ASSESMENT OF ANTIDERMATOPHYTE ACTIVITY OF OIL FROM *CURCUMA LONGA* L. *IN VITRO*

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ABSTRACT: *Curcuma longa* L. is a rhizomatous herbaceous perennial plant, and its essential oil showed antifungal, antibacterial, antioxydant, antivenom, and antitumor effects. The antidermatophytes of oil from turmeric was demonstrated. This study was carried out in order to evaluate inhibition activity of oil from Vietnamese turmeric against two strains dermatophyte, *Trychophytol mentargrohytes* and *Candida albicans*. The oil from Vietnamese turmeric was obtained by hydrodistillation method and its chemical composition was determined by GC/MS. By comparing with authentic reference compounds in mass spectra library Nist 98.1 and Wiley 275.L, 13 compounds of oil from Vietnamese turmeric were identified. The received results showed that, the oil is rich in turmerones (about 44%), which has been recognized as major constituent attributing to antimicrobial activity. By disc diffusion method, the antifungal activity of the turmeric essential oil against *T. mentargrophyte* and *C. albicans* was demonstrated. The MICs of the turmeric oil for *T. mentargrophytes* và *C. albicans* were 0.5%. The minimal killing time for *C. albicans* was 60 minutes and for *T. mentargrophyte* was 90 minutes after treatment with the turmeric oil.

Keywords: Curcuma longa, dermatophytes, inhibition, turmeric oil, turmerones.

INTRODUCTION

Dermatophytoses is the common forms of fungal infection found in most countries. The diseases are caused by dermatophytes, the molds that require keratin for nutrition and must live on stratum corneum, hair, or nails to survive. The infection can be transmitted from man to man, animal to man and even from soil to man.

Trichophyton mentagrophytes is a pathogenic agent causing tinea cruris, tinea pedis, tinea capitis..., meanwhile *Candida albicans* causing dermatitis and diseases of nail, corners of nail. Dermatophytose is still a public health problem around the world and imported drugs used for the treatment of this disease are expensive.

Medicinal plants are widely used as an integral part of primary health care in many asian countries and important natural sources of anti-infectious agents. *Curcuma longa* is a rhizomatous herbaceous perennial plant, of the family Zingiberaceae and widely used as a spice and colouring agent, as well as pharmaceutical properties in traditional medicine [5, 12]. In Vietnam, genus *Curcuma* comprises at least 16 species and many of them have been used in ethnical medicines, such as *C. longa* L., *C. aeruginosa* Roxb., *C. zedoaria* (Berg.) Rosc., and *C. pierreana* Gagnep. [4]. The biological properties of extracts of Zingiberaceae species have been investigated by many workers and their biological activity were reported including antiemetic, anticancer, antiinflammation, hypolipidemic, antioxidant, antibacterial and antifungal [1, 10].

In the last years, research in essential oils of aromatic and medicinal plants has attracted many investigators. Recently, several studies demonstrated potential use of these natural products as antifungal agents, their use in a number of pharmaceutical, food and cosmetic products [8, 16]. Turmeric essential oil showed antifungal, antibacterial, antioxydant, antivenom, and antitumor effects. The inhibitory effect of turmeric essential oil against microorganisms has been reported by many authors [1, 2, 9, 10, 14]

Keeping in view the antifungal activity of turmeric oil, the present study was conducted to demonstrate it's inhibition on growth of dermatophyte *Trichophyton mentagrophyte* and the yeast-like fungi *Candida albicans.* The

chemical composition of oil extracted by hydro-distillation was determined by GC-MS.

MATERIALS AND METHODS

Rhizomes of *C. longa* collected from Hung Yen province were washed, dried and then ground. One kilogram of turmeric powder (about 4mm particles) were put into steam distillation unit, add distilled water at ratio 2:1 (v/w), some pumice stone, and the process was carried out on electric cooker for 8 hours. The essential oil was condensed by cooling water at room temperature.

Two strains of fungi were used: *Trichophyton mentagrophyte* and *Candida albicans* obtained from Dermatology hospital (Hanoi, Vietnam). The Sabouraud medium was used for all experiments.

Determination of antifungal activity: Inhibition zone diameter was determined by the disc diffusion method as decribed elsewhere [13]. The clear zone surrounding each disc was interpreted as minimum inhibitory concentration (MIC). In order to determine minimum killing time, one milliliter of the medium supplemented with the oil (final concentration was 0.5% for *C. albicans* and 0.7% for *T. mentagrophyte*) was prepared and inoculated with 0.1 ml of freshly grown test fungi and incubated at appropriate temperature. After 0, 5, 10, 15, 20, 25, 30, 60, 90, 120 min intervals, 10 µl of the sample from above test tubes were subcultured onto Sabouraud plates and incubated overnight. Count the forming colonies.

Statistical analysis: Each experiments were carried out in triplicate. The data were statistically analysed using software SPSS 11.5. A least significant difference (LDS 0.05) was used to test effect of essential oil through a general linear model. The test was statistically significant at $p < 0.05$

RESULTS AND DISCUSSION

Chemical composition of obtained turmeric oil

Percentage yield of *C. longa* rhizome hydrodistillation was 0.93%. The essentiol oil was analysed by GC-MS system and the components are given in fig 1. The identity of the components was assigned by comparing their GC retention time and the mass spectra with those of authentic reference compounds in mass spectra library Nist 98.1 and Wiley 275.L (≥ 90%). By this method, 13 compounds were identified in the obtained oil.

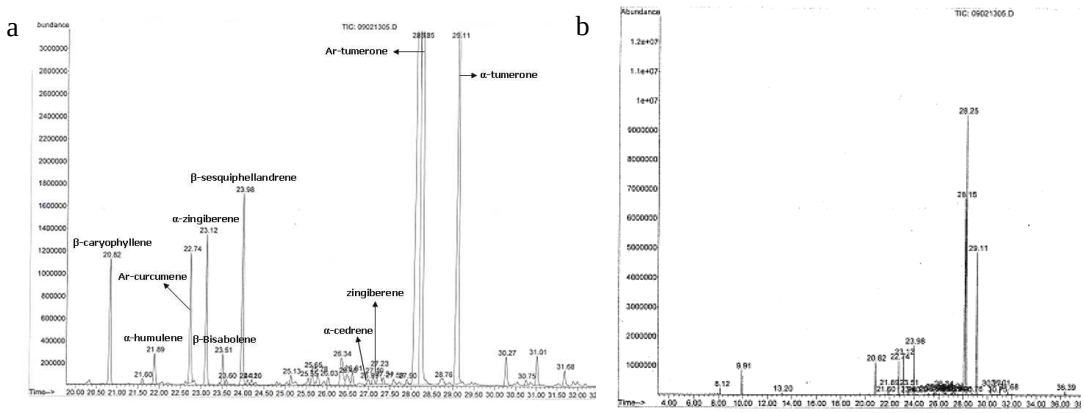

Fig. 1. Volatile compounds of turmeric oil obtained by hydrodistillation (a) and monoterpenes (b).

The major peaks were turmerones (ar-turmerone and α-turmerone) composed more than 44%, followed by other sesquiterpenes such as β-sesquiphellandrene, α-zingberene and β-caryophyllene (more than 10%). In this oil, two monoterpenes were identified, 1,8-cineole and α-terpinolene at the rentention times 8.13 and 9.91, respectively (fig.1b). There are 37%

of unidentified compounds in Vietnamese turmeric oil.

Antifungal activity *in vitro*

By disc diffusion method on Sabouraud's dextrose agar, the presence of inhibition zone and zone diameter were evaluated for assessment of antifungal activity of turmeric oil. Sterile 6 mm diameter filter paper discs were impregnated with the oil diluted by ethylene glycol to desired concentration. Negative control were prepared using the same solvent. Experimental discs were incubated at 30°C overnight for *C. albicans* and 36 hours for *T. mentagrophyte* (table 1).

Table 1. Antifungal activity of Vietnames turmeric oil*

Fungi	Concentration of turmeric oil (%)				
	0.3	0.5	0.7	1.0	2.0
Candida albicans	0.3 ± 0.4	1.6 ± 0.3	1.7 ± 0.5	1.8 ± 0.3	2.6 ± 0.7
Trychophytol mentargrohytes	0.2 ± 0.1	0.9 ± 0.2	1.4 ± 0.2	1.6 ± 0.2	2.2 ± 0.1

* Inhibition zone (mm). Mean ± standard deviation where n = 3 and data is significant at $p < 0.05$.

There are great number reports concerning antimicrobial activity of turmeric oil [12, 9, 10, 4, 15]. Depending on the oil source, method of extraction and the nature of tested microorganisms, varying degree of inhibition of turmeric oil was observed. MICs of Hexane extract of rhizome turmeric against *T. mentagrophytes* and *T. rubrum* varied from 230-919 μg/ml, depending on the strains [1]. The MIC of freshly distilled oil from Thailand turmeric against some clinical dermatophyte was 7.8 mg/ml [15]. Using the whole plant bioassay, Lee et al. (2003) [7] evaluated the control values of methanol extract of the *Curcuma* rhizome to six plant disease organisms. This extract exhibited good fungicidal activity against rice sheath blight caused by *R. solani*, late blight caused by *P. infestans* and barley powdery mildew caused by *E. graminis*. Hydrodistillation turmeric oil in India had MICs against *C. albicans*, *A. niger* and *Staphylococcus aureus* at 5.5, 6.7 and 1.95 μg/ml, respectively [13]. From table 1, it was obviously that MIC of oil from Vietnamese turmeric against investigated fungi was 0.5%. *T. mentagrophytes* strains seems to be more resistant to the turmeric oil than *C. albicans*.

Determination of minimum killing time

The forming colonies on Sabouraud agar were determined after defined intervals of time of fungal incubation in the turmeric oil (table 2; fig. 2, 3). The results showed that *C. albicans* was killed after 1 hour incubation in turmeric oil, and *T. mentagrophytes* was more resistant toward oil. This strain was killed only after 90 min incubation in the oil.

Table 2. Surviving numbers of fungi after incubation in turmeric oil

Time (min)	*C. albicans* (CFU/ml)	*T. mentagrophytes* (CFU/ml)
0	8.6×10^3	7.5×10^3
15	2.1×10^3	3.0×10^3
25	1.5×10^3	1.9×10^3
45	1.2×10^2	6.0×10^2
60	0	4.0×10^2
90	0	0

Experimental examination for biological properties and use of medicinal plants *in vitro* and *in vivo* has been one of the principal criteria of drug discovery since centries. Although turmeric rhizome powder is popularly used in Vietnam for human ailments, but there was lack of experimental verification. Curcuma oil showed positive activity against fruit spoiling

fungi (*Cladosporium cladosporioides* (Fres.) de Vries, *Cladosporium tenuisimum* Cooke, *Aspergillus japonicus* Saito, *Aspergillus tubingensis* Mosserary and *Aspergillus versicolor* Vuill. Tiraboschi [3]. According to many reports, main component in turmeric oil responsible for antibacterial activity are turmerones, especially ar-turmerone [9, 10, 13]. It is also recognised that synergistic effect of some active components in the oil contributes to antifulgal activity. The varying degree of sensitivity of the microbial organisms toward turmeric oil may due to both the intrinsic tolerance of microorganisms and the nature and combination of compounds present in the essential oil [10]. The mechanism of action of essential oils was reported by some authors and remains somewhat controvesial. Some studies suggest that the compounds may penetrate the microorganisms and react with active sites enzymes and/or interfere with cellular metabolism; most evidence supports direct disruption of cellular membranes and concentration-dependent pro-oxidant cytotoxic effects [2, 16]. The antifungal activity may due to its ability to disrupt the permeability barrier of the plasma membrane, mitochondrial dysfunction-induced ROS accumulation in fungi [6].

The essential oil of many plants show a broad spectrum of activity against pest insects and plant pathogenic fungi, ranging from insecticidal, antifeedant, repellent, oviposition deterrent, growth regulatory and antivector activities. Keeping this in mind, plant oils can be used as "green pesticides" in developing countries and they ultimately have great impact in integrated pest management programmes due to their safety to non-target organisms and the environment [11].

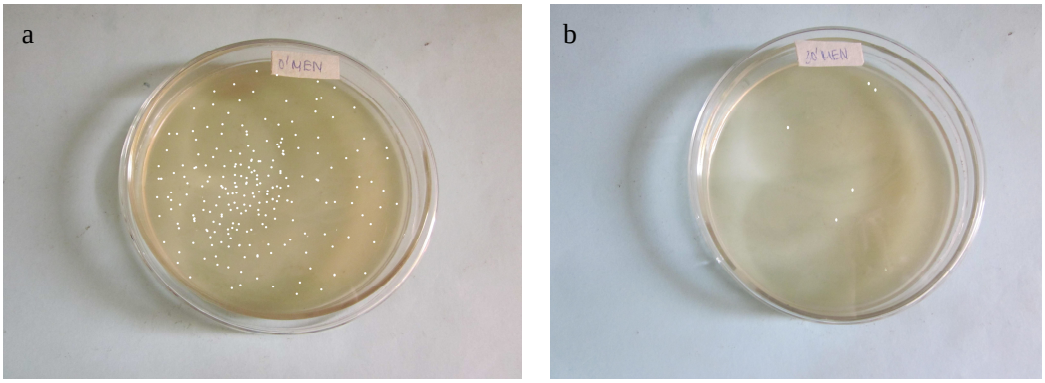

Fig. 2. Candida albicans (a. 0 min; b. 30 min)

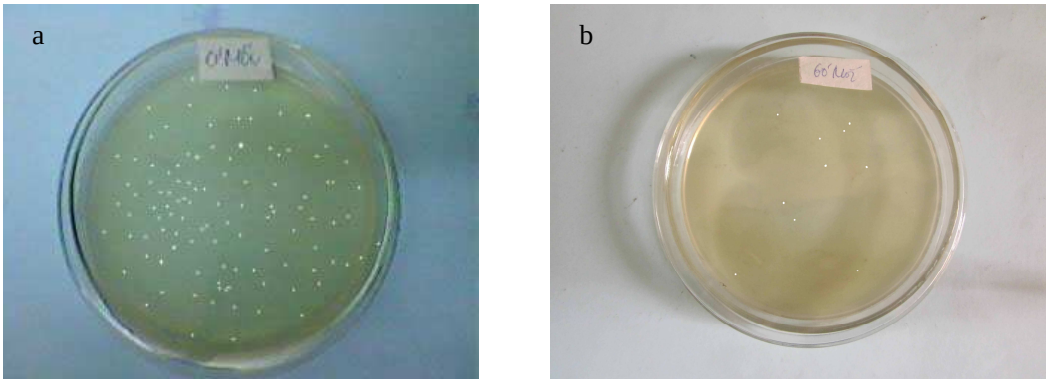

Fig. 3. Trychophytol mentargrohytes (a. 0 min; b. 60 min)

CONCLUSION

The essential oil from Vietnamese *Curcuma longa* had high level of turmerones and exihibited not only fungistatic, but also fungicidal effect on dermatophytes *T. mentagrophyte* and *C. albicans*. The obtained results demonstrated the potential use of turmeric oil in treatment of dermatomycosis. The development of natural antifungals will help to decrease the negative effect of synthetic drugs.

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ĐÁNH GIÁ HOẠT TÍNH KHÁNG NẤM GÂY BỆNH TRÊN DA CỦA DỊCH CHIẾT NGHỆ (*Curcuma longa* L.) *IN VITRO*

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TÓM TẮT

Curcuma longa L. là loại cây thuốc thân rễ lâu năm, tinh dầu của nó được xác định có hiệu quả kháng nấm, kháng khuẩn, chống oxy hóa, chống độc và chống khối u. Hoạt tính kháng nấm hại da của tinh dầu nghệ đã được chứng minh. Nghiên cứu này được tiến hành nhằm đánh giá hoạt tính ức chế của tinh dầu từ cây nghệ vàng của Việt Nam đối với 2 loại nấm hại da là *Trychophytol mentargrohytes* và *Candida albicans*. Tinh dầu nghệ thu được bằng phương pháp cất lôi cuốn hơi nước và thành phần hóa học của tinh dầu được xác định bằng GC/MS. Bằng cách so sánh với các hợp chất chuẩn trong thư viện khối phổ Nist 98.1 và Wiley 275.L, có 13 hợp chất đã được nhận dạng trong tinh dầu từ nghệ vàng Việt Nam. Kết quả nhận được cho thấy tinh dầu rất giàu thành phần turmerones (khoảng 44%), hợp chất được công nhận là phần tử chủ yếu cho hoạt tính kháng vi sinh vật.

Bằng phương pháp khuếch tán trên đĩa thạch, hoạt tính kháng nấm của tinh dầu nghệ đối với *T. mentargrophyte* and *C. albicans* đã được chứng minh. Nồng độ ức chế tối thiểu cho *T. mentargrophyte* và *C. albicans* là 0,5%. Thời gian tối thiểu để diệt *C. albicans* là 60 phút và đối với *T. mentargrophyte* là 90 phút ủ trong tinh dầu.

Từ khóa: Curcuma longa, dermatophytes, tinh dầu nghệ, turmerones, ức chế.

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