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Effects of heartwood extractive of *Terminalia spinosa* on wood degradation by fungi

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ABSTRACT

Terminalia spinosa species have very high durability and could survive for long while in use, even in areas prone to attack by termites or fungi. Extractives have been found to have different inhibition rates against fungi. Wood extractives play an important role in the natural durability. The objective of this study was to investigate the durability of *Terminalia spinosa* when exposed to wood decay fungi. Three brown-rot and three white-rot wood decay fungi were screened for their capacity to degrade *T. spinosa* .Samples were evaluated for decay by weight loss measurements using a modified E7-93 standard (AWPA) for block test method, growth inhibition by solvent extract of *T. spinosa* was also tested. Brown rot fungi *P. placenta* and *C. puteana* caused the highest weight losses of 16% and 15% on heartwood extracted with dichloromethane and acetone, respectively. *P. sanguines* a member of white rot fungi was the most aggressive in reducing weight by 24% on toluene/ethanol extracted heartwood. The later was comparable to weight loss of *P. patula* wood which was used as a positive control. All the test fungi species caused negligible weight loss of less than 2% on un-extracted heartwood of *T. spinosa* implying that extractives in the heartwood played an important role in preventing fungal infestation and hence damage. Efficacy of extracts increased with increase in concentration.

Introduction

Green plants act as a reservoir of in-exhaustible source of innocuous fungicides/pesticides, which are mammalian non-toxic and easily biodegradable than synthetic chemicals. To develop eco-friendly wood preservatives, many studies have been conducted. Most of the reported work is on extractives from heartwood (Onuorah, 2000; Gupta and Indra Dev, 1999). However, very few reports are available in which other components of tree for example the leaves of *Ipomea carnea* (Saxen *et al.,* 2002) and *Azardirachta indica* A. Juss (Swathi *et al.,* 2004) possess a number of toxic constituents exhibiting high toxicity against wood-destroying microbes. Efforts have been made by many workers to use the plant products with the amendment of toxic metals and to test for durability against termites or fungi (Jain *et al.,* 1989 and 1997; Indra Dev and Nautiyal, 2004).

Natural resistance to fungi and termites is primarily attributed to the content of secondary metabolites present in heartwood, since these compounds frequently exhibit antifungal (Gomez-Garibay *et al.,* 1990) and antitermitic properties (McDaniel 1992; Scheffrahn, 1991; Reyes-Chilpa *et al.,* 1995). Bell *et al.,* (1980) suggested that plants synthesized a greater variety of secondary natural compounds than did animals because plants cannot rely on physical mobility to escape from their predators.

Some wood species have very high durability and could survive for long while in the use even in areas prone to attack by termites or fungi. According to wood type and nature of the extraction solvent, extractives present different inhibition rates against fungi. Wood extractives play an important role in the natural durability (Reyes Chilpa *et al.,* 1998; Celimene *et al.,* 1999; Mori *et al.,* 1997; Haiput *et al.,* 2003; Windeisen *et al.,* 2002; Neya *et al.,* 2002; Gerardin *et al.,* 2004). The natural durability of wood has been defined as its degree of resistance to deterioration by a whole range of biological, chemical, mechanical and physical wood-destroying agents (Wong *et al.,* 2005). Extraction of secondary metabolites with organic solvents and water renders heartwood susceptible to wood-destroying organisms (Deon, 1983; Reyes-Chilpa *et al.,* 1987).

Tremendous wood destruction results annually from the ravages of fungi and insects in the forests and the wood yard or buildings/ structures though different species exhibit wide variation in their resistance to attack (Gerardin *et al.,* 2004). The main groups of wood destroying fungi include soft rot, white rot and brown rot fungi (Anke *et al.,* 2006). White rot fungi produce lignases and peroxides that are able to cleave the ring structures of lignin as well as cellulases that break down the linear cellulose molecules. White rots degrade all components of the wood structure leaving behind bleached strands of cellulose (Celimene *et al.,* 1999). Certain white rot fungi have also been found to have mutualistic associations with bacteria (Blanchette *et al.,* 1990). Brown rot fungi are only equipped with cellulases that break down the linear structure, which leaves the brown cubical remnants of lignin behind (Rayner and Boddy, 1988).

Most of the brown rot fungi have shown apparent tolerance to copper, an important, component in the latest generation of wood preservatives (Woodward and Degroot, 1999; Clausen *et al.,* 2000). Over the past four decades, there has been substantial global concern to develop eco-friendly wood preservatives to replace inorganic with those which do not cause any ill effect on

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the health of mammals (Onuorah, 2000). There is now a continuous search for new materials and methods for wood preservation.There is therefore a need for continuous quest to look in to other technologically appropriate alternative control measures by developing new biocides based on natural formulations exhibited by durable wood species such as *T. spinosa* to ameliorate fungal problem.

The objectives of this study were (1) to determine which extractive components in *T. spnosa* most affect attack by three species of the Brown rot fungi namely; *Poria placenta* (Pp) , *Coniophora puteana* (Cp) , *Gloephyleum trabeum* Pers.ex Fries (Gt) and another three species of White rot fungi namely; *Coriolus versicolor* L.ex Fr (Cv), *Antrodia species* (Asp) and *Pycnoporus sanquineus* L. ex Murr (Ps) and (2) to assess the relationship between naturally occurring variations in heartwood extractive content and resistance to bio-deterioration.

Materials And Methods

Plant material

The plant species, *T. spinosa* was identified at the herbarium in the department of Biological sciences (Botany) of Moi University and voucher specimen deposited. Ten mature trees of *T. spinosa* were selected randomly from Wei Wei forest, felled, debarked, seasoned and converted into 5 metre long logs. The wood logs **were** transported to the Moi University, Wood Science Laboratories where the sapwood and heartwood were separated by band sawing. The heartwood was used in this study. Heartwood was cut and converted into blocks of various sizes based on a modified **E7-93** standard **(AWPA),** Longitudinal, Radial and Tangential (L.R.T) respectively for use in different tests later.

Fungi : Three brown rot fungi, *Poria placenta* (Pp) , *Coniophora puteana* (Cp) , *Gloephyleum trabeum* Pers. ex Fries (Gt) and three white rot fungi, *Coriolus versicolor* L. ex Fr (Cv), *Antrodia species* (Asp) and *Pycnoporus sanquineus* L. ex Murr (Ps) was used in these study.

Extraction

The heartwood of *T. spinosa* weighing 1 kg were ground separately to fine powder using a vibrating hammer mill and passed through a 115 – mesh sieve and dried separately at 60^0C. Extraction using hexane, dichloromethane, methanol, acetone, toluene/ethanol (2:1v/v) mixture and water in a soxhlet extractor was carried out using samples of 10g for each solvent.

Test on natural durability of *T. spinosa*

This part of the study was to determine the natural durability of the plant species to resist and tolerate fungal infections. Heartwood samples of *T. spinosa* were cut into 25 X 25 X 5mm longitudinal, radial and tangential (L.R.T) blocks, respectively. Ten samples with their inherent chemicals were compared to 40 samples that had been extracted as above and were conditioned at room temperature, 20^0C to 22^0C and a relative humidity of 60 – 70% until they achieved a constant weight.

The test assumed correctness of theoretical dry mass of wood because for natural durability test, samples should not be dried at 103^0C to avoid degradation of the wood components. Theoretical dry mass (M_{to}) of test samples was determined by calculating the average percent (%) moisture of similar samples dried at 103^0C to get µ, Where;

µ is the average % humidity after oven drying at 103^0C, using the formula

$$M_{to} = \frac{100M\mu}{100 + \mu}$$

Where

M_{to} – theoretical dry mass of test samples

Mµ- Mass of test samples after conditioning at room temperature.

µ- Percentage average humidity of samples after conditioning.

The extract samples were exposed to fungal inoculation using petri dishes with potato dextrose agar medium which had been each inoculated with test fungi seed mycelium at the centre to grow and to colonise the media. The Petri dishes had been incubated at 25^0C and relative humidity of 85% for 10 days to achieve total colonization prior to introduction of test wood samples.

Two extracted and unextracted test samples of each sterilized by UV light were placed in the petri dishes containing fully grown fungi and untreated *Pinus patula* used as positive control for 3 species each of brown rot and white rot fungi. A total of 264 samples were involved in the assessment. Assessment was done by determination of weight loss (%) using the following formula.

$$\text{Mass loss (\%)} = \frac{(M_{to} - M_f)}{M_{to}} \times 100$$

M_{to} –Theoretical weight of test samples

M_f – Final weight of dry samples.

This was to yield the deterioration in wood confirmation after deleterious effects of infection by pathogens as happens in the field.

Bioassay on fungal growth inhibition by extractives

The bioassay in fungal growth inhibition was done to show the ability of extractives to deter infection by fungus. The test on fungal growth inhibition by extractives was carried out in the laboratory prior to the inoculation as follows: - potato dextrose agar medium was prepared by mixing 30g potato dextrose and 40g agar in 1 litre of distilled water. The solution was warmed and stirred continuously until harmonised. The pH of the solution was made to 4.8 by addition of 0.1N HCl. It was sterilized in the autoclave for 25 minutes at 120^0C.

The potato dextrose agar medium was poured into empty 100 ml conical flasks which had been sterilised in the autoclave using the same procedure inside the sterilised chamber while still hot. Different wood extractives from *T. spinosa* heartwood extracted by hexane, dichloromethane, methanol, acetone, mixture of toluene/ethanol (2:1v/v) and water was dissolved in 5 mls of methanol to make-up different concentrations of 50 ppm, 100 ppm, 500 ppm and 1000 ppm and then mixed with 100 ml of malt agar in the flasks. After putting the extract, each flask was properly shaken, then poured into 6 petri dishes and allowed to cool and solidify. In the same sterilised chamber, a small portion of fully-grown test fungi was placed at the centre of each petri dish and allowed to colonise the media. Three replicates were prepared for each concentration and the petri dishes were placed in the incubation chamber at 25^0C and relative humidity of 85%.

The rate of inhibition (T) was determined for each concentration by applying the formula.

$$T = 100 \times [1- \frac{\text{Surface area covered by fungi at each concentration}}{\text{Surface area covered by the control}}]$$

This was to yield the thresh hold efficacy that is inherently present in the extracted material.

Natural durability of *T. spinosa* (extractives) against fungal biodegradation

The detailed results in **Figures 1** and **2** illustrates the effect of fungal damage on solvent extracted heartwood compared with unextracted heartwood of *T. spinosa* and that of *Pinus patula* which was used as a positive control. A general decrease in resistance on extracted *T. spinosa* heartwood against white

rot 2-14%, 2-18%, 1-23% (**Figure 1**) and brown rot fungi 5-17%, 3-13% and 1-16% (**Figure 2**) were observed as not significantly different from each other (p=0.064) and while using different solvents yielded different weight loss (p-value =0.001).

Brown rot fungi caused the highest weight loss on test heartwood whose extractives had been removed using different solvents. Dichloromethane extracted heartwood recorded the highest weight loss of 16% from *P. Placenta*, *C. puteana* was the most effective against wood extracted with acetone solvent at 15% **(Figure 1)**

Weight loss was comparable for heartwood extracted with solvent of medium polarity methanol, acetone, toluene/ ethanol. Of the treated wood, *P. sanguineus* white rot fungi was the most aggressive in reducing the weight by 24% on toluene/ethanol extracted heartwood comparable to that of the positive control wood of *P. Patula* **(Figure 2)**, white rot fungi species used did not result in much damage to the extracted heartwood compared with the brown rot fungi damage.

Unextracted heartwood of *T. spinoasa* showed a consistenntly very low weight loss (<2%) for all fungi species tested. This is an indication that chemicals in heartwood play a significant role in preventing white and brown rot fungi.

(i) Brown-rot fungi

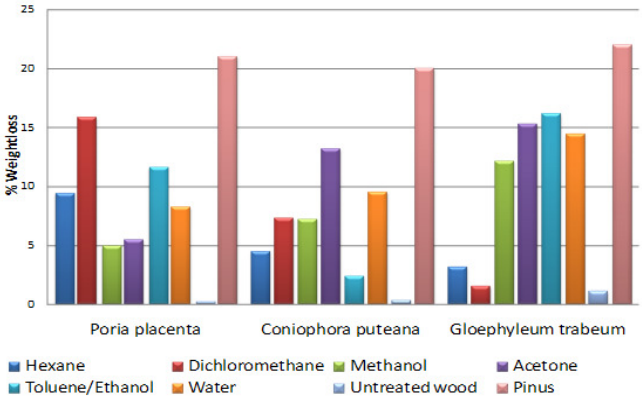

Figure 1: Weight loss (%) results of *T. spinosa* heartwood from Brown-rot fungi damage

(ii) White-rot fungi

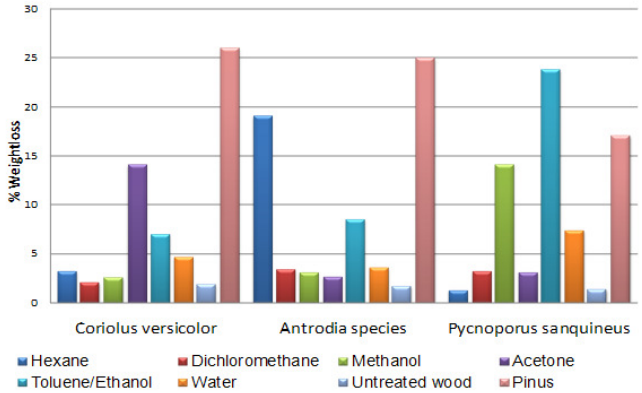

Figure 2: Weight loss (%) results of *T. spinosa* heartwood from white-rot fungi damage

Results of inhibition on fungal growth on the heartwood of *T. spinosa*

Fungal growth inhibition was significantly different from each other (P-value = 0.024), similarly inhibition rates compared differently using different concentrations (P-value =0.001which is less than 0.05, and that different solvents yielded different inhibition rates (P-value =0.001).

Generally *T. spinosa* extractives inhibited growth of all test fungi to varying degrees **(Figures 3 and 4).** However, acetonic, methanolic and distilled water extractives were the most effective in inhibiting growth of *P. placenta*, *P.Sanguines* and *C. versicolor* on the heartwood of *T. spinosa* at over 85% (P=0.001 *). Antrodia species* was the least inhibited at 78%. (P=0.0024).

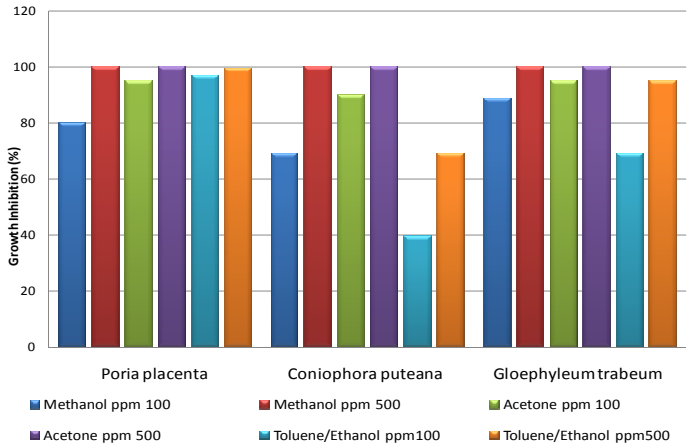

Figure 3: Fungal growth inhibition percentage (Brown rot)

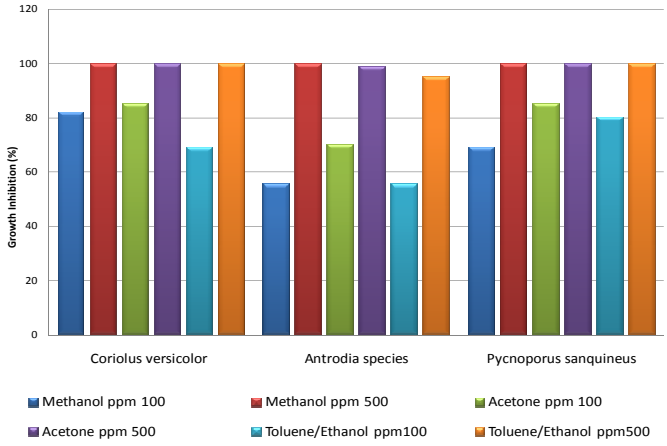

Figure 4: Fungal growth inhibition percentage (White rot)

These results confirm that extractives from *T. spinosa* had strong antifungal properties. Efficacy of the extracts increased with increase in concentration upto 200 ppm where any further increase in concentration had no significant effect.

Generally, acetone extract showed the highest inhibition rate for all fungi that were tested.

Inhibition reduced with time with some instances having the fungal growth long after the control filled the petri dish **(Figure 5).** Brown rot colouration was observed in the petri dishes and increased with extrative concentration. The colouration intensity reduced with time as fungal growth progressed.

Discussion

There were no obvious variations in percentage (%) weight loss of extracted *T. spinosa* heartwood when exposed to the test species of brown and white rot fungi. However marked variations in weight loss were noted on heartwood extracted with different solvents. Brown rot fungi *P. placenta* and *C. puteana* caused the highest weight losses of 16% and 15% on heartwood extracted with dichloromethane and acetone, respectively. On the hand *P. sanguines* a member of white rot fungi was the most aggressive in reducing weight by 24% on toluene/ethanol extracted heartwood.

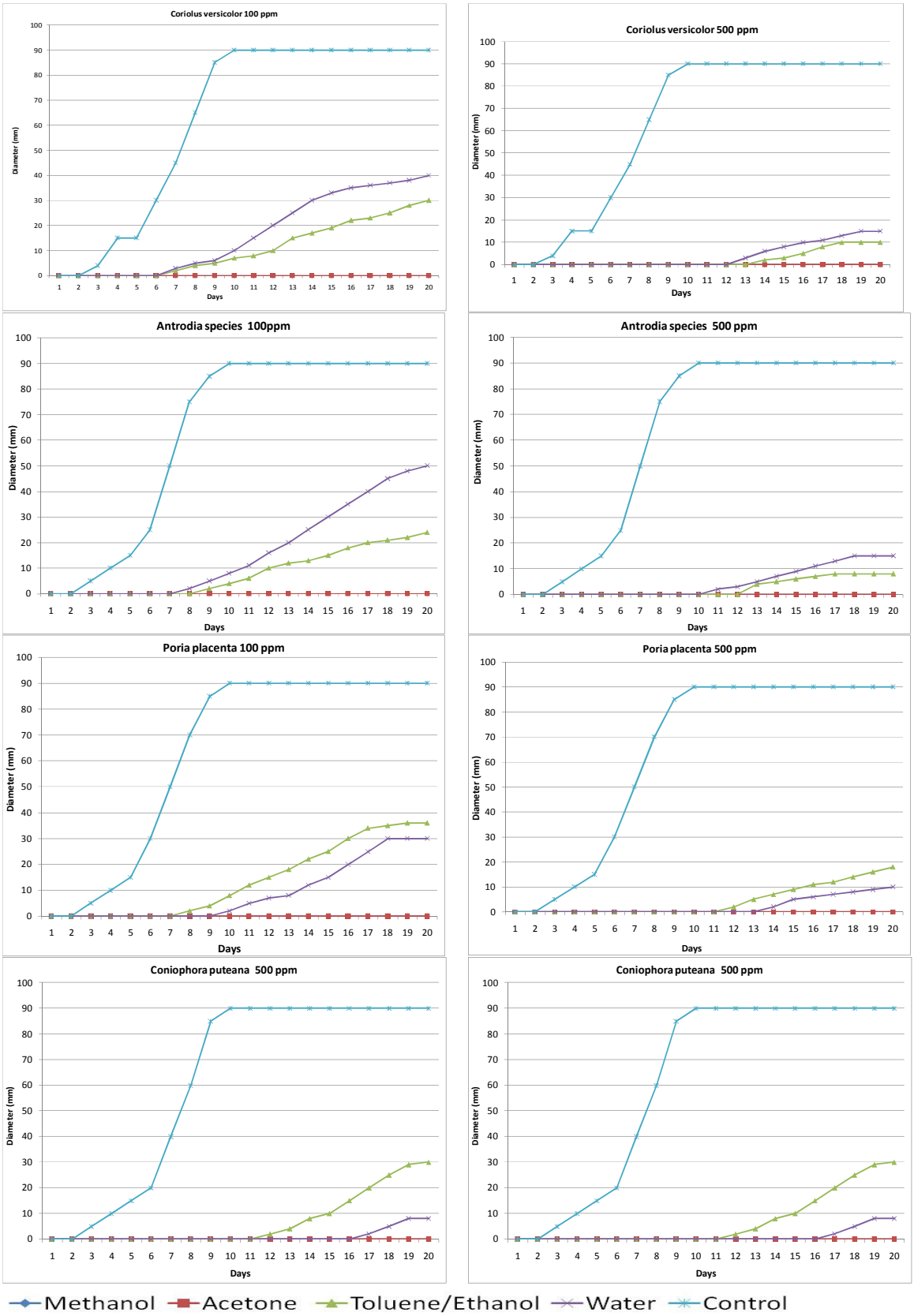

Figure 5: Effects of extractives on fungal growth

The later was comparable to weight loss of *P. patula* wood which was used as a positive control.

All the test fungi species caused negligible weight loss of less than 2% on unextracted heartwood of *T. spinosa* implying that extractives in the heartwood played an important role in preventing fungal infestation and hence damage.These findings are consistent with the study by Hart and Hillis (1974) and Kishino *et al.,* (1995) who reported on the resistance to fungal decay and antifungal activity properties of durable wood attributable to the content of extractives in wood. Schultz *et al.,* (1988) also confirmed that extractives possed fungicidal activity and were excellent free radical scavengers because they produced antioxidants. Many other workers have also corroborated the same findings (Back *et al.,*1992; Tanaka *et al.,*1999).

Great variations were also observed in fungal growth inhibition using the different extractives at varying concentrations. Acetone extracts showed the highest inhibition rate of all the fungi that were tested.

Efficacy of extracts increased with increase in concentration. At low concentrations (200 ppm) all extractives were able to completely inhibit growth for all species of fungi tested indicative of substantial deterrent activity. These findings were consistent with the work done by Gerardin *et al.,* (2004) who reported that wood extractives inhibited fungal growth and therefore contributed to the natural durability of wood. Fungal growth inhibition reduced with time; similarly, colouration intensity also reduced with time as fungal growth progressed. This observation could be explained by the detoxification of the extractives by free radicals which produce oxaloacetic acid in fenton reaction in wood decay as reported by Green *et al.,* (1991) and Hyde and Wood (1997). These findings were also corroborated by Schultz and Nicholas (2000) who reported that heartwood extractives protected wood against fungal colonization and subsequent degradation by having both fungicidal and antioxidant properties. This dual defense mechanism for both white and brown rot fungi use same type of free radical species in order to initially disrupt cell walls (Tanaka *et al.,* 1999; Lu *et al.,* 1994) so as to increase the pore size so that the relatively larger extracellular fungi enzymes could penetrate through the cell wall (Flourney *et al.,* 1993). However the phenolic extractives in the heartwood were excellent antioxidants (Larson, 1988).

Conclusion

The results showed that *T. spinosa* heartwood was very resistant to fungal infestation. Wood durability against fungus correlated to the concentration of compounds in extractives, more so in dichloromethane and acetone extracts. The above active extracts presented higher fungicidal activity allowing total inhibition of all tested species at the concentration of 500 ppm with the rest of the test extractives being less inhibitive.

The presence of extractives in the heartwood conferring apparent durability was shown to inhibit the growth of several fungi species include *Poria placenta, Coniophora puteana* and *Pycnoporus sanguineus* were the three most important rot-causing fungi. Extracted heartwood exhibited a lower durability while unextracted heartwood had enhanced durability. Higher susceptibility to termites was observed for acetone extracted heartwood which indicated the importance of the extractives present. Additionally, *T. spinosa* heartwood possess high lignin content, high dimensional stability and low water uptake which partly explains natural durability**.**

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