Evaluation of Extracts of *Jatropha curcas* and *Moringa oleifera* in Culture Media for Selective Inhibition of Saprophytic Fungal Contaminants

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Most fungi occur in nature and utilize simple sources of carbohydrates and nitrogen for growth. Sabouraud's dextrose agar has been an ideal medium for primary isolation of fungi from clinical specimens, but for specimens from nonsterile sites or heavily contaminated ones, it has been necessary to include inhibitory substances such as antibiotics like chloramphenicol (antibacterial) and cycloheximide (antifungal). The problems we have in the our laboratory owing to frequent contamination of cultures and the delays in the procurement of cycloheximide have stimulated a search for alternatives in our local environment to enhance effective laboratory diagnoses of fungal infections. Purified extracts of the leaves and bark of *Jatropha curcas* and *Moringa oleifera* (common plants in our locality) were tested against clinical isolates of fungi at various concentrations

to determine the minimum inhibitory concentration at which common fungal contaminants are inhibited, without affecting the growth of the pathogenic fungi sought for. At a concentration of 0.75 mg ml⁻¹ contaminants were totally inhibited by the leaf extracts. The bark extracts did not inhibit any fungus even at higher concentrations. From the results it was evident that the leaf extracts of both plants have potentials for use as inhibitory substances in culture media against contaminant fungi including *Aspergillus* spp., *Penicillium* spp., etc. *J. curcas* and *M. oleifera* are very common plants in our locality. They can be obtained at almost no cost and at any time needed. The benefits of these findings to mycology laboratories in a developing country are enormous. J. Clin. Lab. Anal. 23:161–164, 2009. © 2009 Wiley-Liss, Inc.

Key words: *Jatropha*; *Moringa*; selective inhibition; contaminant fungi

INTRODUCTION

All fungi require specific elements for growth and reproduction but most are difficult to isolate from infected tissues owing to the rapid and often antagonistic development of contaminant fungi, which have faster growth rates. The incorporation of inhibitory substances such as cycloheximide (antifungal) and chloramphenicol (antibacterial) in isolation media to achieve selective exclusion of contaminant fungi has often been necessary. Cycloheximide has been the standard agent used for the control of fungal contaminants in our laboratory for several years but its procurement has also been problematic. The high cost

of this antibiotic, coupled with long delays before delivery to our laboratory, has been a major setback in effective and prompt diagnosis. Faced with a seemingly endless contention with contaminant fungi, a search for a readily available and affordable alternative was thus stimulated.

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Jatropha curcas (physic nut, purging nut) is known in Nigeria by various local names as butuje, lapapa, ologbo and *Moringa oleifera* (horse radish, benzolive, drumstick) is known locally as zogale, ewe ile, ikwe oyibo. Both are common plants in locality. These two multipurpose hedge plants are widely distributed all over the world, especially in the tropics. *J. curcas* (family Euphorbiaceae) is a drought-resistant small tree or large shrub (up to 5 m tall), which probably originated from Mexico and Central America. *M. oleifera* (family Moringaceae) is a perennial soft wood native to the Sub-Himalayan tracts of India, Pakistan, Bangladesh, and Afghanistan (1–3). These plants are now cultivated in many locations in the tropics where they are known for traditional medicinal and industrial uses.

Numerous investigations have been carried out to determine the antimicrobial and medicinal properties of various parts of these plants (4–7) but little attention has been given to their use in mycological culture media for selective inhibition of fungal contaminants. This study attempted to evaluate the extracts of leaves of these plants in this respect.

MATERIAL AND METHODS

Plant Materials

The plant materials (leaves and barks) were obtained from the permanent site quarters of the University of Jos and the New Layout, Farin Gada, Jos, Nigeria, and authenticated by Ampitan Titus Ayodele (Lecturer, forest biology) of the Federal College of Forestry, Jos, Nigeria. The plant materials were dried at room temperature for 2 weeks and then ground into fine powder in a blender (Philips, Mexico City, Mexico). A total of 200 g (leaves) and 60 g (bark) of powder were steeped in 500 and 250 ml of ethanol, respectively, for 48 hr at room temperature and then filtered by a vacuum pump and an aspirator. The filtrate was distilled using a vacuum distiller. All the solvent was removed, leaving the extracts, which were then placed in desiccators to remove moisture.

The extracts were fractionated by accelerated gradient chromatography (AGC). All separation steps were monitored by thin layer chromatography (TLC). Five separate fractions were obtained and labeled as A–E for *J. curcas* and four, labeled as A–D for *M. oleifera*.

Organisms

The test organisms included clinical isolates and contaminants obtained from the Mycology Laboratory of the Medical Microbiology Department of the University of Jos, Jos, Nigeria. They included *Trichophyton soudanense*, *T. verrucosum*, *T. tonsurans*, *Cladosporium*

sp., *Microsporum ferrugineum*, *Candida albicans*, *Aspergillus terreus*, *A. niger*, and *Penicillium expansum*.

Preparation of Selective Medium

Preliminary evaluation of the activity of the bark extracts of both plants yielded negative results; therefore, further work was done only with the leaf extracts. Graded concentrations of each extract, 0.25, 0.5, 0.75, and 1.0 mg ml⁻¹, in the medium were constituted as follows: 0.25 g of extract was dissolved in 10 ml of methanol (*Jatropha* extracts) or 10 ml of 2% tween 80 (*Moringa* extracts). This was then added to 100 ml of Sabouraud's dextrose agar to obtain a final concentration of 0.25 mg ml⁻¹ of extract in the medium, dispensed into 18 × 180 mm test tubes (Baiel, Tokyo, Japan) and sterilized at 121°C in the autoclave for 15 min, and then slanted to cool. Slants of sabourad¹⁵ dextrose agar (SDA) devoid of extracts were prepared as controls. Slants of SDA containing 0.5 mg ml⁻¹ of cycloheximide were also set up as standards for comparison. The above procedure was repeated for all the concentrations.

Evaluation of Selective Inhibition

The method of Brigham et al. (8) was slightly modified. Using a flamed wire loop, a ring (5 mm) was made in the center of each slant of the leaf extract–SDA medium. It was then seeded with approximately 4 mm plug of fungal hyphae from a pure culture of a test organism. Each organism was tested on all concentrations. A control was included for each. Hyphal growth was observed 48 hr after incubation and subsequently at 72-hr interval for 3 weeks. Growth was determined by extension of hyphae outside the borders of the ring, and the extent indicated by the plus (+) sign preceding the numbers (1–5) depending on the distance (in millimeters) from the ring. The absence of growth (no visible extension of hyphae or inward shrinking of inoculum in the ring) was indicated by the minus (–) sign.

RESULTS

The extracts of the bark of both plants had no inhibitory effect on the fungi. At the lowest concentration (0.25 mg ml⁻¹) of the leaf extracts of both plants there was no observable inhibition of growth. At the highest concentration of extracts of both plants there was poor growth, no sporulation. Generally, the leaf extracts of *Jatropha* exhibited stronger antifungal activity than *Moringa* extracts. The concentration of extracts at which growth of contaminants was completely inhibited was 0.75 mg ml⁻¹ for *Jatropha* and 1.0 mg ml⁻¹ for *Moringa* as given in Table 1. The effects

TABLE 1. Effect of Various Concentrations of (a) SDA+Jatropha Extract Medium and (b) SDA+Moringa Extract Medium on Fungal Growth at 21 Days

						Cycloheximide
Fungi	0.25 mg ml ⁻¹	0.5 mg ml ⁻¹	0.75 mg ml ⁻¹	1.0 mg ml ⁻¹	Control	0.5 mg ml ⁻¹
(a)						
<i>Microsporum ferrugineum</i>	5+	5+	5+	1+	5+	5+
<i>Trichophyton soudanense</i>	5+	5+	5+	1+	5+	5+
<i>Trichophyton tonsurans</i>	5+	5+	4+	1+	5+	5+
<i>Trichophyton verrucosum</i>	5+	5+	5+	1+	5+	5+
<i>Candida albicans</i>	5+	5+	3+	1+	5+	5+
<i>Cladosporium</i> sp.	5+	4+	4+	–	5+	4+
<i>Aspergillus terreus</i>	5+	2+	–	–	5+	1+
<i>Aspergillus niger</i>	5+	2+	–	–	5+	1+
<i>Penicillium expansum</i>	5+	2+	–	–	5+	1+
(b)						
<i>Microsporum ferrugineum</i>	5+	5+	5+	3+	5+	5+
<i>Trichophyton soudanense</i>	5+	5+	5+	3+	5+	5+
<i>Trichophyton tonsurans</i>	5+	5+	5+	3+	5+	5+
<i>Trichophyton verrucosum</i>	5+	5+	5+	3+	5+	5+
<i>Candida albicans</i>	5+	5+	5+	3+	5+	5+
<i>Cladosporium</i> sp.	5+	5+	5+	2+	5+	4+
<i>Aspergillus terreus</i>	5+	4+	2+	1+	5+	1+
<i>Aspergillus niger</i>	5+	4+	4+	1+	5+	1+
<i>Penicillium expansum</i>	5+	4+	2+	1+	5+	1+

5+, Maximum growth; 1+, minimum growth.

TABLE 2. Effects of (a) Fractions (A–E) of *Jatropha curcas* at Various Concentrations and (b) Various Fractions (A–D) of *Moringa oleifera* at Different Concentrations on the Growth of Fungi

Fungal isolates	0.25 mg ml ⁻¹					0.5 mg ml ⁻¹					0.75 mg ml ⁻¹					1.0 mg ml ⁻¹				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
(a)																				
<i>Trichophyton verrucosum</i>	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2	4	2	3
<i>Microsporum ferrugineum</i>	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	1	2	2	1	3
<i>Trichophyton soudanense</i>	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	2	2	2	1	2
<i>Candida albicans</i>	5	5	5	5	5	5	5	5	5	5	2	2	5	4	5	1	1	1	1	1
<i>Trichophyton violaceum</i>	5	4	4	5	5	4	3	3	4	5	5	3	3	2	3	1	0	0	0	0
<i>Penicillium expansum</i>	5	4	4	3	5	3	2	4	3	5	1	0	1	0	1	0	0	0	0	0
<i>Cladosporium</i> sp.	5	4	4	4	5	4	4	5	3	5	2	2	4	3	4	0	0	0	0	0
<i>Aspergillus terreus</i>	4	4	4	3	5	3	2	4	2	5	1	0	1	0	1	0	0	0	0	0
(b)																				
Fungal isolates	0.25 mg ml ⁻¹				0.5 mg ml ⁻¹				0.75 mg ml ⁻¹				1.0 mg ml ⁻¹							
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D				
<i>Trichophyton verrucosum</i>	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5				
<i>Microsporum ferrugineum</i>	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	3				
<i>Trichophyton soudanense</i>	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5				
<i>Candida albicans</i>	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5				
<i>Penicillium expansum</i>	5	5	5	5	5	5	5	5	5	5	5	5	5	5	3	4				
<i>Cladosporium</i> sp.	5	5	5	5	5	5	5	5	5	5	5	5	4	1	0	0				
<i>Aspergillus terreus</i>	5	5	5	5	5	5	5	5	5	5	5	5	4	4	4	4				

5, Maximum growth; 1, minimum growth; 0, no growth.

of different fractions of the plant extracts on the test fungi are presented in Table 2. The growth rate of the fungi in the SDA+leaf extract medium was comparable

to that in SDA+cycloheximide medium (Table 1). The thick black waxy fraction (D) was the most active against the test fungi, followed by the oily fraction (A).

The oily fractions of the plant extract were particularly active against *C. albicans* (Table 2).

DISCUSSION

The minimum inhibitory concentration (MIC) at which the extracts of *J. curcas* and *M. oleifera* prevented the growth of laboratory contaminants, while allowing good growth of clinical pathogens, was determined. The enhancement of growth of some fungi and the inhibition of others, at different concentrations of extracts of these plants, have been reported by other workers (5,7,9,10). At a concentration of 0.75 and 1.0 mg ml⁻¹ of the extracts of *Jatropha* and *Moringa*, respectively, the contaminants, *A. niger*, *A. terreus*, and *P. expansum*, were inhibited. Dahot (5) reported inhibition of these fungi by *Moringa* fractions at MICs greater than 100 µg ml⁻¹. The dermatophytes exhibited luxuriant growth at these concentrations. *C. albicans* was fairly inhibited by the oily fractions (A) of both plants. The oil of the seed extracts of these plants has been found to exhibit strong antifungal activity, particularly against yeasts (3). Fraction D (black, waxy) was the most effective in enhancing the growth of dermatophytes, whereas preventing that of contaminants. This fraction at higher concentration also had relatively stronger inhibitory effect on all the fungi. *M. oleifera* fractions were less active against fungal contaminants, especially *A. niger*, which is among the most frequently encountered contaminants in our laboratory.

Comparable results with that of cycloheximide for selective inhibition of contaminants were obtained, particularly with *Jatropha* extracts, although at a slightly higher concentration. *J. curcas* and *M. oleifera* are very common plants in our locality. They can be obtained at almost no cost and at any time needed. Their use as alternatives to cycloheximide in culture media for selective inhibition of contaminants may as well be considered.

Further purification of the extracts is necessary to determine the actual components of individual fractions. There is need for further investigation to determine the

shelf life of these fractions and their effectiveness after long storage.

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REFERENCES

1. Heller J. Physic nut. *Jatropha curcas* L. Promoting the conservation and use of underutilized and neglected crops. 1. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, 1996.
2. Church World Service. *Moringa oleifera*—The miracle tree [www document], 2000. Available from: <http://www.churchworldservice.org/moringa/index.html>.
3. Fahey JW. *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic and prophylactic properties. Part 1. Trees for life [www document], 2005. Available from: <http://www.TFLjournal.org/article.php/2005>.
4. Lans C, Harper T, Georges K, Bridgewater E. Medicinal and ethno veterinary remedies of hunters in Trinidad. BMC Complement Altern Med 2001;1:10.
5. Dahot MU. Antimicrobial activity of small proteins of *Moringa oleifera* leaves. J Islamic Acad Sci 1998;11(1):27–32.
6. Ogbebor AO, Adekunle AT, Enobakhare DA. Inhibition of *Coleotrichum gliosporoides* (Penz) Sac. causal organism of rubber (*Hevea brasiliensis* Muell Arg.) leaf spot using plants extracts. Afr J Biotechnol 2007;6:213–218.
7. Roland DA, Patrick UU, Thomas AF, Michael JH, Scott K, Bret JS. Partial purification and properties of lipase from germinating seeds of *Jatropha curcas* L. J Am Oil Chem Soc 2002;79:1123–1126.
8. Brigham LA, Michaels PJ, Flores HE. Cell-specific production and antimicrobial activity of naphthoquinones in roots of *Lithospermum erythrorhizon*. Plant Physiol 1999;119:417–418.
9. Aiyelagbo OO, Adesogan EK, Ekundayo O, Adeniyi BA. The antimicrobial activity of roots of *Jatropha prodigrica* (Hook). Phytother Res 2000;14:60–62.
10. Ayanbimpe GM, Fagbemi O. Antifungal activity of extracts from a hedge plant—*Jatropha curcas* on some pathogenic fungi. Niger Ann Nat Sci 2005;6:18–22.