ANTIMICROBIAL EFFECT OF TURMERIC OIL (*CURCUMA LONGA* L.)

NGUYEN THỊ KIM CUC, TRAN THI KIM DUNG, PHAM VIET CUONG

ABSTRACT

Essential oil was extracted from turmeric rhizome by steam distillation and it's composition was determined by GC-MS method. In total, 36 volatile compounds were identified and 13 of them had ≥ 90% identity with reference compounds. The main constituent of obtained turmeric oil was ar-turmerone (30.33%), followed by alpha-turmerone (14.14%).

The antimicrobial activity of turmeric oil was evaluated by observing microbial growth on solid medium supplementing with different concentration of oil. The result showed that Gram positive bacteria were more sensitive to the oil than Gram (-) bacteria. *B. cereus* was inhibited at 1%, while *L. damsella* lost it's growth at essential oil concentration 3% only.

The test-fungi exhibit different sensitivity to turmeric oil. The most resistant was MNh14 (*Aspergillus sydowi*) and MNh19 (*Aspergillus japonicus*) isolated from *Litchi chinensis*, these strains can tolerate 4% of oil in growth medium.

Considering the importance of essential oils as ecofriendly agents, and their antimicrobial activity, the essential oils have potential as environmentally safe alternatives and as components in integrated pest management programs.

Keywords. Curcuma longa, hydrodistillation, microbial activity, essential oil, turmeric rhizome.

1. INTRODUCTION

Naturally derived compounds and other natural products may have applications for controlling pathogens in foods. The essential oils in some spices are known to possess antimicrobial activity, such as eugenol in cloves, allicin in garlic, and cinnamic aldehyde and eugenol in cinnamon [Hsieh et al, 2001]. Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties. Some oils have been used in cancer treatment and others in food preservation, aromatherapy and fragrance industries. Nowaday, interest in looking at antimicrobial properties of extracts from aromatic plants, particularly essential oils is increased [Satish et al., 1999; Prabuseenivasan et al, 2006].

Curcuma longa L. belongs to Zingiberaceae family and has long been used as a common household medicine and as a spice in Southeast Asia. In Vietnam, the genus *Curcuma* is widespread and represented by at least 16 species, many of them have been used in the Vietnamnes folk medicine [Phan Minh Giang et al, 2000]. In recent years, there are several reports concerning the composition and/or the biological properties (antimicrobial, antioxidant, anticancer and a stimulated effect on the immune system) of Zingiberaceae extracts [Apisariyakul et al, 1995; Negi et al, 1999; Phan Minh Giang et al, 2000; Prabuseenivasan et al,

2006; Norajit et al, 2007]. These studies have emphasized the existence of marked chemical differences among oils extracted from different species or varieties. These variations are likely a function of three factors: genetically determined properties, the age of the plant and the environment.

In this report, we have assessed antimicrobial activity of turmeric oil extracted from Vietnames *Curcuma longa* L. against common fruits pathogens, evaluating minimal inhibitory concentrations and the main components of the extracts by GC/MS, in an attempt to contribute to the use of the oil as alternative products for microbial control of the fruits after havest.

2. MATERIALS AND METHODS

2.1. Extraction of turmeric oil

Rhizomes of *Curcuma longa* L from Khoai Chau, Hung Yen province were collected, cut into pieces and pulverised, average particle size approximately 4 mm. The moisture content was about 90%. 1 kg of material was put into steam distillation apparatus and the process was carried out on electric cooker for 8 hours. The essential oil was condensed by cooling water at room temperature.

2.2. Characterization of essential oil

The chemical composition of turmeric oil was determined using model GC-System HP6890 MSD 5973 Aglilent on a 30.0 m 9 250 lm HP-5MS-coated fused silica capillary column with 0.25 lm film thickness. One microliter of essential oil sample was injected into the GC-MS system using split mode (50:1). The purge flow was 1.0 ml/min. The injector temperature was 250^{o}C. The column temperature was programmed as follows: initial temperature 65^{o}C for 2 min, 5^{o}C/min to 90^{o}C, 90^{o}C for 3 min; 20^{o}C/min to 103^{o}C; 103^{o}C for 3 min; 8^{o}C/min to 150^{o}C; 150^{o}C for 15 min; 20^{o}C/min to 280^{o}C. Mass conditions were as follows: electron impact (EI) ionization, electron energy 70.1 eV; interface temperature, 280^{o}C; ion source temperature, 230^{o}C; detector voltage, 1435 V; solvent delay, 3 min. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. All data were obtained by collecting the full-scan mass spectra within a scan range of 45 – 550 amu.

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 ($\geq$ 90%).

2.3. Microbial isolates

Bacteria and fungi were isolated from spoiled fruits such as blue dragon (*Hylocereus undatus/polyrhizus*), mango (*Mangifera indica*), orange (*Citrus sinensis*), *Litchi chinensis*, *Dimocarpus longan*, avocado (*Persea americana*), *Annona squamosa*. The bacterial isolates were identified by KIT API 20 NE to *Micrococcus* sp. (VN9), *Listonella damsela* (VNh1), KIT API 50 CHB to *Bacillus cereus* (VX23). The fungal isolates were identified based on their morphological characteristics to *Valsa* sp.Fr. (MC3), *Cladosporium cladosporioides* (Fres.) de Vries (MTL2), *Cladosporium tenuisimum* Cooke (MTL4) *Aspergillus japonicus* Saito (MNh19), *Aspergillus tubingensis* Mosserary (MB24) and *Aspergillus versicolor* Vuill. Tiraboschi (MX25) (Institute 69, Command for Ho Chi Minh president tomb guard).

2.4. Antimicrobial activity determination

Prior to the antimicrobial activity assay, the microbial cultures were plated in meat-peptone agar, or Czapex-Dox and grown for 24 h (for bacteria) or 48 h (for fungi) at 30^0C. From each plate, a loop was used to inoculate corresponding agar plates supplementing with turmeric oil at different concentrations. The growth of investigated strains was observed after 24h or 48 h culture.

Ethylene glycol was used for oil dilution.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of turmeric oil

The gas chromatographic method is used almost exclusively for the qualitative analysis of the volatiles. The primary criterion for peak identification were retention times. The mass spectrometer used as a chromatographic detector offers additional data for the identification of the separated compounds. For identification of compounds, the comparison of the recorded spectra with an MS library and/or reference standard compound is the most frequent used method.

The chemical composition of essential oil from fresh rhizome of Vietnames turmeric was determined by GC method. In total, 36 volatile compounds were identified and 13 of them had ≥ 90% identity with reference compounds (table 1), contributing more than 60% of the oil.

Table 1. Chemical composition of Vietnames turmeric oil obtaining by distillation method

No	Compounds	Rentention time	Area (%)
1	1,8-cineole	8.13	0.45
2	Terpinolene	9.91	1.87
3	Benzene	13.20	0.19
4	β-caryophyllene	20.83	3.02
5	α -humulene	21.88	0.79
6	Ar-curcumene	22.74	3.02
7	α-zingiberene	23.12	3.44
8	β -bisabolene	23.51	0.72
9	β -sesquiphellandrene	23.98	4.42
10	Di-epi-alpha-cedrene	26.99	0.15
11	Zingiberene	27.10	0.27
12	Ar-tumerone	28.25	30.33
13	α -tumerone	29.11	14.14
14	Other compounds		37.19

The main constituent of turmeric oil extracted by hydrodistillation was ar-turmerone (30.33%), followed by alpha-turmerone (14.14%). 26 unidentified compounds contributed about 37.19% of the oil. The obtained essential oil composed mainly sesquiterpenes (beta-caryophyllene, alpha-humulene, ar-curcumene, alpha-zingiberene, β –bisabolene, β – sesquiphellandrene, zingiberene, ar-tumerone, α –tumerone). Only two compounds were monoterpenes (1,8-cineole, terpinolene).

Chemical composition of turmeric oil extracted by hydrodistillation from different parts and from various geographic regions of *Curcuma* have been reported [Leela et al., 2002; Garg et al., 1999; Manzan et al., 2003; Norajit et al., 2007; Phan Minh Giang et al., 1998, 2000; Wilson et al., 2005]. The essential oil from Indian rhizomes of *Curcuma longa* composed 47 constituents, 24 of which could be identified. The major components were ar-turmerone (31.1%), turmerone (10.0%), curlone (10.6%) and ar-curcumene (6.3%) [Leela et al., 2002]. The essential oil profile from Thai Lan *Curcuma longa* shows turmerone as the main compound (50%), other major constituents were curlone, α-farnesene and α-zingiberene [Norajit et al., 2007]. The main components of *Curcuma kwangsiensis* (China) essential oil including β-elemene, curzerenone, curcumol, curdione, germacrone, and β-elemenone [Zeng et al., 2008]. Turmeric oils from rhizomes of Vietnames *Curcuma aff. aeruginosa* Roxb. extracted by petroleum ether composed ten sesquiterpene hydrocarbons and eight oxygen containing sesquiterpenoids; and of *C.aromatica* Salisb. were six oxygenated sesquiterpenes [Phan Minh Giang et al., 1998, 2000]. It was obviously that, there was considerable quantitative variation in the main components depending upon the cultivars from which the oil was produced. Manzan et al. (2003) determined the best processing conditions to maximize the yields of essential oil and pigments, as well as their content of ar-turmerone, (α and β)-turmerone, and the curcuminoids. The highest yields of essential oil (0.46 wt%) and pigment (0.16 wt%) were obtained at a pressure of $1.0x10^5$ Pa and a time of 2 h.

3.2. Determination of antibacterial activity in vitro

The antibacterial activity of turmeric essential oil was evaluated by growth of the investigated strains on appropriate media supplementing with different amount oil. The result showed that Gram positive bacteria were more sensitive to the oil. *B.cereus* was completely suppressed at the concentration 1%, and growth of *Micrococcus* sp. (VN9) was inhibited at this concentration, while *L. damsella* growed well. *L. damsella* lost it's growth at essential oil concentration 3% only (Table1, Fig. 1).

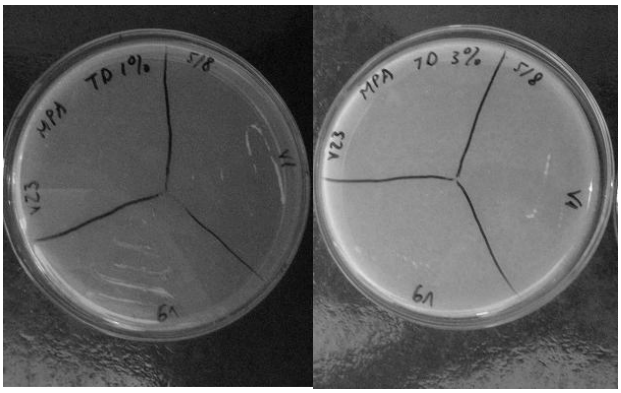

Figure 1. Antibacterial (VN9, VX23 and VNh1) activity of turmeric oil at different concentration

Table1. Growth of invertigated bacteria on medium supplemented with turmeric essential oil

Concentration of oil / Bacteria	1%	3%
VNh1 (*L. damsella*)	+	-
VN9 (*Micrococcus* sp.)	±	-
VX23 (*B.cereus*)	-	

Note: - No growth; ± weak growth; + good growth

This result is consistent with those reported by other workers (Negi et al, 1999; Phan Minh Giang et al, 2000; Alzoreky and Nakahara, 2003; Norajit et al, 2007). According to Alzoreky and Nakahara (2003), Gram negative bacteria were not susceptible to plant extracts due to lipopolysaccharide in their outer membrane. In another viewpoint was the work of Prabuseenivasan et al. (2006), which reckoned that Gram-positive bacteria were more resistant to the essential oils than gram-negative bacteria. To this disagreement may contribute many facts such as the nature and combination of bioactive compounds present in the essential oil (Norajit et al, 2007), nature of growth medium, physical and chemical properties of antibacterial compounds, differences in test methodologies and etc. (Negi et al, 1999).

3.3. Antifungal activity of turmeric oil in vitro

The ability of investigated fungi developed after incubation on medium adding essential oil was taken as the index of growth inhibition. It was obviously that fungi were more resistant to turmeric oil than bacteria. Two strains (MTL4 and MB24) were inhibited at oil concentration 2% (Table 2). One strains could not develop at oil concentration 3% (MC3). At concentraion 4%, MX25 was lost their growth and the most resistant from investigated strains were MTL2 and MNh19 (table 2).

Table 2. Growth of fungal strains on Czapek-Dox with turmeric oil obtained by hydrodistilation

Concentration of oil / Fungi	2%	3%	4%	5%
MC3 (*Valsa* sp.)	+	-		
MTL2 (*C.cladosporioides*)	++	+	±	-
MTL4 (*C.tenuisimum*)	-			
MNh19 (*A.japonicus*)	++	+	±	-
MB24 (*A.tubingensis*)	-			
MX25 (*A.versisolor*)	+	±	-	

Note: - No growth; ± weak growth; + good growth; ++ very good growth.

Interestingly that *Aspersgillus* sp. isolated from diffenrent sources had various sensitivity to turmeric oil. MNh19 (isolated from *Litchi chinensis*) was most resistant, it can grow at oil concentration 4%, while MB24 (from *Persea americana*) was most susceptible, it's growth was inhibited completely at oil concentration 2%, while strain MX25 was lost it's growth at concentration 4%. Even two *Cladosporium* strains isolated from *Hylocereus undatus/polyrhizus* exhibited various behaviour to turmeric oil. Growth of MTL4 was depressed at oil concentration 2%, but strain MTL2 was tolerated 4% of oil in culture media and it's growth was inhibited completely only at oil concentration 5%

The antimicrobial activity of essential oil of turmeric was reported by others scientists (Apisariyakul et al., 1995; Phan Minh Giang et al, 2000; Dhingra et al., 2007; Pawar and Thaker, 2007; Garcia et al., 2008). *In vitro* system, turmeric oil could inhibit dermatophytes and pathogenic molds and *in vivo*, it markedly reduced erythema and scale induced by *Trichphyton rubrum* in guinea pigs (Apisariyakul et al., 1995). Varying sensitivity of the test-fungi in this work was confirmed by works of Wilson et al., 2005; Sacchetti et al., 2005; Dhingra et al., 2007.

In view of hydrophobicity of essential oils and their components, it is generally considered that essential oil can partition the lipids of the bacterial cell membrane and mitochondria, confusing the cell structures and causing them more permeable (Prabuseenivasan et al., 2006). Extensive leakage of critical molecules and ions from bacterial cells will lead to death. Several authors concluded that as lipophilic agents essential oils execute their action at membrane integrity level, affecting embedded enzymes and fatty acid composition. The inhibitory action was manifested as depression of mycelium germination and hyphal wall synthesis (Sharma and Tripathi, 2006; Garcia et al,. 2008)

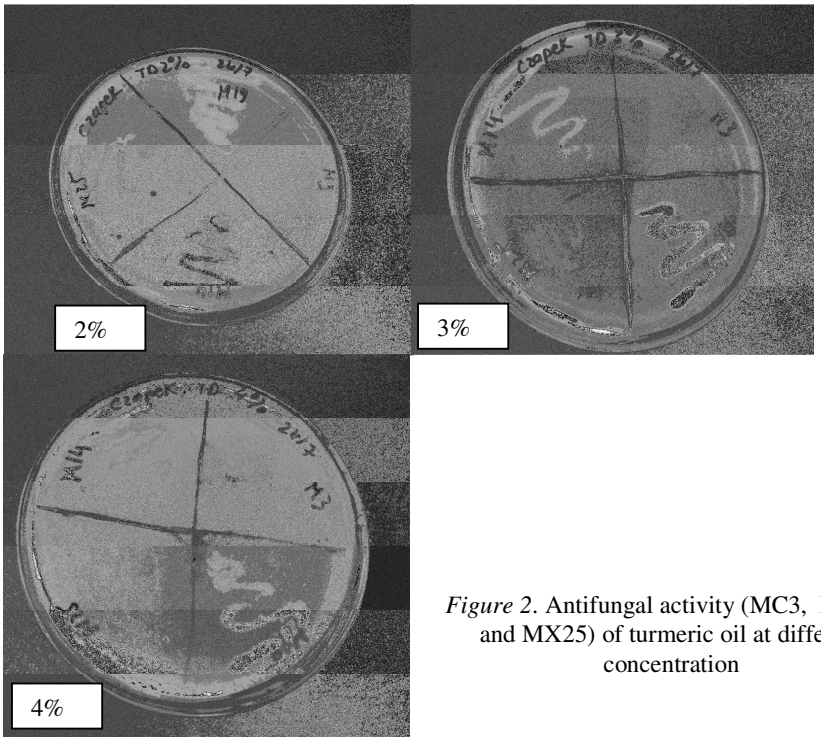

Figure 2. Antifungal activity (MC3, MNh19 and MX25) of turmeric oil at different concentration

The dried rhizome of *C. longa* contains 2- 6% of essential oil, of which up to 58% comprises turmerones (Negi et al., 1999; Chowdhury et al., 2000; Chattopadhyay et al., 2004; Norajit et al., 2007). According to these authors, antimicrobial activity was mainly contributed by turmerones. The discrepancy in the reported microbial growth inhibitory concentration of the turmeric rhizome oil, may be related to the composition, and proportion of the turmerones of the oil extracted from rhizomes sourced from different eco-systems or cultivars (Norajit et al., 2007; Dhingra et al., 2007).

Recently the use of agrochemicals for plant protection has fallen into disfavour because of their detrimental effects on nontarget organisms and environment pollution. Considering the importance of essential oils as ecofriendly agents, and their antimicrobial activity (reducing populations of soil-borne plant pathogens and control disease development), the essential oils have potential as environmentally safe alternatives and as components in integrated pest management programs. Thus these essential oils are potent antimicrobial agents with broad spectrum activity with possible potential for the control of microbial infections in plants as well as that of post-harvest spoilage of many crops and crop products.

4. CONCLUSION

Essential oil extracted from *C. longa* by hydrodistillation contains 44.47% turmerones, followed by β–sesquiphellandrene (4.42%), α-zingiberene (3.44%) and terpinolene (1.87%). This oil exhibits antimicrobial activity toward Gram (-) and Gram (+) bacteria as well some fungi. The most sensitive microorganisms were *B.cereus* (was inhibited at oil con. 1%) and MA24 (*Cladosporium* sp.) which was inbibited at oil con. 2%. Based on biological properties of turmeric oil, the compound can be used as tropical fruits preservatives and in integrated pest management programs.

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TÓM TẮT

HIỆU QUẢ KHÁNG VI SINH VẬT CỦA TINH DẦU NGHỆ (*CURCUMA LONGA* L.)

Tinh dầu nghệ được chiết bằng phương pháp cất lôi cuốn hơi nước và thành phần của nó đã được xác định bằng phương pháp GC/MS. Tổng số 36 thành phần bay hơi đã được xác định,

trong đó 13 thành phần có độ tương đồng ≥ 90% so với các chất chuẩn. Thành phần chính của tinh dầu nghệ là ar-turmerone (30,33%), tiếp theo là alpha-turmerone (14,14%).

Hoạt tính kháng vi sinh vật của tinh dầu nghệ đã được đánh giá bằng cách quan sát sự sinh trưởng của vi sinh vật trên môi trường đặc có bổ sung tinh dầu ở các nồng độ khác nhau. Kết quả nhận được cho thấy, vi khuẩn Gram dương mẫn cảm hơn so với vi khuẩn Gram âm. Sinh trưởng của *B.cereus* bị ức chế ở nồng độ tinh dầu 1%, trong khi đó *L. damsella* không sinh trưởng được khi bổ sung 3% tinh dầu.

Các chủng nấm sợi thử nghiệm thể hiện sự mẫn cảm khác nhau với tinh dầu nghệ. Chủng MNh19 (*Aspergillus japonicus*) được phân lập từ *Litchi chinensis* có tính kháng mạnh nhất, có thể chịu được nồng độ tinh dầu 4% trong môi trường nuôi cấy.

Tinh dầu là một chất thay thế tiềm năng an toàn cho môi trường và là thành phần quan trọng trong chương trình quản lí dịch hại tổng hợp nhờ đặc tính thân thiện môi trường và hoạt tính kháng vi sinh vật của tinh dầu.

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