Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 98 (2007) 232–236
BIORESOURCE TECHNOLOGY

Short Communication

Anti-fungal activity of crude extracts and essential oil of *Moringa oleifera* Lam

Ping-Hsien Chuang [a,b], Chi-Wei Lee [b], Jia-Ying Chou [a], M. Murugan [a], Bor-Jinn Shieh [b], Hueih-Min Chen [a,*]

[a] *Institute of Bioagricultural Sciences, Academia Sinica, Taipei 115, Taiwan, ROC*
[b] *Graduate Institute of Chemistry, Chung Yuan Christian University, Taoyuan 320, Taiwan, ROC*

Received 14 June 2005; received in revised form 30 October 2005; accepted 5 November 2005
Available online 6 January 2006

Abstract

Investigations were carried out to evaluate the therapeutic properties of the seeds and leaves of *Moringa oleifera* Lam as herbal medicines. Ethanol extracts showed anti-fungal activities in vitro against dermatophytes such as *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, and *Microsporum canis*. GC–MS analysis of the chemical composition of the essential oil from leaves showed a total of 44 compounds. Isolated extracts could be of use for the future development of anti-skin disease agents.

Keywords: Anti-fungal activity; Crude extract; Essential oil; *Moringa oleifera*

1. Introduction

Many skin diseases such as tinea and ringworm caused by dermatophytes exist in tropical and semitropical areas. In general, these fungi live in the dead, top layer of skin cells in moist areas of the body, such as between the toes, the groin, and under the breasts. These fungal infections cause only a minor irritation. Other types of fungal infections could be more serious. They can penetrate into the cells and cause itching, swelling, blistering and scaling. In some cases, fungal infections can cause reactions elsewhere in the body. For example, a person may develop a rash on the finger or hand after coming into contact with an infected foot. The dermatophytes, *Trichophyton*, *Epidermophyton* and *Microsporum canis* are commonly involved in such infections. However, their clinical differentiation is difficult. The clinical care is required by a physician or other healthcare professional in the treatment of these diseases (Beentje, 1994).

Moringa oleifera is a shrub and small deciduous tree of 2.5–10 m in height. When matured, the fruit becomes brown and has 10–50 seeds inside (Vlahof et al., 2002). The plant was reported to contain various amino acids, fatty acids, vitamins, and nutrients (Nesamani, 1999) and its constituents such as leaf, flower, fruit and bark have been anecdotally used as herbal medicines in treatments for inflammation, paralysis and hypertension. Many reports described *M. oleifera* as highly potent anti-inflammatory (Ezeamuzle et al., 1996), hepatoprotective (Pari and Kumar, 2002), antihypertensive (Faizi et al., 1995) and anti-tumor (Murakami et al., 1998). Also, its seed has strong coagulative and antimicrobial properties (Eilert et al., 1981). The seed oil has physical and chemical properties equivalent to that of olive oil and contains a large quantity of tocopherols (Tsaknis et al., 1999). The leaf extracts in rats were found to regulate thyroid status and cholesterol levels (Tahiliani and Kar, 2000; Ghasi et al., 2000). In recent years, many people in Taiwan or China have been using the seed of *Moringa* as an herbal medicine to treat athlete's foot and tinea

* Corresponding author. Tel.: +886 2 27855696x8030; fax: +886 2 27888401.
E-mail address: robell@gate.sinica.edu.tw (H.-M. Chen).

0960-8524/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2005.11.003

and found that it is effective. For the first time, in this communication we provide the evidence that extracts of *M. oleifera* have anti-fungal properties.

2. Methods

2.1. Materials and fungal strains

M. oleifera was grown and collected from Taichung, Taiwan. All materials were lyophilized and powdered before experiments. The dermatophytes used in this study were obtained from the Food Industry Research and Development Institute (FIRDI) in Taiwan. *Trichophyton rubrum* (BCRC 32805), *Trichophyton mentagrophytes* (BCRC 32066), *Epidermophyton floccosum* (BCRC 30531) and *Microsporum canis* (BCRC 30541) were maintained by monthly sub-culturing on Sabouraud dextrose agar (SDA) at 28 °C.

2.2. Anti-fungal assays

Anti-fungal assays were followed as per the National Committee for Clinical Laboratory Standards, USA. Samples (crude extracts and sub-fractions) were stocked in solvent DMSO (<1%). The sample solution was further diluted to 1:10 with RPMI1640 medium prior to test. Each sample was then 1:2 diluted and divided into 10 tubes. The four strains were grown to 10^4 CFU per ml and then co-incubated with crude extract or essential oil samples for 72 h at 28 °C. The anti-fungal agent, ketoconazole, was used as the positive control. For the conventional micro-dilution procedure, the growth in each sample well was compared with that of growth control with the aid of a reading mirror. Each micro-dilution well was then given a "numerical score" shown as following: 4 meant no reduction in growth; 3 indicated a slight reduction in growth or approximately 75% the growth of the growth control (drug-free medium); 2 implied a prominent reduction in growth or approximately 50% the growth of the growth control; 1 was a slight growth or an approximately only 25% growth relative to the growth control and 0 showed optically clear or absence of growth. The minimal inhibitory concentration (MIC) was then determined for each test sample.

2.3. Extraction of essential oil

Steam distillation and analyses were conducted as previously described (Brophy et al., 1991) for essential oil collection. About 16.9 g (yield = 0.24%) of a clear brown essential oil was finally obtained from 7.04 kg of washed and air-dried *M. oleifera* leaves. A total amount of 500 mg of essential oil was then chromatographically separated over a silica gel column (230–400 mesh, Merck) and eluted by one liter *n*-hexane and one liter diethyl ether to yield a essential oil hydrogenated fraction (named as EHF, yield = 15.75%) and an oxygenated fraction (named as EOF, yield = 84.25%), respectively. The EOF fraction was used in the following experiments.

2.4. Extractions of seed and leaf

About 1 kg of *M. oleifera* seeds that had been powdered was extracted with one liter of 70% EtOH (repeated 5 times) and incubated for 15 days at room temperature. The yield was about 64 g per 1000 g (yield = 6.4%) of seed weight. Ten grams of seed extract was then re-suspended in 250 ml of 70% EtOH and then diluted with 750 ml dd H_2O. The solution was extracted for three times serially with *n*-hexane, ethyl acetate and then *n*-butanol. These organic solvent extracts were then completely dried under reduced pressure. The dried sub-fractions: seed hexane fraction (SHF), seed ethyl acetate fraction (SEF), seed butanol fraction (SBF) and seed water fraction (SWF) were obtained.

Washed, air-dried *M. oleifera* leaf powder (1 kg) was extracted using a similar procedure as described above for seed extraction. A total of 52 g crude extract (collected from the extraction, repeated 5 times, each time with one liter of 70% EtOH, yield = 5.6%) was obtained. Ten grams of leaf extract was re-suspended in 1000 ml 70% EtOH and decolorized with charcoal. After filtration and lyophilization, the decolorized crude extract was suspended again in 100% dd H_2O (500 ml) and stirred for 10 min. The solution was centrifuged and the supernatant collected. Subsequently, the supernatant was completely dried under reduced pressure to give two fractions: (1) a water dissoluble fraction LWF; (2) a water indissoluble fraction of which precipitate was collected and dried. This indissoluble fraction was named LEF.

2.5. Chemical characterization of essential oil

The total neutral essential oil from *M. oleifera* leaves was analyzed by an Agilent 6890N Network GC (Gas chromatograph) system with an Agilent 5973 Network mass selective detector. The machine was equipped with a HP-5MS (Mass spectroscopy) column (30 × 0.25 mm (5%-phenyl) – methylpolysiloxane capillary column, film thickness × 0.25 μm), 250 °C temperature injector and 240 °C temperature transfer line. The oven temperature was programmed as follows: initial temperature; 50 °C for 15 min, increase 2 °C/min up to 150 °C, 10 min at 150 °C, and then increase 2 °C/min up to 220 °C, 20 min at 220 °C. The carrier gas was H_2. The amount of sample injected was 5 μl (split ratio 1:20) and the ionization energy was 70 eV. Qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds or by comparison of their retention indices and mass spectra with those shown in the literature (Adams, 1995). For this purpose, probability merge search software and the NIST MS spectra search program were used. The relative amounts (RA) of individual components of the essential oil were expressed as percentages of the peak area relative to the total peak

Table 1
Minimum inhibitory concentration (MIC) of *Morninga oleifera* extracts against specific fungi

Samples (mg/ml)	Essential oil		Seed				Leaf			EOF + SEF[c]	Kitoconazole[d]
	Crude[a]	EOF	Extract[b]	SEF	SBF	SWF	Extract[b]	LEF	LWF		
Trichophyton rubrum	1.6	0.8	2.5	0.625	2.5	>10	>10	>10	>10	0.8	1.000
Trichophyton mentagrophytes	0.8	0.4	2.5	1.250	2.5	>10	>10	>10	>10	1.6	0.250
Epidermophyton floccosum	0.2	0.1	2.5	0.625	2.5	>10	>10	>10	>10	0.8	0.125
Microsporum canis	0.4	0.2	2.5	0.156	2.5	>10	>10	>10	>10	1.6	1.000

[a] Essential oil before partition.
[b] 70% EtOH crude extract.
[c] EOF:SEF = 1:1 (w/w).
[d] μg/ml.

Table 2
Constituents of the essential oil of *Morninga oleifera*

No.	Component	RT[a]	RI[b]	%RA[c]
1	Toluene	3.76	765	0.03
2	5-*tert*-Butyl-1,3-cyclopentadiene	5.90	788	0.07
3	Benzaldehyde	13.16	889	0.55
4	5-Methyl-2-furaldehyde	13.69	896	0.27
5	Benzeneacetaldehyde	22.22	1028	2.16
6	Linaloloxide	25.00	1071	0.24
7	2-Ethyl-3,6-dimethylpyrazine	25.67	1080	0.12
8	Undecane	27.79	1100	0.12
9	α-Isophoron	29.06	1132	0.10
10	Benzylnitrile	30.94	1161	1.10
11	2,6,6-Trimethyl-2-cyclohexane-1,4-dione	31.28	1166	0.05
12	2,2,4-Trimethyl-pentadiol	32.03	1177	0.09
13	2,3-Epoxycarane	34.41	1213	0.16
14	*p*-Menth-1-en-8-ol	35.04	1223	0.08
15	2,6,6-Trimethylcyclohexa-1,3-dienecarbaldehyde	35.63	1232	0.23
16	Indole	42.68	1340	1.20
17	Tridecane	43.62	1300	0.16
18	α-Ionone	45.37	1381	0.03
19	1,1,6-Trimethyl-1,2-dihydronaphthalene	46.52	1398	0.41
20	α-Ionene	46.78	1401	0.09
21	β-Damascenone	48.90	1435	0.28
22	β-Ionone	48.93	1436	0.13
23	Ledene oxide	49.22	1440	0.60
24	2-*tert*-Butyl-1,4-dimethoxybenzene	51.66	1477	0.39
25	(E)-6,10-dimethylundeca-5,9-dien-2-one	53.42	1503	0.26
26	4,6-Dimethyl-dodecane	54.02	1513	0.29
27	3,3,5,6-Tetramethyl-1-indanone	56.69	1555	0.23
28	Dihydro-actiridioide	57.60	1568	1.21
29	2,3,6-Trimethyl-naphthalene	59.36	1594	0.37
30	Megastigmatrienone	59.66	1599	0.57
31	1-[2,3,6-Trimethyl-phenyl]-2-butanone	61.04	1621	3.44
32	1-[2,3,6-Trimethyl-phenyl]-3-buten-2-one	62.70	1647	0.75
33	Isolongifolene	63.29	1656	0.56
34	Hexahydrofarnesylactone	79.93	1910	1.30
35	Farnesylacetone	85.00	1988	0.08
36	Methyl palmitate	85.77	1999	0.08
37	*n*-Hexadecanoic acid	88.62	2044	1.08
38	[6E,10E]-7,11,15-trimethyl-methylene-1,6,10,14-hexadeca-tetraene	91.71	2090	0.11
39	(E)-phytol	96.55	2165	7.66
40	Docosane	100.69	2200	0.28
41	1-Docosene	109.23	2359	0.41
42	Teracosane	109.51	2400	1.45
43	Pentacosane	114.52	2500	17.41
44	Hexacosane	128.24	2600	11.20

[a] RT indicates the retention time on the column in minutes.
[b] RI indicates the retention indices calculated on the HP-5MS column.
[c] RA indicates relative area (peak area relative to the total peak area).

area. Retention indices (RI) of the components were determined relative to the retention times (RT) of a series of *n*-alkanes with linear interpolation on the HP-5MS column.

3. Results and discussion

The MICs of various fractions and sub-fractions of *M. oleifera* extracts are shown in Table 1. Results showed that both essential oil (crude and sub-fraction of EOF) and seed extracts (sub-fractions SEF and SBF) have anti-fungal effect on *T. rubrum*, *T. mentagrophytes*, *E. floccosum* and *M. canis*. However, both leaf crude extract and sub-fractions had little effect on dermatophytes. The crude essential oils showed different MICs on different fungi (ranging from 0.2 mg/ml (*E. floccosum*) to 1.6 mg/ml (*T. rubrum*)). The EOF fraction showed equal ratios of 1/2 MICs (ranging from 0.1 mg/ml (*E. floccosum*) to 0.8 mg/ml (*T. rubrum*) as compared with those for the crude essential oil and had the lowest MIC (0.1 mg/ml) on *E. floccosum*, which was classified as anthropophilic dermatophyte. This fungus is restricted to human hosts and produces a mild, chronic inflammation. It is a worldwide disease and usually infects humans via glabrous skin, groin, hands, feet or nails. Although the MIC of ketoconazole (0.125 μg/ml) was lower than that of EOF (100 μg/ml), ketoconazole has been only used as an anti-fungus agent for the optical application. For EOF extract, the main advantage could be that it could be developed for both oral and optical applications. Furthermore, the complex form like EOF extract is usually less toxic than the pure compound like ketoconazole. Since *M. oleifera*, leaf has been orally tested by local people in Asia and Africa for many years and, therefore, the development of EOF fraction as an anti-dermatophyte agent via oral treatment might have a huge profit in the future. The cheaper source obtained from this plant for making the EOF fraction would be another advantage. Components of the leaf essential oil were analyzed by using GC–MS. The results revealed a total of 44 compounds (Table 2). In general, pentacosane (no. 43; 17.41%), hexacosane (no. 44; 11.20%), (E)-phytol (no. 39; 7.66%) and 1-[2,3,6-trimethylphenyl]-2-butanone (no. 31; 3.44%) were the major components in the leaf essential oil.

The MIC (0.156 mg/ml) of seed extract SEF showed the strongest anti-fungal activity against *M. canis*, a zoophilic dermatophyte causing marked inflammatory reactions in humans. Infected areas usually include human beard, hair, glabrous skin and hand. Since EOF had a similarly low MIC (0.2 mg/ml) to *M. canis*, both SEF and EOF extracts could be individually developed as a treatment agent for *M. canis* infections. The combination of SEF and EOF, however, had less effect than either fraction individually used (Table 1). These non-additive observations could be due to the different anti-fugal compounds included in these two fractions.

It is vitally important to know about the cell lysis mechanisms of *M. oleifera* extracts on fungal cells so that further development of disease treatment can be conducted accordingly. A study of the morphological change of the cell induced by these extracts would therefore be the preliminary in understanding the lysis mechanism. *M. oleifera* seed crude extract was used as an example to study the shape change of *T. rubrum* cells using transmission electron microscopy (Chen et al., 2003). The TEM images of fungal cells, which were treated with 70% EtOH crude extract of *M. oleifera* seed for 24 h, showed that the cytoplasmic membrane of the fungal cell was ruptured and the intracellular components were seriously damaged after treatment with seed crude extract (results not shown). However, the intracellular components did not leak out. Based on previous studies of the cell lysis pathways of anti-microbial peptides on bacteria using TEM and immuno-gold TEM (Chan et al., 1998; Chen et al., 2003), this indicated that extracted compounds interacted with the lipid bilayers in membranes leading to the separation of the two membranes (outer and inner membranes). Subsequently, water dips into the cell, which causes cell to swell more and leads cell to death.

Acknowledgement

This work was partially supported by a grant from National Science Council (NSC 93-2311-B-001-069), Taiwan, ROC.

References

Adams, R.P., 1995. Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy. Allured Publishing, Carol Stream, IL.

Beentje, H.J., 1994. Moringaceae. In Kenya Trees Shrub and Lianas. Majestic Printing Works Ltd., Nairobi, Kenya. Chapter 37.

Brophy, J.J., House, A.P.N., Bolandand, D.J., Lassak, E.V., 1991. Digests of the Essential Oil of III Species from Northern and Eastern Australia in Eucalytpus Leaf Oils—Use Chemistry Distillation and Marketing. Inkata Press, Melbourne/Sydney.

Chan, S.C., Yau, W.L., Wang, W., Smith, D., Sheu, F.S., Chen, H.M., 1998. Microscopic observations of the different morphological changes by the anti-bacterial peptides on *Klebsiella pneumoniae* and HL-60 leukemia cells. Journal of Peptide Science 4, 413–425.

Chen, H.M., Chan, S.C., Lee, J.C., Chang, C.C., Murugan, M., Jack, R.J., 2003. Transmission electron microscopic observations of membrane effects of antibiotic cecropin B on *Escherichia coli*. Microscopy Research and Technique 62, 423–430.

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

Ezeamuzle, I.C., Ambadederomo, A.W., Shode, F.O., Ekwebelem, S.C., 1996. Antiinflammatory effects of *Moringa oleifera* root extract. International Journal of Pharmacognosy 34, 207–212.

Faizi, S., Siddiqui, B.S., Saleem, R., Siddiqui, S., Aftab, K., Gilani, A.H., 1995. Fully acetylated carbonate and hypotensive thiocarbamate glycosides from *Moringa oleifera*. Phytochemistry 38, 957–963.

Ghasi, S., Nwobobo, E., Ofili, J.O., 2000. Hypocholesterolemic effects of crude extract of leaf of *Moringa oleifera* Lam in high-fat diet fed Wistar rats. Journal of Ethnopharmacology 69, 21–25.

Murakami, A., Kitazono, Y., Jiwajinda, S., Koshimizu, K., Ohigashi, H., 1998. Niaziminin, a thiocarbamate from the leaves of *Moringa oleifera*, holds a strict structural requirement for inhibition of tumor-promoter-induced Epstein–Barr virus activation. Planta Medica 64, 319–323.

Nesamani, S., 1999. Medicinal Plants (vol. I). State Institute of Languages, Thiruvananthapuram, Kerala, India. p . 425.

Pari, L., Kumar, N.A., 2002. Hepatoprotective activity of *Moringa oleifera* on antitubercular drug-induced liver damage in rats. Journal of Medicinal Food 5 (3), 171–177.

Tahiliani, P., Kar, A., 2000. Role of *Moringa oleifera* leaf extract in the regulation of thyroid hormone status in adult male and female rats. Pharmacological Research 41, 319–323.

Tsaknis, J., Lalas, S., Gergis, V., Dourtoglou, V., Spilotis, V., 1999. Characterisation of *Moringa oleifera* variety Mbololo seed oil of Kenya. Journal of Agricultural and Food Chemistry 47, 4495–4499.

Vlahof, G., Chepkwony, P.K., Ndalut, P.K., 2002. ^{13}C NMR characterization of triacylglycerols of *Moringa oleifera* seed oil: an Oleic-Vaccenic acid oil. Journal of Agricultural and Food Chemistry 50, 970–975.

Miracle tree moringa