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POTENTIALS OF *Tridax procumbens* Linn EXTRACTS AS PRESERVATIVE AGAINST WOOD DECAY FUNGI

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ABSTRACT

All over the world, researches are focused on wood bio-preservatives that are environmentally friendly, locally available and also serve as alternative to chemical preservatives which have hazardous effects on the environment and their users. 15x25x50mm test-blocks of *Aningeria robusta* and *Ceiba pentandra* were exposed to *Trametes versicolor* having been brushed with both aqueous and ethanolic extracts of *Tridax procumbens.* Another set was completely immersed in both extracts for twelve hours prior exposure to *Trametes versicolor* for sixteen weeks. 2x2x2 factorial experimental design was adopted; data obtained analysed using descriptive statistics and analysis of variance. Preservative absorption was very poor; it varied from 0.02 to 0.22Kg/m^3. The phytochemical screening revealed the presence of phenols, flavonoids, tannins and saponin. Both the species and preservatives used had significant effect on the absorption at 5% probability level. Visual observation of the test blocks showed that the control (untreated) and those treated with aqueous extract were completely covered with fungal mycelia. However, those treated with ethanolic extract had poor and scanty fungal growth. Untreated test blocks had the highest weight loss (89.58%) for *Aningeria robusta* and 91.06% for *Ceiba pentandra.* Dipping in ethanolic extract had the least weight loss of 13.20% and 23.88% respectively for *Aningeria robusta* and *Ceiba pentandra.* The species used, method of application and the type of preservative used significantly affected weight loss at 5% probability level. In conclusion, the preservative threshold of the extract was 0.19Kg/m^3 and 0.22Kg/m^3 for *Aningeria robusta* and *Ceiba pentandra* while the weight loss was 13.20% and 23.88% respectively; complete immersion being preferred.

Keywords: Phytochemical screening, immersion, preservative, brushing, *Aningeria robusta*

Introduction

The tropical forest is extremely vast in terms of species. It comprises of thousands of species; the properties of which greatly differ. Despite the versatility of wood for construction purposes, it is limited in application due to infestation by deteriorating agents such as termite, fungi and marine borers (Ajala, 2014). Large quantities of timber are destroyed annually by these agents particularly in the humid coastal areas where the warm and moist climatic areas are very favourable for their rapid development (Ogunsanwo *et al.*, 2002). Some timbers are reported to possess excellent resistance to various agents of deterioration, thus referred to as being durable and are therefore preferred to the non- durable ones. Many others have only moderate resistance or non-resistance at all hence making wood preservation a necessity (FAO, 1986). Wood preservation is the chemical conditioning of wood to increase its resistance to invading destructive organisms and deterioration caused by unfavourable environmental condition to prolong the life span of wood in service.

Some of the chemicals used as preservatives have restrictions; they are harmful to the environment and the users. There is the need therefore to search for environmental friendly preservatives which can increase the service life of wood and also less harmful to the environment and the users (Aldo and Orlando, 2009).

Currently, new preservative systems are being evaluated as a replacement for the synthetic. When available, bio-preservative on the other hand is environmentally friendly, locally available and cheap to acquire. Ability of wood and natural plant extractives to protect wood against wood degrading fungi and insects has been one possible approach for developing new wood preservatives (Kabir and Alam, 2007). Several scientists have reported that plant extracts are good source of fungicides. Most reported works on the use of ecofriendly wood preservatives is on extractives from heartwood, leaf, root and oil from herbaceous plants (Onuorah, 2000; Saxena and Dev, 2002; Adetogun *et al.*, 2009 and Ajala, 2014). Moreover, there are also reports on the use of oil to preserve different wood species (Venmalar and Nagaveni, 2005). Despite the extensive studies on the use of preservatives of plant origin to protect wood, there is little or no information on the use of *Tridax procumbens* extractives to preserve *Aningeria robusta* and *Ceiba pentandra* which are tropical species and timbers of commerce that are used in many wooden applications.

Tridax procumbens belongs to the family Asteraceae. It is native of tropical America, naturalized in tropical Africa, Asia, and Australia (Kushwaha *et al.*, 2019). This plant can be found in fields, meadows, croplands, disturbed areas, lawns, and roadsides in areas with tropical or semi-tropical climates (Wikipedia, 2021). The plant bears daisy-like yellow-centered white or yellow flowers with three-toothed ray florets. The leaves are toothed and generally arrowhead-shaped. Its fruit is a hard achene covered with stiff hairs and having a feathery, plume-like white pappus at one end. Calyx is represented by scales or reduced to pappus. The plant is invasive in part because it produces so many of achenes, (up to 1500 per plant) and each achene can be dispersed by wind via its pappus (Wikipedia, 2021). Traditionally, *T. procumbens* has been known for its anti-coagulant, anti-fungal and insect repellent activity (Sharma and Kumar, 2009; Kushwaha *et al.*, 2019 and Wikipedia, 2021). However, FAO (1986) and Adetogun (1998) noted that before a new preservative could be accepted as a better alternative to the proprietary chemicals, information about its properties and effectiveness should be obtained by laboratory and field test.

This study therefore investigated the potentials of *Tridax procumbens* extract using its threshold and weight loss values to determine its preservative against wood fungi attack.

Materials and Method

Preparation of Extract

The whole plant of *Tridax procumbens* Linn were collected from Federal College of Forestry, Ibadan, Oyo State, Nigeria and was taken to Forestry Research Institute of Nigeria's (FRIN) herbarium for identification. The plant was air dried at room temperature in the laboratory for 15 days and then ground to powder with a grinding machine. Aqueous extract of the sample was prepared by soaking 50g of the powered sample in 500ml of distilled water for 24hours. It was then filtered with Whatman filter paper No.42 (125µm), the liquid extract was transferred into beakers and refrigerated until required for use.

The ethanolic extract of the sample was prepared by soaking 100g of the powdered sample in 1 litre of 95% ethanol for 24 hours. It was filtered and the extract was transferred into beakers, labelled and refrigerated until required for use.

Preparation of culture medium

The fungi strains were obtained from Pathology Department, FRIN. The fungi was cultured using Potato Dextrose Agar (PDA) as the culturing medium, thirty-nine (39) g of PDA was dissolved in 1litre of distilled water, homogenized and sterilized in the autoclave at 1.05kg/cm^2 for 15 minutes. After sterilization, the medium was allowed to cool and maintained at 45^{o}C and later dispensed into petri dishes. The PDA was incorporated with streptomycin to avoid bacteria contamination. Pure isolates of *Trametes versicolor was* used for the study**.** The isolates were then sub-cultured into fully solidified PDA with a 4mm corkborer. The plates were incubated at room temperature. After full ramification, the test blocks were introduced into the fungi species for sixteen weeks (AWPA, 1997).

Determination of Absorption

Ten test-blocks of dimension 15 x 25 x 50mm for each of *Aningeria robusta* and *Ceiba pentandra* in accordance with Arora (2006) and Sarker *et al., (*2006) were used for each of the treatments viz: control, dipping in ethanolic extract, dipping in aqueous extract, brushing with ethanolic extract and brushing with aqueous extract.

Ten test blocks each of the species were completely immersed (FAO, 1986) in 100% ethanolic extract and aqueous extract of *Tridax procumbens* for 48 hours in accordance with (BCMAFF, 1993). Another set of ten test blocks for each species were brushed with ethanolic extract and aqueous extract of *Tridax procumbens.* After treatment, the blocks were drained and reweighed to determine the level of absorption. Treated wooden blocks were conditioned inside a desicator for two weeks. Absorption of preservative was determined using BS (1961) formula thus:

$$\text{Absorption} = \frac{\text{Total absorption} \times \text{Concentration} \times 10\ \text{Kg/m}^3}{\text{Vol. of wood} \times \text{Number of pieces}} \quad (1)$$

Determination of Moisture Content

After inoculation, moisture content of the test blocks was determined using BSI (1961) and Adetogun *et al.,* (2009) proposed method. They wet weight of the test blocks after inoculation was determined after which they were oven- dried at 100^{o}C for 20 hours, the test blocks were allowed to cool before reweighing. Moisture content was calculated using the formula:

$$\%\ \text{Moisture Content} = \frac{\text{Final wet weight} - \text{Final dry weight} \times 100}{\text{Final dry weight}} \quad (2)$$

Visual examination of the test blocks was done to assess the extent of fungi infestation after incubation for sixteen weeks. The rating was done in accordance with Greaves *et al.,* (1988) and Adetogun (1998) where:

5.... Completely (100%) covered with fungal growth

4--- Almost (75%) covered with fungal growth

3---Moderate (50%) fungal growth

2---Poor (25%) fungal growth

1---Very poor (5%) fungal growth

0—No fungal growth

Preliminary Phytochemical Screening

Preliminary phytochemical screening and quantitative test of *Tridax procumbens* for the presence of phenols, tannins, flavonoids, alkaloids, terpenoids, anthraquinones, steroid, and saponins was carried out in accordance with Kushwaha *et al*., (2019)

procedures; these phytochemicals were identified by characteristics colour changes.

Experimental Design

A 2x2x2 factorial experimental design was adopted; data obtained analysed using descriptive statistics and analysis of variance

Results and Discussion

Absorption of preservative by wood sample

The mean preservative absorption is presented in Table 1. For both species, dipping in ethanolic extract had the highest absorption, followed by dipping in aqueous extract while the least is brushing with aqueous extract. Results of dipping was significantly higher ($p<0.05$) than that of brushing because the test blocks were completely immersed in the extract for a longer period hence, allowed the extract to penetrate the test blocks unlike the brushing that was on the surface. The ethanol used in the extraction being an organic solvent also aided the penetration of the preservative into the pores of the test blocks. With brushing, the preservative did not penetrate deep into the sapwood and definitely not reaching the heartwood because it is superficial, hence, the result in Table 1. Brushing is not a good treating method for preserving wood exposed to high risk of decay such as for ground contact uses as noted by Milton (1995). Brushing is most widely used for protecting areas of previously treated wood that have been cut or machined, thereby exposing untreated surfaces or joints (OSUE, 1995).

The characteristics of the wood species involved in the study; variation in the porosity of wood species, proportion of sapwood to heartwood, moisture content of the wood may have been responsible for the differences in absorption (Kazemi *et al.*, 2006; Viitanen *et al.*, 2006 and OSUE, 1995). The type of solvent coupled with the viscosity of the chemicals used must also have influenced the penetration of the preservatives (Kazemi *et al.*, 2006). Both timber species and the type of preservative used had significant effect on the absorption at 5% probability level whereas interactions between species used, methods adopted and the preservatives used had no significant effect on the absorption at the probability level (Table 2).

Table 1: Mean Values of Absorption of Preservative by the Wood Sample

Species / Method	Dipping in ethanolic extract (Kg/m^3)	Dipping in aqueous extract (Kg/m^3)	Brushing with ethanolic extract (Kg/m^3)	Brushing with aqueous extract (Kg/m^3)
Aningeria robusta	0.19 ± 0.05	0.18±0.01	0.03±0.02	0.02±0.01
Ceiba pentandra	0.22 ±0.01	0.19±0.01	0.05 ±0.02	0.03± 0.02

Table 2: Analysis of Variance for Absorption of the Wood Sample

S V	DF	SS	MS	F Cal	F Tab
Species	1	994.76	994.76	82.89*	3.96
Method	1	6.56	6.56	0.55ns	3.96

Preservative	1	48.53	48.83	4.07*	3.96
Spp. x Method	1	4.28	4.28	0.36ns	3.96
Spp. x Preservative	1	32.13	32.13	2.86ns	3.96
Method x Preservative	1	0.00	0.001	0.00001ns	0.00
Spp. x Method x Preservative	1	4.01	4.01	0.33ns	3.96
Error	72	864.08	12.001		
Total	79	1954.64			

Legend: * Significant at α = 0.05 ns: Not Significant Spp. =Species

Visual rating of mycelia growth of the fungus

Result in Table 3 reveals the visual rating of the mycelia growth of *Trametes versicolor* on the test blocks. Total immersion in ethanolic extract had very poor (5%) mycelia growth, indicating that the extract prevented the growth of the fungus while brushing with ethanolic extract had moderate (50%) fungal growth meaning that the preservative could prevent the growth to some extent. Brushing with aqueous extract and the control resulted in total covering (100%) of the test blocks by the fungus mycelia. Total immersion as a method had a better result than dipping just as 100% ethanolic extract had a better result than aqueous extract.

Table 3: Visual rating of the mycelia growth of the fungus on the test blocks

Species/Method	Dipping in ethanolic extract	Dipping in aqueous extract	Brushing with ethanolic extract	Brushing with aqueous extract	Control
Aningeria robusta	5%	75%	50%	100%	100%
Ceiba pentandra	5%	75%	25%	100%	100%

The degree of fungi infestation of the woodblocks was categorized base on the percentage weight loss as follows:
Sound –---------- 0-10%
Slightly decayed----------11-20%
Fairly decayed----------21-30%
Badly decayed---------- above 30%

Moisture content of the test blocks after inoculation

The growth and effectiveness of the test fungus mycelia in bio-deterioration are in agreement with the assertion of Arora (2006) and Sarker *et al.*, (2006). They reported that agar-block test is a useful technique for evaluating the effects of potential wood preservatives on wood biodegradation by rot fungi. The mean moisture content of test blocks subjected to the fungus is presented in Table 4. It shows that the test blocks were exposed to optimum moisture content above fibre saturation point (average 30%) which is required for fungal growth and decay as reported by Kollman and Cote, (1968) and Flaete *et al.*, (2006). This implies that moisture content ranged from 43.95% to 65.40% were adequate for fungus growth hence, provided conducive environment for it to cover the test blocks.

Table 4: Mean moisture content of the test blocks after inoculation

Species/ Method	Dipping in ethanolic extract	Dipping in aqueous extract	Brushing with ethanolic extract	Brushing with aqueous extract	Control
Aningeria robusta	49.13±1.70	65.40±3.79	57.61±2.54	46.05±4.64	43.95±6.29
Ceiba pentandra	50.65 ±4.10	63.23±0.95	55.61±2.54	47.73±1.82	51.64±4.29

Weight loss and condition of test blocks after inoculation

The result presented in Table 5 reveals the percentage weight loss after inoculation; untreated test blocks (control) had 89.58% and 91.06% for *Aningeria robusta* and *Ceiba pentandra* respectively. By implication, *A. robusta* had 10.42% soundness whereas *C. pentandra* had 8.94% soundness. It can then be said that the control test blocks were badly affected. Brushing with aqueous extract was also badly attacked by the fungus; 92.60% and 90.58% were recorded against the treatment for *A. robusta* and *C. pentandra* respectively. Total immersion in aqueous and brushing with ethanolic extract were badly affected because their percentage weight loss was above 30%. Ethanolic extract imparted extra durability to the test blocks hence, *A. robusta* had 13.20% therefore categorising it as slightly decayed with 86.80% part of it still sound while 23.88% was recorded for *C. pentandra* thereby categorising it as fairly decayed with 76.12% part of it still sound for use . In a study using *Gliricidia sepium* heartwood extract to preserve *A. robusta,* Ajala (2014) reported a weight loss of 18.65% when exposed to *Lentinus sajor-caju* and 19.10% when exposed to *Trichoderma viride.* Olajuyigbe (2007) reported a weight loss of 16% for *Triplochiton scleroxylon* exposed to *Pleurotus squarrosulus* and *Lentinus subnudus* white rot fungi after treated with cuprinol clear, a chemical preservative. It can be inferred that total immersion in preservative gives a better result than brushing, also, using aqueous extract to preserve wood does not confer protection against fungal attack on the wood. Dipping in ethanolic extract confer greater protection against fungal attack on *A. robusta* than it does on *C. pentandra.*

The weight loss, despite preservative treatment confirms the suggestions of Kazemi *et al.,* (2006) and Humar *et al.,* (2006) that treatment may only reduce and not stop degradation, unless applied at extremely toxic levels. It could also be deduced from the study that the extract had antifungal effect on the species used. This is in agreement with the assertion of Bowyer *et al*., (2003) that before an extractive could impart decay resistance, it must have antifungal properties. The species used, the preservative used and the method of application had significant on weight loss at 5% probability level (Table 6).

Table 5: Mean percentage weight loss and condition of test blocks after inoculation

Treatment	*Aningeria robusta*		*Ceiba pentandra*	
	Weight Loss (%)	**Condition of test blocks**	**Weight Loss (%)**	**Condition of test blocks**
Control	89.58±6.29	badly decayed	91.06±4.29	badly decayed

Brushing with aqueous extract	92.60±4.64	badly decayed	90.58±1.82	badly decayed
Brushing with ethanolic extract	78.72±2.54	badly decayed	74.98±2.45	badly decayed
Dipping in aqueous extract	87.96±3.79	badly decayed	90.90±1.95	badly decayed
Dipping in ethanolic extract	13.20±1.70	slightly decayed	23.88±4.10	fairly decayed

Table 6: Analysis of Variance for Weight loss of the Wood Sample after inoculation

S V	DF	SS	MS	F Cal	F Tab
Species	1	781.20	781.20	23.97*	4.03
Method	1	2345.00	2345.00	71.95*	4.03
Preservative	1	48.53	48.83	4.07*	3.96
Spp. x Method	1	4.28	4.28	0.36ns	3.96
Spp. x Preservative	1	32.13	32.13	2.86ns	3.96
Method x Preservative	1	0.00	0.001	0.00001ns	0.00
Spp. x Method x Preservative	1	4.01	4.01	0.33ns	3.96
Error	72	864.08	12.001		
Total	79	1954.64			

Legend: * Significant at $\alpha = 0.05$ ns: Not Significant Spp. =Species

Phytochemical Screening

Results in Table 7 show the results of phytochemical analysis of *Tridax procumbens*. Phytochemicals generally exert their antimicrobial activities through different mechanisms and are known to be biologically active because they protect the plants against infections (Scalbert, 1991). From the present study, on addition of 0.5ml of $FeCl_3$ solution to 2ml of test solution, an intense colour was formed which indicated the presence of phenol in *Tridax procumbens*. This is in conformity with the findings of Kushwaha *et.al*, (2019). When 2 drops of NaOH was added to 3ml of *Tridax procumbens*, deep yellow colour was formed. On addition of 2 drops of dilute HCl, the colour remain which indicated the presence of flavonoid in the test solution.

This was in agreement with the finding of Adebawo (2019) who reported it in the oil extract of *Azadirachta indica* seeds. Also, addition of 10% lead acetate solution into 5ml of *Tridax procumbens* extract formed a yellowish substance that indicated the presence of tannin in *Tridax procumbens.* Whereas when the extract was diluted with distilled water and thoroughly shaken for 15 minutes, a thick layer was formed indicating the presence of saponin in the test solution. These findings were in conformity with those of Kushwaha *et.al*, (2019).

Table 7: Phytochemical constituents of *Tridax procumbens*

Constituent	Result
Phenols	+
Flavonoids	+
Tannins	+
Saponins	+

+ indicating the presence of phytochemical

Conclusion

The aqueous and ethanolic extracts used as preservative against wood destroying fungi (*Trametes versicolor*) imparted some levels of durability to the test blocks though, preservation with ethanolic extract was much better. The phytochemical screening revealed the presence of phenols, flavanoids, tannins and saponin. These have been reported to have antimicrobial properties which may be responsible for the little preservation the test blocks enjoyed. The species used as well as the method of application and the preservative had significant effect on the weight loss, hence the durability of the species. The preservative threshold of the extract was 0.19Kg/m^3 and 0.22Kg/m^3 for *Aningeria robusta* and *Ceiba pentandra* while the weight loss was 13.20% and 23.88% respectively; complete test blocks immersion and 100% ethanolic extract being preferred. It is recommended that further studies be carried out using other bio-preservatives.

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