ANTIFUNGAL POTENTIALS OF STEM EXTRACTS OF *EUPHORBIA HIRTA* AGAINST WHITE ROT FUNGI IN CEIBA *PENTANDRA* WOOD

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Abstract
The susceptibility of wood to deterioration from different organisms and longtime effects on global economic is enormous. One important aspect of alternative preservatives is bio-preservatives plants in which locally available plants or its parts can be used which are economically viable and environmentally friendly. This research was undertaken to establish the potency of the extracts from *Euphorbia hirta* species on strains of white rot fungi (*Pleurotus ostreatus*) on *Ceiba pentandra* wood for percentage weight loss. The test samples then inoculated using white rot fungi (*pleurotus ostreatus*), then, it was exposed to a susceptibility test for 12 weeks using three extraction duration (8, 16 and 24 hours) of aqueous extracts of stems that were individually steeped in (boiled, un-boiled, and ethanol) extract of *Euphorbia hirta.* A 3×3×6 factorial in a *complete randomised block design* was adopted for the experiment. The *obtained data were subjected to analysis of variance (ANOVA). DMRT was used to determine the significance of the treatment means at* α 0.05. *The findings indicated that* Un-boiled *aqueous extract* had the highest retention (2.71 kg/m^3), and lowest weight loss (2.32%), It also, *exhibited the highest inhibitory potency against white rot fungi,* followed by boiled extract (1.18 kg/m^3) and ethanol extract (1.30 kg/m^3) after the six weeks of inoculation. Therefore, the study has established that the stem extract of *Euphorbia hirta* has antifungal activities against white rot fungi isolates and could be a suitable bio-preservative for the control of fungi attack in wood.

Keywords: *Ceiba pentandra*, white rot fungi, *Euphorbia hirta*, weight loss, *Pleurotus ostreatus*

Introduction
Wood is the hard, fibrous biological substance found beneath bark in the stems and branches of trees and shrubs which is renewable and biodegradable. Wood is a renewable natural resource. It is widely used in our daily lives and economy, in wood-frame homes and furniture, newspapers, books, and magazines, railroad ties, fences, posts, and poles, textile textiles, and organic compounds. (Bandana and Bhupender, 2018). Approximately 50% of wood's elements are carbon, 6% are hydrogen, 44% are oxygen, and numerous metal ions are in trace quantities (Rowell, 2013). Wood as biological materials, proned to attacked by bio-deteriorating agents such as bacteria, fungi and termites is pronounced. Wood extractive have been found to be effective against fungi and insect damage (Schultz and Nicholas, 2002, Teaca *et al*, 2019). Due to the high extractive content in the heartwood and presence of phenolic compounds, most of the attack on wood is usually limited to the sapwood. As such, heartwood has greater deteriorating resistance than sapwood (Bandana and Bhupender, 2018). The hemicelluloses are principally in charge of moisture sorption, but the available cellulose, noncrystalline cellulose, lignin, as well as surface of crystalline cellulose are also important (Rowell *et al.*, 2008). Fungal attack on wood mainly pronounced in outdoor wooden structures. It reduces wood physical, mechanical and aesthetic properties and expressively reduces its service life (Magdalena, 2020). Unfriendly ecosystem substances used as chemical preservatives have been prohibited due to environmental restrictions and thus, there is a search for alternative techniques which can extend wood service life, and at the same time less harmful to the environment and man (Faruwa *et al.*, 2015).

Euphorbia hirta *commonly known as asthma weed or milk weed is widely distributed in the temperate or tropical parts of India, Asia, Australia, and Africa. It grows in dry and wet land, and mainly found in lowland, rice paddy fields, refuse places, most gardens and often in the roadsides (Ghosh,* et.al. *2018, 2019,* Manorma, et.al.*2011 and* Abdul et. al. *2007). According to Essiett* et al. *(2013); Asha* et al. *(2014), (2015), the phytochemical analysis of leaf extract from E. hirta reveals the presence of carbohydrates, reducing sugars, terpenoids, alkaloids, steroids, tannins, proteins, fats, oils, mucilages, glycoside, saponin, coumarin, and anthroquinones. These substances were also isolated from the aerial parts of the plants. Again, according to (Huang* et al. *2012, Shih* et al. *2012, Kausar* et al. *2016; Chen, 1991 and Pioro* et al. *2011), these plants also include alkaloids, saponins, protein, amino acids, and minerals.*

Ethanol extract of Euphorbia hirta also showed marked anti-microbial properties against the growth of Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus subtilis. Based on the studies that compared the antibacterial capability of the methanol, hexane, and water extract in Escherichia coli, Klebsiella pneumoniae by (Akinrinmade,*et.al* 2010, Michael, *et.al.*, 2010 and Ogbulie, *et.al.*, 2007). Meanwhile, it was found that aqueous decoctions provided more anti-microbial properties than organic solvent decoctions and the leaves extract was found to regulate the growth of all examined microbes with large proportion of inhibition. Okanlawon and Olaoye (2020) also reported the successful utilization of biological products for preserving wood against bio-deteriorating agents. Hence, plant extracts are

found to be economical, environmentally friendly, and very effective against organisms that degrade wood. Therefore, this study was carried out to authenticate the anti-fungal potency of *Euphorbia hirta* extracts as potentials preservative in perishable wood species (*Ceiba pentadra).*

Materials and Methods

Aqueous Extractions procedure of *Euphorbia hirta:* The stems of *E. hirta* harvested within the University of Ibadan, Nigeria and identified at the Botany Department of the University were used for this study. The crude extraction process was done through mechanical extraction process. The stems were weighed, cut into smaller sizes and macerated following the procedure by (Adedapo *et al.,* 2004). The macerated materials were subsequently shaken and filtered using Whatman filter paper (0.037mm) directly placed on top of funnel to obtain the aqueous crude extract, and it was later measured using a measuring cylinder to obtain the volume. The total volume of the total aqueous crude extract obtained for the plant was divided into three equals portions where one portion served as the un-boiled extract, another portion was boiled at 100 ^{O}C for 2 hours so as to be used for the boiled extract of *E hirta* and the third portion was used for the ethanol extract.

$$\%\ stem\ extract = \frac{volume\ collected}{weight\ of\ initial\ material} \times 100\% \quad \ldots\ldots (1)$$

Ethanol extractions procedure of *Euphorbia hirta:* One hundred gram (100g) of *Euphorbia hirta* was dissolved into 500ml of 80% ethanol and was kept for 72 hours at room temperature and shaken to get a better extraction. Then the extract was filtered with cotton wool. The preparations of dilutions of crude extract for anti-fungal assay was adopted following Akujobi *et al*. (2004) and Esimone *et al*. (1998) methods.

Preparation of wood test sample: *Ceiba pentandrapentandra* wood (was chosen because it has been classified as a perishable timber) was selected for this study and because of it is susceptible to biodeterioration. *Ceiba pentandrapentandra* tree was harvested from Amina way within the premises of University of Ibadan campus, and were converted into planks. A cntre plank was obtained and further converted into square block sizes of (2.0 × 2.0 × 2.0) cm following the method of Faruwa *et al*. (2015). Prepared samples were oven dried at 103 ^{O}C until they attained a constant weight. The weights obtained were recorded as initial dry weight for each of the test sample. The 24 block of wood samples were then arranged into different jars based on the three different soaking periods 8, 16 and 24 hours and extract type (fresh extract, boiled extract and extract prepared with ethanol given total block samples, respectively.

Treatment of test block: Soaking of the wooden blocks using a non-pressure method was adopted. The wooden blocks of 2.0 cm × 2.0 cm × 2.0 cm prepared were soaked in the crude extract of un-boiled, boiled and ethanol-made *Euphorbia hirta* for 8, 16 and 24 hours respectively. After the treatment, the blocks were then conditioned for 72 hours and then reweighed to determine the retention of the extract.

$$Retention\ (Kg/m^3) = \frac{10^6 \times weight\ of\ preservative\ absorbed}{1000\ \times volume\ of\ wood \times 100} \quad \ldots\ldots (2)$$

Procedures for culturing fungi: Cultured white rot fungus was obtained from pathology section of Forestry Research Institute of Nigeria. The wood samples were then inoculated with the fungus (*Pleurotus osteatus)* at room temperature (27± 2 ^{O}C) in the laboratory.

Infestation of test sample: The block samples placed in the bottle were inoculated with fungi. Inoculation was made such that the entire wooden blocks in different jars were in contact with the mycelium of the test fungus and not in contact with the medium. This is done to prevent some preservatives escaping or leaching out. The bottles were then incubated at 27 ± 2 ^{O}C for 2, 4, 6, 8, 10 and 12 weeks. After incubation, the block samples were removed from the bottles for cleaning of mycelium that may adhere to its surface and weighed to determine the weight gain or moisture absorbed. The weighed samples were thereafter oven dried for 18 hours at 103 ^{O}C to constant dry weight (Sarker *et al.,* 2006).

Moisture content after incubation:

At the conclusion of each incubation period, the moisture absorbed by each wood sample was determined using weighing balance. The blocks were measured for their wet weight before being dried in an oven for 18 hours at 103 ^{O}C. Final weighing of the test wood block was done after test samples were allowed to properly cool.The moisture content was calculated thus:

$$\%\ Moisture\ content = \frac{Wet\ weight - Oven\ Dried\ Weight}{Oven\ Dried\ Weight} \ldots\ldots (3)$$

Weight Loss Determination: At the end of each incubation period, wood test sample was carefully removed from each bottle, oven dried and reweighed to determine weight loss after 2-week interval for 12 weeks. Weight loss method was used to evaluate the decay resistance of the wood species against *Pleurotus ostreatus.*

$$\%\ weight\ loss = \frac{initial\ dry\ weight - final\ dry\ weight}{initial\ weight} \quad \ldots\ldots (4)$$

Statistical analysis

The data obtained were analyzed using the analysis of variance (ANOVA) and a 3×3×6 factorial in a *complete randomized block design* was adopted. DMRT was used to determine the separation of means at *α0.05*.

Where; = test blocks (*Ceiba pentandrapentandra)* and fungi (*Pleurotus ostreatus)*

Factor A: 3 = extract types (boiled, unboiled, and ethanol)

Factor B: 3 = period of soaking (8, 16 and 24 hours)

Factor C: 6 = weeks of inoculation (2, 4, 6, 8, 10 and 12).

Results and Discussion

Retention Rate of Extracts in *C. pentandrapentandra* wood

The descriptive analysis of extract type retention is presented in Table 1. The un-boiled extracts of *Euphorbia hirta* showed the highest retention in *Ceiba pentandrapentandra* wood samples after a 24-hour soaking period, with a mean value of 2.7128kg/m^3. It was followed by the boiling extracts after a 16-hour soaking time with a mean value of 1.4310 kg/m^3. The ethanol extract had mean (1.1572 kg/m^3) and the boiling extract had a mean (1.1371 kg/m^3), hence, both had the lowest retention after an 8-hour soaking time.

Table 1: Descriptive analysis of retention rate of *Euphorbia hirta* extract by *Ceiba pentandra*

Treatment	Time (Hours)	Mean (kg/m^3)
Boiled	8	1.1371±0.6^b
	16	1.4123±0.8^a
	24	1.1845±0.1^b
Un-boiled	8	1.1612±0.1^b
	16	1.4310±0.8^b
	24	2.7128±0.6^a
Ethanol extract	8	1.1572±0.1^b
	16	1.1880±0.6^b
	24	1.3030±0.7^a

*means with the same alphabet are not significantly different from each other ($p < 0.05$).

Table 2 shows the ANOVA for the retention of boiled extract and it reveals that there was no significant difference ($p > 0.05$) in the retention of boiled and ethanol extract of *Euphorbia hirta* after subjecting it to different soaking period (8, 16 and 24 hours) and this indicates that at 8 hours soaking period, *Ceiba pentandra* wood samples would still retain sufficient preservative chemical of the extract as much as that of 16 and 24 hours soaking period. But for un-boiled extract, there was significant difference ($p < 0.05$) with respect to the different soaking period.

Table 2: Analysis of variance of retention rate of *Euphorbia hirta* extract by *Ceiba pentandra*

Source of variation	Sum of square	Df	Mean square	F	Sig
Soaking period (Boiled Extract)	1.039	2	0.519	1.572	0.215^{ns}
Error	22.792	69	0.330		
Total	23.831	71			
Soaking period (Un-boiled extract)	32.98	2	16.492	48.890	0.215*
Error	23.276	69	0.337		
Total	56.260	71			
Soaking Period (Ethanol)	0.283	2	0.142	0.434	0.650^{ns}
Error	22.558	69	0.327		
Total	22.842	71			

Note: ^{ns} insignificant, * significant

Percentage Weight Loss: The effect of extract types of *Euphorbia hirta* on weight loss of *Ceiba pentandra* after exposure to the fungi (*Pleurotus ostreatus)* is presented in Table 3. The lowest weight loss was observed in un-boiled extract (2.3%), followed by boiled extract (3.6%) and ethanol extract (3.8%) while the highest weight loss was observed in the control having (7.4%)

Table3: Potency of extraction type on the percentage weight loss

Types of extract	Mean value (%)
Boiled	3.6146
Un-boiled	2.3181
Ethanol	3.8190

Control	7.4376

Note: means with the same alphabets are not significantly different from each other

The result presented in Table 4 showed that, there was a significant difference ($p<0.05$) in the percentage weight loss after 12 weeks of exposure of *Ceiba pentandra* wood samples to *Pleurotus ostreatus* (white rot basidiomycetes) as well as type of extract used. Also, boiled, un-boiled and ethanol extract of *Euphorbia hirta* has a significant effect on *Pleurotus ostreatus* with reference to weight loss on wood sample when compared to control after 12 weeks of exposure. Significant differences also exist with respect to the soaking period; 8, 16, and 24 hours. But, no significant difference ($p>0.05$) in weight loss was obtained among the interaction effect weeks and extract types, weeks and soaking periods, Extract types and Soaking period as well as Weeks, Extract and Soaking period.

Table 4: Analysis of variance for treatment effects on percentage weight loss of samples

Source	Sum of square	df	MSS	F	Sig
Inoculation Weeks	45.233	6	14.564	5.491	0.02*
Soaking Period	0.311	2	0.155	8.990	0.000*
Extract Type	0.145	3	0.048	2.789	0.041*
Extract type * Soaking Period	0.270	6	0.045	2.606	0.018*
Extract type* i inoculation Weeks	88.565	10	8.857	1.230	0.275^{ns}
Weeks* Soaking period	74.278	5	14.250	1.246	0.107^{ns}
Extract type* inoculation Weeks* Soaking period	69.458	10	16.393	2.276	0.702^{ns}
Total	5.496	287			

*significant (p>0.05), ns- not significant (p>0.05)

The result of the overall weight loss difference after 12weeks of exposure of both the treated and untreated *Ceiba pentandra* wood samples to fungi infestation was presented in Table 5. At 6th week, wood samples of *Ceiba pentandra* had the lowest percentage weight loss with (2.3%) after inoculation while the 2nd week with (6.4%) has the highest percentage weight loss. More so there was no significance difference in the percentage weight loss (4.82% and 4.64%) for 8th and 12th week respectively.

Table 5: Follow up test on period of exposure (Duncan) for white rot fungi (*Pleurotus ostreatus*)

Weeks	Mean value
2	6.4942^{d}
4	6.1249^{d}
6	2.3180^{a}
8	4.8286^{b}
10	5.3140^{c}
12	4.6429^{b}

**means with the same alphabets are not significantly different from each other*

The quality of wood impregnation is being demonstrated by the quantity of the preservative that is retained in the wood. (Dong, *et al.*, 2020). The concentrations of preservatives as well as the condition under which the treatment is done such as type of preservatives as well as the duration are the primary determinant of retention. The result obtained from this study shows that the degree of retention of *Euphorbia hirta extract by Ceiba pentandra* is directly proportional to period of soaking irrespective of the extract type. But then, from the result of the analysis of variance, there is no significance difference in the period of soaking of boiled and ethanol extract on the retention by *Ceiba pentandra*. The woods that were soaked for 24 hours in each of the extract have the highest retention rate similar to what Areo *et al.* (2016) reported for *T. grandis* wood.

According to (Salami *et al.*, 2019), opine that the retention level of an extract is a function of the plant from which the extract was gotten as well as the degree of viscosity and chemical constituent of extracts with respect to their genetic make ups. Retention value can be impacted by a lot of factors which include texture, density as well as wood porosity. Penetration efficiency rate on the other hand can be determined by the particle size of the extractive (*Gupta et al., 2021).*

The highest and lowest weight loss after 2 and 6 week inoculation respectively is similar to those reported of Adegeye *et al* (2009) and Okon-Akan *et al*, (2019) on the effect of two white rot fungi weight loss of wood samples. This may be as a result of the reduction in the quantity of the fungi due to the potency of the extract. Meanwhile, the untreated wood (control) had the highest weight loss. This is in accordance with the report of Okon-Akan *et al.* (2019), Okanlawon and Olaoye, (2020) and Ogutuga *et al*. (2020). This shows that *Euphorbia hirta* extract is potent in protecting the wood against the attack of the fungi. In another research, Salami *et al*. (2019), and Yayaha *et al.* (2021) observed that plant extract has been recorded to have success as an alternative for wood preservatives to chemical preservatives which are harmful to human and the environment.

Conclusion

Based on the findings of this research, both boiled, un-boiled and ethanol extract of *Euphorbia hirta* has a significant effect on *Pleurotus ostreatus* with reference to weight loss on wood sample when compared with control after 12 weeks of exposure. This shows that the extract of *Euphorbia hirta* has a great potential to be used as a preservative for wood against fungi attack. Hence, it is recommended that more research should still be carried out by scientist on other plants as well as other natural source of preservatives in other to harness the potential of this eco-friendly resource. This will help in reducing the adverse effect of chemical preservatives and thereby makes the world more habitable for both fauna and flora.

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