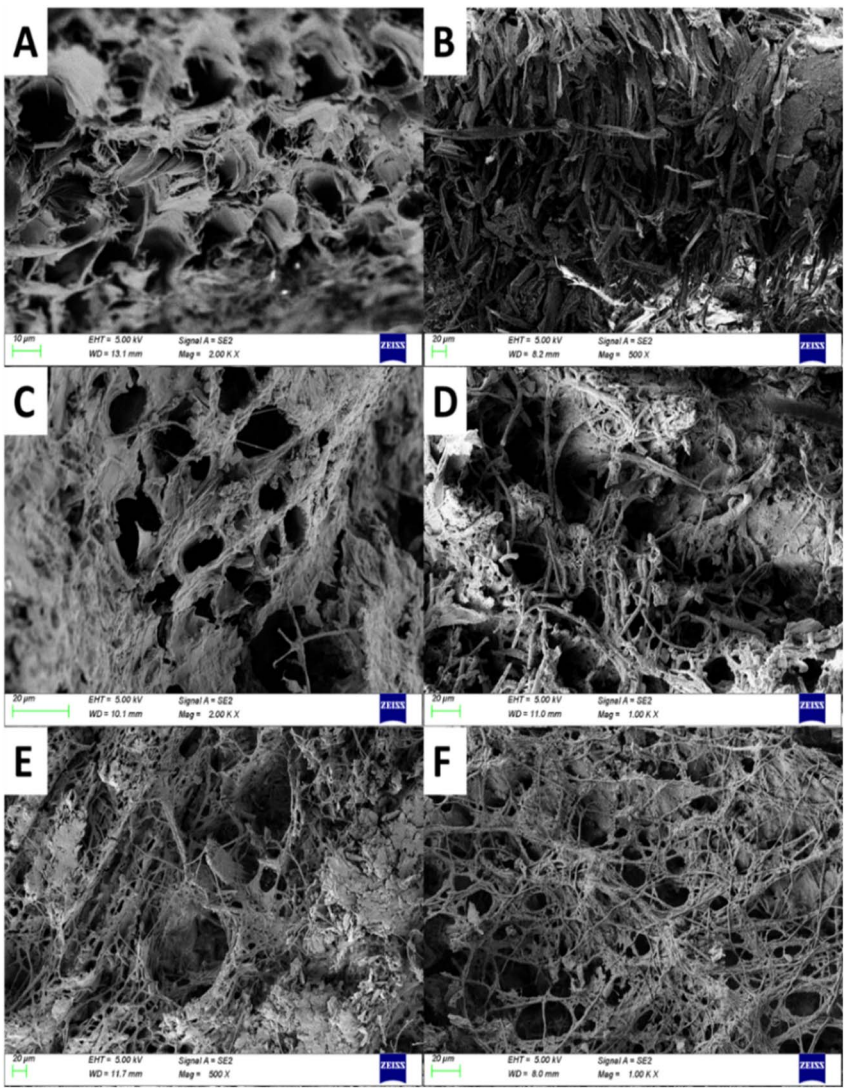

Fig. 5 SEM images of control samples against (left column) white-rot fungi and (right column) brown-rot fungi in (A and B) rubberwood, (C and D) mango wood and (E and F) melia wood specimens.

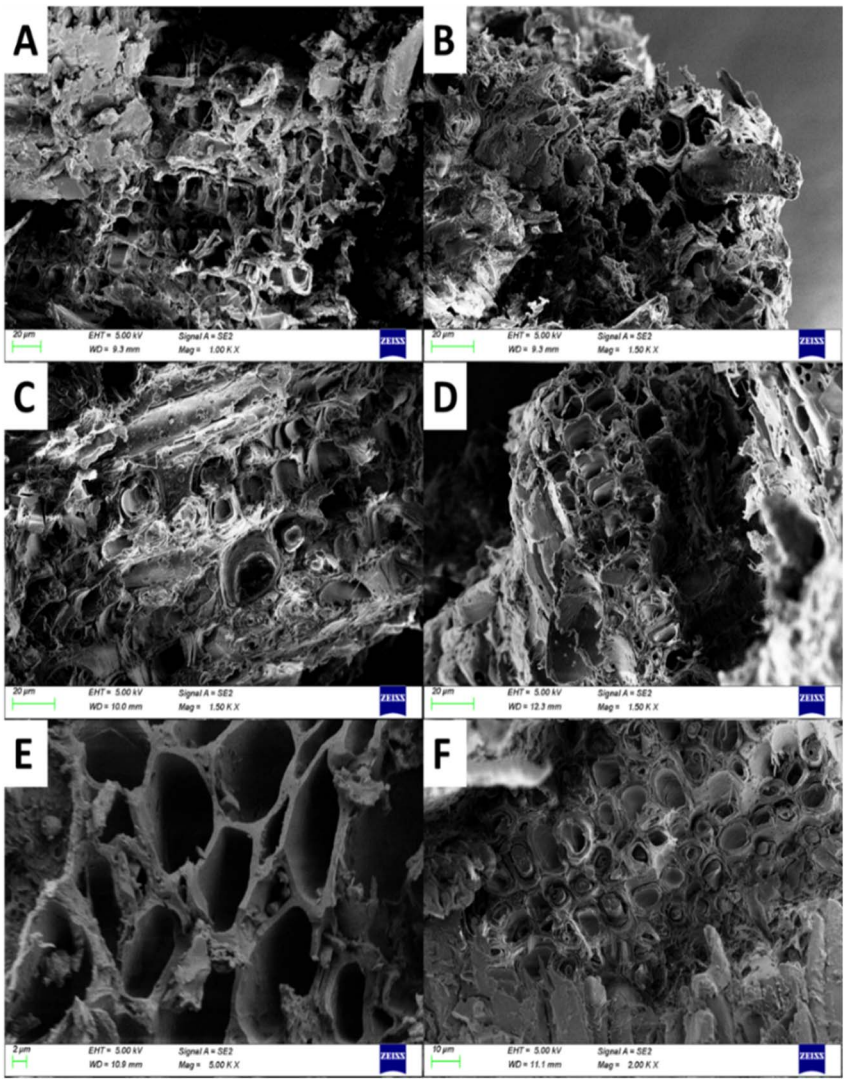

Fig. 6 SEM images of methanol extract treated wood samples against (left column) white-rot fungi and (right column) brown-rot fungi in (A and B) rubberwood, (C and D) mango wood and (E and F) melia wood specimens.

wood. The untreated wood samples exposed to decaying fungi were also studied and shown in Fig. 5A–F. As seen in the images, the cell walls undergo damage and result in complete breakdown of the internal structures. Moreover, the white rot decayed samples become fibrous and somewhat pale in colour. In comparison, the methanol treated samples had intact their structures intact when compared to the control samples.

The presence of hyphae in the wood along with fibrous structure is also seen. The hyphae of the fungi encase the microstructures and degrade the whole structure. White rot fungi preferentially degrade lignin, hemicellulose and cellulose.[61] The white rot fungi utilise hydrolysable and oxidative enzymes to degrade cellulosic components, but the lignin components are completely broken down by enzymatic activity of fungi such as manganese peroxidase, lignin peroxidase, volatile peroxidase, and laccase.[62] In contrast, a two-step oxidative-enzymatic process is seen for brown rotting fungi.[63] The brown rot fungi degrade the hemicelluloses and cellulose, leaving the lignin undigested which gives it the brown hue after disintegration. Wood degraded by brown rot fungi disintegrates into a powder due to complete carbohydrate degradation.

However, internal structures of the methanol treated wood are found to have microstructures intact or have undergone slight changes. Moreover, there was the presence of solidified particles inside the vessels. The methanol extract treated wood samples exposed to decaying fungi were also studied and are shown in Fig. 6A–F. There is almost no presence of fungal hyphae in the wood, and it was also seen that the vessel in the wood is filled with the extracts. The internal microstructure is also found to be intact, and breakdown of the cell wall is also not found. This conforms with the FTIR results seen in Fig. 4 and with the mass loss data after exposure. However, there were no significant changes to the wood microstructure in the treated wood specimens as shown in Fig. 6A–F. Thus, it can be said that the red sanders bark extract can be used as a biopreservative against wood decaying fungi.

4. Conclusion

The present study demonstrated that red sanders bark extract contains bioactive phytochemicals that exhibit significant antifungal and anti-termite properties. The wood samples treated with the bark extract showed a marked reduction in fungal growth compared to untreated controls, particularly against *Trametes versicolor* and *Oligoporous placentus*. Similarly, the anti-termite tests indicated reduced wood mass degradation, confirming its potential as a natural termiticide. Moreover, the termite testings done in outdoor exposed conditions suggested the fixation of the preservatives. Impregnation studies showed that increasing the treatment duration improved extract fixation within the wood matrix, resulting in lower leaching losses and enhanced durability. These findings emphasize that optimizing treatment parameters can maximize the preservative performance of the bark extract. Overall, this research provides a sustainable alternative to synthetic wood preservatives by utilizing red sanders bark, an underutilized forest residue, as an eco-friendly bio-preservative.

Author contributions

Souvik Ray: experimentation; investigation; writing – original draft, review & editing; Rakesh Kumar: conceptualization; methodology; project administration; resources; writing – review & editing; supervision; validation; Mithila NS: experimentation; SR Shukla: conceptualization; methodology; resources; manuscript review & editing.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data will be made available on request. Also the data supporting this article have been included as part of the supplementary information (SI). Supplementary information is available. See DOI: https://doi.org/10.1039/d5su00478k.

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