

## *Acorus calamus* L. and *Parthenium hysterophorus* L. plant extracts potential as wood preservative against *Gloeophyllum striatum* decay fungus

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This study aimed to assess the antifungal resistance of *Acorus calamus* and *Parthenium hysterophorus* plant extracts. Wood samples were treated with plant extracts at different concentrations: 0.25, 0.50, 1.00, 1.50, and 2%. For a period of 12 weeks, treated wood samples (*Pinus roxburghii*) were tested for resistance to the brown rot decay fungus *Gloeophyllum striatum*. Wood samples treated with petroleum ether extract of *A. calamus* at 2% concentration showed the lowest mass losses (10.75%), and wood samples treated with the methanolic extract of *P. hysterophorus* showed the lowest mass losses (13.61%) at 2% concentration. Plant extracts of *A. calamus* and *P. hysterophorus* showed the highest antifungal activity and percentage fungus growth inhibition at 2.0% concentration. Maximum colonization was noticed for untreated wood samples, and the lowest was noticed at 2.0%. Antifungal properties of *A. calamus* and *P. hysterophorus* extracts were confirmed by a decay index test. After the decay test, chemical properties of wood samples were evaluated to confirm the efficiency of plant extracts, and it was observed that minimum losses of soluble extractives, lignin, and holocellulose of treated wood samples occurred at 2.0% concentration of extract. As per the findings of the present investigation, selected botanicals can be used for wood preservation and reducing the mass losses.

**Keywords:** Antifungal index, Bio-preservation, Brown rot fungus, Chemical properties, Decay test, *Pinus roxburghii*

Wood is a significant natural rejuvenation product made primarily of cellulose, hemicelluloses, lignin, gum, and wax, among other ingredients<sup>1-3</sup>. These chemical constituents of wood can be attacked by different wood degrading agencies like wood rotting fungi, termites, marine borers, insects pests, etc.<sup>5-7</sup>. Wood's robust mechanical qualities, ease of processing, and pleasing aesthetics have not only made it popular for creating furniture, building materials, ornamental objects, railway sleepers, and tools<sup>8-11</sup>. Wood has enough natural durability for these applications. However, several biotic agents like termites, insect's bugs, and wood-rotting fungus impair the natural durability<sup>6</sup>. Abiotic factors such as moisture content, temperature, UV rays, fire, heat, cold, pollutant chemicals, and so on affect the natural durability of wood<sup>5,7</sup>.

Several tropical and subtropical trees, including teak, sisso, mahogany and deodar have good inherent durability and resistance to wood-degrading organisms<sup>13</sup>. However, the demand of wood-based

industries is not met by current production<sup>14,15</sup>. Due to the decrease in raw material supply, it ultimately affects the growth of wood-based industries<sup>14</sup>. Furthermore, to continue supplying the raw materials for the wood-based industries, one of the best solutions would be introducing less-used species (LUS) into the market<sup>16</sup>. LUS species are found in tropical and subtropical forests. In the present investigation, *Pinus roxburghii*, a less used wood species, is used for the experiment. However, pine wood is considered non-durable and not resistant to wood decay<sup>17,18</sup>. In such cases, chemical wood preservatives are able to increase the durability of wood. There are numbers of chemicals developed by the different wood preservative industries<sup>1,19</sup>. However, due to their harmful effects on people, animals and environment many nations have banned the use of chemical wood preservatives<sup>20</sup>. As a result, demand of wood preservatives that are natural, eco-friendly, easily degradable and do not affect human and environment health growing. As eco-friendly wood preservatives such as essential oil plants, flowers, fruits, leaves, roots and wood extractives from heartwood, sap wood, bark and root are now being used<sup>21</sup>. Several studies have been conducted to

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determine the potential of essential oils against molds<sup>22-24</sup> and reported that the essential oil of *Eucalyptus citriodora* is able to control the growth of moulds. Panek *et al.*<sup>23</sup> tested the essential oils of 10 different species and found that they were effective against wood decay fungi and molds. The attack of *Postia placenta* and *Trametes versicolor* fungi was suppressed by *Sternbergia candida* extract<sup>25</sup>. *Cinnamaldehyde chitosan* emulsion is found effective against the *Gloeophyllum trabeum* and *Trametes versicolor* fungi, and the emulsion is also environmentally friendly<sup>26</sup>.

Lignans, terpenes, terpinoids, stilbens, flavonoids, and tannins can be identified as primary chemicals to protect the wood from wood degrading agents<sup>27</sup>. Considering previous studies, two plant species, *A. calamus* and *P. hysterophorus*, were selected for the present investigation. Selected plant species have antifungal properties due to the presence of different phytochemicals. The major phytochemicals of *A. calamus* are tannins, flavonoids, polyphenolics, mucilage, and glycosides<sup>28,29</sup>. While saponins and glycosides, lactone, hysterin, ambrosin, flavonoids, fumaric acid, vanillic acid, and caffeic acid are the major phytochemicals of *P. hysterophoru*<sup>30</sup>. is study aimed to assess the efficacy of selected plant extracts against the *Gloeophyllum striatum* (Fr.) Murrill Brown rot decay fungi.

## Materials and Methods

### Experiment Location

The current study was carried out at the Department of Forest Products and Utilisation, Dr. Yaswant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh), located at 1275 meters above sea level (30.8613° N, 77.1708° E).

### Preparation of wood samples

Wood samples were prepared in the department workshop from over-dried sapwood of *Pinus roxburghii*. all wood samples were cut into an equal size 5.0 cm longitudinal × 2.5 cm radial × 2.5 cm radial. Before treatment, the wood samples were oven-dried at 105°C until they were attained a constant weight. The wood samples were completely free of defects, fungal infections, and any type of injury. Oven-dried wood sample density varied from 0.4868 to 0.5388<sup>32-34</sup>. Three replicates, each six wood samples were cut for the each treatment, along with control samples.

### Collection of plants materials and extraction preparation

*Acorus calamus* L. and *Parthenium hysterophorus* L. extracts were chosen for the investigation since both plants species have antifungal characteristics. By comparing herbarium specimens with accession numbers, such as 8812 *Acorus calamus* and 12639 *Parthenium hysterophorus*, and a voucher specimen, the botanical identity was established. *A. calamus* rhizomes were procured from Nauni and Khaltu Village, while the mature *P. hysterophorus* aerial portions were procured from the university campus. The samples were initially dried individually for 20 days in open areas with shade. By manually crushing the dried material into powder, mechanical crushing alters the chemical makeup of plants.

Petroleum ether and methanol solvents were used for plant extraction. Hence, petroleum ether is very well known for its ability to remove fats, oils, terpenes, sterols, etc. and its low polarity<sup>31,35</sup>. Furthermore, methanol is well known for its high polarity, low toxicity, and ability to extract different polar phytochemicals such as phenolics, flavonoids, alkaloids, glycosides, steroids etc.<sup>36-39</sup>.

### Extractive solutions

The crushed aerial part of *P. hysterophorus* and rhizomes of *A. calamus* rhizomes (100.0 g each) were extracted with refluxing petroleum ether in a soxhlet apparatus extractor for 10 cycles. The crushed, pre-extracted plant material with petroleum ether was extracted with methanol solvent in a soxhlet apparatus in a boiling water bath. Following extraction, solvents were extracted from the respective solution using rotary evaporation and stored at 4°C. The extracts were named E1 (*A. calamus* petroleum ether extract), E2 (*A. calamus* methanol extract), E3 (*P. hysterophorus* petroleum ether extract), and E4(*P. hysterophorus* methanol extract).

### Treatment method and retentions

Previously conditioned and weighted wood samples were treated through the dipping method with petroleum ether and methanol extracts of *A. calamus* and *P. hysterophorus* at different concentrations: 0.25, 0.50, 1.00, 1.50, and 2.00 % (w/v) by diluting the respective amounts of extract in 5% methanol (v/v). Oven-dried wood samples were dipped in a solution of each concentration of extract for 72 hat room temperature 25°C. Immediately after being removed from the extractive solution, the treated wood samples were weighed to assess the gross solution uptake. The

retention of wood samples  $\text{kg/m}^3$  for each extractive solution was calculated using the formula suggested by<sup>40</sup>.

All wood samples were dried at  $103 \pm 2^\circ\text{C}$  for 24 hs. Treated and untreated wood samples were stored in a conditioning room at  $20 \pm 2^\circ\text{C}$  and  $65 \pm 5\%$  relative humidity until the wood samples reached a constant weight before the decay resistance tests. After the wood samples were dried, their weight percent gain (WPG) was calculated using the given equation (1).

$$WPG = \frac{(W_3 - W_1)}{W_1} \times 1000 \quad \dots (1)$$

[Where as: WPG = Weight Percent gain;  $W_1$  = Weight (g) of oven dried wood sample before treatment;  $W_3$  = Weight (g) of oven dried wood sample after treatment]

#### Fungal strain

For the testing of treated wood samples, *Gloeophyllum striatum* (Fr.) Murrill Brown Rot (culture catalogue number: 916) fungus was used. Fungus culture was procured from the Forest Pathology Division, Forest Research Institute, Dehra Dun (U.K.). Furthermore, Fresh brown rot fungus colonies were raised in a BOD chamber at  $20 \pm 2^\circ\text{C}$  and  $65 \pm 5\%$  RH using 4% malt powder and 2% agar culture media.

#### In vitro antifungal activity

The efficacy of plant extracts, *viz.*, *A. calamus* and *P. hysterophorus* in different solvents, *viz.*, petroleum ether and methanol, at different concentrations, *viz.*, 0.25, 0.50, 1.00, 1.50, and 2.00%, were tested against the fungus by the poisoned food technique suggested by<sup>41</sup>. Furthermore, plant extract solution was added separately to the zation of potato dextrose agar and placed in a Petri dish (dia. 9 cm). As per the concentration, a control treatment was also maintained. Petri dishes of potato dextrose agar were inoculated with the 5-day-old mycelium strain of fungi. Each sample was repeated 3 times. The petri dish was cultivated at  $27 \pm 1^\circ\text{C}$  and 65% RH in the BOD incubator for 5-7 days. The test pathogen's colony diameter was measured up until the point at which the control plates were completely covered in the pathogen's mycelium. The antifungal index was calculated using the equation (2).

$$\text{Antifungal index} = (1 - D_a/D_b) \times 100 \quad \dots (2)$$

[Where:  $D_a$  = Growth zone diameter in experimental plate (cm);  $D_b$  = Growth zone diameter in control plate (cm)]

#### Decay resistance evaluation test

The EN 113 (TS 5563)<sup>42</sup> standard was followed when conducting the block decay test. Growth medium (100 mL in each jar), 2% malt powder, and 2% agar were all present in the 500 mL glass jars. Growing media was inoculated with one plug (5.5 mm) of the actively growing *Gloeophyllum striatum* (Fr.) Murrill Brown Rot (Forest Research Institute culture catalogue number: 916) under sterile conditions. Parafilm was used to seal the jars, and then they were incubated. at  $25 \pm 1^\circ\text{C}$  and  $65 \pm 5\%$  relative humidity until the fungus colonies did not reach the jars' edges. Both treated and untreated wood samples were sterilised in a hot air oven for 24 h at  $105 \pm 2^\circ\text{C}$  before being exposed to fungi. Wood samples (treated and untreated) were placed in jars containing mycelia and kept in the BOD at  $25 \pm 1^\circ\text{C}$  and  $65 \pm 5\%$  relative humidity for 21 days to expose them to the brown rot fungus. The growth of the fungus was then visually assessed by eye using the following scale: Per cent Fungus Colonization Index was calculated according to McKinney<sup>43</sup> and Per cent fungus growth inhibition was estimated as per Vincent<sup>44</sup>.

#### Decay test

The EN 113 (1996)<sup>42</sup> standard test method was used to perform the decay test. Wood specimens were exposed with *Gloeophyllum striatum* fungi continued to be incubated under similar conditions for twelve weeks. After being oven-dried for 24 h at  $103 \pm 3^\circ\text{C}$ , the exposed wood samples were weighed and the mass loss percentage was calculated.

#### Decay index

Wood samples were evaluated for the new decay susceptibility index (NSDI) and decay susceptibility index (DSI), as per by Curling<sup>45</sup>. These assessments compare the condition of the species under investigation to that of a control.

#### Chemical properties analysis after biological treatment

Wood samples for chemical properties analysis were taken after completion of decay test. The T1M-59 procedures was used to evaluate the hot and cold water-soluble extractives<sup>46</sup>. T6M 59<sup>47</sup>, T12M 59<sup>48</sup>, and T9M 54<sup>49</sup> were used to measure the extractives of alcohol and benzene, kalson-lignin concentration, and holocellulose, respectively.

#### Statistical analysis

Statistical Software Package for Agricultural Research Workers was utilised as a statistical tool<sup>50</sup>. The analysis was carried out using a completely

randomized design (CRD) and one-factor analysis of variance (ANOVA). The means were expressed as mean  $\pm$  SE. Duncan's multiple range test (DMRT) was used to separate the means in order to test the level of significance ( $P < 0.01$ ).

## Results

### Retentions and weight percent gain

Table 1 displays the mean extract retentions of treated wood specimens that were determined using the concentration and gross solution absorption data. The retention values increased as the solution concentration increased from 0.25% to 2%, as was to be predicted. The retention percentage and weight gain percentage data were significantly different at ( $P < 0.01$ ) significance level. The wood samples treated with the highest concentration of plant extracts (2.00%) retained 11.68 kg/m<sup>3</sup> E4; 11.23 kg/m<sup>3</sup> E3; 11.13 kg/m<sup>3</sup> E2, and 10.40 kg/m<sup>3</sup> E4. The lowest retention was noticed in the wood samples treated with the lowest concentration of 0.25%, whereas, 1.25 kg/m<sup>3</sup> was observed for all extractives (E1, E2, E3, and E4). In general, increasing the concentration of extract leads to an increase in weight percent gain. The weight gain percentage data was found to be

statistically significant at ( $P < 0.01$ ) Table 1. According to the stated retention data, the wood samples treated with the highest concentration of 2.00% reported the maximum weight gain percentage of 5.28 % E4, 3.76 % E3, 3.70% E2, and 3.01% E1, while the lowest weight percent gain was recorded for wood samples treated with the lowest concentration of 0.25%. In contrast, the minimum WGP's were 1.19%, 1.33%, 1.70%, and 1.78% for E2, E1, E3, and E4, respectively.

### *In vitro* antifungal activity

The antifungal index shows the antifungal activity of the selected plant extracts of *A. calamus* (E1, E2) and *P. hysterophorus* (E3, E4) against the wood-rotting fungus *G. striatum*. The *in vitro* antifungal assay results presented in the Table 2, which were determined to be statistically significant at the ( $P < 0.01$ ) level of significance. According to the findings, at 2.0% concentrations, E1, E2, E3, and E4 had the highest activity against *G. striatum* and E1 and E2 had the highest antifungal activity, with results of 97.41 and 97.22%, respectively. The antifungal activity levels of the other extracts, E3 (94.44%) and E4 (91.29%), were lower. Despite a larger variance between doses, the extracts showed dose-dependent

Table 1 — Mean extraction retention (Kg./M<sup>3</sup>) and weight percentage gain (%) of *Pinus roxburghii* wood treated with *A. calamus* and *P. hysterophorus* extracts

Treatments	Concentration	Retention (Kg/m <sup>3</sup> )	Weight Gain (%)
<i>A. Calamus</i> extract with Methanol (E1)	0.25	1.25 $\pm$ 0.12 <sup>i</sup>	1.33 $\pm$ 0.03 <sup>jk</sup>
	0.50	2.60 $\pm$ 0.14 <sup>h</sup>	1.86 $\pm$ 0.31 <sup>i</sup>
	1.0	5.58 $\pm$ 0.25 <sup>f</sup>	2.08 $\pm$ 0.06 <sup>hi</sup>
	1.5	8.53 $\pm$ 0.21 <sup>c</sup>	2.31 $\pm$ 0.12 <sup>gh</sup>
	2.0	11.68 $\pm$ 0.35 <sup>a</sup>	3.01 $\pm$ 0.03 <sup>ef</sup>
<i>A. Calamus</i> extract with Petroleum ether (E2)	0.25	1.25 $\pm$ 0.10 <sup>i</sup>	1.19 $\pm$ 0.07 <sup>k</sup>
	0.50	2.36 $\pm$ 0.16 <sup>h</sup>	1.71 $\pm$ 0.11 <sup>ij</sup>
	1.0	5.18 $\pm$ 0.14 <sup>f</sup>	2.64 $\pm$ 0.09 <sup>fg</sup>
	1.5	8.08 $\pm$ 0.04 <sup>cd</sup>	2.74 $\pm$ 0.26 <sup>fg</sup>
	2.0	11.13 $\pm$ 0.25 <sup>a</sup>	3.70 $\pm$ 0.10 <sup>c</sup>
<i>P. hysterophorus</i> extract with Methanol (E3)	0.25	1.25 $\pm$ 0.10 <sup>i</sup>	1.70 $\pm$ 0.10 <sup>ij</sup>
	0.50	2.43 $\pm$ 0.10 <sup>h</sup>	2.14 $\pm$ 0.16 <sup>hi</sup>
	1.0	5.13 $\pm$ 0.36 <sup>f</sup>	2.65 $\pm$ 0.05 <sup>fg</sup>
	1.5	7.49 $\pm$ 0.21 <sup>e</sup>	3.19 $\pm$ 0.05 <sup>de</sup>
	2.0	11.23 $\pm$ 0.08 <sup>a</sup>	3.76 $\pm$ 0.30 <sup>c</sup>
<i>P. hysterophorus</i> extract Petroleum ether (E4)	0.25	1.25 $\pm$ 0.06 <sup>i</sup>	1.78 $\pm$ 0.11 <sup>i</sup>
	0.50	2.76 $\pm$ 0.16 <sup>h</sup>	2.58 $\pm$ 0.08 <sup>fg</sup>
	1.0	4.37 $\pm$ 0.24 <sup>g</sup>	3.59 $\pm$ 0.08 <sup>cd</sup>
	1.5	7.71 $\pm$ 0.08 <sup>de</sup>	4.84 $\pm$ 0.02 <sup>b</sup>
	2.0	10.40 $\pm$ 0.23 <sup>b</sup>	5.28 $\pm$ 0.14 <sup>a</sup>

[Data represent the mean of three replications  $\pm$  standard error. Different superscript letters in a column specify a statistical difference between the mean ( $P \leq 0.01$ , DMRT test)]

Table 2 — Antifungal index (%) of different extracts from *A. calamus* and *P. hysterothorus* against to *Gloeophyllum striatum* brown rot fungus

Treatments	Concentration	Antifungal Index (%)
<i>A. Calamus</i> extract with Methanol (E1)	0.25	84.44±0.32 <sup>fh</sup>
	0.50	85.93±0.18 <sup>efg</sup>
	1.0	87.22±0.32 <sup>e</sup>
	1.5	96.85±0.37 <sup>a</sup>
	2.0	97.41±0.19 <sup>a</sup>
<i>A. Calamus</i> extract with Petroleum ether (E2)	0.25	93.70±0.49 <sup>bc</sup>
	0.50	95.00±0.00 <sup>b</sup>
	1.0	94.44±0.00 <sup>b</sup>
	1.5	95.19±0.19 <sup>b</sup>
	2.0	97.22±0.00 <sup>a</sup>
<i>P. hysterothorus</i> extract with Methanol (E3)	0.25	46.85±0.18 <sup>j</sup>
	0.50	87.22±0.56 <sup>e</sup>
	1.0	85.93±0.67 <sup>ef</sup>
	1.5	91.11±0.32 <sup>d</sup>
	2.0	94.44±0.32 <sup>b</sup>
<i>P. hysterothorus</i> extract Petroleum ether (E4)	0.25	36.67±0.64 <sup>k</sup>
	0.50	82.78±0.64 <sup>i</sup>
	1.0	91.11±0.32 <sup>d</sup>
	1.5	92.41±0.37 <sup>cd</sup>
	2.0	91.29±1.52 <sup>d</sup>

[Data represent the mean of three replications ± standard error. Different superscript letters in a column specify a statistical difference between the mean ( $P \leq 0.01$ , DMRT test)]

antifungal activity that increased with increasing concentrations from 0.25 to 2.00%. All of the extracts (E1, E2, E3, and E4) showed the least antifungal activity at the lowest concentration of 0.25%. E4, E3, E1, and E2 had the lowest antifungal indices, measuring 36.67%, 46.75%, 84.44%, and 93.70%, respectively.

#### Visual observations antifungal assay

*A. calamus* and *P. hysterothorus* extracts treated wood blocks were evaluated for their antifungal properties by visually observing the growth of mycelium on the wood surface. The findings of the fungus inhibition analysis after 21 days of incubation. On untreated wood blocks, *G. striatum* expanded swiftly and covered the surface entirely (rating scale 5), indicating total fungal colonization (100%) of the surface. The types and concentrations of extract data presented in Table 3 significantly impacted the mycelial linear growth of the tested fungus. Data related to the colonization percent found significant at ( $P < 0.01$ ) level of significance. Whereas maximum (100%) colonization was noted for untreated wood samples and minimum colonization was recorded at 2.00% concentration (12.42% E4; 12.10% E3;

17.01% E2; and 18.15% E1). They demonstrated remarkably stronger inhibition than the control. Moreover, as extract concentrations were raised, a greater degree of fungal inhibition was seen. The extracts of both plants significantly suppressed the test fungus growth. *P. hysterothorus* extracts were found more effective than *A. calamus* extract (Table 3) and data related to inhibition percent was found significant. Fungus growth was considerably suppressed by the methanolic extracts of both plants (E1 and E3), with the maximum fungal inhibition (88.41%) for E3 at 2.00% concentration and a minimum observed for E1 (27.65%). Although the effects of the petroleum ether extracts (E2 and E4) were less significant, maximum fungal inhibition was recorded for E4 (87.58%) at 2.00% concentration and the minimum was noticed for E2 (18.57%) at 0.25% concentration.

#### Mass losses

Table 3 ( $P < 0.01$ ) displays the mean percentage mass loss (ML) of extract-treated *P. roxburghii* wood samples that were examined after being infected for 12 weeks by the *G. striatum* brown rot fungus and data related to mass losses was found statistically

Table 3 — Effect of plant extracts on fungal inhibition, fungal colonization and mass losses of treated and untreated wood samples of *Pinus roxburghii* after exposed with *Gloeophyllum striatum* Brown rot fungus

Treatments	Concentration (%)	Colonization (%)	Inhibition (%)	M L (%)
Control	0	100±0.00 <sup>a</sup>	/	30.66±0.10 <sup>a</sup>
<i>A. Calamus</i> extract with Methanol (E1)	0.25	72.85±0.52 <sup>c</sup>	27.65±0.52 <sup>n</sup>	21.66±0.83 <sup>e</sup>
	0.50	64.98±0.02 <sup>f</sup>	35.53±0.01 <sup>k</sup>	21.03±0.38 <sup>e</sup> <sup>f</sup>
	1.0	58.20±0.31 <sup>h</sup>	42.30±0.32 <sup>i</sup>	19.33±0.83 <sup>g</sup>
	1.5	33.13±0.34 <sup>l</sup>	67.37±0.36 <sup>f</sup>	16.33±0.81 <sup>i</sup>
	2.0	18.15±0.31 <sup>p</sup>	82.36±0.35 <sup>b</sup>	11.55±0.49 <sup>l</sup>
<i>A. Calamus</i> extract with Petroleum ether (E2)	0.25	81.43±0.62 <sup>b</sup>	18.57±0.62 <sup>o</sup>	27.74±0.07 <sup>b</sup>
	0.50	66.46±0.60 <sup>e</sup>	33.55±0.60 <sup>l</sup>	26.60±0.10 <sup>c</sup>
	1.0	53.35±0.23 <sup>i</sup>	46.65±0.23 <sup>h</sup>	18.21±0.05 <sup>h</sup>
	1.5	28.08±0.39 <sup>m</sup>	71.47±0.22 <sup>e</sup>	14.58±0.11 <sup>jk</sup>
	2.0	17.01±0.29 <sup>d</sup>	83.00±0.29 <sup>b</sup>	10.75±0.88 <sup>l</sup>
<i>P. hysterothorus</i> extract with Methanol (E3)	0.25	67.51±0.00 <sup>e</sup>	32.99±0.00 <sup>l</sup>	28.63±0.10 <sup>b</sup>
	0.50	58.08±0.39 <sup>h</sup>	42.42±0.39 <sup>i</sup>	20.38±0.20 <sup>fg</sup>
	1.0	36.46±0.60 <sup>j</sup>	64.05±0.60 <sup>g</sup>	17.42±0.03 <sup>h</sup>
	1.5	23.95±0.11 <sup>o</sup>	76.55±0.12 <sup>c</sup>	15.43±0.07 <sup>ij</sup>
	2.0	12.10±0.49 <sup>f</sup>	88.41±0.49 <sup>a</sup>	13.61±0.13 <sup>k</sup>
<i>P. hysterothorus</i> extract Petroleum ether (E4)	0.25	71.03±0.13 <sup>d</sup>	28.97±0.13 <sup>m</sup>	25.71±0.16 <sup>c</sup>
	0.50	62.80±0.55 <sup>g</sup>	37.20±0.55 <sup>j</sup>	24.38±0.01 <sup>d</sup>
	1.0	34.91±0.05 <sup>k</sup>	65.09±0.05 <sup>g</sup>	20.89±0.07 <sup>ef</sup>
	1.5	26.51±0.57 <sup>n</sup>	73.50±0.57 <sup>d</sup>	17.73±0.05 <sup>h</sup>
	2.0	12.42±0.67 <sup>f</sup>	87.58±0.67 <sup>a</sup>	14.49±0.21 <sup>jk</sup>

[Data represent the mean of three replications ± standard error. Different superscript letters in a column specify a statistical difference between the mean ( $P \leq 0.01$ , DMRT test)]

significant. The highest mass loss (30.66%) was noticed in the untreated wood samples. Both plants increased wood resistance because treated wood blocks showed mass loss of less than 30.66%. Fungal resistance increased as the concentration rose from 0.25 to 2.00%. The mass losses for the wood samples exposed to methanol and petroleum ether extracts of *A. calamus* and *P. hysterothorus* ranged from 10.75 to 28.63%. The minimum mass loss was recorded at 2.00% concentration for E2, E1, E3, and E4: 10.75%, 11.55%, 13.61%, and 14.49%, respectively.

#### Decay index

Antifungal activity of the plant extract was confirmed through decay susceptibility index and new decay susceptibility index tests. The treated wood samples show less susceptibility to *G. striatum* brown rot fungus. Results of DSI and NDSI were significant at ( $P < 0.01$ ). Fungal susceptibility of treated wood samples was reduced with the reduction of the concentration of plant extract. Furthermore, wood samples treated with petroleum ether extracted from *A. calamus* were found to be less susceptible to *G. striatum* brown rot fungus than methanol-extracted

treated wood samples. Similarly, *P. hysterothorus* methanol-extracted treated wood samples were less susceptible to *G. striatum* brown rot fungus than petroleum ether extract-treated wood samples. The results indicate the potential of selected plant species against *G. striatum* brown fungus.

#### Soluble extractive

To better understand the effect of *G. striatum* brown rot fungi on wood samples of *P. roxburghii*, examine the soluble extractives, viz., hot water, cold water, and alcohol-benzene soluble extractives, of treated wood after decay test data presented in Table 4 and data found statistically significant ( $P < 0.01$ ) at level of significance. The extractives of wood are directly related to the durability of wood. The soluble extractive is significantly affected by the concentration and type of extractive. The highest soluble extractives were found in treated wood samples at 2.0% concentration, and the lowest was found in untreated wood samples. The maximum hot water-soluble extractives were recorded at 2.00% concentration for E2, E4, E1, and E4: 7.76%, 7.75%, 7.28%, and 7.23%, respectively, whereas a minimum

Table 4 — Decay susceptibility index (DSI) and new decay susceptibility index (NDSI) of treated wood samples of *Pinus roxburghii* with *Acorus calamus* and *Parthenium hysterophorus* plant extracts

Treatments	Concentration (%)	DSI (%)	NDSI (%)
<i>A. Calamus</i> extract with Methanol (E1)	0.25	70.65±2.48 <sup>e</sup>	89.32±4.25 <sup>ab</sup>
	0.50	68.60±0.99 <sup>e</sup>	86.34±2.97 <sup>abc</sup>
	1.0	63.06±2.92 <sup>fg</sup>	79.21±2.74 <sup>d</sup>
	1.5	53.30±2.83 <sup>ij</sup>	68.06±1.31 <sup>e</sup>
	2.0	37.7±1.74 <sup>m</sup>	50.44±1.70 <sup>fg</sup>
<i>A. Calamus</i> extract with Petroleum ether (E2)	0.25	90.49±0.52 <sup>ab</sup>	88.94±4.76 <sup>ab</sup>
	0.50	86.76±0.64 <sup>bc</sup>	86.11±3.63 <sup>abc</sup>
	1.0	59.40±0.02 <sup>gh</sup>	79.02±3.35 <sup>d</sup>
	1.5	47.55±0.52 <sup>kl</sup>	67.38±0.80 <sup>e</sup>
	2.0	35.03±2.77 <sup>m</sup>	49.17±1.78 <sup>g</sup>
<i>P. hysterophorus</i> extract with Methanol (E3)	0.25	93.37±0.04 <sup>a</sup>	90.00±4.49 <sup>ab</sup>
	0.50	66.47±0.88 <sup>ef</sup>	87.03±3.59 <sup>abc</sup>
	1.0	56.83±0.09 <sup>hi</sup>	81.93±3.33 <sup>cd</sup>
	1.5	50.33±0.06 <sup>jk</sup>	70.63±1.68 <sup>e</sup>
	2.0	44.38±0.28 <sup>l</sup>	53.31±1.62 <sup>fg</sup>
<i>P. hysterophorus</i> extract Petroleum ether (E4)	0.25	83.84±0.22 <sup>c</sup>	92.71±3.78 <sup>a</sup>
	0.50	79.53±0.28 <sup>d</sup>	88.65±0.08 <sup>abc</sup>
	1.0	68.12±0.09 <sup>e</sup>	83.76±0.61 <sup>bcd</sup>
	1.5	57.84±0.37 <sup>h</sup>	72.87±0.65 <sup>e</sup>
	2.0	47.25±0.83 <sup>kl</sup>	56.69±0.63 <sup>f</sup>

(2.24%) was noticed for untreated wood samples. Although cold-water-soluble extractives were found to be maximum for E1, E3, E2, and E4 at the highest concentration of 2.00% (7.20%, 6.75%, 6.72%, and 6.70%, respectively), the untreated wood sample had the lowest (2.24%). While alcohol and benzene soluble extractives were also recorded at 2.00% concentration in the treated wood sample, a minimum was noticed for the untreated wood sample. The highest soluble extractive was observed for E3 (8.78%); E2 (8.41%); E1 (4.57%), and E4 (7.41%), with a minimum (3.60%) recorded for the untreated wood sample.

#### Lignin and holocellulose content

The mean present lignin and holocellulose content of an extract-treated *P. roxburghii* wood sample after 12 weeks of *G. striatum* exposure is shown in Table 5 and data found statistically significant at ( $P < 0.01$ ) level of significance. Types and concentrations of extracts significantly affected the lignin and holocellulose content in the treated wood samples. The lignin and holocellulose of treated wood samples increased as the extract concentration increased. The highest lignin and holocellulose content were recorded at 2.0% concentration, and the lowest was noticed in untreated wood samples. The maximum

lignin content was noticed for E3 (26.16%), E4 (25.45%), and E1 (24.67%) at 2.0% concentration in treated wood samples, and the minimum (18.51%) was observed for untreated wood samples. Similarly, the maximum holocellulose content was recorded at 2.0% concentration (63.20%, 61.77%, 59.01%, and 58.13%) for E4, E3, E1, and E2, respectively, and the minimum (51.17%) was recorded for the untreated wood sample.

#### Discussion

Treatment parameters (such as duration and preservative type) and concentration are the main determinants of retention. With respect to extract type, there was a variance in retention, with E1 having the best retention. Wood retention and weight gain percent growth statistics were consistent. Retaining the preservative is an important indicator of how well the impregnation worked<sup>51-52</sup>. Gupta *et al.*<sup>14</sup> reported a positive relationship between the retention present and concentration when wood blocks of *Lannea coromandelica* were treated with *Ageratum conyzoides* and *Lantana camara* extracts. Several studies have explored the linear link between rising preservative retention and rising preservative concentration<sup>54</sup>. Goktas *et al.*<sup>55</sup> reported that retention of wood blocks increased with increasing plant extract

Table 5 — Variation in Soluble extractives, Holocellulose and Lignin percent of *Pinus roxburghii* Sargent wood after exposed with *Gloeophyllum striatum* (Fr.) Murrill brown rot fungus

Treatments	Concentration (%)	Hot water-soluble extractives (%)	Cold water-soluble extractives (%)	Alcohol-Benzene soluble extractives (%)	Holocellulose Content (%)	Lignin Content (%)
Control	0	2.24±0.11 <sup>k</sup>	2.24±0.11 <sup>k</sup>	3.61±0.05 <sup>p</sup>	51.17±0.07 <sup>n</sup>	18.51±0.06 <sup>k</sup>
<i>A. Calamus</i> extract with Methanol (E1)	0.25	2.91±0.08 <sup>j</sup>	2.60±0.03 <sup>j</sup>	3.73±0.05 <sup>o</sup>	54.58±0.03 <sup>l</sup>	20.71±0.08 <sup>h</sup>
	0.50	2.84±0.17 <sup>j</sup>	3.01±0.01 <sup>i</sup>	4.84±0.05 <sup>l</sup>	55.82±0.11 <sup>j</sup>	21.60±0.13 <sup>g</sup>
	1.0	4.49±0.04 <sup>g</sup>	4.51±0.26 <sup>f</sup>	5.87±0.05 <sup>j</sup>	56.99±0.04 <sup>h</sup>	22.93±0.09 <sup>e</sup>
	1.5	5.33±0.13 <sup>e</sup>	5.59±0.21 <sup>c</sup>	6.82±0.05 <sup>h</sup>	57.85±0.07 <sup>fg</sup>	23.88±0.09 <sup>d</sup>
	2.0	7.28±0.06 <sup>b</sup>	7.20±0.01 <sup>a</sup>	7.57±0.05 <sup>d</sup>	59.01±0.14 <sup>e</sup>	24.67±0.09 <sup>c</sup>
<i>A. Calamus</i> extract with Petroleum ether (E2)	0.25	3.50±0.25 <sup>i</sup>	3.66±0.06 <sup>h</sup>	4.13±0.03 <sup>n</sup>	52.99±0.14 <sup>m</sup>	18.84±0.04 <sup>j</sup>
	0.50	4.92±0.02 <sup>f</sup>	3.30±0.04 <sup>i</sup>	5.25±0.04 <sup>k</sup>	54.54±0.07 <sup>l</sup>	20.01±0.06 <sup>i</sup>
	1.0	5.87±0.07 <sup>d</sup>	4.06±0.03 <sup>g</sup>	6.85±0.00 <sup>h</sup>	54.56±0.17 <sup>l</sup>	20.70±0.09 <sup>h</sup>
	1.5	6.77±0.02 <sup>c</sup>	5.19±0.05 <sup>d</sup>	7.93±0.03 <sup>c</sup>	56.62±0.14 <sup>i</sup>	22.21±0.13 <sup>f</sup>
	2.0	7.76±0.01 <sup>a</sup>	6.72±0.12 <sup>b</sup>	8.41±0.04 <sup>b</sup>	58.13±0.07 <sup>f</sup>	23.05±0.04 <sup>e</sup>
<i>P. hysterophorus</i> extract with Methanol (E3)	0.25	3.82±0.03 <sup>h</sup>	3.12±0.06 <sup>i</sup>	4.78±0.00 <sup>l</sup>	54.70±0.10 <sup>l</sup>	21.51±0.04 <sup>g</sup>
	0.50	5.42±0.03 <sup>e</sup>	4.79±0.03 <sup>e</sup>	5.25±0.02 <sup>k</sup>	55.44±0.10 <sup>k</sup>	23.76±0.09 <sup>d</sup>
	1.0	5.68±0.03 <sup>d</sup>	5.15±0.07 <sup>d</sup>	6.94±0.02 <sup>g</sup>	56.92±0.07 <sup>h</sup>	24.61±0.09 <sup>c</sup>
	1.5	6.84±0.04 <sup>c</sup>	6.75±0.09 <sup>b</sup>	7.47±0.01 <sup>e</sup>	58.04±0.04 <sup>f</sup>	25.31±0.11 <sup>b</sup>
	2.0	7.23±0.03 <sup>b</sup>	6.78±0.09 <sup>b</sup>	8.78±0.02 <sup>a</sup>	61.77±0.11 <sup>b</sup>	26.16±0.09 <sup>a</sup>
<i>P. hysterophorus</i> extract Petroleum ether (E4)	0.25	3.72±0.09 <sup>h</sup>	3.72±0.04 <sup>h</sup>	4.30±0.01 <sup>m</sup>	54.46±0.10 <sup>l</sup>	21.63±0.07 <sup>g</sup>
	0.50	5.26±0.04 <sup>e</sup>	4.27±0.07 <sup>fg</sup>	5.96±0.02 <sup>j</sup>	57.70±0.07 <sup>g</sup>	22.17±0.08 <sup>f</sup>
	1.0	5.83±0.03 <sup>d</sup>	5.80±0.05 <sup>c</sup>	6.41±0.02 <sup>i</sup>	59.49±0.08 <sup>d</sup>	23.80±0.13 <sup>d</sup>
	1.5	6.77±0.04 <sup>c</sup>	6.65±0.06 <sup>b</sup>	7.10±0.01 <sup>f</sup>	60.51±0.15 <sup>c</sup>	25.35±0.09 <sup>b</sup>
	2.0	7.75±0.04 <sup>a</sup>	6.7±0.04 <sup>b</sup>	7.41±0.02 <sup>e</sup>	63.20±0.12 <sup>a</sup>	25.45±0.09 <sup>b</sup>

[Data represent the mean of three replications ± standard error. Different superscript letters in a column specify a statistical difference between the mean ( $P \leq 0.01$ , DMRT test)]

concentration when wood blocks of *Fagus orientalis* and *Pinus sylvestris* were impregnated with the plant extracts of *Muscari neglectum* and *Gynandris sisyrinchium*. Differences in moisture content, variances in wood density, and absorption and intensity of interaction of extracts with various wood constituents present in the microstructure of *P. roxburghii* wood are all responsible for variations in weight present gain. Previous studies reported the positive relation between weight percent gain and concentration. After treating *Lannea coromandelica* wood samples with extracts of *Ageratum conyzoides* and *Lantana camara*, the WPG increased with increasing concentration<sup>14</sup>.

Due to the detrimental effects of chemical wood preservatives, focus has shifted to natural wood preservatives. The present investigation evaluated *A. calamus* and *P. hysterophorus* extracts as wood preservatives against brown rot fungi. Bi *et al.*<sup>56</sup> reported the antifungal activity of konjac flying powder from waste material *Amorphophallus konjac* against white-rot fungus, *Trametes versicolor*, and brown-rot fungus, *Gloeophyllum trabeum*. Fuchtn

*et al.*<sup>57</sup> reported the antifungal properties of heartwood extracts of a moderately durable conifer, *Larix gmelinii*, and a non-durable species, *Picea abies* against brown-rot fungus. Ljunggren *et al.*<sup>58</sup> reported the antifungal properties of different chemicals of Norway spruce. Shiny *et al.*<sup>59</sup> found that various plant extracts have antifungal properties against brown and white rot decay fungi. Silva *et al.*<sup>60</sup> reported that the essential oil and dehydro fukinone of *Nectandra grandiflora* inhibited the mycelial growth of *Gloeophyllum trabeum* and *Pycnoporus sanguineus*. Xie *et al.*<sup>61</sup> reported the antifungal effects of 41 distinct monoterpenes on the wood rot fungus *Trametes hirsuta*, *Schizophylhls commune*, and *Pycnoporus sanguineus*. The essential oil of *Syzygium aromaticum* flower buds was found to have antifungal activity against three wood decay fungi. The essential oil inhibited the growth of fungi by 88.0%, 100.0%, and 87.1% of *Trametes hirsuta*, *Schizophylhls commune*, and *Pycnoporus sanguineus*, respectively, at a concentration of 400 µg/mL<sup>62</sup>. Poonia *et al.*<sup>8</sup> reported *Acacia auriculiformis* sawdust inhibited the growth of *Trametes versicolor* (15.32%) and

*Oligoporus placenta* (47.80%) at 1% concentration. The white-rot fungus *Coriolus versicolor*, as well as the brown-rot fungi *Gloeophyllum trabeum* and *Polyporus vaporaria*, were significantly inhibited by hexane and ethyl acetate extracts of *Pinus elliottii* needles<sup>53</sup>. Saha *et al.*<sup>13</sup> investigated heartwood extracts from five different tree species (Ayous, Moabi, movingui, padouk, and tali) and found that extracts from all species inhibited fungal growth up to concentrations of 250 g/mL, but tali and movingui extract inhibited fungal growth completely at 500 g/mL. Tomak *et al.*<sup>63</sup> reported that four different tannins efficiently thwarted brown rot fungal attacks. Meena *et al.*<sup>64</sup> reported that methanol and petroleum ether extracts of *A. calamus* and *P. hysterophorus* improved the resistance against white rot fungus *Trametes versicolor*. Chen *et al.*<sup>65</sup> reported the n-hexane-soluble fraction of *Taiwania cryptomerioides* heartwood extracts inhibited the growth of the brown rot fungus *Phellinus noxius*. Vek *et al.*<sup>66</sup> reported the growth of white and brown-rot fungi were effectively inhibited by extracts of scots pine knot wood and black locust heartwood.

A negative linear relationship between concentration strength and mass losses was discovered when *A. calamus* and *P. hysterophorus* extract treated wood specimens. Meena *et al.*<sup>67</sup> investigated the impact of natural extracts on the white rot fungus *Trametes versicolor* and discovered that treated wood samples degraded least (13.46%) with methanol extract of *P. hysterophorus* and (13.63%) with petroleum ether extract of *A. calamus*-treated wood samples, while untreated wood samples degraded most (maximum). According to Bernardis & Popof<sup>68</sup> when retention levels were raised to 9-25 kg/m<sup>3</sup>, mineral salts and quebracho extract treated wood specimens showed a considerable decrease in mass losses against *Pcynoporus sanguineus* and *Gloeophyllum sepiarium*. Shiny *et al.*<sup>69</sup> reported that *Lantana camara* leaf extract inhibited the growth of *Trametes hirsuta* and *Oligoporus placenta* fungi, and *Nerium oleander* leaf extract inhibited the growth of *Oligoporus placenta* fungus. Poisonous ethyl alcohol plant extracts of *Muscari neglectum* and *Gynandris sisyrinchium* were tested for antifungal activity. The wood blocks of *Fagus orientalis* and *Pinus sylvestris* were impregnated with the plant extracts and evaluated for wood decay resistance through the soil block method for 12 weeks, and it was noticed that the selected extracts were able to suppress attacks by

*Postia placenta* and *Trametes versicolor*<sup>55</sup>. Bahmani & Schmidt<sup>70</sup> observed that resistance improved against *Aspergillus niger*, *Penicillium commune* (moulds), *Coniophora puteana* (brown rot), *Trametes versicolor* (white rot), and *Chaetomium globosum* (soft rot) when wood samples of *Fagus orientalis* and *Pinus taeda* were vacuum impregnated with essential oils of lavender, lemongrass, and thyme. Okon-Akan *et al.*<sup>9</sup> reported antifungal property of *Cola nitida* and *Musanga cecropioides*. Yildiz *et al.*<sup>71</sup> discovered that methanol extracts of lichen (*Usnea filipendula*) and mistletoe (*Viscum album*) leaves have antifungal properties against the brown rot fungus *Coniophora puteana*. Ahmed *et al.*<sup>72</sup> found that termite activity was significantly reduced by *Pongamia pinnata* heartwood extract. Teak heartwood extract had potential as a wood preservative<sup>73-74</sup>. DSI and NDSI are useful tools to interpreting the effect of concentrations on decay fungi. DSI and NDSI are also helpful to understand the effect of plant extracts on fungi<sup>75</sup>. Curling & Murphy<sup>45</sup> also reported similar findings. *Mimosa caesalpinifolia*, *Mimosa ophthalmocentra*, and *Mimosa tenuiflora* showed the highest natural durability against biodeterioration examined through DSI and NDSI tests<sup>76</sup>.

Soluble extractives are directly related to the quality and durability of wood<sup>77</sup>. Huge quantities of wood preservatives show the higher durability of wood and its resistance to wood degrading agents. In the present investigation, treated wood samples showed higher wood extractives than untreated wood samples, and the quantity of wood-soluble extractives also increased with the increase in extract concentration. Similar studies conducted by other researchers and reported similar results. Meena *et al.*<sup>67</sup> reported higher water-soluble extractives after a decay test on *Pinus roxburghii*-treated wood with natural extractives *A. calamus* and *P. hysterophorus*. Shang *et al.*<sup>78</sup> reported that 1% NaOH extractive high in compression to control after wood decayed. Silveira *et al.*<sup>79</sup> reported that high extractives after decay test in *Acacia mearnsii* treated wood with tannic extract. The treated wood sample had higher holocellulose and lignin content, while the untreated wood sample had the lowest. While the correlation between concentrations (lower to higher), holocellulose, and lignin content was observed to be positive. The higher holocellulose and lignin content in the treated wood sample indicated that the plant extracts could save the wood. No study was conducted on the effect of wood

decay fungus on the chemical composition of treated wood with natural wood preservatives. Meena *et al.*<sup>67</sup> reported higher lignin and holocellulose content in treated wood samples of *Pinus roxburghii* with *Acorus calamus* and *Parthenium hysterophorus* (methanol and petroleum ether) extracts when exposed to *Trametes versicolor* white rot fungi. Silveira *et al.*<sup>79</sup> studied the effects of white rot fungi (*Pycnoporus sanguineus*) on lignin, holocellulose, cellulose, and hemicelluloses of *Acacia mearnsii* wood treated with its own tannin concentration at 5% and 10% and reported that a higher percent of chemical constituents was present in the treated wood sample compared to the untreated wood sample. Monrroy *et al.*<sup>80</sup> reported that changes in chemical composition and structural modification for *Pinus radiata* infected with brown rot fungi (*Gloeophyllum trabeum*) after eight weeks of biodegradation, including the highest losses of weight (13%) and hemicellulose (31%). Schilling *et al.*<sup>81</sup> reported that brown rot causes significant lignin losses. Wood rotting is closely related to the intricacy of the cell wall, its chemical makeup, and the various enzymatic methods of the rotting fungus<sup>82</sup>. Harju *et al.*<sup>83</sup> reported a variation in hemicellulose,  $\alpha$ -cellulose and total lignin between the decay-resistant and susceptible Scots pine trees (heartwood).

### Conclusion

Wood samples of *Pinus roxburghii* were treated with *A. calamus* and *P. hysterophorus* extract at different concentrations, viz. 0.25, 0.50, 1.0, 1.5, and 2%. It was concluded that *A. calamus* and *P. hysterophorus* extracts were found effective against *G. striatum* decay fungus. Selected plant extracts can be utilized as a substitute wood preservative. Furthermore, *A. calamus* extract was found to be more effective against *G. striatum* than *P. hysterophorus* extracts. Short-term studies at low concentrations of plant extract reveal the significant effects against wood decay fungi. However, further research is required on a long-term and higher-concentration basis to develop effective wood preservatives.

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