RESEARCH NOTE

COMPARATIVE ANTIBACTERIAL ACTIVITIES OF OIL PALM-*ELAEIS GUINNEESIS* JACQ. (PALMAE) NUT AND COCONUT-*COCOS NUCIFERA L.* (PALMAE) SHELLS PYROLYSATES

G.C. UKOH[1], B.A. AYINDE[1,+], C.O. USIFOH[1], G.A. BADMUS[2], N.A. ADEYEMI[2] and A. MOHAMMED[2]

1. Faculty of Pharmacy, University of Benin, Benin City, Nigeria.
2. Research Engineering Division, Nigerian Institute for Oil Palm Research, Benin City, Nigeria.

(*Submitted*: 10 March 2004; *Accepted*: 22 June 2004)

Abstract.

There are many industrial or plant waste products which can be examined for their probable use(s) medicinally.This work was carried out to examine the probable anti-bacterial effects of liquid pyrolysates obtained from oil palm nut and coconut shells. The antimicrobial activities liquid pyrolysates (obtained by destructive distillation), their fractions (obtained by successive partitioning of each pyrolysate with hexane, chloroform and ethyl acetate) were tested against clinical isolates and type bacteria. The anti microbial activities of the pyrolysates were compared with phenol.

All the organisms used showed remarkable sensitivities to the pyrolysates at 0.5ml (i.e.469mg of oil palm nut shell and 484mg of coconut shell). MIC and MBC of 0.02 ml (i.e.18.78mg of oil palm nut shell pyrolysate and 19.34mg of coconut shell pyrolysate) was obtained for the pyrolysates while their activities were observed to be comparable with phenol (0.01ml). The antibacterial activities of the samples appeared to be due to the components of their respective chloroform fractions which showed higher activities than the crude pyrolysates.

Thus, the pyrolysates obtained from oil palm kernel and coconut shells have a substantial antibacterial potential which are yet to be fully examined and made into appropriate useful products.

1. Introduction

Many agricultural waste products are usually regarded as useless and are therefore dumped or discarded indiscriminately in our society. If such materials are treated properly, it is possible to convert them to useful products, especially in formulating medicinal compounds. Oil palm nut and coconut shells are examples of such materials. With enormous oil palm plantations and palm oil production as well as extensive cultivation and use of coconut for various purposes in Nigeria, the annual yield of oil palm nut shell can be estimated at 2 million metric tones, while that on coconut shell is equally substantial. Although, palm kernel oil as well as coconut oil are well known to have pharmaceutical importance (Evans, 1989) while their shells are used by blacksmiths to generate energy to melt irons, the medicinal importance of the shells have received little or no attention.

Destructive distillation of these shells were reported to contain phenol and other phenolic derivatives like O-cresol, guaicol,2,6-dimethoxyphenol e.t.c. although in varying concentrations. (Chan *et al.*, 1976a,b, 1980). This present work was carried out to examine the effects of the pyrolysates yielded by these shells using destructive distillation equipment designed by Badmus (1999). The antimicrobial effects of the organic solvent fractions were also examined and compared with phenol.

2. Materials and Methods

After harvesting and processing of oil palm and coconut fruits, 1.81kg and 1.52kg of their respective shells were separately reduced to pieces by passing through a cracking machine. Each was subjected to destructive distillation by heating in a steel-made closed chamber for 1 1/4 hr using pyrotechnic distillation equipment designed by Badmus (1999) to yield charcoal and volatile compounds. These were collected after passing through a condenser. The condensates consisted of pyroligneous and tarry layers.

Partitioning of the pyrolysates with organic solvents

After a thorough mixing of the pyroligneous and the tarry layers, 500ml each of the pyrolysates (i.e. 469.3g of oil palm nut shell pyrolysate, 484g of coconut shell pyrolysate) was separately subjected to partitioning in succession using hexane, chloroform and ethyl-acetate.

The organic solvent fractions obtained were concentrated using rotavapor order to absolutely remove traces of the solvent. Each of them was measured, weighed and kept in a refrigerator until needed.

Bacterial isolates

All the bacteria used were obtained from Medical Microbiology laboratory, University of Benin Teaching Hospital. The organisms included *Pseudomonas aeruginosa* ATCC 27857, *Escherichia coli* NCTC 10418, *Staphylococcus aureus* NCTC6571, which were all type strains while *Klebsiella pneumoniae, P. aeruginosa, Salmonella typhimurium, E. coli and Bacillus subtilis* were isolated locally.

+ *corresponding author*

All the organisms were cultured on agar slant of buffered peptone water and sub cultured onto deoxychocolate citrate agar incubated at 37°C for 24h before use.

Determination of Antibacterial Activities

The agar-cup (7mm) diffusion method was used in determining the antibacterial activities of the samples using isosensitest agar medium. For sensitivity tests, 0.5ml each (939mg/ml for oil palm nut shell and 967mg/ml for coconut shell pyrolysates) of the pyrolysates were used to challenge the overnight cultures of the organisms while the MIC and MBC of the pyrolysates were determined using 0.01 – 0.16ml (0.01ml is equivalent to 9.39mg of oil palm nut shell pyrolysate and 9.67mg of coconut shell pyrolysate) and observed for 72hrs. The organic solvent fractions of the pyrolysates as well as their respective residues were tested at concentrations of 0.01, 0.015 and 0.02ml. For oil palm nut shell pyrolysate, 0.01ml is equivalent to 8.9mg of hexane fraction, 10.3mg of chloroform fraction, 9.9mg of ethyl acetate fraction and 8.7mg of residue. For the coconut shell pyrolysate, 0.01ml is 6.69mg of hexane fraction, 9.47 of chloroform fraction, 8.87mg of ethyl acetate fraction and 8.9mg of the residual fraction obtained after the partitioning. The antibacterial activities of the pyrolysates and the fractions were compared with phenol at the same concentrations.

3. Results and Discussion

At the end of the destructive distillation process, 1.81kg of oil palm nut shell yielded 654.0ml (613.8g, 36.09%) liquid pyrolysate while 1.52kg coconut shell produced 614ml (596.6g, 40.4%). It was observed that 0.5ml of each of the pyrolysates absolutely inhibited the growth of all the bacteria after incubation for 24h (Table 1). After 24h, a minimum inhibitory concentration of 0.02ml (i.e. 18.78mg of oil palm nut shell pyrolysate and 19.34mg of coconut shell pyrolysate) was observed for the two crude samples. This concentration was found to be same for the minimum bactericidal concentration observed after 72h (Tables 2 and 3).

Partitioning of samples with solvents of increasing polarities ensures separation of components of such samples based on their solubility potentials in the respective solvents used. Table 4 shows the effects of various fractions of the pyrolysates on the microorganisms. The hexane fractions as well as the residue obtained after the final partitioning with ethyl acetate did not exhibit antibacterial effects. In both samples, the chloroform fractions seem to be more potent than the crude pyrolysates since at a concentration of 0.01ml (i.e. 10.30mg of chloroform fraction of oil palm nut shell pyrolysate, 9.47mg of chloroform fraction of coconut shell pyrolysate) the fraction, particularly that of former, inhibited the growth of *P.aeruginosa* ATCC 27857, *E.coli* NCTC 10418, *S.aureus* NCTC6571 and *S. typhimurium*.

The antibacterial activities of the pyrolysates and their chloroform fractions were relatively similar to those observed in phenol 0.01 – 0.02ml. The higher activities observed in the chloroform fractions of the pyrolysates suggest that the bulk of the antibacterial components are contained in the fractions. However, the fact that the oil palm nut shell pyrolysate as well as its chloroform fraction showed higher activities than the coconut shell pyrolysate indicate the higher concentration of the antimicrobial constituents in the former than later. The similarities in the pattern of activities of the two pyrolysates as well as their organic solvent fractions suggest that the two of them contain similar constituents (as reported in literature) although the con-

Table 1: Antibacterial activities of oil palm nut shell (ons) and coconut shell (cs) pyrolysates at 0.5ml.

ORGANISMS	Oil palm nut shell pyrolysate (0.5ml of 939mg/ml)	Coconut shell pyrolysate(0.5ml of 967mg/ml)
Pseudomonas aeruginosa ATCC 27853	-	-
Escherichia coli NCTC 10418	-	-
Staphlococcus aureus NCTC 6571	-	-
Klebsiella pneumoniae	-	-
Staphlococcus aureus	-	-
Pseudomonas aeruginosa	-	-
Salmonella typhimurium	-	-
Escherichia coli	-	-
Bacillus subtilis	-	-

+ Growth at 0.5ml
- No growth at 0.5ml

Table 2: Minimum inhibitory concentrations (MIC) of crude oil palm nut shell (ons) and coconut shell (cs) pyrolysates.

ORGANISMS	Oil palm nut shell pyrolysate (939mg/ml)							Coconut shell pyrolysate (967mg/ml).						
	0.01	0.02	0.04	0.08	0.1	0.12	0.16	0.01	0.02	0.04	0.08	0.1	0.12	0.16
E.coli NCTC 10418	+	-	-	-	-	-	-	+	-	-	-	-	-	-
S. aureus NCTC 6571	+	-	-	-	-	-	-	+	-	-	-	-	-	-
P.aeruginosa ATCC 27853	+	-	-	-	-	-	-	+	-	-	-	-	-	-
**B.subtilis*	+	-	-	-	-	-	-	+	-	-	-	-	-	-
**P. aeruginosa*	+	-	-	-	-	-	-	+	-	-	-	-	-	-
**K.pneumoniae*	+	-	-	-	-	-	-	+	-	-	-	-	-	-
**S. aureus*	+	-	-	-	-	-	-	+	-	-	-	-	-	-
**E. coli*	+	-	-	-	-	-	-	+	-	-	-	-	-	-
**S.typhmurium*	+	-	-	-	-	-	-	+	-	-	-	-	-	-

* Clinical isolates.
\+ Growth
\- No growth

Table 3: Minimum bactericidal concentration (MBC) of oil palm nut shell and coconut shell pyrolysates after 72hrs .

ORGANISMS	Oil palm nut shell pyrolysate (939mg/ml)			Coconut shell pyrolysate (967mg/ml).		
	0.01	0.015	0.02	0.01	0.015	0.02
P.aeruginosa ATCC 27853	+++	+	-	+++	+	-
Esch. coli NCTC6571	+++	+	-	+++	+	-
S. aureus NCTC 6571	+++	+	-	+++	+	-
S. aureus	+++	+	-	+++	+	-
K. pneumoniae	+++	+	-	+++	+	-
S.typhimurium	+++	+	-	+++	+	-
P. aeruginosa	+++	+	-	+++	+	-
E. coli	+++	+	-	+++	+	-
B. subtilis	+++	+	-	+++	+	-

+++ Significant growth
\+ Insignificant growth
\- No growth

Table 4: Antibacterial effects of organic solvent fractions of oil palm nut shell (ons) and coconut shell (cs) pyrolysates.

Organisms	Conc. (ml)	Zones of inhibition (mm)										
		Crude		Hexane fraction		Chloroform fraction		Ethylacetate fraction		Residue		Phenol
		ons	cs	ons	cs	ons	cs	ons	cs	ons	cs	
P.aeruginosa ATCC 27857	0.01	-	-	-	-	15	-	18	-	-	-	21
	0.015	15	-	-	-	18	18	18	-	-	-	22
	0.02	20	20	-	-	20	20	12	-	-	-	24
E.coli NCTC 10418	0.01	-	-	-	-	15	10	-	-	-	-	23
	0.015	15	-	-	-	20	18	12	-	-	-	24
	0.02	20	20	-	-	22	22	18	20	-	-	25
S.aurens NCTC 6571	0.01	-	-	-	-	15	-	-	-	-	-	25
	0.015	15	-	-	-	18	10	15	-	-	-	27
	0.02	20	20	-	-	20	23	18	20	-	-	28
K.pneumoniae	0.01	-	-	-	-	-	-	-	-	-	-	22
	0.015	15	-	-	-	17	18	-	-	-	-	22
	0.02	20	18	-	-	20	20	-	16	-	-	23
S. aureus	0.01	-	-	-	-	-	-	-	-	-	-	25
	0.015	18	-	-	-	20	18	15	-	-	-	26
	0.02	22	20	-	-	22	22	18	20	-	-	28
P.aeruginosa	0.01	-	-	-	-	-	-	-	-	-	-	21
	0.015	10	-	-	-	10	10	-	-	-	-	22
	0.02	20	20	-	-	20	18	-	-	-	-	24
S.typhimurium	0.01	-	-	-	-	15	10	10	-	-	-	24
	0.015	-	-	-	-	18	18	15	-	-	-	25
	0.02	20	18	-	-	20	20	18	-	-	-	26
E.coli	0.01	-	-	-	-	-	-	-	-	-	-	20
	0.015	20	-	-	-	20	18	18	-	-	-	23
	0.02	22	20	-	-	22	22	18	20	-	-	25
B.subtilis	0.01	-	-	-	-	-	-	-	-	-	-	12
	0.015	-	-	-	-	-	18	-	-	-	-	14
	0.02	20	19	-	-	20	20	16	20	-	-	14

centrations of each of such components may vary. Many plants as well as their secondary metabolites have been reported to exhibit inhibitory effects on bacterial growth and proliferations. For instance, *Garcinia cola, Aframomum melegueta, Nauclea latifolia,* etc., have been investigated and confirmed to have significant inhibitory effects on many bacteria as well as fungi although the constituents responsible for the activities vary (Iwu *et al.*, 1999).

The antibacterial activities of the pyrolysates reported here are of importance as the shells from which they were obtained were hitherto not known to be of medicinal value. As their distillation requires simple locally made equipment, this research work suggests their probable incorporation into soaps and creams for dermatological infections. Further works are being considered to ascertain the actual nature of the constituents of both pyrolysates and their respective chloroform fractions.

REFERENCES

Badmus, G.A., 1999. Development of the Shell Pyrotechnic Distillation Equipment Prototype for the Extraction of Gas, Char and Liquid (pyrolysate) from Shell, Report to the Nigerian Institute for Oilpalm Research Directorate, NIFOR.

Chan, K.C., Goh, S.H. and Tan, W.I., 1976. Utilisation of Oilpalm Nut Shell, *The Planter*, 52: 127.

Chan, K.C., Goh, S.H. and Tan, W.I., 1980. Destructive Distillation of Oilpalm (*Elaeis guineensis*) Nut Shell, Analysis of Phenolic Components, *Malaysian J. Sci.* 6(B): 139-144.

Chan, K.C., Goh, S.H., Tan, W.I., Tang, T.S. and Toh, H.T., 1976. Production and Use of activated Carbon from Oilpalm Nut Shells, British Patent- Provisional Specification No. 46861/76.

Evans, W.C., 1989. In:Trease and Evans Pharmacognosy, 13th Edition. Bailliere Tindall, London. 335pp.

Iwu, M.W., Duncan, A.R. and Okunji, C.O., 1999. New Antimicrobials of Plant Origin. In Perspectives on New Crops and New Uses. J. Janick (Ed.), ASHS Press, Alexandria, VA. pp. 457-462.