Efficacy of Oil Extracts from Sweet Orange (*Citrus Sinensis*) Peels and Seeds as Natural Wood Preservative

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Abstract
Oil extracts of sweet orange (*Citrus sinensis*) seeds and peel were examined for their effects on *Gmelina arborea* wood as preservative chemicals. Wood samples of *Gmelina arborea* were Oven dried and treated with oil extracts from the seeds and peels of *Citrus sinensis* at 0%, 25%, 50%, 75%, 100% concentration levels and exposed to fungi attack for a period of 12 weeks. The percentage weight loss of the wood samples after fungi attack was assessed and data were processed by Analysis of variance. The absorption of preservative varied in respect to concentration levels in the wood samples which ranged with a mean value of between 13.98±3.17 and 15.83±2.20. The percentage weight loss was significantly high with the samples without treatment (the control) compare to wood treated with preservative level. The concentration level of preservative was significant at ($p \leq 0.05$). The highest concentration (100%) had the lowest mean values of weight loss of 11.75±4.44 and 8.19±1.44 with both seed and peel oil extracts respectively. The level of effectiveness exhibited by the bio preservative *Citrus sinensis* oil extract thus shows the need to exploit the development of other forms of natural based plant preservatives for protection of wood and wood products in service.

Introduction

Wood is one of the most frequently used materials for construction purposes world-wide. However, it can easily be attacked by biodegrading agents due to its inherent properties, hence there is the need for its adequate protection for an extended service life span. Wood chemical preservatives have been successful in preventing both fungi and termite attack, however, because of toxicity, risk to human health and the environment, the use of these chemicals have also been restricted (Kartal *et al.*, 2004, Verma *et al.*, 2009). In addition, because metals cannot be broken down in the environment, the disposal of any wood treated with a metal-based preservative will be more expensive and difficult in the future. Presently, different researches are ongoing on the use of plant extracts as natural preservatives. Natural preservative are cheaper, readily available, require low skill application and are eco-friendly compared to chemical preservatives. Phenolic compounds present in essential oils have been recognized as the bioactive components for the antimicrobial activities in plants. Series of medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value (Nostro *et al.*, 2000) and also some have been used to treat wood against fungi attack in the recent decades.

Sweet orange (*Citrus sinensis*) belongs to the family *Rutaceae*. The tree could be up 7.5m in height or with great age, up to 15 m high. It is grown throughout the world, including Nigeria, and belongs to the *Plantae* kingdom, *Citrus* genera (Karoui and Marzouk 2013). In terms of volume in production, citrus ranks as the world second fruit crop with more than 108 million tons (FAO Statistics 2006). The Citrus peel is in abundance and at large extent, being used as a top note component in some perfumes and colognes (Glen *et al*, 2007). One of the important products of citrus fruits is the essential oil, which is obtained from its peels (Mondello *et al.*, 2005). Series of investigation has been conducted on the efficacy of the essentials oil derived from sweet orange and some other plant extracts on inhibitions against microbial attacks. Due to the active ingredients in sweet orange fruit extract, it is considered to be one of the potential sources for the screening of anticancer, antimicrobial, antioxidant, and free radical scavenging agents (Shabbir *et al.*, 2009). This work was carried out to investigate the efficacy of the oils obtained from the peel and seeds of sweet orange as wood preservatives.

Materials and Methods

Method of Collection and Preparation of *Citrus Sinensis* Samples

Sweet orange (*Citrus sinensis*) was obtained from a standing tree in Ile-Ife, Osun state (ranging from lat 7 28'N, long 4 34'E), and identified at the Department of Botany, University of Ibadan, Ibadan, Nigeria. Three hundred (300) pieces of oranges were peeled and the seeds were manually removed. The number of the seed per orange fruit ranged from 8-17. The seeds were air dried for two weeks to reduce the moisture content, shelled and air dried again for one week before they were grinded to reduce the surface area in accordance with the method employed by Olabanji *et al.* (2016)

Determination of Moisture Content of the Peels and Seeds

A small portion of the samples was used for the determination of moisture present in the seeds and peels. A crucible was washed and dried in the oven, after cooling in the desiccator and weighed (W1) of the sample. The weight of the crucible with the sample was taken as (W2). The crucible containing the sample was oven-dried at 102 ± 3°C as described by AOAC (1990) for one hour. It was reweighed after cooling. The process was repeated until a constant weight was obtained (W3) (Ibrahim and Yusuf, 2015). Percentage moisture content was calculated with equation 1:

$$percentage\ moisture\ content = \frac{W2-W1}{W1}\ x\ 100$$

…… Equation 1

Extraction Process of Oil Extract

One hundred grammes (100g) of grounded sample was used for the extraction of oil. The extraction was carried out at 60ºC for 16 hours using n-hexane (BDH annalar grade) as the extracting solvent (AOCS 2001). After extraction, the oil was stored in amber bottle to reduce the effect of radiation on the extracted oil before usage. The oil was concentrated to 100% using rotary evaporator at 65°C. This process continued for three hours, to completely remove the solvent for the extraction. The oil was concentrated and analyzed for its active ingredients.

Determination of Percentage Yield of the Sample

The residue from the extraction process was air dried, weighed. The oil extract was also weighed and noted. The results of the respective sample weight (g) of the orange peels and seeds were used in the calculation of percentage yield and was calculated with the formula equation 2:

$$percentage\ yield = \frac{weight\ of\ the\ sample\ residue}{weight\ of\ sample\ before\ extraction}\ x\ 100$$

……Equation 2

Preparation of Wood Samples for Impregnation

Gmelina arborea wood was purchased at Sango wood plank market in Ibadan, Oyo State and taken to the Department of Forests Products Development and Utilization (F.P.D.&U), Federal Research Institute of Nigeria (F.R.I.N) for identification. The wood samples were planed and cut into 2 (tangential) x 2 (radial) x 6 (longitudinal) mm (Nurudeen *et al*, 2012). A total of seventy-two (72) wood samples were prepared for the experiment. The wood samples were oven dry at a temperature of 103ºC for 24hrs.

Determination of Moisture Content

The moisture content of the wood samples was determined in accordance to ASTM D 4442-16. The process involved the weighing of the wood samples before and after oven drying. Oven drying was at a temperature of 103^0C until a constant weight was obtained.

$$Moisture\ content = \frac{Wet\ weight-Oven\ dryweight}{Oven\ dry\ weight} \times 100$$

…… Equation 3

Preparation of Preservative Concentration

The volume-to-volume method was used to dissolve the seed and the orange extracts in kerosene. That is, 1ml of the extract in 99ml of Kerosene (diluent) is equivalent to 1% dilution. Hence, 0%, 25%, 50%, 75% and 100% of dilution were obtained.

Impregnation of Preservative

The wood samples were impregnated with oil extract through cold soaking. During cold soaking, wood samples tend to float but a glass block was used to immerse it in the oil for uniform absorption of preservatives. The wood samples were soaked for seventy-two (72) hours at room temperature in different concentration level 0%, 25%, 50%, 75%, and 100% of the seed and peel extracts.

Absorption Test

The rate of absorption of the wood samples when immersed in preservative was based on the difference between the weight before and after immersion. According to Owoyemi (2008), preservative absorption was estimated as:

$$P.A\% = \frac{W3-W2}{W2}\ x\ 100$$

…… Equation 4

P.A = preservative absorption
W3 = Weight of after wood sample after immersion
W2 = Weight of wood after oven dry.

Decay Test

The wood samples were inoculated with white and brown rot wood decay fungi namely *Pleutus florida* and *Pleutus sajor-caju* respectively. The inoculated plates were then incubated at room temperature (28±2°C) and observations on weight loss were made at the end of twelve (12) weeks in a modified test according to ASTM D 1413-76 test method for solid wood. At the end of the incubation, blocks were removed from the test Petri-dishes and the mycelia were carefully brushed off the samples. The initial and the final weights were taken to determine the weight loss.

$$Percentage\ weight\ loss = \frac{initial\ weight-final\ weight}{initial\ weight} \times 100$$

…….. Equation 5

Experimental Design and Statistical Analysis

The experimental design for this experiment was (2 × 2 × 3 ×6) factorial experiment in Completely Randomized Design with replicates to gives a total of six (72) samples for the wood samples. Analysis of variance (ANOVA) was used to estimate the significance difference of sources of variation at concentration levels (control, 0%, 25%, 50%, 75%, and

100%) absorption rates as well as the percentage weight loss.

Results and Discussions

The initial moisture content of the *C. sinensis* peels and seeds were 60.7% and 57.6% respectively. The peel and the bark were dried below 10% moisture before blending and extraction. Low moisture content enhanced the extraction of oil as also noted by Adejumo *et al.*, (2013). At very low moisture, oil yield is high. The percentage oil yields of the peel were noted to be 4.1% and 31% for the seed. The colour of the oil peel was brown and that of seed was yellow. The percentage seed oil yield was in line with the report of Madhuri *et al.*, (2014). The results of the percentage oil yield of the seeds was in line with Nwobi *et al*., 2006 who reported the oil yield of seed to be 36%.

Table 1: The physico-chemical properties of oil of the seeds and peels of *Citrus sinemsis*

Variables	Seed Oil	Peel Oil
% Moisture Content	1.10	12.45
Specific Gravity	0 .93	0.95
Acidic value	5.3	1.6
Peroxide Value (meq/kg)	13.59	13.40
Iodine Value (g/100g)	54.00	56.85
Saponification value (mgKOH/g)	190.00	190.65

The mean absorption rate of the preservatives was found to be 14.98±2.68 as shown in table 2. The highest value of absorption of the peel oil extract was 16.69±1.89 while the lowest value was 12.25±.94. The mean absorption rate of wood samples in citrus seed oil extract as preservatives was found to be 15.10±2.83 and citrus peel extract was found to be 14.87±2.56. It was observed that seed oil extract penetrated more into the wood than peel extract. The absorption rate at each concentration level had different values which showed the level of penetration. At 25% level of concentration, the value of absorption rate of seed oil was noted to be 14.73±3.98 which is higher than peel oil extract 12.25±0.94. At 100% concentration level, it was observed that wood samples had absorption rate of 14.97±2.31 which is lower than absorption in peel oil extract 16.69±1.88. Variation occurred in the absorption rate of the preservatives at different levels of concentration and with different preservatives which might be due to their viscosity. The nature of preservative chemicals affects the quantity absorbed by wood samples (Owoyemi and Kayode, 2007). Owoyemi (2010) reported in his study that the viscosity of preservatives is a major consideration when applying them in wood protection since it determines the rate of penetration.

Table2: Mean absorption of the preservatives by *Gmelina arborea* (± Standard Deviation)

Preservative conc. (%)	Seed Oil (%)	Peel Oil (%)	Mean (%)
0	13.71±3.38067	14.25±3.23992	13.98±3.16958
25	14.72±3.98331	12.25±.94314	13.49±3.04836
50	15.39±1.68434	16.34±1.23918	15.86±1.49407
75	16.69±2.27742	14.83±2.50663	15.76±2.48209
100	14.97±2.31240	16.69±1.88626	15.83±2.20330
Mean	15.10±2.82623	14.87±2.56031	14.98±2.67604

Table 3 shows the mean weight loss of the wood samples after their exposure to both white and brown rot fungi (*P. florida* and *P. sajor-caju* respectively) using *C. sinensis* seed oil extracts as preservative. The highest mean weight loss (22.75±4.75) was observed in the wood samples that were not treated (the control) compared to other samples treated with the different concentration levels. The least weight loss (11.75±4.44) was observed with the wood samples treated with 100% preservative. The mean weight loss caused by brown rot decay fungi was lower with a value of 12.80±5.98 compare to a mean value of 15.97±4.98 exhibited by the samples attacked by white rot fungi. At 100% preservative concentration, the seed oil extract exhibited the highest preservative properties compared to 0%, 25%, 50%, 75% and the control.

Table 4 shows the results of the effects the preservative levels of the orange peel oil extract against white rot fungi (*P. florida*) and brown rot fungi (*P. sajor-caju*). It was observed that as the concentration levels of the extracts increased the resistance of treated wood samples to the fungi attacks also increased. The highest weight loss of 21.20±1.31 was observed in the untreated wood samples (the control)

compared to the other samples treated with the preservative at different concentration levels. The samples treated with 100% preservative level were the most effective in the control of attack by both white and brown rot fungi with value of 8.32±1.61 and 8.06±1.60 respectively with a mean value of 8.19±1.44. The attack of white rot fungi in respect to percentage weight loss is higher compare to brown rot fungi. The range of concentration of orange peel oil extract that displayed maximum resistance against fungi attack in respect to weight was 75%-100% concentration. Madhuri *et al.,* (2014) reported the antimicrobial activity of peel oil extract of orange which was reflected in this study against fungi attack.

Table 3: Weight Loss in the Wood samples with *Citrus sinensis* Seed Oil Extract as Preservative

Preservative conc. (%)	White rot fungi(%)	Brown rot fungi(%)	Mean value(%)
0	17.05±2.68	13.64±5.69	15.35±4.39
25	14.53±3.35	11.42±2.08	12.97±3.02
50	13.31±2.74	8.32±6.59	10.81±5.28
75	14.24±2.41	11.17±3.72	12.70±3.27
100	11.92±3.75	11.57±5.94	11.75±4.44
Control	24.79±1.88	20.71±6.35	22.75±4.75
Mean value	15.97±4.98	12.80±5.98	14.39±5.65

Table 4: **Weight Loss Variation in the Wood samples with *Citrus sinensis* Peel Oil Extract as Preservative**

Preservative/concentration (%)	White rot fungi (%)	Brown rot fungi (%)	Mean value (%)
0	13.66±0.11	13.64±0.08	13.65±0.08
25	15.69±7.20	10.96±0.07	13.33±5.24
50	15.19±8.51	11.54±1.95	13.36±5.88
75	9.76±0.31	8.51±1.90	9.13±1.40
100	8.32±1.61	8.06±1.60	8.19±1.44
Control	21.20±1.86	21.20±.93	21.20±1.31
Mean value	13.97±5.84	12.32±4.66	13.14±5.27

Conclusion

The study revealed that *Citrus sinensis* seed and peel oil extract showed considerable effectiveness against white rot fungi (*Pleutus florida*) and brown rot fungi (*Pleutus sajor-caju*) attack on *Gmelina arborea* wood at varying concentration levels. The maximum range of effective concentration level was found to be between 75% and 100% of the orange seed and peel oil extract. Though the level of efficacy as observed from the study was lower compared to other plant extracts and synthetic chemical, the level of effectiveness exhibited by the oil extract showed that it could minimally be used as an environmentally friendly wood preservative.

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