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Phytochemical screening of the bark of *Chlorophora excelsa* (WELW.) BENTH and HOOK F

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ABSTRACT

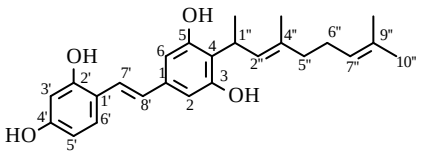
Phytochemical screening of the bark extract showed the presence of carbohydrates, and saponins. Cytotoxic test using brine shrimps (*Artemia salina*) lethality assay was carried out on methanol, petroleum ether [60-80^{0}C] and ethylacetate extracts. The LC_{50} (lower-upper limits) obtained by the Finney computer programme were 54µg/ml [9.90 – 100.70µg/ml], 165 µg/ml [117.58 – 217.42µg/ml] and 63µg/ml [34.03 – 87.77µg/ml] respectively at 95% confidence interval and 3 degrees of freedom. Antimicrobial sensitivity tests of the same extracts were carried out on microorganisms such as *Escherichia coli*, *Staphylococcus epidermis*, *Salmonella* and *Candida albicans*.

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INTRODUCTION

The *Chlorophora* species belong to the family Moraceae. *Chlorophora* comprises of three species – *C. sim*, *C.regia* and *C. excelsa*. *Chlorophora* is a very large dioecious tree of both evergreen and mixed forests. *C. excelsa* has some African names such as African teak, Swamp mahogany, e.t.c. [1]. It's Nigerian names include *ioko* (Hausa), *iroko* (Yoruba) and *Oji* [Igbo] e.t.c. [2]]. The bark of *C. excelsa* is grey to dark brown or blackish, usually fairly rough and flaking off in small scales.

It is one of the most valued timber trees throughout tropical Africa. The barks, ashes, leaves, wood and latex have uses in traditional medicine. From the wood, a substance with fungicidal properties has been isolated and identified as stilbene, chlorophorin [3], excelsa-octaphenol, a stilbene dimer from



Chlorophora excelsa. Molecular mass 380.49.

4 – geranyl – 3,5,2',4' – tetrahydroxy – trans – stilbene (Chlorophorin 1) $C_{24}H_{28}O_4$.

The bark extract is sometimes used as an expectorant. It is an ingredient in a mixture used in a hip-bath for venereal sores and as a wash for chancre. The crushed bark in water is taken for heart troubles, general fatigue and lumbago. The ashes from the bark with palm-oil are rubbed on swellings and fragments of the bark are added to local remedies to increase their therapeutic action [3].

EXPERIMENTAL

The Plant Material

The bark of *C. excelsa* was collected from the matured iroko tree in Ijumu Local Government Area of Kogi State. It was air-dried and pounded into powder and preserved well in an air-tight container.

Extraction procedure

250g of the powdered bark of *C. excelsa* was packed into a soxhlet extractor. The solvents used for continuous extraction were petroleum ether [60 – 80^{o}C], ethyl acetate and methanol respectively. After exhaustive extraction, each extract was evaporated to dryness using the rotary evaporator (Table 1).

Preliminary phytochemical screening

This was carried out using known procedures [6,7].

Cytotoxicity

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Table 1: Amount of Extract from 250g Powdered Bark

Solvent	Weight of Extract (g)	Percent Extract
Petroleum ether	1.56	0.62
Ethyl acetate	1.18	0.47
Methanol	1.79	0.72

were incubated at 37ºC aerobically for 24 hours. Following this, the plates were examined for inhibition or otherwise around the discs. The inhibition zones were measured from the edge of the discs to the end of the inhibition zones (Tables 4 and 5).

RESULTS AND DISCUSSION

Table 2: Effect of Crude Extracts on Brine Shrimps

Concentration µg/ml	No. of shrimps added	Methanol extract		Petroleum ether extract		Ethyl acetate extract	
		No. of dead shrimps	% mortality	No. of dead shrimps	% mortality	No. of dead shrimps	% mortality
1000	30	28	93	29	97	30	100
500	30	25	83	17	57	30	100
250	30	21	70	16	53	26	87
125	30	19	63	15	50	24	80
62.5	30	17	57	13	43	14	47

Table 3: Brine shrimp lethality assay: LC_{50} value obtained at 95% confidence limits

Extracts	LC50 (µg/ml)
Methanol	54
Ethyl acetate	63
Petroleum ether (60-80^{0}C)	165

Brine Srimp lethality bioassay

This was carried out using the standard procedure [5,8]. The LC_{50} values in µg/ml were determined using the Finney computer programme (Tables 2 and 3).

Antimicrobial screening

Extracts of petroleum ether, ethyl acetate and methanol were tested against clinical isolates of microorganisms, obtained from Veterinary Medicine Teaching Hospital, A.B.U., Zaria. Plates of blood agar and nutrient agar were lawn inoculated with broth cultures of the tested microorganisms. Discs of about 1mm diameter bearing the plant extracts were transferred with a loop onto the plates and the plates

Continuous extraction of the bark was carried out in a soxhlet apparatus using petroleum ether (60-80^{0}C), ethyl acetate and methanol (Table 1).

The phytochemical screening of the petroleum ether, ethyl acetate and methanol were carried out. The general tests for carbohydrates were positive as well as for the general tests for the presence of alkaloids, tannins, glycosides and anthracene derivatives. Positive results were obtained for the presence of saponins, deoxysugars, cardiac glycosides. Cytotoxicity test was determined using the brine shrimp lethality bioassay [5] from which the LC_{50} values in µg/ml of extracts were obtained. Microbial screening to test for the inhibitory effects of the extracts on the growth of pathogenic microorganisms was also carried out. The results provide some scientific basis for the traditional use of the bark of *C. excelsa* as an antimicrobial agent.

Petroleum ether, ethylactate and methanol extracts displayed cytotoxicity in the brine shrimp bioassay. From the LC_{50} values (µg/ml) methanol extract with 54 is the most active, followed by ethyl acetate extract with 63 and petroleum ether extract the least active with 165 (Tables 2 and 3).

The disc-method was employed in investigating the antimicrobial activity in the extracts. For gram-positive bacteria such as *Staphylococcus aureus* and Beta hemolytic *Streptococcus*, the petroleum ether

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Table 4: Microbial testing for inhibitory activity against common pathogens

S/NO	Microorganism	PE	EA	ME
1	*Staphylococcus aureus*	6+	-	-
2	*Escherichia coli*	5+	7+	3+
3	Beta hemolytic *Streptococcus*	9+	9+	3+
4	*Salmonella*	7+	5+	-
5	*Enterobacter*	7+	+	+
6	*Candida albicans*	6+	6+	3+
7	*Pasteurella multocida*	7+	6+	4+
8	*Corynebacterium*	5+	6+	-
9	*Proteus* species	6+	4+	-
10	*Citrobacter*	7+	6+	-

PE – Petroleum ether extract; EA – Ethyl acetate extract; ME – Methanol Extract - = resistant to drug; + = sensitive to drug; 5+ and above = extremely sensitive

Table 5: Antimicrobial activity Test of the Pure Component

S/No	Microorganism	Sensitivity
1	*Staphylococcus epidermis*	-
2	*Corynebacterium* species	-
3	*Escherichia coli*	2+
4	*Streptococcus* (Beta) haemolytic	4+
5	*Citrobacter* species	-
6	*Salmonella typhi*	5+
7	*Proteus* species	-
8	*Pseudomonas* species	5+
9	*Candida albicans*	4+
10	*Pasteurella multocida*	-

extract exhibited the greatest inhibitory activity, followed by the ethylacetate extract. Inhibition of the growth of the following gram-negative bacteria was also observed; *E coli*, *Citrobacter*, *Proteus* species, *Pseudomona* species and *Pasteurella multocida* by petroleum ether and ethylacetate extracts, as well as on yeast – like fungi *Candida albicans* (Table 4).

Chromatography was used to separate the chemical components in the petroleum ether extract using petroleum ether: ethyl acetate (9:1) as the solvent system. The major component exhibited a bright florescence at λ 366nm and had a R_f value of 0.71.

CONCLUSION

The antimicrobial screening gave some justification of the use of the bark extract of *C. excelsa* in the use of traditional medicine, and for the treatment of gastrointestinal system, urinary tract and skin infections. Spectroscopic analysis shall help to characterise and determine the molecular structure and mass of the component of petroleum ether extract of *C. Excelsa*.

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