ELSEVIER

Journal of Ethnopharmacology 49 (1995) 163–169

Journal of ETHNO PHARMACOLOGY

Antifungal activity of turmeric oil extracted from *Curcuma longa* (Zingiberaceae)

Amphawan Apisariyakul*[a], Nongnuch Vanittanakom[b], Duang Buddhasukh[c]

[a]*Department of Pharmacology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand*
[b]*Department of Microbiology, Faculty of Medicine, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand*
[c]*Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand*

Received 27 September 1993; revision received 3 August 1995; accepted 17 October 1995

Abstract

Turmeric oil and curcumin, isolated from *Curcuma longa* L., were studied against fifteen isolates of dermatophytes, four isolates of pathogenic molds and six isolates of yeasts. The inhibitory activity of turmeric oil was tested in *Trichophyton*-induced dermatophytosis in guinea pigs. The results showed that all 15 isolates of dermatophytes could be inhibited by turmeric oil at dilutions of 1:40–1:320. None of the isolates of dermatophytes were inhibited by curcumin. The other four isolates of pathogenic fungi were inhibited by turmeric oil at dilutions of 1:40–1:80 but none were inhibited by curcumin. All six isolates of yeasts tested proved to be insensitive to both turmeric oil and curcumin. In the experimental animals, turmeric oil (dilution 1:80) was applied by dermal application on the 7th day following dermatophytosis induction with *Trichophyton rubrum*. An improvement in lesions was observed in 2–5 days and the lesions disappeared 6–7 days after the application of turmeric oil.

Keywords: *Curcuma longa* L.; Antifungal activity; Dermatophytes; Miconazole

1. Introduction

Dermatophytoses (dermatomycosis, tinea, ringworm) are the most common forms of fungal infections found in most countries (Ribbon, 1988). The diseases are caused by keratinophilic fungi called dermatophytes. The dermatophytes can cause diseases of the skin, hair and nails. There are three groups of dermatophytes depending on their ecology: geophilic, zoophilic and anthropophilic species. The infections can be transmitted from animal to man, from soil to man and from man to man. Therefore, it is still a public health problem and imported drugs used for the treatment of this disease are expensive. In Thailand, there are many medicinal plants which exhibit antimicrobial activity and may be used for the treatment of infectious diseases (Imwidthaya et al., 1983; Avirutnant et al., 1983; Soyton et al., 1985). We are interested in the antifungal activity of turmeric oil, extracted from *Curcuma longa* L. (Zingiberaceae) whose inhibitory effect against bacteria has already been

* Corresponding author.

0378-8741/95/$09.50
SSDI 0378-8741(95)01320-D

reported earlier (Krishnamurthy et al., 1976; Banerjee and Nigam, 1978).

Curcuma longa L. (turmeric) has long been used as a common household medicine and as a spice in Southeast Asia. Turmeric contains essential oil, yellow pigments (curcuminoids), starch and oleoresin (Leung, 1980).

In this investigation the antifungal activity of turmeric oil and curcumin was tested against dermatophytes, yeasts and pathogenic molds. The minimum inhibitory concentration (MIC) of these compounds was determined. Following the completion of in vitro antifungal tests, an in vivo study in guinea pigs was performed.

2. Materials and methods

2.1. Extraction of turmeric oil and crude curcumin from turmeric

The rhizomes of turmeric (*Curcuma longa* L., Zingiberaceae) were peeled, cut into pieces and dried at the temperature of 50–60°C, then pulverised and 500 g dried powder was steeped twice overnight in hexane. The combined hexane solution was evaporated at a reduced pressure to produce turmeric oil in the form of a yellow oil (18.4 g; 3.7% w/w). The powder from the hexane extraction was further steeped overnight in 95% ethanol and this process was repeated. The combined alcoholic extract was concentrated and the residue was treated with ether. The mixture was well stirred, then filtered to give crude curcumin in the form of a yellow-orange powder after washing with diethyl ether. The yield of crude curcumin was 5.4 g (1.1%), m.p. 171–182°C. When mixed with an authentic sample (m.p. 175–180°C), it gave a m.p. of 173–180°C. TLC of the isolated product and the authentic curcumin (Merck) were virtually identical, showing one major orange spot and two other minor orange spots. Since this crude curcumin was inactive against tested dermatophytes, it was not fractionated further. The above procedure was repeated several times to yield more material for testing.

2.2. Determination of in vitro antifungal activity

Sabouraud's dextrose agar was used to culture fifteen isolates of dermatophytes, six isolates of yeasts and four isolates of other pathogenic molds. The species of all tested fungi are listed in Table 1. These fungi were isolated from patients at Maharaj Nakorn Chiang-Mai Hospital. The agar well diffusion method was used to test the susceptibility of the fungi to turmeric oil and the diffusion disc method (Shadomy et al., 1985) was modified to test curcumin.

Seven different dilutions (1:10, 1:20, 1:40, 1:80, 1:160, 1:320 and 1:640) of turmeric oil were prepared in ethylene glycol. Twenty-five μl of each dilution was pipetted into each well of the agar plates seeded with each tested fungus. Twenty-five μl of ethylene glycol and 25 μl of miconazole (20 μg/ml) were used as a solvent control and positive control, respectively.

Another set of plates was tested with various concentrations of curcumin in dimethyl sulfoxide (1000, 100, 10 and 1 μg/ml). Paper discs containing 25 μl of each concentration of curcumin were plac-

Table 1
List of fungi used in this study

Group	Number of isolates	Microorganisms
Dermatophytes	6	*Trichophyton rubrum*
	5	*Trichophyton mentagrophytes*
	3	*Epidermophyton floccosum*
	1	*Microsporum gypseum*
Yeasts	4	*Candida albicans*
	1	*Candida tropicalis*
	1	*Candida stellatoidea*
Other pathogenic molds	1	*Trichophyton rubrum*
	1	*Trichophyton mentagrophytes*
	1	*Epidermophyton floccosum*
	1	*Sporothrix schenckii*

Trichophyton rubrum (Castellani, 1910) Sabouraud, 1911
Trichophyton mentagrophytes (Roobin, 1853) Blanchard, 1896
Epidermophyton floccosum (Hartz, 1871) Langeron et Micochevitch, 1930)
Microsporum gypseum (Bodin, 1907) Guiart et Grigorakis, 1928
Candida albicans (Robin) Berkhout, 1923
Candida tropicalis (Castellani) Berkhout, 1923
Candida stellatoidea (Jones et Marin) Langeron et Guerra, 1939
Exophiala jeanselmei (Langeron) McGinnis et Padhye, 1977
Fonsecaea pedrosoi (Brumpt) Negroni, 1936
Scedosporium apiospermum (Saccardo) Castellani et Chalmers, 1919
Sporothrix schenckii Hektoen et Perkins, 1900

ed on the tested plates. The plates were incubated at 28°C for 10 days. The clear zone surrounding each well or disc was observed and interpreted as minimal inhibitory concentration (MIC) of the turmeric oil or curcumin.

2.3. Antifungal test in vivo in guinea pigs

All infections were performed in guinea pigs of either sex weighing between 200 and 300 g. (4–8 weeks old). Inocula were made from a culture of *Trichophyton rubrum*. The method used to study antifungal activity in guinea pigs was modified by the method of Greenberg et al., (1976).

Guinea pigs, aged 4–8 weeks, weighing 300–400 g, were kept in each animal cage for 3 days before the experiment. The hair on the hip of the experimental animal was pulled out on both the left and right sides in order to obtain a circular area of 3 cm in diameter. Spore suspension of dermatophytes was prepared in sterile water and counted in a hemacytometer. Twenty-five μl of the suspension containing 10^4–10^5 spores were applied to the skin on the prepared area by an automatic pipette on the day of preparation of the skin area. The skin of the guinea pigs was observed daily for 3 weeks.

The experiments were divided into three parts as follows:

(1) induction of dermatophytosis and observation of the lesions : — erythema, scale, crusts and scoring;

(2) after observation of dermatophytosis, the drug canesten was applied and observed daily by scoring. This will be compared with the effect of turmeric oil on the same dermatophytosis;

(3) the lesions were scored after induction of dermatophytosis. After the cream base was applied to the lesion, we then observed and recorded the results over a period of 2 weeks.

3. Results

3.1. Antifungal activity of turmeric oil in in vitro systems

Turmeric oil and curcumin are the two extracts isolated from *Curcuma longa* L. (Zingiberaceae). Both were tested for antifungal activity in in vitro system by studying the minimum inhibitory concentration (MIC) of these two extracts on two groups of fungi, namely, fifteen isolates of dermatophytes and four isolates of other pathogenic molds.

Table 2
The minimum inhibitory concentration (MIC) of turmeric oil and crude curcumin which produced the inhibition growth of dermatophytes

Dermatophytes	Strains	Minimum inhibitory concentration	
		Turmeric oil (μg/ml)	Crude curcumin (μg/ml)
Microsporum gypseum	MMC 23	459.6	>10 000
Epidermophyton floccosum	MMC 41	229.8	>10 000
	MMC 74	229.8	>10 000
	MMC 92	114.9	>10 000
Trichophyton mentagrophytes	MMC 73	919.2	>10 000
	MMC 86	229.8	>10 000
	MMC 91	459.6	>10 000
	MMC 96	459.6	>10 000
	MMC 98	229.8	>10 000
Trichophyton rubrum	MMC 72	229.8	>10 000
	MMC 90	459.6	>10 000
	MMC 93	919.2	>10 000
	MMC 94	459.6	>10 000
	MMC 95	459.6	>10 000
	MMC 97	229.8	>10 000

Table 3
The minimum inhibitory concentration (MIC) of turmeric oil and crude curcumin in inhibition growth of pathogenic molds, four isolates

Pathogenic molds	Strains	Minimum inhibitory concentration	
		Turmeric oil (μg/ml)	Crude curcumin (μg/ml)
Exophiala jeanselmei	MMC 17	459.6	>10 000
Sporothrix schenckii	MMC 38	114.9	>10 000
Fonsecaea pedrosoi	MMC 42	459.6	>10 000
Scedosporium apiospermum	MMC 70	114.9	>10 000

Table 4
Inhibition zone of turmeric oil and crude curcumin in comparison to a standard drug, miconazole, effective against dermatophytes

Dermatophytes	Strains	Inhibition zone (mm) induced by:								
		Turmeric oil (dilution)						Miconazole (μg/ml)		
		Und	1:10	1:20	1:40	1:80	1:160	50	25	12.5
Microsporum gypseum	MMC 23	20.5	14	13.5	8.5	0.0	0.0	17	15.5	0.0
Epidermophyton floccosum	MMC 41	23.0	20.5	17.5	17.5	15.0	9.0	58	42.0	21.0
	MMC 74	29.0	23.0	20.0	16.0	14.0	6.5	28	23.0	21.5
	MMC 92	30.5	27.0	19.5	18.5	16.5	14.0	43	38.0	25.0
Trichophyton	MMC 73	26.5	20.5	18.5	0.0	0.0	18.0	14	0.0	0.0
mentagrophytes	MMC 86	29.5	26.5	22.5	18.5	15.5	5.0	30	18.0	10.0
	MMC 91	20.5	16.5	14.0	9.5	7.0	0.0	34	27.0	20.0
	MMC 96	34.0	26.0	19.0	17.5	12.0	0.0	33	31.5	19.0
	MMC 98	21.0	19.0	14.5	14.0	13.5	13.0	40	36.0	25.0
Trichophyton rubrum	MMC 72	32.5	28.0	25.0	22.5	16.0	5.5	25	16.0	8.0
	MMC 90	39.0	32.5	26.5	13.0	22.5	0.0	54	34.0	14.0
	MMC 93	27.5	24.0	15.0	10.5	0.0	0.0	38	16.0	0.0
	MMC 94	22.5	19.5	15.0	6.0	6.0	0.0	38	36.0	16.5
	MMC 95	29.5	29.0	27.5	19.5	19.0	0.0	54	37.5	15.5
	MMC 97	34.0	32.5	27.5	22.0	14.0	7.0	25	18.0	10.0

Und, 36 mg/ml; 1:10, 3676.8 μg/ml, (3.67 mg/μl); 1:20, 1838.4 μg/ml, (1.84 mg/μl); 1:40, 919.2 μg/ml; 1:80, 459.6 μg/ml; 1:160, 229.8 μg/ml.

It was found that turmeric oil could inhibit the growth of dermatophytes. The MIC against *Microsporum gypseum* was at a dilution of 1:80. The MIC against *Epidermophyton floccosum* was at a dilution of 1:60–1:320. The MIC against *Trichophyton mentagrophytes* was at a dilution of 1:40–1:160 and the MIC against *Trichophyton rubrum* was 1:40–1:160 as shown in Table 2. Curcumin was shown to have no antifungal activity.

The MIC of turmeric oil on pathogenic molds is shown in Table 3. The inhibition zone of turmeric oil in comparison to miconazole (antifungal drug) on dermatophytes and other pathogenic molds is shown in Tables 4 and 5, respectively. The antifungal activity of turmeric oil which was compared to that of the control drug, miconazole, against *Epidermophyton floccosum* (MMC 92) *Trichophyton mentagrophytes* (MMC 96) and

Table 5
Inhibition zone of turmeric oil in comparison to miconazole in inhibition of other pathogenic molds

Pathogenic mold	Strains	Inhibition zone (mm)							
		Turmeric oil (dilution)					Miconazole (μg/ml)		
		Und	1:10	1:20	1:40	1:80	50	25	12.5
Exophiala jeanselmei	MMC 17	28.0	18.0	16.0	13.0	8.0	36.5	21.0	10.0
Sporothrix schenckii	MMC 38	19.0	16.5	5.5	3.0	0.0	20.0	17.5	0.0
Fonsecaea pedrosoi	MMC 42	28.0	20.0	18.5	15.0	5.0	31.0	25.0	14.0
Scedosporium apiospermum	MMC 70	17.5	10.5	9.5	7.0	0.0	20.0	18.0	0.0

Und, 36 mg/ml; 1:10, 3676.8 μg/ml, (3.67 mg/μl); 1:20, 1838.4 μg/ml, (1.84 mg/μl); 1:40, 919.2 μg/ml; 1:80, 459.6 μg/ml.

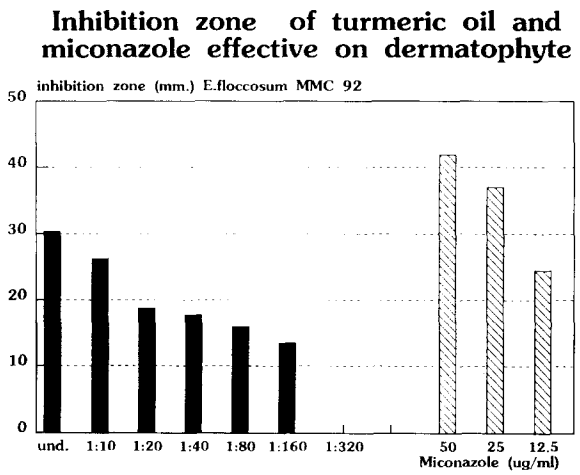

Fig. 1. The histogram showing inhibition zone (mm) produced by tumeric oil and miconazole effective against *Epidermophyton floccosum* MMC 92.

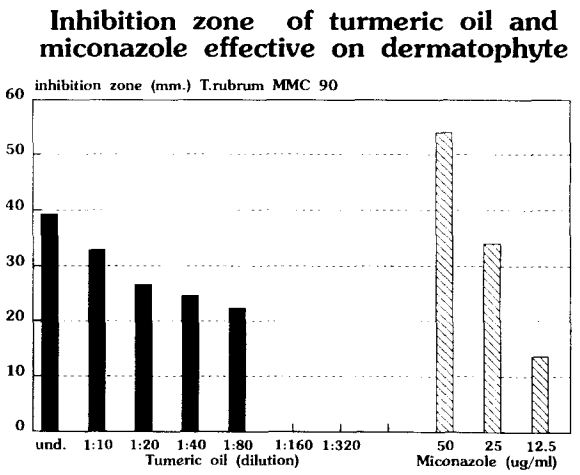

Fig. 3. The histogram showing inhibition zone (mm) produced by turmeric oil and miconazole effective against *Trichophyton rubrum* MMC 90.

Trichophyton rubrum (MMC 90) are shown as histograms in Figs. 1,2 and 3.

3.2. Antifungal activity of turmeric oil in in vivo system in guinea pigs

The lesion sites of infected area induced by dermatophytes of the first group of guinea pigs were studied. After induction of the infection by

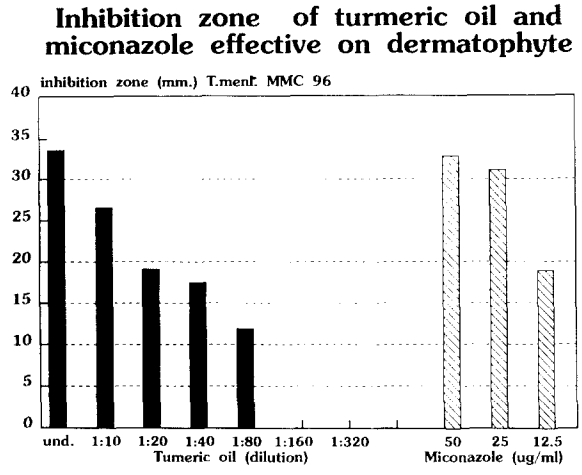

Fig. 2. The histogram showing inhibition zone (mm) produced by tumeric oil and miconazole effective against *Trichophyton mentagrophytes* MMC 96.

Trichophyton rubrum, the infection site was observed daily for 3 weeks as control. Lesion scoring was measured as the following parameters: — erythema, scale, crust and scar. Erythema was graded on a 1–4 basis: 1 was pink, 2 was rose, 3 was red and 4 was crimson red. Scale and crust were graded on a 1–4 basis. If a few punctated areas covered up to 25% of lesion areas it was 1; 25–50% was 2; 50–80% was 3 and 80–100% was 4.

The erythema began to develop score 1 on the first day after the induction of *Trichophyton rubrum* and developed score 4 in 6–12 days after the induction. The scale developed score 1 at 2–6 days after *Trichophyton rubrum* induction, and score 4 at 8–12 days after the induction. Crust score 1 developed 8–9 days after the induction of *Trichophyton rubrum* and a maximum score 3 in 12–14 days. The representative of a lesion site is shown in Fig. 4.

It was shown that erythema, scales and crust induced by *Trichophyton rubrum* was clearly seen on the fifth day after the induction.

The second group of guinea pigs was used for studying the effect of a control drug, canesten, on the infected area. It was found that canesten could decrease the erythema on the sixth day after the induction by *Trichophyton rubrum* in guinea pigs.

The third group of guinea pigs was used for

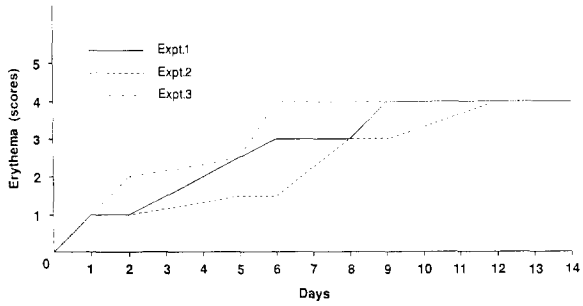

Fig. 4. Erythema induced by *Trichophyton rubrum* in the guinea pigs.

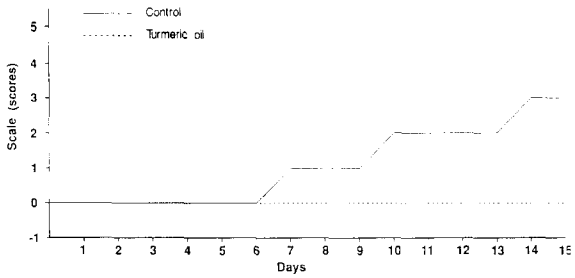

Fig. 6. After application of turmeric oil at day 7, the scale induced by *Trichophyton rubrum* in the guinea pig was decreased.

studying the effect of turmeric oil on the infected area. It was found that turmeric oil noticeably decreased erythema and scale as shown in Figs. 5 and 6, respectively.

4. Discussion and conclusions

From this study, it was found that curcumin has no antifungal activity. Turmeric oil could inhibit dermatophytes and pathogenic molds in in vitro systems. Turmeric oil markedly decreased erythema and scale induced by *Trichophyton rubrum* in guinea pigs.

This experimental infection induced in the experimental animals is a quantitative technique and we repeated each experiment. This quantitative infection model in guinea pigs is a parallel to the quantitated human infection model. The investigation is a part of drug development from natural sources. Turmeric oil itself is not harmful or irritable to the skin. Formulation from turmeric oil will be further studied.

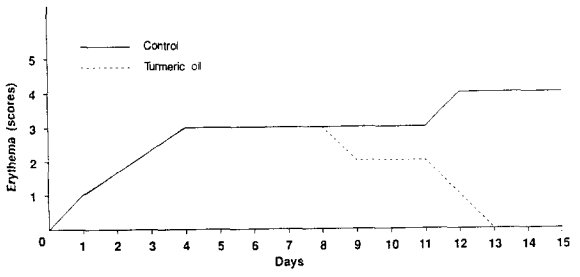

Fig. 5. After application of tumeric oil at day 7, the erythema induced by *Trichophyton rubrum* in the guinea pig was decreased.

Acknowledgements

The authors wish to thank The Regional Network for the Chemistry of Natural Products in Southeast Asia for supplying the research grant for this study. We also wish to thank the Faculty of Sciences and the Faculty of Medicine, Chiang Mai University for their permission and support for providing all the facilities necessary for the successful completion of this project.

References

Avirutnant, W. And Pongpan, A. (1983) The antimicrobial activity of some Thai flowers and plants. *Journal of Pharmaceutical Sciences* 10, 81–86.

Banerjee, A. and Nigam, S.S. (1978) Antimicrobial efficacy of the essential oil of *Curcuma longa*. *Indian Journal of Medical Research* 68, 864–866.

Greenberg, J.H., Robert, D.K., Krebs, S. and Field, R. (1976) A quantitative dermatophytes infection model in the guinea pig, a parallel to the quantitated human infection model. *Investigative Dermatology* 67, 704–708.

Imwidthaya, S., Sripathomswat, N. and Nilvises, N. (1983) Pharmacological activity of Thai medical plants for the treatment of dermatophytosis. *Bulletin of Infectious Disease Group of Thailand* 6, 1–8.

Krishnamurthy, N., Mathew, A.G., Nambudiri, E.S., Shivashankar, S., Lewis, Y.S. and Natarajan, C.P. (1976) Oil and oleoresin of turmeric. *Tropical Science* 18, 37–45.

Lennette, E.H., Balows, A., Hausler, W.J. and Shadomy, S. (1985) Manual of Clinical Microbiology, 4th Ed., *American Society for Microbiology*, Washington DC, 967.

Leung, A.Y. (1980) *Encyclopedia of Common Natural Ingredients Used in Foods, Drugs and Cosmetics*. John Wiley & Som, New York, pp. 313–314.

Punyarajun, S. (1981) Determination of the curcuminoid con-

tent in curcuma, *Journal of Pharmaceutical Science* 8, 29–31.

Ribbon, J.W. (1988) The pathogenic fungi and the pathogenic actinomycetes. *Medical Mycology*. 2nd Ed. W.B. Saunders Company, Philadelphia, London, Toronto.

Shadomy, S. Espinel-Ingroff, A. and Cartwright R.Y. (1985) Laboratory Studies with Antifungal Agents: Susceptibility Tests and Bioassays. In: E.H. Lennette et al. (Eds.) *The Manual of Clinical Microbiology*, 4th edition.

Soyton, K. and Rakvidhyasastra, V. (1985) Antifungal properties of some medicinal plants. *Thai Phytopathology* 5, 38.

Stransky, E., (1979) U.S. Patent, 4138 212 (Cl, 8–80, A 23 Li/28), 06 Feb.

The Merck Index, 10th Edition, Merck & Co., Inc., NJ, 1983, p. 2666 (Curcumin).