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Antifungal Activity of *Lagenaria breviflora* Fruit Extracts Against Wood Rotting Fungi on *Vitex doniana* Wood

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Abstract

As a result of contemporary environmental concerns, a number of studies from plants' tissues as one of the alternatives to conventional chemicals are increasingly investigated. In tandem with these trends, *Lagenaria breviflora* (LB) fruit, reputed as antiviral and depilatory agents in the Yoruba folkloric medicine was examined on *Vitex doniana* wood to ascertain its antifungal activity. Fungicides of 25%, 50%, 75%, and 100% LB fruits formulations (concentrations) were developed through simple one-step mechanical-forming process, including control. In this study, the yield, the chemical compositions, the absorption capacity of the fungicides and wood weight losses (WWL) analysis were evaluated to investigate the antifungal activity of LB fruit on wood. The fruit extract yielded 35.4% of fresh juice weight. LB fruits contained total: alkaloids (8.78±0.21 mg/mL), flavonoids (2.01±0.02 mg/mL), phenol (7.42±0.09 mg/mL), saponins (11.00±0.10 mg/mL) and tannins (5.47±0.05 mg/mL) contents. All the formulations provided effective protection against the tested wood fungi compared to control. Interestingly, the antifungal activity of 50% and 25% formulations of 6.8% WWL and 9.9% WWL satisfied the excellent fungal resistance class description against white rot fungus (*Ganoderma lucidum*) and brown rot fungus (*Fibroporia vaillantii*), respectively according to ASTM D 2017. These results thus, support LB fruit as a strong potential source of natural antifungals for industrial wood production.

Key Words: antifungal, *Lagenaria breviflora*, *Fibroporia vaillantii*, *Ganoderma lucidum*, *Vitex doniana* wood

Introduction

Recently, compounds extracted from plants have been extensively studied worldwide. In fact, these flora compounds play a crucial role due to their potent biological properties which extended their application in particular, to wood protection field or industry. Environmentally friendly approaches have recognized phyto-compounds as one of the major active ingredients, which is responsible for various biological activities, such as antitermites and antifungal (Kartal et al. 2006; Abbas et al. 2013; Bento et al. 2016; Adedeji et al. 2017).

Lagenaria breviflora (LB) is a tropical plant naturally grown in Nigeria and is widely distributed in Africa (Oridupa et al. 2011). Its fruit is a prime item of trade in Western region of Nigeria with a long traditional history of use for preventing and treating viral infections (human chickenpox, smallpox, measles and New Castle Disease in fowl) (Sonaiya et al. 1992; Ajayi et al. 2002; Tomori et al. 2007; Oridupa and Saba 2013; Arowosegbe et al. 2015), as

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well as depilatory agent (Aiyeloja et al. 2015). The ethnomedicinal use of LB fruit as an antiviral agent in Nigeria has been scientifically investigated and reported (Oridupa et al. 2011). Many studies have also reported the beneficial activities of LB fruit such as antimicrobial, including antibacterial (Adesina and Akinwusi 1984; Tomori et al. 2007; Oridupa et al. 2011), anti-inflammatory (Elujoba et al. 1985; Onasanwo et al. 2011; Oridupa and Saba 2012), parasiticidal (Ajayi et al. 2002; Abstracts 2015), anti-ulcerogenic and anti-oxidant (Onasanwo et al. 2010), anti-nociceptive (Onasanwo et al. 2011), hepatoprotective (Saba et al. 2012), relaxant (Oridupa and Saba 2013), and insecticidal (Emerhi et al. 2015). Potential use of phyto-compounds is strongly related to their quantitative and qualitative contents. Concerning LB fruit, its active ingredients has been explored to contain alkaloids, anthraquinine, flavonoids, phenols, saponins, tannins, terpenoids and triterpenoids (Elujoba et al. 1990; Elujoba et al. 1991; Banjo et al. 2013; Eyitayo et al. 2014).

The impacts that the LB has made in various biological activities, more importantly in microbial inhibition and health improvement have been good, with interesting evidences. Given how extensively the important active tissue, essentially juice extracts from the fruit have attracted the interest of many researchers, no detailed study is available on its yield, quantification of chemical compositions and their antifungal activity on wood. In this study, however the whole fruit juice extract yield, chemical compositions and their antifungal activity on non-durable tropical wood (*Vitex doniana*) were investigated.

Materials and Methods

Samples collection, production of extracts and percentage yield estimation

Fresh LB fruits ranging between 175.5 g and 237.2 g were collected from Abuja Campus, University of Port Harcourt (UNIPORT), Nigeria in December 2015. The fruits' samples were cleaned with water manually to remove dirt and dust. The juice extract from the whole fruits was obtained through mechanical separation. Briefly, fruits were cut into small pieces, homogenized by a blender and sieved using white sieve cloth. The extracts were bottled and kept in fridge until further used. The juice obtained as extract or yield was quantitatively measured in percentage using Eqn. 1:

$$\text{Percentage (\%) yield} = \frac{\text{Weight of the whole fruits juice extract}}{\text{Weight of the whole fruits}} \times 100 \cdots\cdots\cdots\cdots \text{Eqn. 1}$$

Preparation of extract for quantification of phytochemicals

The whole fruit was rinsed with distilled water, and extracted mechanically as described above. Chemical compositions of LB fruit such as total: alkaloids, flavonoids, phenol, saponins and tannins contents were quantitatively determined by Spectrophoto-metric and Forlin-Ciocalteu methods (Padmaja 1989; Singh et al. 2004; Chan et al. 2007; Makkar et al. 2007; Kale et al. 2010) with changes in extraction method. All the quantifications were performed in duplicate and presented as mean values (n=2)±Standard Deviation (SD) in mg/mL.

Total alkaloid content quantification

The total alkaloid content of the samples was measured using 1,10-phenanthroline method described by Singh et al. (2004) with slight modifications. A portion of 1 mL of the extract was mixed with 1 mL of 0.025M $FeCl_3$ in 0.5M HCl, and 1 mL of 0.05M of 1,10-phenanthroline in ethanol. The mixture was incubated for 30 minutes in hot water bath with maintained temperature of 70±2°C. The absorbance of red coloured complex was measured at 510 nm against reagent blank. Alkaloid content was expressed as quinine equivalent in mg/mL.

Total flavonoid content quantification

Total flavonoid content was determined using aluminium chloride method as reported by Kale et al. (2010). Aliquot (0.5 mL) of the extract was dispensed into test tube, followed by 1.5 mL of methanol, 0.1 mL of aluminium chloride (10%), 0.1 mL of 1M potassium acetate and 2.8 ml of distilled water. The reaction mixture was shaken, allowed to stand at room temperature for 30 minutes, before absorbance was read at 514 nm. Total flavonoids content was expressed as quercetin equivalent (QE) in mg/mL.

Total phenol content quantification

The total phenol content of the extract was determined according to the Folin-Ciocalteu method reported by Chan et al. (2007). Briefly, 300 μL of extract was dispensed into test tube (in triplicates). To this was added 1.5 mL of Folin–Ciocalteu reagent (diluted 10 times with distilled water), followed by 1.2 mL of Na_2CO_3 solution (7.5% w/v). The reaction mixture was shaken, allowed to stand for 30 min at room temperature before the absorbance was measured at 765 nm against a blank prepared by dispensing 300 μL of distilled instead of sample extract. Total phenolic content was expressed as gallic acid equivalent in mg/mL.

Total saponin content quantification

Total saponin was determined by the method described by Makkar et al. (2007). An aliquot (0.25 mL) of the extract was mixed with 0.25 mL vanillin reagent (8% vanillin in ethanol) and 2.5 mL of 72% aqueous H_2SO_4. The reaction mixtures in the tubes were heated in a water bath at 60°C for 10 min. Then tubes were cooled in ice for 4 min and then allowed to acclimatize to room temperature. Subsequently, the absorbance was measured in a UV/Visible spectrophotometer at 544 nm. Diosgenin was used as a standard and the results obtained were expressed as diosgenin equivalent in mg/mL.

Total tannins content quantification

Tannin content of the extract was determined according to the method of Padmaja (1989). Small quantity 0.1 mL of the extract was added with 7.5 mL of distilled water, 0.5 mL of Folin-Denis reagent, 1 mL of 35% sodium carbonate solution and diluted to 10 mL with distilled water. The mixture was shaken well, kept at room temperature for 30 min and absorbance was measured at 760 nm. Blank was prepared with water instead of the sample. Tannins content was expressed as tannic acid equivalent in mg/mL.

Development of formulations and treatment of test wood samples

From juice extract obtained, four formulations (0.25 L, 0.50 L, 0.75 L and 1 L) to mean 25%, 50%, 75% and 100% concentrations were developed following the method described by Kadir et al. (2015). Standard wood blocks (2×2×6 cm in dimension) of *V. doniana* (n=60) were obtained from Analytical Wood Laboratory, University of Ibadan. The wood samples were oven dried to constant weight at 103°C±2 for 24 hours according to ASTM D-1413 (2007) specification, conditioned and weighed as W_1. Thereafter, 12 replicates were soaked in 1 L of formulations for 3 days. After 3 days of soaking, the wood sample were drained, reweighed as W_2 and assessed for absorption using Eqn. 2.

Four formulations developed thus:

i. (25%)=0.25 L of extract+0.75 L of distilled water=1 L
ii. (50%)=0.50 L of extract+0.50 L of distilled water=1 L
iii. (75%)=0.75 L of extract+0.25 L of distilled water=1 L
iv. (100%)=1 L of extract=1 L
v. (Control)=100% distilled water

$$Absorption,\ kg/m^3 = 1000\ (G)/V \quad \cdots\cdots \text{Eqn. 2}$$

Where $\mathbf{G}=(\mathbf{W_2}-\mathbf{W_1})$=amount of the treating solution absorbed by the test wood blocks (g),

$\mathbf{W_1}$=is the oven dried weight of the conditioned wood blocks before treatment (g),

$\mathbf{W_2}$=is the weight after treatment,

$\mathbf{V}$=volume of wood test block (24 cm^3).

Culturing of Ganoderma lucidum and Fibroporia vaillantii

A nutrient medium (39 grams) of Potato Dextrose Agar (PDA LAB098) powder with 1 L of distilled water was prepared and sterilized by autoclaving at 0.1 N/mm^2 (121°C) for 15 minutes. After sterilization, the molten Agar solution was immediately poured (25 cL/ bottle) sideways into inoculation bottles. The poured bottles were left at room temperature to solidify. Actively growing *Ganoderma lucidum* (white rot fungus) was isolated from live *Azadirachta indica* in UNIPORT, while *Fibroporia vaillantii* brown rot fungus was obtained from the Microbiology Department, UNIPORT, cultured and sub-cultured on solidified PDA medium. Sub-cultured fungi in 750 mL glass bottles were maintained at room temperature 26±2°C in 75±5% relative humidity (RH) at the Wood Science Laboratory Unit of the Department of Forestry and Wildlife Management, UNIPORT prior to decay test.

Decay test

The laboratory decay test was conducted according to method described by Ogunsanwo and Adedeji (2010) using mixed blocks of *Vitex doniana* heartwood and sapwood. Six replicates were tested according to ASTM D-2017 (2008) standard after oven drying, conditioning, and weighing of samples as W_3. The treated wood sample were exposed to actively growing cultures of white rot fungus, *G. lucidum* and brown rot fungus, *F. vaillantii* on Potato Dextrose Agar (PDA) medium in glass bottles at 26±2°C in 75±5% RH for six months.

Determination of antifungal activity of LB fruit extracts

After six months of exposures, the test woods were carefully cleaned to remove adhering mycelia and stains, oven dried and finally weighed as W_4 to determine percentage weight loss. The weight loss was calculated in percentage using Eqn. 3:

$$\text{Wood block weight loss } \% = [(W3 - W4/W3)] \times 100 \quad \cdots\cdots\text{Eqn. 3}$$

Where,

W_3=oven dry weight before decay exposure test,

W_4=oven dry weight after decay test.

Statistical analysis

Data from the experiments were analysed by analysis of variance (ANOVA) using Duncan's Multiple Range Test to examine significant differences among the treatments (formulated fungicides) at $p < 0.05$. All results were expressed as means±Standard Deviation (SD).

Results and Discussion

Extract yield and chemical compositions

LB yielded high quantity (35.4±8.9%) of juice extract, given as a percentage of the original weight of whole fruit (n=20). Chemical investigation of LB fruit quantified by Spectrophoto-metric and Forlin-Ciocalteu analyses revealed appreciable quantity of saponins>alkaloids>phenol >tannins and>flavonoids (Table 1). The need to protect wood products under threshold approaches that ensure human safety is a global goal. Research into plant resources as alternative environmentally friendly replacers of conventional wood preservatives requires those that can rapidly grow, compatible with forest and species conservation. In this study, the fruits of LB were obtained freely and around 14 pieces of fruits (2,888.9 g) were required to produce 1 L of juice extract with low inputs. Yield is of great interest to meet the specific biocide quantity requirement to treat a unit volume of wood against the target agent(s). Although, no available information was found on LB fruit juice yield but 12.6% of its leaf yield reported by Adedapo et al. (2013) was expectedly lower than the result found in this study. However, its family (Cucurbitaceae) member, watermelon (*Citrullus lanatus*) yielded higher quantity of 52.2% (Aremu and Ogunlade 2016) reflecting its higher moisture content composition than LB fruit. Interestingly, the juice extract appeared to be stable without any indication of spoilage under room storage conditions for ten months. The perceived stability property exhibited by the processed LB fruit juice extract under ten months storage was an indication that the bioproduct can have long shelf life.

The potential use of plant extracts as wood biocide is strongly related to their chemical quantitative contents. The appreciable quantity of phytochemicals found in this study corroborated the results published by Elujoba et al. (1990, 1991), Banjo et al. (2013) and Eyitayo et al. (2014) that LB

Table 1. Phytochemicals' constituents of LB fruit

Material	Total phyto-chemical constituents in (mg/mL)				
	Saponins	Alkaloids	Phenol	Tannins	Flavonoids
LB fruit	11.00±0.10	8.78±0.21	7.42±0.09	5.47±0.05	2.01±0.02

Values are presented as means (n=2)±S.D.

fruits revealed the presence of triterpenoids, saponins, phenols, alkaloids, anthraquinine, flavonoids, tannins and terpenoids. These phytochemicals are renowned for their antimicrobial activity (Augusti and Cherian 1995; Harborne 1998; Okigbo et al. 2009; Kasolo et al. 2010). Saponins content being highest quantity was known to posses antimicrobial, insecticidal and cytotoxic activity (Sodipo et al. 2000; Podolak et al. 2010). Similarly, alkaloids are reputed to have antimicrobial effects (Almahy and Nasir 2011; Garba and Okeniyi 2012; Maatalah et al. 2012). Phenols and phenolic compounds are widely present in plants and their presence is often associated with decay resistance (Hart and Hillis 1974; Scheffer and Cowling 1966). Their toxicity and inherent antioxidant properties have been reported to impart potent termiticidal (Ragon et al. 2008), antifungal (Adaskaveg 1992; Celimene et al. 1999; Czemplik et al. 2011) as well as antibacterial and antiviral activity (Maddox et al. 2010; Czemplik et al. 2011). Tannins toxicity to bacteria, fungi and yeast (Harborne 1973) has found applications in various fields of research such as food science, wood science, plant pathology (Scalbert 1991). Flavonoids are antioxidant and free radical scavengers (Almahy and Nasir 2011) known to possess strong antimicrobial, antifungal and antiviral activities (Orhan et al. 2010). Though, the total content of the phytochemicals markedly varied, it can be explained that the synergy and/or additive effects of these FB chemicals are responsible for the beneficial activities previously reported by Adesina and Akinwusi (1984), Elujoba et al. (1985), Ajayi et al. (2002), Tomori et al. (2007), Oridupa et al. (2011), Emerhi et al. (2015).

Table 2. Absorption of LB formulations by test wood

Concentrations	Mean±S.D (kg/m^3)
Control	374.21±31.05^a
25%	370.14±53.15^a
50%	340.97±65.03^{ab}
75%	308.68±44.72^{bc}
100%	295.14±38.76^c

Means with superscripts in common are not significantly different from each other at α=0.05.

Absorption of LB extracts

LB extracts absorbed by the test block samples during 3 days soak treatment are shown in Table 2. Untreated control and treated samples easily absorbed water and formulations, respectively and absorption was inversely proportional to the concentration (Table 2). The uptake of extractives is a critical parameter for evaluating the treatment potential of wood biocide formulations. The decreasing absorption trend with increasing fruit extract was expectedly the interference of higher concentrations to reduce the uptake of the formulations. This result then indicated that higher concentrations probably increased the viscosity and acted as physical barrier to formulations uptake by wood pores. In this study, the results obtained were considerably higher than the results (190-206 kg/m^3) of LB pulp extract absorption documented by Emerhi et al. (2015) on *Triplochiton scleroxylon* wood. The variation was likely resulted from the difference in soaking period and variation in wood density.

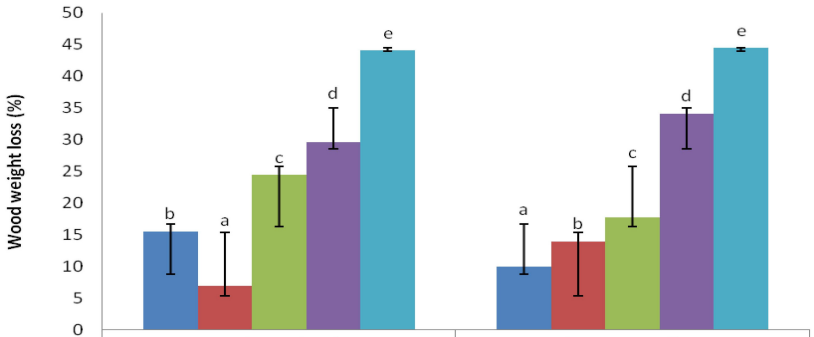
Antifungal activity

The degree of wood weight losses (WWL) of LB fruit treated wood samples by test wood rotting fungi compared to control, as shown in Table 3 and Fig. 1 indicate that LB fruit has antifungal property. Reports on the antimicrobial activity of LB have been mainly focused on animal degradative agents (pathogens). From the main effect analysis, formulations from FB fruit provided similar protection against the two types of test wood fungi with formulation ii

Table 3. Effect ofdecay fungi and LB extraction concentrations

Parameters	Mean wood weight loss (%)
Test fungus type	
Ganoderma lucidum	24.04±13.41^a
Fibroporia vaillantii	24.00±13.54^a
Extract concentrations	
25%	12.73±5.55^d
50%	10.37±4.85^d
75%	21.02±4.13^c
100%	31.81±4.19^b
Control	44.18±2.59^a

Means with superscripts in common are not significantly different from each other at α=0.05.



	Ganoderma lucidum	*Fibroporia vaillantii*
25%	*15.5*	*9.9*
50%	*6.8*	*13.9*
75%	*24.4*	*17.7*
100%	*29.5*	*34.1*
Control	*44*	*44.4*

Fig. 1. Antifungal activity of FB fruit. Means with same alphabet within fungus type are not significantly different from each other at $\alpha=0.05$.

Table 4. Decay resistance class ASTM D 2017

Average weight loss (%)	Average residual weight (%)	Resistance class description
0-10	90-100	Highly resistant
11-24	76-89	Resistant
25-44	56-75	Moderately resistant
Above 45	55 or less	Slightly resistant/ non resistant

Source: Kartal and Green III, 2003.

(50% concentration) showing strongest antifungal activity of 10.4% WWL compared to control with 44.2% WWL (Table 3). According to ASTM D 2017 (2008) in Table 4, when the WWL caused by fungi on treated wood sample is less than 10%, the antifungal activity of such treatment biocide is classed highly resistant or excellent. Concerning formulations and fungi interaction factors, their effect significantly resulted into variable classes of antifungal activities (Fig. 1) with 50% and 25% concentrations of LB fruit exhibiting excellent biological activity of 6.8% and 9.9% WWL compared to controls (44.0% and 44.4% WWL) against *G. lucidum* and *F. vaillantii*, respectively. This result shows the moderate and low consumption of LB fruit for effective protection of tropical non-durable wood against white and brown rots. While lower concentrations of LB fruit (25% and 50%) tended to enhance the antifungal activity of the non-durable wood, in contrast higher concentrations (75% and 100%) tended to reduce the antifungal activity. This trend of antifungal activity could be linked to seeds inclusion enhancing lipid (fat) aggregation (Taiwo et al. 2016) at higher concentrations which might add consumption value to test wood samples. Previously, results about strong antimicrobial activities of LB fruit mainly antibacterial and antiviral as well as parasiticidal were published (Adesina and Akinwusi 1984; Tomori et al. 2007; Oridupa et al. 2011; Banjo et al. 2013; Abstracts 2015). This study reporting the detailed antifungal activity of LB fruit juice extracts on tropical non-durable wood is a new biological profile of the plant.

Conclusion

In this study, the results indicate that LB fruit extract can be a viable alternative to traditional wood protection chemicals. Further research to examine the exact threshold concentration for both fungus types using different strains as well as indoor termites is required to explore the use of LB fruit beyond the local utilisation for industrial practice and wood protection technology. It is suggested that such research should be conducted with the exclusion of the seeds from the fruit to remove the possibility of (antagonizing effect) adding eaten value to the test wood (Taiwo et al. 2016). While this study is the first, reporting yield estimation, chemical quantification and antifungal activity of the plant on wood, wider spread sampling is required to con-

firm its biocide preparation.

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