

Preservation of wet-blues (Leather) using cocos oil and organotin compounds

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Abstract

The effects of oil extracts of seeds of three varieties of *Cocos nucifera* were investigated on bacterial and fungal isolates from exposed “wet blues” of unfinished leather. The oil was extracted using soxhlet extraction hexane. The bacterial isolated from the “wet blues” were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Bacillus subtilis*, while the fungal isolates were *Aspergillus niger* and *Penicillium chrysogenum*. The effect of the oil extracts on the isolates were compared with that of organotin compounds. All the oil extracts and organotin compounds treated samples had very low microbial load. The sample treated with orange dwarf coconut oil had the least microbial load which was still minimal at the end of the experiment ($0.39 \pm 0.029 \times 10^6$ CFU/g). The zones of inhibition of the organotin compounds on *S. aureus* was a bit higher than those of the oil extracts, though not significant. DBT was more effective on *P. aeruginosa* with a zone of inhibition of $7.6\text{mm} \pm 0.45$; yellow dwarf oil had a zone of inhibition of $4.5\text{mm} \pm 2.50$; Orange dwarf oil 2.5 ± 0.50 . Green dwarf oil and Yellow dwarf oil were quite effective against *E. coli* with zones of inhibition of $10.3\text{mm} \pm 1.65$ and $13.5\text{mm} \pm 0.65$, respectively. ODO and YDO had no effect on *A. niger*. ODO, GDO, DBT and TPT were effective against *P. chrysogenum* with zone of inhibition of $7.0\text{mm} \pm 3.0$, $13.5\text{mm} \pm 1.50$, $9.8\text{mm} \pm 0.41$ and 9.3 ± 1.2 , respectively. *Bacillus* species was susceptible to ODO but resistant to GDO and YDO. The oil extracts showed good antimicrobial activity against the organisms, so they can be used as an alternative to organotin compounds in the preservation of leather materials.

Keywords: Wet-blues, *Cocos nucifera*, cocos oil, organotin compounds, preservation.

INTRODUCTION

Leather is animal skin/ hide that has been treated to preserve and make them suitable for use. It is the general term for hide or skin which retain its original fibrous structure more or less intact, and which has been treated so as to be non-putrescible even after treatment with water (Chrysteb and Mavine, 2000). Wet-blue is leather that has not been finished and is the form that leather is exported from most countries such as Nigeria. When these wet-blues are attacked by microorganisms, it sometimes result in pigmentation which eventually gives a poor finished leather. Vegetable tanned leathers have been found to be more susceptible to growth of moulds than chrome tanned leather, but chrome tanned leathers

have also been deteriorated by microorganisms. It is required that microbicides be effective against a wide range of organisms that bring about deterioration of the products (Admnis *et al.*, 2001).

Chrome-tanned leathers have been found to be biodeteriorated by microorganisms thus impairing the aesthetic, functional and other properties (Orlita, 2004). Glucose, reduced chrome, vegetable tannages, natural fats and liquors are retained in the leather which encourages the growth of bacteria and fungi (Muthusubramana *et al.*, 2008) bringing about deterioration. Some countries such as Nigeria export leather in the chrome-tanned form so it is required that microbicides be added which are effective against a wide range of microorganisms that bring about deterioration of the product.

Akpomie *et al.* (2006) reported the effectiveness of Dubutyltin salicylate and triphenyl tin acetate against

Aspergillus, *Mucor*, *Penicillium* and *Rhodotorula* at different concentrations. The use of aminoquinoline derivatives has been demonstrated against *A. niger*, *A. flavus*, *P. chrysogenum* and *Paecilomyces variola* (Peruwal *et al.*, 2004). The drawbacks in the use of some of these chemicals are the cost-implication, limited use for long-term preservation, the recalcitrance in the environment when used in excess, thus constituting environmental concern and the development of tolerance by some organisms. Some can cause discoloration on the leather, others damage the leather fibers matrix (Akpomie *et al.*, 2011). The stringent limitations placed on some of these chemicals as a result of their attending problems calls for the need to research into naturally occurring microbicides which would be cost-effective and environmentally friendly. This study therefore reports the antimicrobial activity of the oil extract of different species of *Cocos nucifera* against organisms isolated from chrome tanned leather.

MATERIALS AND METHODS

Wet-blue samples were obtained from the Federal College of Chemical and Leather Technology, Zaria, Nigeria, and kept in a refrigerator at 4°C. Some of the skins were exposed at room temperature (26°C) for aerial inoculation of bacteria and fungi.

Coconut (*Coco nucifera*)

Various varieties of the coconut seeds were collected from Nigerian Institute of Oil Palm research (NIFOR), Benin City, Nigeria. Five seeds each of three varieties, *Coco nucifera* (Green dwarf, yellow dwarf and orange dwarf) were obtained and botanically identified at the taxonomical unit of the plant Breeding Division, NIFOR, Benin City.

Extraction of Coconut Oil

This was done using the modified method of Ejechi and Akpomedaye (2006). Three seeds each of the different varieties were selected for the extraction. The covering of the seed was removed using a knife and dried in an oven at 100°C for 24 hours to remove the moisture content and yield copra. After complete drying, the endosperms were removed from the shell and grated using a manual grater. The oil of the grated endosperms was extracted with hexane by continuous hot extraction using soxhlet apparatus. The extracts were concentrated to remove the solvent using Rotary evaporator then dried in a desiccator. The pure coconut oil was stored in a dark sterile container and labeled as ODO for orange dwarf oil, YDO for yellow dwarf oil and GDO for green dwarf oil.

Determination of Minimum Inhibitory Concentration (MIC)

This was done using the agar well diffusion method. 100%, 50%, 25% and 12.5% v/v concentration of the oil extracts were placed into 8.0mm agar wells made on inoculated plates. The NA plates were incubated at 26°C for 24 hours while the SDA plates were incubated at 26°C for 72 hours. The diameter of zones of inhibition were measured while the least concentration that inhibited the growth of each organism was recorded as the minimum inhibition concentration.

The minimum inhibitory concentrations of the organotin compounds were also determined using 0.01mg/ml, 0.02mg/ml, 0.05mg/ml and 0.1mg/ml.

Isolation and Identification of Isolates from Skin Samples

This was done using the method of Akpomie *et al.* (2006). Cut portions from different parts of the tanned leather were soaked in water. This was used as inoculum on Nutrient Agar plates incubated at 26°C for 24 hours for isolation of bacterial isolates and on Sabouraud Dextrose Agar incubated at 26°C for 72 hours for the isolation of fungal isolates.

Identification of the bacterial isolates was done using morphological characteristics and reaction to biochemical test while the fungal isolates were morphologically characterized using observation under the microscope when stained with lactophenol blue.

Trial preservation of tanned leathers using the oil extracts and organotin compounds

This was done using minimum inhibitory concentrations of the organotin compound and oil extract. Small portions of the tanned leather were cut using specification of SLP₂ (SLTC, 1996). They were soaked in different concentrations. A small portion not soaked in the oil extract nor organotin was used as control. The leathers were left in the open for seven days for physical observation of microbial growth.

Physical testing of the leather

The shrinkage temperature and tensile strength of each sample was determined according to the official methods of analysis (SLTC, 1996; IUP, 1976). This was done before and after preservation trial test.

RESULT AND DISCUSSION

Using morphological characteristics and reaction to

Table 1. Assessment of growth of microorganisms in the preservation trials using the oil extracts and organotin compound

Preservation material	Days						
	2	4	6	8	10	12	14
DBT	-	-	-	-	++	+++	+++
TPT	-	-	-	+	++	+++	+++
ODO	-	-	-	-	-	+	+
GDO	-	-	-	-	-	+	+
YDO	-	-	-	-	+	++	++
Control	+	+	+	+++	++++	++++	++++

ODO- Orange dwarf coconut oil; GDO - Green dwarf coconut oil; YDO- Yellow dwarf coconut oil ; DBT- Dibutyl Tin Salicylate; TPT- Triphenyl Tin Salicylate.

- No growth

+ scanty growth

++ moderate growth

+++ Heavy growth

++++ Very heavy growth.

Table 2. Microbial counts of the chrommed tanned wet-blue leathers with the preservative material (10^6 CFU/g)

Preservative material	4	6	8	10	12
DBTSA	0.005±0.0007	0.002±0.002 ^a	0.0035±0.00006 ^{ab}	0.32±0.0004 ^b	0.32±0.11 ^{ac}
TPTASA	-	0.008±0.001 ^a	0.011±0.002 ^{ab}	1.1±0.064 ^b	23.3±4.37 ^{ac}
GDO	-	1.00±1.15 ^a	0.0011±0.0002 ^{ab}	0.001±0.0003 ^b	0.39±0.060 ^{ac}
ODO	-	0.003±0.00007 ^a	0.0017±0.0003 ^{ab}	0.002±0.0003 ^b	0.39±0.060 ^{ac}
YDO	0.003±0.001	0.0037±0.0003 ^a	0.0088±0.0006 ^{ab}	1.1±0.058 ^b	1.5±0.29 ^{ac}
Control	0.007±0.002	11.00±0.58 ^a	91.67±5.24 ^{ab}	15.3±8.5 ^b	1643±80.9 ^{ac}

a, ab, b, c – differences between the means that have common letters are significant ($p < 0.05$).

biochemical tests, the organisms isolated from the leather samples were *S. aureus*, *P. aeruginosa*, *E. coli*, *A. niger*, *P. chrysogenum* and *Bacillus subtilis* as shown in Table 1. These organisms might have been deposited on the samples from the environment, the tannery workers and even from the raw material. The microorganisms utilize the tanning products for their growth and development where they cause fermentation of the tanning material and thus bring about decomposition of the material.

The microbial counts of the samples treated with the extracted oils is shown in Table 2. The microbial counts of the samples treated with the extracted oils were quite low compared with that of the organotin compounds. The load in the control was quite high showing that the extracted oils had inhibitory potentials on the organisms. The result of the visual observation as shown in Table 3 conforms to that of the microbial load. As the days progressed there was visible growth in form of coloured pigments on the leather which would have led to a corresponding increase in the microbial load. This could be attributed to contamination from the environment since the leathers were exposed. Some of these organisms might be resistant to the extracts.

The result of the zones of inhibition and minimum inhibitory concentrations are shown in Tables 3 and 4. The zones of inhibition of ODO on *P. chrysogenum* and *E. coli* was very pronounced while the inhibition of *S. aureus* and *P. aeruginosa* was minimal. There was no inhibition by ODO on *A. niger* and *B. subtilis* growth. GDO had significant zone of inhibition for all organisms apart from *S. aureus* and *B. subtilis* while YDO inhibited *P. aeruginosa* and *E. coli* significantly. There was no inhibition of growth of *A. niger* and *B. subtilis* with YDO. The monoglycerides and free fatty acids present in the coconut oil might have exerted antimicrobial activities by disrupting the cell membrane permeability barrier and inhibiting the amino acid uptake. The result conforms to that of Kabara (2009) that saturated fatty acids; lauric acid among others confers antiviral, antifungal, antibacterial and antiprotozoal activities in coconut oil. Coconut oil contains monolaurin and some other antimicrobials which are active against *E. coli*, α and β -haemolytic streptococci, *Aspergillus fumigatus* and *S. aureus* (Ekpa and Ebana, 2006).

DBT was able to inhibit the growth of *P. aeruginosa*, *A. niger* and *P. chrysogenum*s. The effect on *S. aureus*, *E. coli* and *B. subtilis* was very minimal as shown in

Table 3. Minimum Inhibitory Concentration of the preservatives (%v/v)

	ODO	GDO	YDO	DBT	TPT
<i>S. aureus</i>	50	-	-	50	6.25
<i>P. aeruginosa</i>	50	25.0	-	25	12.50
<i>E. coli</i>	6.25	12.5	12.5	50	12.50
<i>A. niger</i>	-	25.0	-	6.25	12.50
<i>P. chrysogenum</i>	12.5	6.25	-	6.25	6.25
<i>Bacillus</i>	6.25	-	-	-	-
Control	-	-	-	-	-

Key:- - no inhibition.

Table 4. Diameter of zones of inhibition of the preservatives against the isolates (mm)

Organism	ODO	GDO	YDO	DBT	TPT
<i>S. aureus</i>	2.5 ± 0.29	0.0 0.00	2.25 ± 0.00	4.9± 0.39	5.4± 0.49
<i>P. aeruginosa</i>	2.5 ± 0.50	5.3 ± 0.63	4.5 ± 2.50	7.6 ± 0.45	3.3± 0.63
<i>E. coli</i>	13.5 ± 0.65	10.3 ± 1.65	13.5 ± 0.65	4.8± 0.59	4.1± 0.39
<i>A. niger</i>	0.0 ± 0.00	8.5 ± 1.55	0.0± 0.00	10.0± 1.17	7.2± 0.49
<i>P. chrysogenum</i>	7.0 ± 3.03	13.5 ± 1.50	4.3 ± 0.48	9.8± 0.41	9.3± 1.21
<i>Bacillus</i>	8.4 ± 1.80	0.0 ± 0.00	0.0± 0.00	3.18± 0.67	3.13± 0.67

Table 5. Shrinkage temperature and Tensile strength of the leather after treatment

Preservation material	Shrinkage temperature(°C)		Tensile Strength (N/M ²)	
	ST(a)	ST(b)	TS(a)	TS(b)
DBTSA	82.0 ± 5.03	86.7 ± 5.93	253.1 ± 2.45	243.3 ± 6.06
TPTASA	93.9 ± 3.71	85.3 ± 1.76	251.8 ± 3.39	246.5 ± 4.77
ODO	96.0 ± 1.15	87.67 ± 5.24	254.5 ± 0.41	251.8 ± 5.39
GDO	95.3 ± 0.67	84.0 ± 3.06	254.5 ± 1.61	247.2 ± 2.12
YDO	88.7 ± 1.76	89.7 ± 4.50	251.8 ± 3.36	249.7 ± 3.87
CONTROL	84.7 ± 0.67	86.3 ± 1.40	254.1 ± 5.25	254.1 ± 5.25

a, Difference between the means that have common letters are significant.

table 5. TPT had slight inhibitory effects on the growth of the organisms apart from *P. chrysogenum* which had a zone of inhibition of 12.1mm. The inhibitory effect of the organotin compounds may be attributed to the inhibition of ATPase of the respiratory organelles (Nesciet *et al.*, 2001).

The minimum inhibitory concentration showed that ODO, DBT and TPT were able to inhibit *S. aureus* even at 50%, 50% and 25% v/v concentration respectively.

P.aeruginosa and *E. coli* and *P. chrysogenum* were inhibited by ODO, GDO and TPT. Only ODO was able to inhibit *Baccillus* sp at a very low concentration of 6.25%v/v. Dibutyltin has shown to have less inhibition effect at lower concentration (Thippayarat *et al.*, 2009). Aricelma *et al* (2008) verified Tri-butyl Tin malleate inhibited 10% of bacterial growth in carpets.

The results of the physical properties of the leather after treatment (table 5) showed that the shrinkage

temperature and tensile strength of the treated leathers compared favourably well with the results before treatment which were within the standard range for good leathers (Shrinkage temp: 82 – 98°C and tensile strength: 225 – 262 N/m². The physical properties of the leather were still within standards for good leathers. The ANOVA analysis of the shrinkage temperature had a significant difference of $P < 0.05$ which showed that there was a significant difference in the values of the shrinkage temperature of leather treated with TPTAS, ODO, GDO and the control.

There was no significant difference in the values of the tensile strength when compared with the leather before treatment.

From the results of the study, all the oil extracts had good preservative effects in being able to inhibit the growth of the organisms isolated from the chrome tanned leather. It can serve as a better alternative to these organotin compounds since it is natural, biodegradable and of little or no environmental hazard. It can thus be incorporated into stages in leather processing or incorporated into formulations prepared for the maintenance of leather.

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