Characterisation of Solvent Components in *Erythrophleum suaveolens* (Guill. & Perr.) Brenan Stem Bark Extracts Treated *Triplochiton scleroxylon* K. Schum Wood

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ABSTRACT

Soxhlet extractions of *Erythrophleum* suaveolens stem barks were carried out using methanol, methanol/chloroform, chloroform and water with the aim of determining the physio-chemical properties and its interaction with *Triplochiton scleroxylon* wood. Chloroform/methanol yielded maximum volume/unit of 37% and was used in the second stage as 5%, 10%, 20% concentration level using kerosene as diluents and 100% water extraction to validate its treatability use. Quantitative analysis of the extract indicated 395.52 mg/g total saponins, 106.16 mg/g total tannins, 91.90 mg/g total phenol, 40.85 mg/g total alkanoids and 1.57 mg/g total flavonoids. Statistical analysis results revealed proportionate interaction between the extracts and absorption/retention. The stem bark of *E. suaveolens* was found to be a staining-drying extract with bioactive phyto-compounds of wood preservative potential, therefore suitable for surface treatment in wood-based processing and utilization industry.

Keywords: *Erythrophleum suaveolens, Nigeria*, Phyto-constituents *Triplochiton scleroxylon*,.

INTRODUCTION

Light organic solvent preservatives (LOSP) treatment of *Triplochiton scleroxylon* ought to have been an important component of the wood processing industry in Nigeria in the production of plywood, furniture, cabinet work and packing cases. This is one of the limitation for the development of wood based processing and utilization industry in Nigeria and most of our commercial timber are exported raw or in semi-processed state. Advantages of LOSP include the ability to treat wood products in final form and the absence of problems associated with other treatment methods such as swelling and bleeding, the need for further drying and disposal of treated wood waste [1]..

The LOSP treatment used in this study was based on the non-pressure method. [2] reported that the LOSP treatment requires complete penetration of sapwood with preservative for timber up to 50mm thick for pressure method. Satisfactory penetration and retention are usually achieved when solution uptake is between 30 and 40L/m^3, this level is often exceeded, particularly with sapwood [3]

Many wood treatment processes involve transient flow of liquid into wood, that is, absorption by a sample under an applied pressure or non-applied pressure method. It seemed appropriate to investigate to what extent this type of flow is affected by solvent-extracts impregnation. The antifungal efficacy of organic solvent stem bark extract of *Erythrophleum suaveolens* in wood has been previously reported [4] while its aqueous extract has competed more favourably than the popular Chromated Copper Arsenate (CCA) against termite attack in Ghana [5]. This work is an investigation of the relationship between concentration level and absorption/retention of organic solvent stem bark extract of *Erythrophleum suaveolens* during transient flow with consumption capacity of 24cm^3 per 20 minutes. *Triplochiton scleroxylon* wood was used because of its wide application and dearth of information on its surface treatability by organic solvent extracts.

MATERIALS AND METHODS

Plant material

The sample stem bark of *E. suaveolens* used for the study was obtained from Odunowo Sawmill Imeko in Imeko/Afon Local Government area of Ogun State Nigeria. The stem barks were peeled from the stacked logs and transported to the Wood Laboratory Unit of the Department of Forest Resources Management, University of Ibadan and dried for one month under an ambient open laboratory conditions.

Preparation of extracts

Five hundred gram (500g) of each of crushed stem bark samples were weighed into four conical flasks and 2000 mL of methanol, chloroform, methanol/chloroform and distilled water were poured into each of the flasks. The contents of the 4 flasks were shaken and the tops covered with aluminium foil and kept at room temperature for 4 days after which the extracts were obtained by filtering using a filter paper. The extracts thus obtained were concentrated and

evaporated under reduced and controlled temperature using a rotary evaporator. The concentrated extracts were the yields. The yields were calculated in percentage thus;

$$\textbf{Yield Estimation} = \frac{\textbf{Quantity of yield X 100}}{\textbf{Quantity of bark sample}}$$

From the final yields (extracts), 5%, 10%, 20% of the best extract and 100% water extraction were used for the study.

Organoleptic properties

Organoleptic properties (colour, texture and odour) of the *E. suaveolens* extracts were determined in respective solvents in their wet conditions.

Preparation of test blocks

Forty-five test blocks of 2cm x 2cm x 6cm were obtained from the base, middle and top of *Triplochiton scleroxylon* trees harvested from Omo Forest Reserve, South Western Nigeria. The blocks were oven-dried for 18 hrs at103^0C, cooled and conditioned to room temperature of 27 ± 2^0C and weighed **W1** before dipping impregnation.

Treatment of test blocks

Dipping impregnation method [6, 4] was used for the treatment of the wood test blocks with the prepared extracts. The diluent used was 2000 ml kerosene for each extract concentration (5, 10 and 20%) and 100% concentration of water extraction. Kerosene was used for control experiment. The test blocks were completely immersed in the prepared extracts for 20 minutes so as to obtain a desirable level of absorption capable of retaining certain amount extracts in the laboratory. After treatment the blocks were removed from the treatment solution, drained and weighed as **W2** to determine the rate and level of absorption. Absorption and retention in kilograms per cubic meter (Kg/m^3) were as follows; **Absorption, kg/m^3 = 1000(G) /V, Retention, kg/m^3 = (G x C/V) x 10.**

Phyto-chemical screening of the bark extract

Quantitative analysis of alkanoids, flavonoids, phenol, tannins and saponins compounds were carried out by using the methods of [7, 8, 9, 10, 11, 12].

Procedure;

Methanolic extract of the samples was prepared following the method of [10] by adding 25 mL of methanol to 0.5g of sample contained in a covered 50 mL centrifuge tube, and shaking continuously for 1 h at room temperature. The mixture was centrifuged at 3,000 rpm for 10 min, and then the supernatant was collected and store at -20^{o}C until analysis was done.

Total Alkaloids Determination

The total alkaloid contents in the samples were measured using 1, 10-phenanthroline method described by [9] with slight modifications. 100mg sample powder was extracted in 10ml 80% ethanol. This was centrifuged at 5000rpm for 10 min. Supernatant obtained was used for the further estimation total alkaloids. The reaction mixture contained 1ml plant extract, 1ml of 0.025M $FeCl_3$ in 0.5M HCl and 1ml 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^{o}C. The absorbance of red coloured complex was measured at 510nm against reagent blank. Alkaloid contents were estimated and it was calculated with the help of standard curve of quinine (0.1mg/mL, 10mg dissolved in 10ml ethanol and diluted to 100mL with distilled water). The values were expressed as g.100g-1of dry weight.

Determination of total flavonoids content (TFC)

TFC was determined with Aluminum chloride method as reported by [12]. 0.5 mL of extract was dispensed into test tube, followed by 1.5 mL of methanol, 0.1 mL of aluminum chloride (10%), 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. The reaction mixture was mixed, allowed to stand at room temperature for 30 minutes, before absorbance was read at 514 nm. TFC was expressed as quercetin equivalent (QE) in mg/g material. The calibration equation for quercetin was $Y = 0.0395x – 0.0055$ ($R^2 = 0.9988$).

Determination of total phenolic content (TPC)

The total phenolic content of samples extracts was determined according to the Folin–Ciocalteu method used (Chan, *et al*., 2006). 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 (7.5w/v). The reaction mixture was mixed, 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. TPC was

expressed as Gallic acid equivalent (GAE) in mg/g material. The calibration equation for Gallic acid was $Y = 0.0645x - 0.0034$ ($R^2 = 0.9997$).

Total Saponins Determination

Total saponins (TS) were determined by the method of [7] as described by [11] with some modifications. 0.5 g of sample was extracted with 25 ml of 80% aqueous methanol by shaking on a mechanical shaker for 2 h, after which contents of the tubes were centrifuged for 10 min at 3,000 rpm. In a test tube an aliquot (0.25 ml) of the supernatant was taken to which 0.25 ml vanillin reagent (8% vanillin in ethanol) and 2.5 ml of 72% aqueous H_2SO_4 were added. 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 mg diosgenin equivalent per g of sample dry matter.

Determination of tannin content

Tannin content of samples was determined according to the method of [8] as follows. Sample (0.1g) was extracted with 5 mL of acidified methanol (1% HCl in methanol) at room temperature for 15 minutes. The mixture was centrifuged at 3,000rpm for 20minutes. 0.1 mL of the supernatant 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. Tannin content was expressed as tannic acid equivalent (TAE) in mg/g material. The calibration equation for tannic acid was $Y = 0.0695x + 0.0175$ ($R^2 = 0.9978$).

Data analysis

The data obtained were analyzed using a Multi-factorial Analysis of Variance ANOVA. Means were separated with the aid of Duncan Follow up Test DFT at 5% level of significance. Tables and barcharts were also used to illustrate variation among the factors and variables. The factors considered were as indicated below: Test wood block at 3 sampling position (Top, middle and base). Five types of preservative (5%, 10%, and 20% chloromethanol extract, 100% water and 100% kerosene)

RESULTS AND DISCUSSION

Physical characterization of the extracts

Extraction yields

Among the four extracts, chloroform/methanol extract was found to have maximum extractive yield followed by the methanol, aqueous and chloroform extracts (Table 1).Appreciable amount of extractives obtained from methanol-chloroform mixture and methanol in Table 1 confirm the suitability of the solvents for removal the bark cell structures. The result showed that soaking technique and if used with suitable solvents, at least 30% yield can be achieved. The best extract yield obtained from methanol/chloroform in Table 1 could be attributed to the mixture power of its polar and non-polar properties which acted to remove both the likely polar and non-polar toxic extractives that either the methanol or chloroform could not remove if not combined [4] From the result, methanol and water also proved effective as it has a very close extract volume but water has drawback of short shelf life and causing dimensional changes in wood. The result showed that polar solvents alone are suitable for extraneous material extraction. Methanol extraction volume obtained is in the range reported by [13, 14].

Table 1.Extractive value of *E. suaveolens* extracts in different solvents.

Solvents	Yield in gram (g)	Yield in %
Methanol	165.16	33.032
Chloroform	45.13	9.026
Chloroform/methanol	188.92	37.789
Aqueous	164.90	32.980

Organoleptic properties

The colour, texture and odour of the *E. suaveolens* extracts in different solvents in wet conditions were characterized (Table 2). The methanolic extract was better than corresponding aqueous and other organic extracts in retaining the natural fragrances of the plants as well as having the finest viscous texture. This may be due to the preservative ability of methanol. Methanol (90%) and ethanol (70%) have been widely reported as being suitable for macromolecules extraction for alkanoids, flavones, tannins, saponins, polyphenol, lectins and quassinoids [15, 16].

Table 2. Organoleptic properties of stem bark extract of *E. suaveolens*.

Solvent extracts	Colour	Texture	Odour
Methanol	Reddish brown	Finest hand feel,slightly slippery/sticky	Alcohol odour
Chloroform	Chocolate	Coarse hand feel	Burning smell
Chloroform/methanol	Brown	Fine coarse hand feel	Sourish smell
Aqueous	Dark brown	Fine coarse hand feel, foamy when shaken	Tobacco like smell

Phyto-constituents composition of *E. suaveolens* stem bark extract

Few studies that have been reported on anti-degradation efficacies of *E. suaveolens* bark extracts against bacteria, fungi and termites lacked quantitative analysis of its phyto-constituents [17, 4, 5]. Table 3 showed Phyto-constituents composition of stem bark extract with total saponins content of 395.52±3.045mg/g, tannins (106.16±0.420mg/g), phenol (91.90±0.335)mg/g, alkanoids (40.85±0.050mg/g) and flavonoids (1.57±0.105). This higher degree of bioactive compounds content of saponins, tannin and phenol might be responsible for the woods preservative efficacies of the bark extracts against fungi and termite as reported by [4, 5] respectively.

Table 3. Phyto-constituents composition of stem bark extract of *E. suaveolens*.

Variables	Alkanoids(mg/g)	Flavonoids(mg/g)	Phenol(mg/g)	Saponins (mg/g)	Tannins(mg/g)
Quantity	40.85±0.050	1.57±0.105	91.90±0.335	395.52±3.045	106.16±0.420

Interaction between extract and the test wood

From the analysis of the extract interaction in *Triplochiton scleroxylon* wood, it was determined that absorption and retention are proportionate though very small quantity of organic solvent extract retained. The highest absorption observed in 100% water extraction is expected, the extract was evenly miscible and evenly distributed in water which must have allowed more extract solution to be absorbed by the wood blocks. Apart from this, water is denser and less mobile as compared with kerosene as diluents, a favourable factor responsible for higher absorption rate [4]. Absorption of the extractives is significantly different at both the wood sampling positions and concentration levels, (Table 4). Duncan follow up test indicated that absorption of extractives at the base and middle woods were not significantly different, while

wood blocks at the two levels were different from wood at the top in absorption of bark extracts (Table 5 and 6).For all bark extracts, absorption was highest in 100% water extraction and lowest in 5% Methanol-Chloroform extraction (figure 1). However, increase in extract concentration did not translate to a corresponding increase in absorption, as absorption of extracts dropped in all wood types when concentration of extracts was increased from 10% to 20%. This is similar to the results obtained by [18], implying that wood of *Triplochiton scleroxylon* has the tendency to repel extracts at higher concentration. Small quantity amount of organic solvent extract retained in Fig 2 showed that the plant has fixative capacity, thus suitable for surface treatment without causing swelling and the need for re-drying.

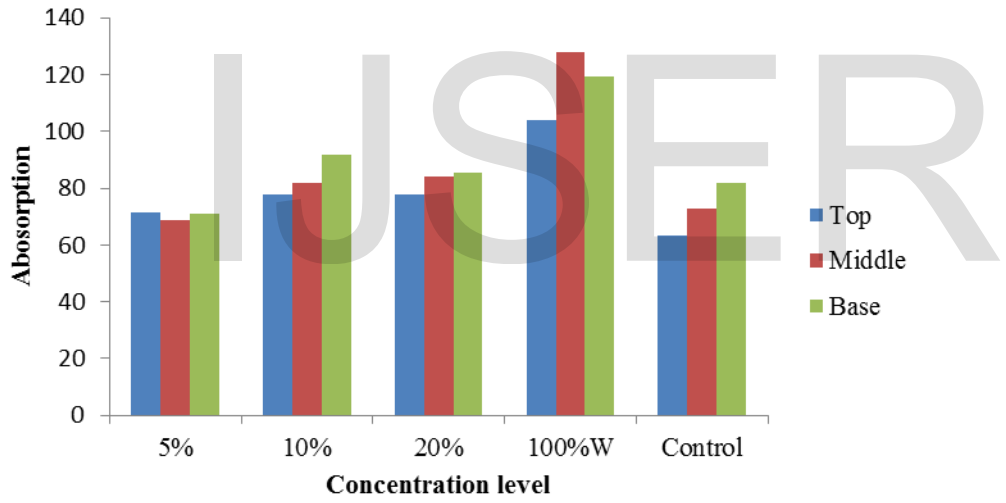

Fig1. Absorption rate of *E. suaveolens* bark extraction concentrations on test wood position

Table 4 Analysis of Variance (ANOVA) for rate of absorption of extract in test block (kg/m^3)

SV	DF	SS	MS	F value	P-level
P	2	1952.546	976.273	4.013	0.022*
C	4	25304.012	6326.003	26.002	0.000*
PC	8	1656.636	207.079	0.851	0.561
Error	75	18246.528	243.287		
Total	89	47159.722			

*** F-ration is significantly different at α = 0.05**

Table 5. Duncan follow up test of absorption on wood position

Wood Position	**Mean Separation**
Base	89.8611 ± 2.848 a
Middle	87.0833 ± 2.848 a
Top	78.8890 ± 2.848 b

Mean with the same alphabet are not significantly different from each other at α = 0.05

Table 6. Duncan follow up test of absorption on Concentration level

Concentration level	**Mean separation**
100%W	117.1296 ± 3.676a
10%	83.7963 ± 3.676b
20%	82.4074 ± 3.676bc
Control	72.6851 ± 3.676cd
5%	70.3704 ± 3.676d

Mean with the same alphabet are not significantly different from each other at α = 0.

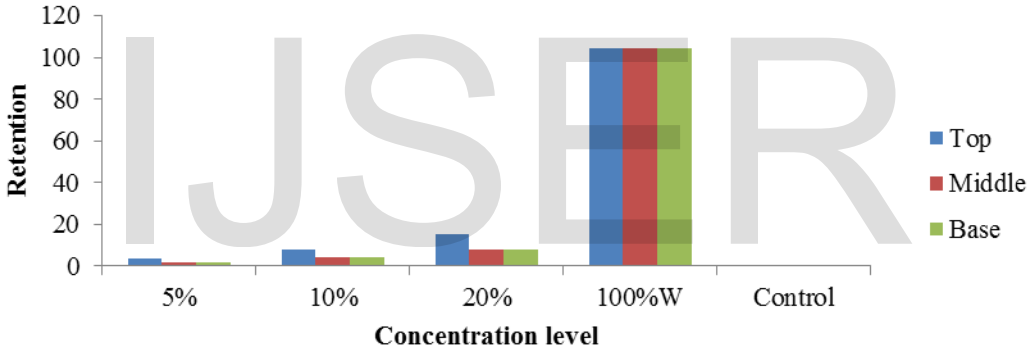

Fig 2. Retention interaction between wood position and concentrations

Table 7. ANOVA for Retention of extract in test block (kg/m^3)

SV	**DF**	**SS**	**MS**	**F value**	**P-level**
P	2	404.221	202.110	3.586	0.033*
C	4	177094.361	44273.590	785.516	0.000*
PC	8	1330.564	166.320	2.951	0.006*
Error	75	4227.185	56.362		
Total	89	183056.331			

*** F-ration is significantly different at α = 0.05**

Table 8. Duncan follow up test of retention on wood position

Wood Position	**Mean Separation**
Middle	31.2445 ± 1.371 a

Base	29.8489 ± 1.371 ab
Top	26.2445 ± 1.371 b

Table 9. Duncan follow up test of retention on Concentration level

Concentration level	**Mean separation**
100%W	117.1296 ± 1.770a
20%	16.4815 ± 1.770b
10%	8.3796 ± 1.770c
5%	3.5185 ± 1.770cd
Control	0.0073 ± 1.770d

Mean with the same alphabet are not significantly different from each other at α = 0.05

CONCLUSION

The organic solvent stem bark of *E. suaveolens* is a brown drying-staining extract containing bioactive phyto-compounds of wood preservative potential. Samples of the organic solvent extract treated woods turned to a more desirable brown colour from white without any dimensional changes and bleeding of the extract, a wood colour that usually attract higher market value in wood utilisation industry worldwide were obtained. The results have shown that organic solvent stem bark extract of *E. suaveolens* is suitable for surface treatment of white woods. Further research should be done to incorporate organic fixative substance or compound into the *E. suaveolens* stem bark extract.

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