Indian Journal of Agricultural Sciences **92** (1): 22–5, January 2022/Article

Assessment of antifungal potential of *Acacia auriculiformis* extracts against wood decay fungi

PAWAN KUMAR POONIA[1,2*], VINAYAK UPADHYA[2], HANUMANTHA M[2] and ANIL KUMAR[3]

College of Forestry, University of Agricultural Sciences, Dharwad, Sirsi, Karnataka 581 401, India

Received: 08 January 2020; Accepted: 16August 2021

ABSTRACT

The bark and heartwood of *Acacia auriculiformis* A. Cunn. are known for toxicity against different organisms. The present study aimed to evaluate the efficacy of leaves and saw dust extract of *A. auriculiformis* against the wood decay fungi like *Trametes versicolor* and *Oligoporus placenta* using bioassay method. The experiment was conducted during 2017-18 at College of Forestry, Sirsi (Karnataka) India. Saw dust extract at 0.5% concentration level showed 46.80% and 29.40% inhibition against *T. versicolor* and *O. placenta,* respectively. *T. versicolor* and *O. placenta* was inhibited by 15.32% and 47.80%, respectively, at 1% concentration of saw dust. The inhibition per cent of saw dust extract against wood decay fungi was higher in comparison to leaf extract. The results indicated that *A. auriculiformis* saw dust extract can be used further for possible wood preservation in field conditions.

Keywords: *Acacia auriculiformis*, Bark, Bioassay, Heartwood, Preservative, Saw dust

The biological damage to wood and wood products is mainly caused by the mould, stain, decay fungi and insects such as beetles and termites. Decaying of wood due to decay fungi is caused by the secretion of digestive action of enzymes by the fungal hyphae (Dhiman and Dutt 2018). Brown rot, white rot (Phylum *Basidiomycota*) and soft rot fungi (Phylum *Ascomycota*) are the most common fungal organisms involved in wood decay. Wood decay fungi are major contributors that damage wood, in timber production and wood used in various applications (Kirker 2018, Poonia *et al.* 2021). Wood decay fungi poses a major threat in the durability of wood leading to estimated loss of $1billion annually (Kirker 2018). Toxic chemical wood preservatives are primarily used for wood preservation to increase the durability of wood. The demand of eco-friendly, non-toxic and easily degradable wood preservative, without any ill-effect on the environment is gradually increasing. Hence, plant based products are being used as eco-friendly wood preservatives. Durability of wood (resistance to fungal decay) is often attributed to extractive content.

Present address: [1]College of Agriculture, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana; [2]College of Forestry, University of Agricultural Sciences, Dharwad, Sirsi, Karnataka; [3]ICAR-The Indian Agricultural Statistics Research Institute, New Delhi. *Corresponding author e-mail: *pooniaforestry@gmail.com.

The extractives from heartwood, leaf, bark, root and oils from herbaceous plants have been evaluated more for its wood preservative activity (Dhiman and Dutt 2018). There are many plants, viz. Neem (Adetogun and Atayese 2006), Pongamia, Eucalyptus (Reddy *et al*. 2009), Lantana (Tripathi *et al.* 2009), which are known for proven toxic effects against wood decay fungi. *Acacia auriculiformis A. Cunn.* is a fast growing plantation species in India and other parts of the world. *Acacia auriculiformis* has diverse uses along with various medicinal properties (Girijashankar 2011). It exhibits a variety of pharmacological properties notably microbicidal, anti-helminthic, anti-filarial activity, antifeedant property etc., (Mandal *et al.* 2005, Drijfhout and Morgan 2010) due to the presence of varied chemical constituents (Mihara *et al.* 2005). Timber of *A. auriculiformis* is used to manufacture furniture, door, window, other construction purposes and fuel wood. Leaf and saw dusts are treated as by-products and many a times considered as waste. The present study was carried out to evaluate the efficacy of leaf and saw dust extracts of *A. auriculiformis* against wood decay fungi like *Trametes versicolor* and *Oligoporus placenta*.

MATERIALS AND METHODS

Collection of plant material: Fresh and healthy leaves and saw dust of *A. auriculiformis* were collected locally in Sirsi region (latitude 14, 62° N, longitude 74, 85° E), Karnataka, India during October 2017. Cleaned leaves and saw dust were shade dried and pulverized to 40–60 mesh size.

Extraction and preliminary phytochemical analysis:

Pulverized leaves and saw dust were subjected to hot extraction method (Poonia *et al.* 2015). Powder of leaves and saw dust (200 g each) were added separately in two different round bottom flasks along with 800 ml methanol. A condenser was attached to the flasks and kept in a water bath at 60°C for 3 days for extraction. After the extraction with the solvent, the leaves and saw dust powder were separated from the solvent by filtration with a filter paper. The solvent was recovered from the extracted solution by distillation.

The extractives were further subjected to assess presence of functional groups, viz. phenols (Ferric chloride test, Folin Ciocalteu's test), saponins (Foam test, Haemolysis test), tannins (Ferric chloride test, Gelatin test, Conc. HCl test), terpenoids (Salkowski test, Liebermann-Burrchard test), flavonoids (Shindola test, NaOH Test), carbohydrates (Molish's test, Fehling's test), Glycosides (Kellar Kiliani test, Conc. H_2SO_4 test), protein (Ninhydrin test, Biuret test) and alkaloids (Wagner's test, Mayer's test, Dragendroff's reagent test) by following standard preliminary phytochemical assessment tests (Vogel 1978).

Extract yield estimation: The yield of leaf and saw dust extract was determined (expressed as per cent) using the formula (Puri 1967):

$$\text{Per cent of extract (Yield)} = \frac{A\,(100 + M)}{Y} \qquad (1)$$

where, A is total weight of the particular extract, Y is weight of the shade dried powder and M is moisture content in powder before extraction.

Bioassay of extracts against wood decay fungi: The efficacy of *A. auriculiformis* leaves and saw dust extracts was measured against brown rot (*Oligoporus placenta*) and white rot (*Trametes versicolor*) fungus using eight different treatment methods with six replicates. Two sets of control i.e. control with solvent (CS), control without solvent (C) and both plant extracts of 0.5%, 1.0%, 1.5%, 2%, 3% and 4% were applied.

Potato dextrose agar bioassay (Hunt and Garrett 1967) method was followed to evaluate the toxicity of *A. auriculiformis* leaves and saw dust extract against the brown rot fungus *Oligoporus placenta* (Fr.) Ryv. & Gilbertson (Strain No. 180) and the white rot species *Trametes versicolor* (Fr.) Lloyd (Strain No. 684) (IS: 4873, 2008). Both the experimental strains were procured from Forest Research Institute, Dehradun. The PDA media with and without the extracts were sterilized in an autoclave maintained at a temperature of 121°C and pressure 103.42 kPa (15 lbs/inch^2) (Datar 1995). Then, 30 ml autoclaved medium was poured into each sterilized Petri plate in sterile condition of laminar air flow chamber. The plates were allowed to cool till the medium was solidified. The plates were inoculated with the test fungi in laminar air flow chamber. For inoculation, one bit of inoculum disc (1 cm) of actively growing 14–16 days old culture of the test fungi was aseptically placed upside down at the center of the Petri plate. For each concentration of extract, control with solvent (CS) and controls (C), six replicates were used. The plates were incubated in dual chamber incubator (Make-Scientek Services) maintained at 25±2°C and 70±4% relative humidity. Inhibition was recorded after 21 days in terms of surface covered by the fungi on the PDA medium with and without extracts. The percent inhibition in growth of fungi (Goyal and Dev 1982) was calculated as:

$$\text{Percent Inhibition} = \frac{A - B}{A} \times 100 \qquad (2)$$

where, A is total distance (cm) covered by test fungus in the control Petri dish and B is total distance (cm) covered by test fungus in Petri dish with extracts.

Grading of surface coverage of mycelia on potato dextrose agar medium was carried out as per cent growth from 0% (None) to >75% (Complete) with the following intervals: 0–5% (Sporadic), 5–25% (Little), 25–50% (Moderate) and 50–75% (Considerable).

Statistics: The effect of saw dust and leaf extract at different concentrations on fungal growth was analysed by means of analysis of variance (ANOVA) using SPSS16.0 version (Shiny *et al*. 2018). Duncan homogeneity test was carried out to test the significance and mean separation of the treatments.

RESULTS AND DISCUSSION

The yield of crude extract in leaf and saw dust was 5.7% and 7.2%, respectively. The saw dust has yielded more amount of extract than leaf. The similar findings of methanol extract were also observed by Shafiei *et al.* (2017), however they followed sequential extraction methodology in the increasing order of solvent polarity. Phyto-chemicals often play an important role in plant defense mechanism against microorganism, stress as well as interspecies protections. Hence, screening of phyto-chemicals serves as an initial step in the prediction of potential active compounds in the plants (Chew *et al.* 2009). The preliminary qualitative assessment of phyto-chemicals carried out with leaf and saw dust revealed the presence of different phyto-constituent groups. The present study revealed the presence of phenol, flavonoids, saponins, carbohydrates and tannins whereas, alkaloids, steroids/ triterpenes and protein showed negative results. Earlier studies revealed the presence of similar preliminary phytochemical groups in ethanolic extract of the bark of *A. auriculiformis* (Sharma *et al.* 2017). Phenolic compounds are one of the largest and most ubiquitous groups of the plant metabolites (Singh *et al.* 2007) and total phenolic and flavonoid contents estimated in bark (Sharma *et al.* 2017) and leaf (Chew *et al.* 2011) of *A. auriculiformis* may influence the bioactivity against wood decay fungi.

In vitro bioassay for antifungal activity of saw dust and leaf: Bioassay in terms of surface covered by *Trametes versicolor* and *Oligoporus placenta* on potato dextrose agar medium containing extracts of saw dust and leaf are represented in Tables 1 and 2, respectively. Saw dust extract at 0.5% concentration level showed 46.80% and 29.40% per cent inhibition against *T. versicolor* and *O. placenta,* respectively. At 1% concentration, the surface coverage by *T. versicolor* and *O. placenta* was 15.32 % and 47.80%.

Table 1 Surface coverage by *Oligoporus placenta (Op)* and *Trametes versicolor (Tv)* on the saw dust extraction agar at different concentrations

Extract concentration (%)	Surface coverage of *O. placenta*		Surface coverage of *T. versicolor*	
	Mean surface coverage (%)	Growth type	Mean surface coverage (%)	Growth type
Control	100[a]	Complete	100[g]	Complete
Control + Solvent	100[a]	Complete	100[g]	Complete
0.5	70.60[b]	Considerable	53.20[h]	Considerable
1.0	47.80[c]	Moderate	15.32[i]	Little
1.5	17.92[d]	Little	4.85[j]	Sporadic
2.0	4.25[e]	sporadic	0.50[k]	None
3.0	0[f]	None	0[k]	None
4.0	0[f]	None	0[k]	None

Mean square error of Op and Tv is 8.90 and 4.62, respectively, at (P≤0.05) level. Different letters denote significantly different groups.

Table 2 Surface coverage by *Oligoporus placenta (Op)* and *Trametes versicolor (Tv)* on the leaves extraction agar at different concentrations

Extract concentration (%)	Surface coverage of *O. placenta*		Surface coverage of *T. versicolor*	
	Mean surface coverage (%)	Growth type	Mean surface coverage (%)	Growth type
Control	100[a]	Complete	100[f]	Complete
Control + Solvent	100[a]	Complete	100[f]	Complete
0.5	100[a]	Complete	100[f]	Complete
1.0	96.66[a]	Complete	96.33[f]	Complete
1.5	90.00[b]	Complete	85.40[g]	Complete
2.0	82.60[c]	Complete	76.80[h]	Complete
3.0	78.20[d]	Complete	70.00[i]	Considerable
4.0	70.30[e]	Considerable	58.40[j]	Considerable

Mean square error of Op and Tv is 12.70 and 13.61 respectively at (P≤0.05) level. Different letters denote significantly different groups.

The comparison of mean surface coverage by the mycelium at 0.5%, 1% and 2% concentrations revealed that increase in concentration of saw dust extract inhibited both fungi significantly as compared to control (P≤0.05). Growth of both fungi were totally inhibited at 3% concentration. This proved the efficacy of *A. auriculiformis* saw dust extract against white rot and brown rot fungus at a lower concentration.

Leaf extracts indicated no significant improvement in fungal inhibition at lower concentration i.e., up to 3%. Only 0-30% inhibition of growth were observed up to 3% concentration level whereas, slight or considerable effects were observed at higher concentration (4%) i.e. 29.70% and 41.60% inhibition of growth of *O. placenta* and *T. versicolor,* respectively. Statistical analysis also indicated no significant difference (P≤0.05) at lower concentration whereas it showed significant difference in growth at higher concentration with respect to controls. It indicated that leaf extract of *A. auriculiformis* did not show much inhibition on the growth of both test fungi.

The marked potential of plant extracts against microorganisms is due to the presence of appreciable amount of alkaloids, terpenoids, phenols, flavonoids and tannins. Polyphenolic and flavonoid compounds accumulate in bark, leaves, heartwood and sapwoods of woody tree species (Hillis 1987). Extracts from bark and heartwood of many woody trees have strong biological activities such as enzyme inhibition (Juntheikki and Julkunen-Titto 2000), antioxidant activity (Chang *et al*. 2001) and antifungal activity (Kishino *et al.* 1995) and are, therefore, known to provide durability of wood against fungi and termites. Saw dust produced during wood processing generally contains bark and heartwood particles. This may be the reason for a higher inhibition activity of sawdust over leaf extracts. Mihara *et al.* (2015) showed inhibition activities of *A. auriculiformis* heartwood extract against *Phellinus* species and found effective at the concentration of 1 mg/ml. They reported the presence of two flavonoid compounds 3,4,7,8-tetrahydroxyflavanone and teracacidin in the heartwood extract and suggested that the antifungal activity may be due to the radical scavenging ability of the above flavonoid components. Quercetin is also one of the major flavonoids found in the seed pods of this plant and is responsible for antifungal activity (Chaki *et al.* 2015). Thus, the presence of large amount of natural products (Barry *et al.* 2005) in *A. auriculiformis* was responsible for present antifungal activity against wood decay fungi.

The results demonstrated that antifungal potency of saw dust extracts against wood decay fungi was higher in comparison to leaf extract. The size of inhibition zones produced by saw dust extract was proportional to the concentration of the extract used against *Oligoporus placenta* and *Trametes versicolor*. It can be used for wood preservation to check the decay caused by fungi.

REFERENCES

Adetogun A C and Atayese A O. 2006. Fungicidal Activity of *Azadirachta indica,Cymbopogon citrates* and *Vernonia amygdalina* on *Penicillium corylophilum*. Presented at the 33rdAnnual Conference of Nigerian Society for Plant Protection. Book of Abstract Paper No.NSPP/06/047.pp36.

Barry K M, Mihara R, Davies N W, Mitsunaga T and Mohammed C L. 2005. Polyphenols in *Acacia mangium* and *A. auriculiformis* wood with reference to heartrot susceptibility. *Journal of Wood Science* **51**(6): 615–21

Chaki S, Ghosh B, Bandyopadyhay S, Mookerjee M, Das S and Dastidar S G. 2015. Detection of various phytochemical compounds from seeds of *A. auriculiformis* for possibilities of

obtaining potent antimicrobial agents. *International Journal of Biological and Pharmaceutical Research* **6**(2): 120–28.

Chang S T, Wu J H, Wang S Y, Kang P L, Yang N S and Shyur LF. 2001. Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. *Journal of Agriculture and Food Chemistry* **49**: 3420–24.

Chew Y L, Goh J K and Lim Y Y. 2009. Assessment of in vitro antioxidant capacity and polyphenolic composition of selected medicinal herbs from Leguminosae family in Peninsular Malaysia. *Food Chemistry* **119**: 373–78.

Chew Y L, Chan E W L, Tan P L, Lim Y Y, Stanslas J and Goh J K. 2011. Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia. *BMC Complementary and Alternative Medicine* **11**(12).

Dhiman B and Dutt B. 2018. Present status and future strategies of wood preservation industry. *Eco-tourism, Environmental Problems and Sustainable Development,* Singh S P, Rathoure A K and Bharti P K (Eds) pp 226-245. Discovery Publishing House Pvt. Ltd., New Delhi, India.

Drijfhout F P and Morgan E D. 2010. Terrestrial natural products as antifeedants. *Comprehensive Natural Products Chemistry*, Vol 3, pp 457-501. Sir Barton D, Nakanishi K, Meth-Cohn O (Eds). Elsevier Science and Technology, India.

Girijashankar V. 2011. Micropropagation of multipurpose medicinal tree *Acacia auriculiformis*. *Journal of Medicinal Plants Research* **5**(3): 462–66.

Goyal P and Dev I. 1982. Effect of extractives on durability of Sal (*Shorea robusta*) wood. *Journal of Timber Development Association (India)* **28**(4): 12–16.

Hillis W E. 1987. *Heartwood and Tree Exudates*, p. 268. Springer-Verlag, Berlin, Germany.

Hunt G M and Garrett G A. 1967. Wood preservation. *The American Forestry Series* (3rd edn). McGraw-Hill Book Inc., New York, NY, USA, 32-33.

IS 4873, 2008. *Methods of Laboratory Testing of Wood Preservatives Against Fungi*. Bureau of Indian Standards. Manak Bhawan, New Delhi.

Juntheikki M R and Julkunen-Titto R. 2000. Inhibition of β-glucosidase and esterase by tannin from Betula, Salix, and Pinus species. *Journal of Chemical Ecology* **26**: 1151–65.

Kirker G T. 2018. Wood Decay Fungi. In: eLS. John Wiley & Sons, Ltd: Chichester. DOI: 10.1002/9780470015902.a0027655

Kishino M, Ohi H and Yamaguchi A. 1995.Characteristics of methanol extractives from Chengal wood and their antifungal properties. *Mokuzai Gakkaishi* **41**: 444–47.

Mandal P, Babu S P and Mandal N C. 2005. Antimicrobial activity of saponins from *Acacia auriculiformis*. *Fitoterapia* **76**(5): 462–65.

Mihara R, Barry K M, Mohammed C L and Mitsunaga T. 2005. Comparison of antifungal and antioxidant activities of *Acacia mangium* and *A. auriculiformis* heartwood extracts. *Journal of Chemical Ecology* **31**(4): 789–04.

Poonia P K, Devi L A and Tripathi S. 2015. The efficacy of methanolic extract of *Eucalyptus tereticornis* sm. Leaves against wood decaying fungi. *Indian Forester* **141**(8): 869–72.

Poonia P K, Deepa S R, Kumar M and Kumar A. 2021. Viability of wood decaying fungal mycelium after microwave radiation of bamboo culm. *Maderas.Ciencia y tecnologia* **23**(4): 1–6.

Puri Y N. 1967. Natural decay resistance of Indian timbers III heartwood extractives of Sal (*Shorea robusta* Gaertn.) and Teak (*Tectona grandis* L.f.). *Indian Forester* **93**(7): 447–54.

Reddy R G, Nirmala R S and Ramanamma C H. 2009. Efficacy of phytoextracts and oils of certain medicinal plants against *Cercospora moricola* cooke of Mulberry (*Morus alba* L.) leaf spot. *Journal of Biopesticides* **2**: 77–83.

Shafiei S N S, Ahmad K, Ikhsan N F M, Ismail S I and Sijam K. 2017. Antibacterial activity of *Acacia* spp. leaves extract against *Xanthomonas oryzae* pv. *oryzae* and screening for active phytochemical contents. *Journal of Agriculture and Veterinary Science* **10**(11): 49–60.

Sharma N, Singh S and Singh S K. 2017. Pharmacognostical standardization and preliminary phytochemical investigations on *Acacia auriculiformis* Cunn. Ex. Benth stem bark. *Journal of Medicinal Plants Studies* **5**(1): 398–402.

Shiny K S, Sundararaj R and Vijayalakshmi G. 2018. Potential use of coconut shell pyrolytic oil distillate (CSPOD) as wood protectant against decay fungi. *European Journal of Wood and Wood Products* **76**: 767–73.

Singh R, Singh S, Kumar S and Arora S. 2007. Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auricliformis* A. Cunn. *Food chemistry and toxicology* **45**(7): 1216–23.

Tripathi S, Rawat K, Dhayani S and Pant H. 2009. Potential of *Lantana camara* L. weed against wood destroying fungi. *The Indian Forester* **135**(3): 403–11.

Vogel I. 1978. *A textbook of Practical Organic Chemistry Including Qualitative Analysis,* Vol 3, pp. 457–501. Longmans, Green and Co., London, New York, Toronto. Elsevier Science & Technology, India.