Pharmacological properties of Moringa oleifera. Part 1. Preliminary screening for antimicrobial activity

ARTICLE *in* JOURNAL OF ETHNOPHARMACOLOGY · AUGUST 1991

Impact Factor: 3 · DOI: 10.1016/0378-8741(91)90078-R · Source: PubMed

Journal of Ethnopharmacology, 33 (1991) 213—216
Elsevier Scientific Publishers Ireland Ltd.

Pharmacological properties of *Moringa oleifera*. 1: Preliminary screening for antimicrobial activity

Armando Cáceres[a,b], Ofyluz Cabrera[a], Ofelia Morales[b], Patricia Mollinedo[a] and Patricia Mendia[b]

[a]*Center for Mesoamerican Studies on Appropriate Technology (CEMAT), P.O. Box 1160 and* [b]*Faculty of Chemical Sciences and Pharmacy, University of San Carlos (USAC), Guatemala City (Guatemala)*

(Accepted December 18th, 1990)

The antimicrobial activities of *Moringa oleifera* leaves, roots, bark and seeds were investigated in vitro against bacteria, yeast, dermatophytes and helminths pathogenic to man. By a disk-diffusion method, it was demonstrated that the fresh leaf juice and aqueous extracts from the seeds inhibit the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* and that extraction temperatures above 56°C inhibited this activity. No activity was demonstrated against four other pathogenic Gram-positive and Gram-negative bacteria and *Candida albicans*. By a dilution method, no activity was demonstrated against six pathogenic dermatophytes. A method was standardized for studying the effect of aqueous extracts on *Ascaris lumbricoides* eggs, but no activity was exibited by any part of the tree in contrast to *Chenopodium ambrosioides* leaf extracts.

Key words: antimicrobials; *Moringa oleifera;* ben oil.

Introduction

Infectious diseases are the main causes of morbidity and mortality in the developing world, especially in children. Traditional medicine is an important source of health and a valuable heritage of the native people, but scientific validation of popular uses is needed in order to achieve a wider application and endorsement by the medical profession.

Moringa oleifera Lam. (family, Moringaceae) is a tree well adapted to most of the tropical world. Its multiple medicinal uses (Council of Scientific Research, 1962; Ramachandran et al., 1980) and coagulating properties (Jahn, 1986) are well known, although the chemistry and pharmacology of the different parts of the plant are little known.

This article reports the in vitro screening of the activity of aqueous extracts of five parts of the tree against several microorganisms pathogenic to man such as Gram-positive and Gram-negative bacteria, yeasts, dermatophytes and helminths.

Correspondence to: Armando Cáceres, Center for Mesoamerican Studies on Appropriate Technology (CEMAT), P.O. Box 1160, Guatemala City, Guatemala.

Material and Methods

Ethnobotanical findings and microorganism selection

In an ethnobotanical survey of the uses of *M. oleifera* in Guatemala, it was demonstrated that the main medicinal uses of this plant are for the treatment of infectious diseases of the skin and mucosa (boils, spots, ringworm rash), digestive system (stomach pains, diarrhea) and respiratory tracts (fever, influenza, cold) (Cáceres et al., unpublished). Similar uses have been reported in India (Council of Scientific and Industrial Research, 1962; Ramachandran et al., 1980; Pushpangadan and Atal, 1986), Pakistan (Dastur, 1977) and Sudan (Jahn et al., 1986).

From this field and from literature information, 12 microorganisms were chosen due to their frequency of isolation from clinical material in Guatemala. Five groups of pathogenic microorganism were selected: Gram-negative (enteropathogenic *Escherichia coli* (ATCC 25922), *Shigella flexneri* (ATCC 12022), *Pseudomonas aeruginosa* (ATCC 27853)) and Gram-positive bacteria (*Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes*

0378-8741/$03.50

(INCAP 8700200)); yeast (*Candida albicans* (CQ/RM-3346)), dermatophytes (*Epidermophyton floccosum* (CQ/RM-936), *Microsporum canis* (IGSS-715), *Microsporum gypseum* (CQ/RM-671), *Trichophyton mentagrophytes* (CQ/RM-1021), *Trichophyton rubrum* (IGSS-174)) and a helminth (freshly isolated *Ascaris lumbricoides* eggs)

Plant materials and extract preparations

All the vegetal material was obtained in February-March of 1989 from Estanzuela, Zacapa, in the dry northeastern part of the country. Botanical samples were identified at the Faculty of Agronomy (USAC) and voucher samples deposited at CEMAT-FARMAYA Ethnobotanical Herbarium (Voucher No. CF-135). Bark, flowers, leaves, root and seeds were collected, washed, dried in the dark at 37°C, and powdered. Freshly collected leaves were used for the preparation of juices.

Extracts were prepared in a manner similar to traditional usage. In this study, up to five different temperatures and extraction methods were used. For maceration, three different settings were tried: at room temperature (25°C) for 6 days, at 37°C for 5 days and at 56°C for 4 days. For percolation using dry leaf material, the setting was 95°C for 60 min. Fresh leaves were mashed and the juice filtered. All extracts were sterilized by Millipore filtration (0.22 μm). Filter paper disks 6 mm in diameter and 0.6 mm thick were impregnated with 100 μl of the extract, dried in a laminar flow hood, stored at 4°C, and used within 5 days; for longer storage, samples were kept at −20°C for 30 days. Extracts for antidermatophyte screening were concentrated in a rotavapor to 1 g/ml.

Antimicrobial bioassays

For antibacterial and anticandidal activity, a disk diffusion method was employed utilizing extract-impregnated paper disks. This methodology has been previously used for demonstration of activity against microorganisms producing infection of the skin and mucosa (Cáceres et al., 1987; Girón et al., 1988), digestive system (Cáceres et al., 1990a) and respiratory tract (Cáceres et al., 1991a). All tests were repeated seven times using Müller-Hinton agar plates, incubated for 24 h at 35°C and the inhibition zones measured in millimeters. Distribution of disks was at random, and results were analyzed by one-way variance with 95% confidence (α 0.05).

For antidermatophyte activity a dilution method was adapted for concentrated aqueous extracts following the methods of Lam (1983), consisting of 1 ml of Sabouraud broth, 1 ml of an aqueous extract and 1 cm^2 of dermatophyte-grown agar followed by incubation at 24°C for 21 days. The test was done in quintuplicate and then interpretated according to the growth at the surface and bottom. Comparison was done with a plant that had previously shown positive antidermatophyte activity (*Solanum nigrescens* leaves). Using this methodology, a group of 44 plants used for the treatment of dermatophytoses was previously screened against six pathogenic dermatophytes, and 22 plants exhibited some antimycotic activity (Cáceres et al., 1991b).

For antihelminthic activity, a dilution method was adapted from the tests used for monitoring contaminated effluents (Kagei, 1986). It consisted of cultivation and hatching *A. lumbricoides* eggs in 5% formalin at 25°C (Hass, 1961), challenge of eggs with a 10% aqueous extract of several parts of the plant, and daily quantitative observation for 15 days for all morphological changes in the eggs. Total count of eggs is little affected by vegetal treatment, but morphological alterations are evident when incubated with a plant with known activity (e.g., ascaridol-containing *Chenopodium ambrosioides* leaves). The percentage of viability was calculated as the number of viable eggs and larvae × 100/total number of eggs. The number of altered eggs and larvae was used to estimate the percentage of inhibition.

Results and Discussion

Five parts of the plant were studied for antimicrobial activity against three Gram-negative and two Gram-positive bacteria and *C. albicans*, using up to four different temperatures for extraction (Table 1). There was a positive inhibition of *P. aeruginosa* by seeds extracted at 56°C (9.04 ± 0.76 mm), at 37°C (7.86 ± 0.36 mm) and juice from fresh leaves at 25°C (7.14 ± 0.12 mm), and

TABLE I

ANTIMICROBIAL ACTIVITY OF EXTRACTS OF *MORINGA OLEIFERA*

Plant parts	Preparation (°C)	A	B	C	D	E	F
Roots	25	—	—	—	—	—	—
Roots	95	—	—	—	—	—	—
Bark	25	—	—	—	—	—	—
Bark	37	—	—	—	—	—	—
Bark	56	—	—	—	—	—	—
Bark	95	—	—	—	—	—	—
Seeds	25	—	—	—	—	—	—
Seeds	37	—	—	+	—	—	—
Seeds	56	—	—	+	—	+	—
Seeds	95	—	—	—	—	—	—
Dry leaves	25	—	—	—	—	—	—
Leaf juice	25	—	—	+	—	—	—
Leaf juice	56	—	—	—	—	—	—

Microorganisms tested: A = *C. albicans;* B = enteropathogenic *E. coli:* C = *P. aeruginosa:* D = *S. flexneri:* E = *S. aureus:* F = *S. pyogenes.* Results: — = negative (<7 mm); + = positive (≥7 mm).

positive inhibition of *S. aureus* by seeds extracted at 56°C (8.25 ± 0.71 mm). Statistical difference between negative and positive results was significant in all four cases ($P < 0.05$).

These results confirm that the popular utilization of the seeds and fresh leaves as an antibacterial in skin and respiratory infections has some scientific justification. Preliminary studies to identify the antibiotic activity have attributed it to pterygospermin, present in *M. oleifera* roots (Raghunandana and George, 1949; Kurup and Narasimha, 1954; Das et al., 1957). Further studies were conducted by Eilert et al. (1981) using a group of non-pathogenic bacteria and fungi; they isolated 4(α-L-rhamnosyloxy) benzyl isothiocyanate which was identified as the active antimicrobial principle. These workers reported a deleterious effect on the active principle by extractions conducted at temperatures higher than 56°C; this phenomenon was confirmed in the present study since the seed extract prepared at 95°C was inactive against both *P. aeruginosa* and *S. aureus.*

No activity was demonstrated by any part of the plant against the six pathogenic dermatophytes, although *Solanum nigrescens* leaf extracts showed a good inhibition of all the strains assayed. These results are partially contradictory to those of Eilert et al. (1981), since they described a positive inhibition (>10 mm) by diffusion methods of several fungi non-pathogenic to humans, such as *Botrytis allii, Candida reukaufii, Phytophthora cactorum* and *Polystictus versicolor.*

No activity was also demonstrated against *A. lumbricoides* eggs, although deleterous activity was demonstrated by the positive control. After seven days of incubation with extracts from *Chenopodium ambrosioides* leaves as a positive control, negative controls and vegetal challenges with different parts of *M. oleifera*, the viability was demonstrated to be 30%, 98% and 99%, respectively. No previous studies of antihelmintic activity using these procedure are known.

In vivo studies are being conducted to show the antibacterial and healing activity of an ointment made with an aqueous extract and the oil of *M. oleifera* seeds using an experimental model of pyodermia induced in mice infected with *S. aureus.* Results will be presented elsewhere.

Acknowledgements

This research was supported by the German Agency for Technical Cooperation (GTZ) under grant 8320830 from the Water Sanitation Project. The authors wish to thank Dr. Samhia A.A. Jahn for technical advice; Ernesto Carrillo (Faculty of Agronomy, USAC) for taxonomical advice; Lidia M. Girón and Edgardo Cáceres (CEMAT) for reviewing the manuscript, and Heidi Logemann and Blanca Samayoa (USAC) for providing the microbial strains. The technical support of Roberto Arenales, Otto Avilés, Virgina Freire and Rebeca Orellana is much appreciated.

References

Cáceres, A. Girón, L.M., Alvarado, S.R. and Torres, M.F. (1987) Screening of antimicrobial activity of plants popularly used in Guatemala for the treatment of dermatomucosal diseases. *Journal of Ethnopharmacology* 20, 223—237.

Cáceres, A., Cano, O., Samayoa, B. and Aguilar, L. (1990) Plants used in Guatemala for the treatment of gastrointestinal disorders. 1: Screening of 84 plants against enterobacteria. *Journal of Ethnopharmacology* 30, 55—73.

Cáceres, A., Alvarez, A.V., Ovando. A.E. and Samayoa, B. (1991a) Plants used in Guatemala for the treatment of respiratory diseases. 1: Screening of 68 plants against Gram-positive bacteria. *Journal of Ethnopharmacology* 31, 193—208.

Cáceres, A., López, B.R., Girón, M.A. and Logemann, H. (1991b) Plants used in Guatemala for the treatment of dermatophytic infections. 1: Screening for antimycotic activity of 44 plant extracts. *Journal of Ethnopharmacology* 31, 263—276.

Council of Scientific and Industrial Research (1962) *The Wealth of India,* Vol. 6. Council of Scientific and Industrial Research, New Delhi, pp. 425—428.

Das, B., Kurup, P. and Narasimha, R. (1957) Antibiotic principles from *Moringa pterygosperma.* VII: Antibacterial activity and chemical structure of components related to pterygospermin. *Indian Journal of Medical Research* 45, 191—196.

Dastur, J.F. (1977) *Medicinal Plants of India and Pakistan.* D.B. Taraporevala and Co, Delhi, pp. 113—114.

Eilert, U., Wolters, B. and Nahrstedt, A. (1981) The antibiotic principle of seeds of *Moringa oleifera* and *Moringa stenopetala. Planta Medica* 42, 55—61.

Girón, L.M., Aguilar, G.A., Cáceres, A. and Arroyo, G.L. (1988) Anticandidal activity of plants used for the treatment of vaginitis in Guatemala and clinical trial of a *Solanum nigrescens* preparation. *Journal of Ethnopharmacology* 22, 307—313.

Hass, D.K. (1961) Extension of a technique of hatching ascarid eggs in vitro. *American Journal of Veterinary Research* 23, 169—170.

Jahn, S.A.A. (1986) *Proper Use of African Natural Coagulants for Rural Water Supplies.* German Agency for Technical Cooperation, Eschborn, 541 pp.

Jahn, S.A.A., Musnard, H.A. and Burgstaller, H. (1986) The tree that purifies water: Cultivating multipurpose Moringaceae in the Sudan. *Unasylva* 38, 23—28.

Kagei, N. (1986) *Use of Ascaris Ova as Indicator for Monitoring Nightsoil.* WHO-Western Pacific Regional Center for Promotion of the Environment, Serdang, p. 5.

Kurup, P. and Narasimha, R. (1954) Antibiotic principle from *Moringa pterygosperma.* IV: Effect of addition of vitamins and amino acids on the antibacterial activity of pterygospermin. *Indian Journal of Medical Research* 42, 101—107.

Lam, S.E. (1983) *Actividad Inhibitoria de Preparados Vegetales Sobre Algunos Dermatofitos.* Thesis, Facultad de Ciencias Químicas y Farmacia, USAC, Guatemala, 44 pp.

Pushpangadan, P. and Atal, C.K. (1986) Ethnomedical and ethnobotanical investigations among some scheduled caste communities of Travancore, Kerala, India. *Journal of Ethnopharmacology* 16, 175—190.

Raghunandana, R. and George, M. (1949) Investigation of plant antibiotics. III: Pterygospermin, the antibacterial principle of *Moringa pterygosperma* Gaertn. *Indian Journal of Medical Research* 37, 159—167.

Ramachandran, D., Peter, K.V. and Gopalakrishnan, P.K. (1980) Drumstick (*Moringa oleifera*): A multipurpose Indian vegetable. *Economic Botany* 34, 276—283.