



EFFECT OF ANTIFUNGAL PROPERTIES OF HONEYBEE PROPOLIS AS PRESERVATIVE ON *Triplochiton scleroxylon* (K. Schum.) WOOD

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ABSTRACT

Conventional wood preservatives are harmful to man and the environment. In the search for environment friendly wood preservatives, Honeybee Propolis, or bee glue, known to possess antimicrobial and wood stabilizing properties, was evaluated as preservative on the wood of *Triplochiton scleroxylon* against wood rot fungi. Propolis was collected from forests and apiaries (bee hives) in Osun State, cleaned and extracted using absolute ethanol. Propolis Extracts (PE) were prepared using hot and cold extraction methods. 2,500g of Propolis was extracted in 5 liters of ethanol (w/v, 1:2), using sox let extractor, to obtain the Hot Ethanol Propolis Extract (HEPE); 2,500g of Propolis was soaked in 5 liters of ethanol (w/v, 1:2) for two weeks, to produce the Cold Ethanol Propolis Extract (CEPE). Simple phytochemical tests were carried out on three different samples of Propolis: HEPE, CEPE and RPS, to determine bioactive constituents. Propolis Extract (CEPE), prepared at different concentrations was subjected to antifungal activity using a white and brown rot fungi (*Coriolopsis polyzona* and *Coniophora puteana*), respectively on wood blocks of *Triplochiton scleroxylon*. Measured parameters in wood block test include Weight Loss and Maximum Compressive Strength. Percentage yield of Propolis Extract was 74.04% and 27.02%, for the HEPE and CEPE, respectively. Phytochemical screening revealed that CEPE was richer in phytochemicals than HEPE. The extract (HEPE) was able to control the two fungi at concentrations of 50% and 75%, respectively, in wood block test. This study confirmed that Propolis possesses antifungal properties that could be exploited in the field of wood preservation.

Key words: Honeybee Propolis, Anti-fungal, Phytochemical analysis, Cold extract, Hot extract

INTRODUCTION

The decay and discoloration caused by fungi, and to a lesser extent by bacteria, are major sources of quality loss in both timber production and the various uses of wood (Hyvonen *et al.*, 2005). In order to ensure a long, useful, and safe life, timber needs protection from the hazards of fungal decay and weathering (Hyvonen *et al.*, 2005). The recent trend in wood preservation is the use of environment friendly materials that are sustainable. The clean wax is that which composes the comb cells where the bees rear brood and store honey, while the other material is Propolis (Hegazi, 1997). The Egyptians knew very well the anti-putrefactive properties of Propolis and used it to embalm their cadavers (Foktet *et al.*, 2010).

(Goktas *et al.*, 2007). Propolis or bee glue is a resinous mixture of complex compounds collected by honeybees (*Apis mellifera*) from tree bark, buds, sapflows, and other botanical sources (Preeti *et al.*, 2012). Abu Ali bin Sinu (Avicenna) distinguishes two kind of wax in his known work, The Canon Medical Science, the clean and the black wax (Propolis).

According to Walker (2009), Propolis is now believed to reinforce the structural stability of the hive (wood), reduce vibration, make the hive more defensible by sealing alternate entrances, and prevent diseases and parasites from entering the hive and to inhibit microbial growth, and prevent

putrefaction within the hive (Foktet *et al.*, 2010 and Walker, 2009). Propolis might serve as a means for colonies of bees to better maintain homeostasis of the nest environment through the reduction of microbial growth on hive walls, prevention of uncontrolled airflow into the nest, waterproofing of walls against external moisture, and protection against invaders (Maria and Maria, 2011). Other uses of Propolis, apart from medical applications, include commercial uses in musical instruments to enhance the appearance of the wood grain, in polishes and varnishes (Gambichler *et al.*, 2004) and for chewing gum production.

Propolis chemical composition is also highly variable, depending on the season of collection, local flora and type of bees foraging around (Bankova *et al.*, 2000; Marucci, 1995; Silici and Kutluca, 2005; Bankova, 2005). Amazingly, samples of different origins can display identical biological activities, (Bankova, 2005). Bankova (2005) proposed that Propolis biological properties should be linked to a detailed investigation of its chemical composition and to its botanical sources. Majority of studies on Propolis were conducted in China and East European countries, but information is difficult to obtain. Even if this is retrieved it is inapplicable to Nigerian situation. More so, not much of such research has been conducted in Nigeria. The voracity and destructive tendencies of fungi on wooden products that necessitate frequent replacement of these structures coupled with the drawbacks associated with the use of conventional proprietary wood preservatives that are costly, scarce and causing environmental pollution prompted the evaluation of the preservative potentials of bee Propolis which is environmental friendly. This study was aimed at assessing the antifungal potentials of Propolis in wood preservation against decay fungi.

MATERIALS AND METHODS

Collection of Propolis

Propolis was collected from two major sources; from nine Top bar hives located in apiaries and wild hives from forests in Osun State. Collection from beehives was achieved from nine Kenyan Top Bar hives, each containing 20 top bars, using improvised Propolis trap placed at the bottom of the

The proportion of the various substances in Propolis is variable and depends upon the time and place of collection (Ghisalberti, 1979). Propolis shows a complex chemical composition, its biological properties such as antibacterial, antiviral, antifungal, insecticidal potentials, among other activities, have attracted researcher's interest (Burdock, 2005; Bankova, 2007; Simone-Finstrom and Spivak, 2010). According to Hegazi (1997), the chemical composition of propolis is still insufficiently known. But in general raw Propolis is composed of about 50% resins, 30% waxes, 10% essential oils, 5% pollen, and 5% of various organic compounds (Burdock, 1998; and Pietta *et al.*, 2002).

hive to stimulate Propolis production, over a period of eighteen months. The trap was frozen in the deep freezer and trapped Propolis was shaken out. Collection from wild hives was achieved by scraping Propolis at the entrance of odd (wild) hives. Both samples were combined and taken to the laboratory for investigation. Propolis was extracted in order to remove the inert material and preserve the desired compounds.

Extraction of Propolis

Propolis was extracted; extracts were prepared using hot and cold extraction methods as showed below:

Sox let (Hot) Extraction Propolis

Crumbs of Propolis were crushed in the laboratory using mortar and pestle. Propolis of 2,500g milled was weighed on a top loading (Mettler Toledo) weighing machine. The weighed sample was placed in a sieve cloth, transferred into a 5 liters capacity sox let extractor using absolute ethanol (Sigma Aldrich Co) as solvent. The extractor was placed on steam bath for 24 hours to ensure complete extraction. The extract was labeled Hot Ethanol Propolis Extract (HEPE).

Cold Extraction

Weighed sample of 2,500g of Propolis was placed in a 10 liter bottle. Five liters of ethanol was added and left for two weeks with daily agitation. At the end of the soaking period, the mixture was sieved using a filter funnel. The extract was labeled Cold Ethanol Propolis Extract (CEPE). Propolis collected

was calculated using equation 1 below, adopted by Adetogun, 1998 and Ajala, 2014:

$$P = \frac{v}{m} \times 100 \text{ -----Eqn 1.}$$

Where:

P is Propolis collected in %,

v is volume of Propolis extract collected, in cm³,

m is the initial weight of Propolis before extraction, in g

Recovering of Propolis through Evaporation in vacuo

In order to recover the Propolis from solvent, both hot and cold extracts, were evaporated in vacuo using a rotary evaporator.

Phytochemical Screening of Propolis

Simple Phytochemical tests were carried out on three different samples of Propolis: Hot Ethanol Propolis Extract, Cold Ethanol Propolis Extract and Raw Propolis Sample. The screening tests were carried out according to the methods described by Trease and Evans (1989) and Sofowora (1993).

Formulation of wood preservative

Test preservative was formulated using the volume to volume method where 1mL of extract (Propolis) in 99mL of ethanol (solvent) is equivalent to 1% dilution (Adetogun, 1998). The preservatives were tested using four concentration levels thus: 25%, 50%, 75% and 100% .

Preparation of Growth Medium for Fungal Test

Synthetic Potato Dextrose Agar (PDA) was used. Thirty nine grams (39g) of PDA was dissolved in 1 liter of distilled water homogenized and sterilized in the autoclave at 1.05kg/cm² for 30 minutes. After sterilization the medium was allowed to cool and maintained at 45°C and later dispensed into Petri dishes. The PDA was incorporated with streptomycin to avoid bacterial contamination and left in the culture room to solidify, according to Ajala, (2014).

A white and brown rot fungus, *Corilopsis polyzona* (Pers) RYV and *Coniophora puteana* (Schum) fries were obtained from the mycology unit of the Forestry Research Institute of Nigeria and used for the study. Stock cultures of test fungi inside McCartney bottles were sub cultured by

transferring bits of the fungi with sterilized picker into clean sterile Petri dishes containing Potato Dextrose Agar and incubated at 25 ± 2°C for 7 days, in accordance with Adetogun (1998).

Experimental Design

The experiment was two factors experiment in a Completely Randomised Design (CRD) with six replications. Factor A: Propolis Extract Concentration (4 levels: 25%, 50%, 75% and 100%); Factor B: Fungi (2 levels: *Corilopsis polyzona* and *Coniophora puteana*).

Determination of Moisture Content of Test wooden Blocks

After incubation the moisture content of test blocks were determined in accordance with BSI (1961) and Olajuyigbe (2007). This was calculated using Equation 2

$$MC = \frac{w_3 - w_4}{w_4} \times 100 \text{ ----- Eqn 2}$$

Where:

MC is moisture content of sample in %,

W₃ is final wet weight of sample in g;

W₄ is final dry weight of sample in g

Treatment of Test wooden Blocks

Dipping impregnation method described by FAO, 1986; Adetogun, 1998; Olajuyigbe (2007) was used to treat test blocks with the preservatives. Test wooden blocks were treated using the Cold Propolis Extract only, because of its higher amount of phytochemicals and ease of dilution with solvents as compared with the Hot Propolis Extract. They were completely immersed in various concentrations of Propolis, CEPE (25%, 50%, 75% and 100%) for 4 minutes according to Adetogun, (1998). Control blocks were not treated with Propolis. The blocks were weighed to determine the rate and level of absorption. The weight obtained was taken as the initial wet weight (W₂). The treated test blocks were conditioned in the desiccator for two weeks.

Determination of the Absorption of Propolis Extract and Solvent

The absorption of PE and solvent by test blocks was determined using Equation 3 adopted by Adetogun,

1998; Adetogun *et al.*, 2006; Adetogun *et al.*, 2009).

$$\text{Absorption} = \frac{t \times c \times 10}{v \times n} \text{ kg/m}^3 \text{ ----- Eqn3.}$$

Where:

t is the total absorption in kg,

c is concentration of fungicide in %,

v is volume of wood sample used in cm³, and

n is number of pieces of wood samples

Data Analysis

Data collected were analysed by 2-way Analysis of Variance (ANOVA). Significant differences among means were determined. Means were separated

using Least Significant Difference (LSD) (at $p < 0.01$).

RESULTS

Percentage Recovery of Propolis

Table.1 shows the percentage recovery of Propolis in hot and cold ethanol extraction. The percentage recovery of Hot Ethanol Propolis Extract was 74.04% while that of Cold Ethanol Propolis Extract was 27.02%, thus representing a ratio 3:1 of Hot Ethanol Propolis Extract to Cold Ethanol Propolis Extract. Hot Ethanol Propolis Extract gave the higher yield compared to Cold Ethanol Propolis Extract.

Table 1: Percentage Recovery of Propolis in Ethanol

Extraction method	Initial Weight of sample (g)	Weight collected (g)	Percentage recovery (%)	Recovery Ratio
Hot	2,500	1,851	74.04	3
Cold	2,500	680	27.02	1

Phytochemical Screening of Propolis

Table 2 showed the result of phytochemical screening of three samples of Propolis: Raw Propolis Sample (RPS), Hot Ethanol Propolis Extract (HEPE), and Cold Ethanol Propolis Extract (CEPE). The highest amount of phytochemicals was observed in Raw Propolis Sample, followed by the Cold Ethanol Propolis Extract. The least amount of

phytochemicals was observed in Hot Ethanol Propolis Extract. Cold Ethanol Propolis Extract was richer in bioactive components, it contained aponins, anthraquinones, flavonoids, tannins, terpenoids and phenol. Tannins, flavonoids and phenol were present in all the three screened extracts, while phlobatanins, cardenolides and steroids were absent in all the three extracts.

Table 2: Phytochemical Screening of Propolis

Phytochemical	Type of Extract		
	Raw Propolis Sample	Hot Ethanol Propolis Extract	Cold Ethanol Propolis Extract
Alkaloids	+	-	-
Phlobatanins	-	-	-
Saponins	+	-	+
Tannins	+	+	+
Anthraquinones	+	-	+
Cardenolides	-	-	-
Flavonoids	+	+	+
Terpenoids	-	-	-
Phenols	+	+	+
Steroids	-	-	-

Table 3 showed that Propolis extract (CEPE) controlled the test fungi (*Coriopsis polyzona* and

Coniophora puteana) at concentrations of 50% and 75%, respectively. There was no weight loss at

100% concentrations of extract which indicated that Propolis extract was efficient in the control of test fungi.

Table 3: Mean percentage weight loss in test blocks of *Triplochiton scleroxylon* after incubation in *C. polyzona* and *C. puteana*

Concentration of Propolis (%)	Weight Loss of test blocks			
	<i>Coriopsis polyzona</i>	<i>Coniophora puteana</i>	LSD	Significance
TC (Control)	50.10	46.25	27.20	NS
0 (Ethanol)	42.20	36.43	23.48	NS
25	24.05	26.43	19.29	NS
50	7.90	15.21	11.50	NS
75	0.00	8.09	8.48	NS
100	0.00	0.00	5.16	NS

Table 4 showed the means separation (averages for the two fungi), using LSD. For the weight loss experiment, it showed that the effect of concentrations on weight loss of wood blocks is not significant, for the two fungi. For the maximum

compressive strength (MCS) test, it showed that the effects of concentrations 75% and 100% on the MCS of wood blocks are the same for the two fungi.

Table 4: Averages of Weight Loss and Maximum Compressive Strength, for the two fungi

Parameters	Concentration of Extract (%)					
	0	25	50	75	100	Control
Weight Loss	23.48 ± 6.0829 ^a	19.29 ± 6.1496 ^b	11.50 ± 7.8911 ^c	8.48 ± 6.16 ^d	5.16 ± 4.29 ^e	27.20 ± 8.82 ^f
Maximum Compressive Strength	18.91 ± 1.7644 ^a	22.12 ± 1.4893 ^b	25.45 ± 0.3838 ^c	18.84 ± 0.57 ^a	18.69 ± 0.51 ^a	17.47 ± 1.25 ^d

DISCUSSION

A higher percentage (74.04%) of extract collected in Hot Ethanol Propolis Extract was due to the use of Soxhlet apparatus, in which most of the bioactive components are soluble under high temperature. The lower yield (27.02%) observed in CE was due to the use of cold solvent in which many bioactive components remain insoluble in the absence of heat. Preeti et al., (2012) observed that Propolis was best extracted using ethanol, giving a percentage yield of 57.2%, indicating that the solvent type also influences yield of extract.

Bioactive components of Propolis are important in predicting its antifungal and utilization potentials. Cowan (1999) reported that wood extractives are rich in a wide variety of secondary metabolites such as phenolic compounds, tannins, terpenoids, alkaloids, and flavonoids which have antimicrobial properties. He stated that the presence of secondary

metabolites in an extract is an indication of its anti-fungal potentials. The screened samples of Propolis contained saponins, tannins, anthraquinones, flavonoids and phenols (in the Cold Ethanol Propolis Extract), tannins, flavonoids and phenols (in the Hot Ethanol Propolis Extract), alkaloids, saponins, tannins, anthraquinones, flavonoids and phenols (in the Raw Propolis Sample). Herbs (2000) stated that tannins usually act as a barrier for microorganisms like bacteria and fungi hence protect the tree. Propolis chemical composition depends on the phytogeographical characteristics of the site of collection, since bees choose different plants as source of Propolis in different habitats (Popova et al., 2010). Propolis chemical composition also depends on the season of its collection, its age and the type of bees foraging at the site of its collection (Bankova et al., 2000; Marucci, 1995; Silici and Kutluca, 2005 and Bankova, 2005). The difference

in the composition of the extracts was due to the extraction methods used.

Alkaloids, saponins and anthraquinones were present in the raw sample but absent in the hot extract. Most active components in the Raw Sample were also present in the Cold Extract, owing to the absence of heat. Vongsak *et al.*, (2003) observed that evaluation of sox let extraction for Moringa leaves resulted in lower yield phenolics and flavonoids content. Anuradha *et al.*, (2010) stated that oxidation and degradation during hot extraction may lead to loss of some active ingredients. Different solvents may also extract different compounds, influencing its biological activity. Farnesiet *al.*, (2009), observed that the fungicidal effect of Propolis was associated with the presence of flavonoids and other phenolic components.

CONCLUSION

It can be concluded from the study, that:

- i. The yield of Propolis is higher in the cold extraction method than in the hot extraction method.
- ii. The Cold Ethanol Propolis Extract is richer in bioactive components than the Hot Ethanol Propolis extract, while the highest amount of bioactive components was found in the R\raw (unextracted) Propolis Sample.

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- iii. The study revealed that Propolis has good potentials to protect wood from fungal degradation because of its richness in bioactive (antifungal) components
- iv. The effect of extract concentrations on weight loss of wood test blocks is not significant for the two fungi, while the effect of extract concentrations 75% and 100% on the maximum compressive strength of wood test blocks is the same (not significant) for the two fungi.
- v. Propolis extract was able to control the test fungi at concentrations 50% and 75%, respectively.

Recommendations

- i. Further investigations are needed, using other solvents and test fungi, apart from those used in this study, for further evaluation of the yield, efficacy and bioactive composition of Propolis.
- ii. Large scale production of Propolis should be encouraged to provide environment- friendly fungicides.
- iii. The use of Propolis as fungicide in wood preservation is new; hence, the need for more research on Propolis extraction and its absorption in wood. Similar study should be carried out to determine the best concentration where Propolis will be most effective.

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