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Antifungal evaluation of shell pyrolysates of oil palm (*Elaeis guineensis* Jacy) and coconut (*Cocos nucifera* L.)

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Abstract
The medicinal values of oil palm and coconut shells are not much known in herbal medicine and the two mostly constitute waste products. The antifungal effects of steam-distilled pyrolysates obtained from the two shells and the respective organic solvent fractions were evaluated against human pathogenic fungi *Trichophyton megnini*, *T. rubrum* and plant pathogenic fungi namely *Penicillium*, *Aspergillus*, *Fusarium*, *Trichoderma* and *Collectotrichum* at concentrations of 0.5-1.5mL. The two pyrolysates completely inhibited the growth of the organisms at 1-1.5mL whereas the growth inhibitory effects of the chloroform and ethyl acetate fractions were observed at 0.5 mL. The MICs of both fractions range between 0.06-0.08 mL for almost all the microorganism. The pyrolysates, chloroform and ethyl acetate fractions showed promising antifungal activities.

Keywords: Antifungal; Coconut shell; Oil palm shell; Pyrolysates.

INTRODUCTION

Oil palm (*Elaeis guineensis*) and coconut (*Cocos nucifera*) are among the crop plants widely cultivated in different parts of the world for various domestic, industrial and pharmaceutical purposes. The palm oil and the palm kernel oil obtained from *E. guineensis* are used in cosmetic and pharmaceutical industries in the preparation of creams and suppositories (Evans, 1989) respectively. Coconut oil is also known to be used locally in the manufacture of some creams. Probable medicinal applications of the shells obtained from these two plants have received little attention, although locally, they are often used by blacksmith for combustion.

Liquid pyrolysates obtained from the destructive distillation of the shells were reported to yield liquid pyrolysates analyzed and discovered to contain phenol and its various derivatives like *o*-cresol, guaicol, and 2,6-dimethoxy-phenol in varying concentrations (Chan *et al.*, 1976a, b; 1980). In our laboratory, we have also reported the remarkable antibacterial potentials of these two pyrolysates against some type specimens and clinical isolates of both Gram positive and Gram negative bacteria (Ukoh *et al.*, 2004). However, there is the need to further explore the probable effects the materials could have on both human and plant pathogenic fungi in order to establish their

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probable spectrum of activities. This step becomes necessary in view of the current world-wide interest in the use of plant-derived products particularly those with antimicrobial activities as traditional antibiotics are becoming ineffective due to disease resistance and emergence of new diseases some of which remain intractable (Cowan, 1999).

EXPERIMENTAL

Sources and extraction of oil-palm nut and coconut shell pyrolysates. The shells of the two plants were obtained at Nigeria Institute for Oil Palm Research (NIFOR), Benin City, Nigeria where 1.81kg and 1.52kg of the respective shells were reduced to pieces using cracking machine. The shells were separately subjected to destructive distillation by heating in a steel-made closed chamber for 1 ¼ h using pyrotechnic distillation equipment designed by Badmus (1999) to yield charcoal and volatile compounds. After passing through a condenser, the volatile compounds formed liquids consisted of pyroligenous and tarry layers as reported earlier (Ukoh *et al*., 2004)**.**

Partitioning of the pyrolysates with organic solvents. This was carried out as described earlier (Ukoh *et al*., 2004). Briefly, 500 mL each of the pyrolysates obtained was subjected to partitioning using n-hexane, chloroform and ethyl acetate in succession. The fractions were concentrated using rotary evaporator to remove all the traces of the solvents. Each concentrate was measured, weighed and preserved in a refrigerator until required.

Sources of the fungal specimens. The human pathogenic fungi *Trichophyton megnini* and *T. rubrum* were collected from the Pathology Department, University of Benin Teaching Hospital Benin City while the plant pathogenic fungi namely *Penicillium, Aspergillus, Fusarium, Trichoderma* and *Collectotrichum* species were collected from the Plant Pathology Department, Rubber Research Institute of Nigeria (RRIN) Iyanomo, Benin City. The *Collectotrichum* species was collected from an infected leaf, while other organisms were obtained from soil samples obtained at a rubber plantation at the Institute. Stock solutions of soil samples containing the organisms as well as $1mm^2$ sizes of *Collectotrichum* infected leaf prices were inoculated on Sabouraud agar containing 0.2ml of 2% w/v streptomycin solution to prevent bacterial growth and contamination. The plates were incubated for 96 h at room temperature after which each organism was sub cultured thrice to obtain pure strains that were eventually used.

Determination of Antifungal Activities of the Pyrolysates and their organic solvent fractions. The agar diffusion method was used in determining the antifungal activities of the pyrolysates and their organic solvent fractions using Sabouraud agar. For sensitivity tests, 0.5, 1.0, 1.5 mL each of the pyrolysates were differently mixed with 20 mL of the agar at 50^0C. For the oil palm nut shell pyrolysate, 1 mL is equivalent to 939 mg whereas for coconut shell pyrolysate, 1 mL is equivalent to 967 mg. After cooling to 37^0C, each plate was inoculated with an organism each using streaking method. The sensitivities of the organisms to all the organic solvent fractions were also determined using the same method. For oil palm nut shell pyrolysate, 1ml of the hexane fraction is equivalent to 890mg; 1ml of the chloroform fraction is 1030mg; 1ml of the ethyl acetate fraction is 990 mg while 1ml of the residual fraction is 870 mg. For the coconut shell pyrolysate; 1ml is equivalent to 669mg for hexane fraction; 947mg for chloroform fraction; and 890mg for the residual fraction. Control plates did not contain any of the test samples but were streaked with a fungus each. All the plates were covered and incubated of room temperature for 96 h. The results were

observed as inhibition or no inhibition to the organism growth.

Determination of the minimum inhibitory concentrations. Following the procedures described above, the minimum inhibitory concentrations of the chloroform, ethyl acetate and the residual fractions of the two pyrolysates against all the organisms were carried out using 0.02 – 0.2ml of each sample. After two weeks of observation, all the organisms treated with 0.2ml of chloroform and ethyl acetate fractions were re-cultured on fresh media to ascertain the fungicidal or fungistatic nature of the samples.

RESULTS AND DISCUSSION

After 1½ h 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%). The two were observed to have tar odour which was more intense in oil palm nut shall than the coconut shell prostates. At the end of 96 h, the crude prostates did not inhibit the growth of most of the organisms at 0.5ml (equivalent to 496.5mg for oil palm nut shell pyrolysate; 483.5mg for coconut shell pyrolysate). However, complete inhibition of growth was observed in all the fungi on the media containing at 1ml and 1.5 ml each of the pyrolysates.

The hexane fractions of the two pyrolysates did not show remarkable inhibition to the growth of the organisms between 0.5 – 1.5ml. The pattern of growth particularly in 0.5ml treated organisms was high and as profuse as the controls. The chloroform and the ethyl acetate fractions of the two samples completely inhibited the fungal growth at 0.5-1.5 ml while the growth inhibitory effects of the residual fractions were remarkable at 1-1.5ml (Table 1).

The results of the tests for minimum inhibitory concentration using the chloroform, ethyl acetate and the residual fractions revealed the potency of the chloroform fractions of the two samples over the other organic solvents and the residual fractions. *Fusarium, Collectotrichum* and *Trichoderma* species seemed to be more susceptible to both the chloroform and ethyl acetate fractions at 0.04ml (i.e. 41.2mg and 39.6mg for chloroform and ethyl acetate fractions of oil palm nut pryolysate respectively; 37.88mg and 35.48mg for chloroform and ethyl acetate fractions of coconut shell pyrolysates respectively) than other fungi. However, complete growth inhibitions to all the organisms were observed at 0.08ml for the chloroform fractions and 0.2ml for the ethyl acetate fractions of the two samples. The residual fractions did not show remarkable activities at the tested concentrations (Table 2).

The organisms recultured from the plates treated with 0.2ml of chloroform or ethyl acetate fractions did not show any sign of growth whereas the *Aspergillus, T. megnini, T. rubrum* and *Trichoderma* species obtained from the residual fractions showed profuse growth. The two pyrolysates were earlier observed to exhibit antimicrobial activities against both Gram positive and Gram negative bacteria (Ukoh *et al.*, 2004) although at lower concentrations of 0.02 ml for both pyrolysates. Higher effective concentrations of the two prostates observed in this investigation suggest the variations in the structural components between bacteria and fungi and hence varying susceptibility or resistance to drugs.

Chloroform fractions of the two samples were again established to possess higher activities than other organic solvent fractions of the pyrolysates. Based on the relatively similar growth inhibitory activities, it is possible both the chloroform and ethyl acetate fractions contain closely related constituents.

Variations in resistance or susceptibilities to the pyrolysates and their

organic solvent fractions were also observed to exist between human and plant pathogenic fungi used. While the growth of all the plant pathogenic fungi were inhibited separately by 0.06ml of the chloroform fractions of the two samples (i.e.61mg of the chloroform fraction of oil palm unit shell; 58.68mg of the chloroform fraction of coconut shell), the growth of the human pathogenic fungi *T. megnini* and *T. rubrum* were completely inhibited at 0.08ml of the fractions (i.e. 82.4mg of chloroform fraction of oil palm unit shell, 75.76mg of coconut palm nut shell).

Table 1: Sensitivities the fungal organisms to the crude pyrolysates of *C. nucifera* and *E. guineensis* and their organic solvent fractions

Pyroly Sates	Vol (ml)	*Penicill-ium*		*Aspergi-llus*		*Fusarium*		*Trichophanton megnini*		*T. rubrum*		*Collecto-trichum*		*Tricho-derma*	
		Ons	Cs	Ons	cs	Ons	Cs	Ons	Cs	Ons	Cs	Ons	cs	Ons	cs
Crude	0.5	++	++	++	++	++	++	++	++	+	+	-	-	+	+
	1.0	-	-	-	-	-	-	-	-	-	-	-	-	+	+
	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hexane fraction	0.5	++	++	++	++	+	+	++	++	+++	+++	+	+	+	+
	1.0	+	+	+	+	+	+	+	+	++	++	-	-	+	+
	1.5	-	-	+	+	-	-	+	+	+	+	-	-	-	-
Chloro-form fraction	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethyl acetate fraction	0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Ons-oil palm nut shell pyrolysate; Cs- Coconut shell pyrolysate; - = growth inhibition; + = fungal Growth;

Table 2: Minimum inhibitory concentrations (MIC) of the organic solvent fractions of oil palm and coconut pyrolysates to fungal growth.

Pyroly-sates	Vol (ml)	*Pen.* Ons	cs	*Asp.* Ons	cs	*Fus.* Ons	cs	*T. meg* Ons	cs	*T. rub* Ons	cs	*Coll.* Ons	cs	*Trich.* Ons	cs
$CHCl_3$	0.02	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	0.04	+	+	+	+	-	-	+	+	+	+	-	-	-	-
	0.06	-	-	-	-	-	-	+	+	+	+	-	-	-	-
	0.08	-	-	-	-	-	-	-	-	-	+	-	-	-	-
	0.20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
EtOAc	0.02	+	+	+	+	+	+	+	+	+	+	+	-	-	+
	0.04	+	+	-	-	-	-	+	+	+	+	+	-	-	-
	0.06	-	-	-	-	-	-	+	+	+	+	+	-	-	-
	0.08	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	0.20	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Residue	0.02	+	+	+	+	+	+		+		+	+	+	+	+
	0.04	+	+	+	+	+	+		+		+	+	+	+	+
	0.06	+	+	+	+	+	+		+		+	+	+	+	+
	0.08	+	-	+	+	-	-		+		+	-	-	+	+
	0.20	+	-	+	+	-	-		+		+	-	-	+	+

Ons= *E. guineensis* pyrolysate Cs = *C. nucifera* pyrolysate - = Growth inhibition + = Fungal growth

The inability of all the organisms to exhibit growth after re-culturing the 0.2ml treated plates (those treated with chloroform and ethyl acetate fractions) showed that the samples have fungicidal and not fungistatic effects. The similarities in the activities of the two pyrolysates and their organic solvent fractions suggest that the two of them relatively have similar constituents as

observed in literature reports and the phytochemical results (not shown).

Although many plants like *Nauclea latifolia*, *Aframomum melegueta*, *and Garcinia cola* have been reported to show significant inhibitory effects against some micro-organism, (Iwu *et al*., 1999), the antifungal effects of the pyrolysates and their organic solvent fractions reported here are of importance in that the shells from which they were obtained were hitherto of little or no medicinal values.

Efforts are being made to establish the probable effects of pharmaceutical adjuvant on the antimicrobial activities of the two pyrolysates and also to ascertain how they can be deodorized to remove the charred smell.

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