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Chemical composition and antifungal activity of oil extracted from leaves turmeric (*Curcuma longa*)

Rini Yanti[1*], Hermina Nurdiawati[1], Puji Wulandari[2], Yudi Pranoto[1], Muhammad Nur Cahyanto[1]

[1] Department of Food and Agricultural Product Technology, Agricultural Technology Faculty, Gadjah Mada University, Indonesia
[2] Department of Food Technology, Agricultural Faculty, Sultan Ageng Tirtayasa University, Indonesia

Abstract

Turmeric rhizomes are commonly used in the culinary, pharmaceutical, herbal medicine, and beverage industries. On the contrary, turmeric leaves are underutilized. The aims of this study were to extract the essential oil from turmeric leaves, characterize the chemical composition of the oil, and determine its antifungal activities against aflatoxin-producing fungi. Steam distillation was used to extract the essential oil from turmeric leaves. The properties of the oil were identified using GC-MS. Antimicrobial activities against *Aspergillus flavus* and *Aspergillus parasiticus* were determined. Spores of the fungi were inoculated into potato dextrose agar plates supplemented with various quantities of turmeric leaves essential oil and incubated at 30°C for 7 days. The oil's primary constituents were α-phelandrene (46.70%), followed by α-terpinolene (17.39%), 1,8-cineole (8.78%), benzene (4.24%), and 2-β pinene (3.64%). At low (<1%) concentrations, the oil delayed mycelia formation and at high concentrations it significantly inhibit fungal growth (at 1%) and completely inhibit colony formation (at 2%). Additionally, the result show that turmeric leaves oil can inhibited fungus growth at the lowest concentration (0.25%) when compared to the control over a seven-day incubation period.

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Essential oil,
Antifungal,
Aspergillus flavus,
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1. Introduction

Curcuma is the largest genus in the *Zingiberaceae* family and is frequently used in the manufacture of spices, pharmaceutical products, dyes, and decorative plants. There are approximately 93–100 *Curcuma* species known (1). Turmeric (*Curcuma longa*) is one of the species that indigenous to South Asia (India, East Indies, Sri Lanka, Pakistan, and Bangladesh), East Asia (China and Taiwan), Southeast Asia (Thailand, Burma, and Indonesia), as well as northern Australia (2). The rhizome of turmeric is widely used in Indonesia's culinary, medicinal, traditional herb, and beverage industries. Turmeric leaves, on the other hand, are underutilized and generate waste during post-harvest processing. Traditionally, the leaves were mostly applied as a flavouring agent in cooking, especially in the Minangkabau cuisine of West Sumatera. Moreover, recent findings show that turmeric leaves and stems have their own biological or chemical activities due to the curcumin and other medicinal components in the leaves and stem (3). Turmeric leaves contain oil approximately 0.53% (weight basis). Several studies (4) demonstrated that turmeric leaves

* Correspondence: **Rini Yanti** riniyanti@ugm.ac.id

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oil was effective against *Escherichia coli*, an antibiotic-resistant bacteria, and oil from *Curcuma longa* leaves possessed antioxidant properties (3,5).

Aspergillus flavus and *Aspergillus parasiticus* were discovered as contaminants in cereals and legumes, respectively (6,7). Both fungi destroy the product's quality and produce mycotoxins. Aflatoxin is a mycotoxic substance generated by *Aspergillus flavus* and *Aspergillus paraciticus*. Aflatoxin B1 is the form of aflatoxin that is most harmful to animals and people health (8).

Aflatoxin is most likely to cause mutagenic, carcinogenic, teratogenic, hepatotoxic, and immunosuppressive effects, as well as to inhibit certain metabolic systems (6). The metabolic systems affected include nutritional disorders such as kwashiorkor and off-balance growth-which is most likely by interfering micronutrient absorption (e.g., zinc, iron, and vitamins), protein synthesis, and metabolic enzyme activities (9).

Aflatoxins have a relatively stable molecular structure. The decomposition temperatures range between 237 and 306°C, and they are extremely resistant to dry heat (melting point 268–269°C) (10). While heating at a higher temperature can reduce aflatoxin levels, it also reduces the nutritional value of the product. To avoid aflatoxin contamination in foods, it is necessary to suppress the growth of *A. flavus* and *A. paraciticus*.

The treatments traditionally used to control the growth of fungi are mostly synthetic fungicides. However, in the past few decades, studies on natural products to replace synthetic additives and preservatives in the food industry have grown tremendously. When used in relatively high concentrations, chemicals such as benzomidazole group and inhibitors of aromatic hydrocarbons biosynthesis have residual risks of being carcinogenic. Natural substances such as essential oils have emerged as a promising and effective compounds to protect food products from microbial contamination. Some essential oils have been classified as GRAS (Generally Recognized as Safe) and have shown antifungal and antibacterial activities against various microorganisms (11). Therefore, the objectives of this study were to extract the essential oil from turmeric leaves, characterize its chemical composition, and determine turmeric oil's antifungal activity against aflatoxin-producing fungi.

2. Materials and Methods

2.1. Preparation of Essential Oil

Turmeric leaves were collected from Yogyakarta's Beringharjo traditional market. The leaves were washed in running water and cut into pieces between 1-2 cm in length and dried in a cabinet dryer. Steam distillation was used to extract essential oils from the leaves. The distillation process lasted four hours; timed when the distillate began to drip. The distillate was in the form of oil-water mixture, which was later separated using a separator funnel. Anhydrous Na_2SO_4 (Merck, Germany) was added in a ratio of 1:10 (g/ml), and the mixture was filtered using Whatman 42 paper (GE Healthcare, UK). The essential oil obtained was stored in a tightly closed dark bottle and kept at a temperature of 4°C for further analysis (12).

2.2. Preparation of Aspergillus Spore

Aspergillus flavus (FNCC 6133) and *Aspergillus parasiticus* (FNCC 6033) were obtained from Gadjah Mada University's Food and Nutrition Culture Collection. *A. flavus* and *A. parasiticus* microbial strains were seeded on slanted Potato Dextrose Agar (PDA) (Merck, Germany) and incubated at 30°C for 7 days. Spores were harvested by adding 3 mL of 0.05% tween 80 solution. The slanted agar surface was scratched carefully to release the spores from the agar media and spores stock in the form of a suspension was obtained. After diluting the stock spores with a 0.05% tween 80 solution, a spore concentration of 107 spores/mL was obtained. The concentration of spores was determined using a Neubauer haemocytometer (Assistant, Germany).

2.3. Identification of Chemical Composition

Gas chromatography (Shimadzu GCMS-QP2010S equipped with an RTX-5MS column) was used to determine the chemical compositions of the oil. Helium was used to transport the gas at a flow rate of 0.5mL/min. Operating temperature was programmed to rise at a rate of 4°C/min from 70 to 280°C. The temperature of the injector was set at 290°C.

2.4. Antimicrobial Activity

The antifungal activity of turmeric leaf essential oil on potato dextrose agar media was investigated using the poisoned food technique (13). The turmeric leaves oil were added in various concentrations (0%; 0.25%; 0.50%; 1%; and 2%) to the PDA liquid media containing 1% Tween 80 (Merck, Germany), and then poured into a petri dish. The PDA media was allowed to solidify for a few minutes before it was inoculated with the fungi spores. Each plate was inoculated at three points and incubated at 30°C. All treatments were compared to PDA with 1% Tween 80. The inhibition area percentage of fungal colony growth was calculated using the following equation (14).

$$\text{Inhibition diameter (\%)} = A = \frac{Dc-Do}{DC}\ x\ 100\ \%$$

Note : Dc = Diameter of the control colony
Do = Diameter of the treated colony

All treatments were carried out in 3 replications. Statistical tests with analysis of variance were used to obtain the difference among the means. If a significant difference existed, the analysis was followed with the Duncan's Multiple Range Test (DMRT) at a significance level of 95%.

3. Result and Discussion

3.1. Chemical Compositions of Essential Oil

The chromatogram obtained from the GC–MS for the essential oils extracted from turmeric leaves is shown in Figure 1 and the chemical compositions of the oil are presented in Table 1.

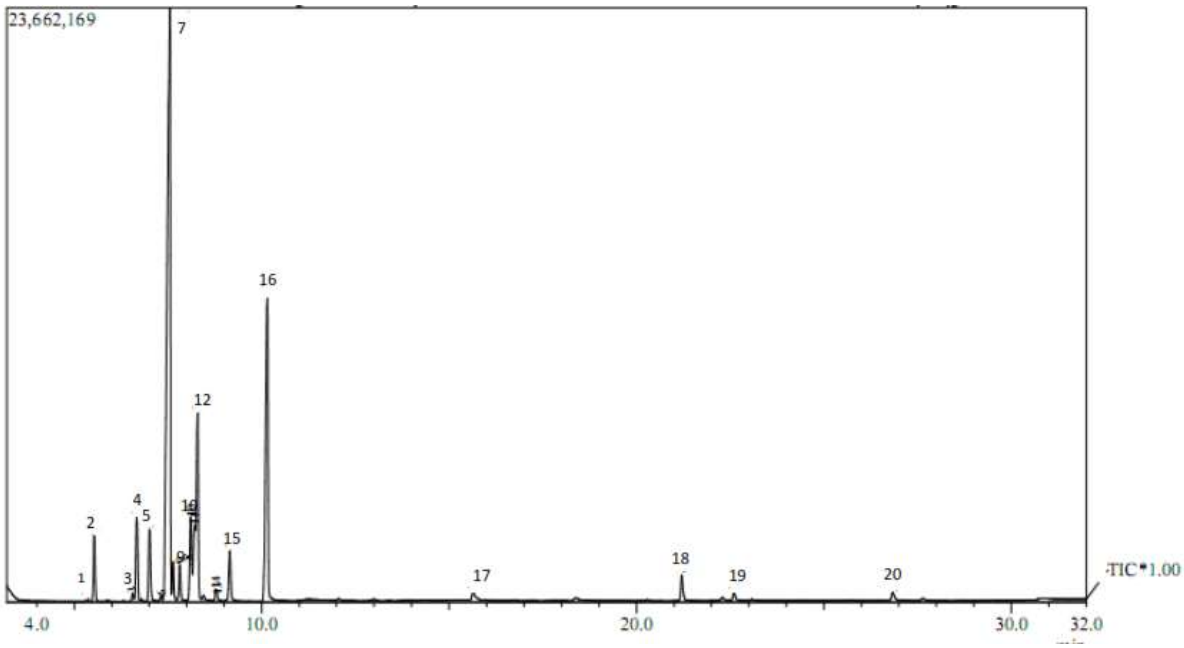

Figure 1. GC-MS chromatogram of turmeric leaves essential oil

Table 1. Chemicals composition of turmeric leaves essential oil

No	Compound	Relative content (%)
1	α- Thujene	0.13
2	α-Pinene	2.65
3	Sabinene	0.32
4	2- β Pinene	3.64
5	β-Myrcene	3.38
6	Delta 4-Carene	0.19
7	α -Phellandrene	46.70
8	Delta 3-Carene	1.48
9	α- Terpinene	1.50
10	Benzene	4.24
11	1-Limonene	3.59
12	1,8-Cineole	8.78
13	cis-Ocimene	0.24
14	1,3,6-Octatriene, 3,7-dimethyl	0.58
15	γ-Terpinene	2.28
16	α-Terpinolene	17.39
17	Limonene dioxide	0.53
18	β-Farnesene	1.37
19	Farnesene	0.47
20	AR-Tumerone	0.52

The results shown in Table 1 reveals that there were twenty compounds identified in the oil with α-phelandren was the largest component (46.70%) followed by α-terpinolene (17.39%), 1,8-cineole (8.78%), benzene (4.24%), and 2-β pinene (3.64%). These findings support the results of previous studies which reported that α-phelandrene is the primary component of turmeric leaves essential oil (14–16). These authors reported that as many as

61 compounds were identified in the essential oil extracted from *C. longa* leaves. McCarron et al (15)reported that α-phellandrene (53.4%), terpinolene (11.5%), and 1,8-cineole (10.5%) were the most abundant. Dixit (16) reported that the primari components of *C. longa* leaf oil were α-phellandrene (24.5%), 1,8-cineole (15.9%), pcymene (13.2%), and β-pinene (8.9%). Similar to the finding reported by McCarron et al (15), Oguntimein et al (17) also found that α-phellandrene (47.7%) and terpinolene (28.9%) were the predominant compounds in *C. longa* leaf oil. Therefore, the results of our study are in accordance with the results reported by these researchers.

3.2. Antifungal Activity

The effects of turmeric leave oil at various concentration on the mycelia growth of *A. flavus* and *A. parasiticus* during incubation are shown in Figure 2 and Figure 3 respectively and the diameter of the colonies are presented in Figures 4 and 5.

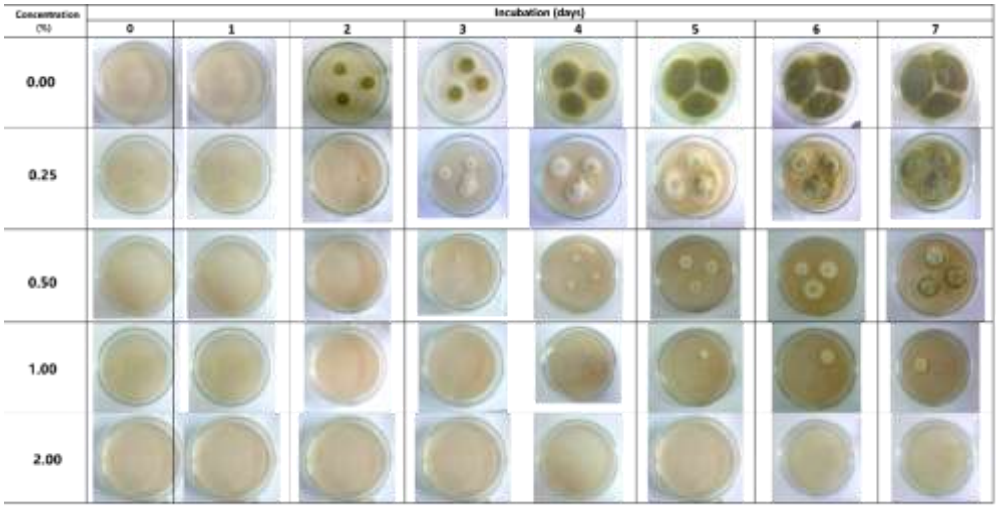

Figure 2. Antifungal activity of essential oil from turmeric leaves against *A. flavus*

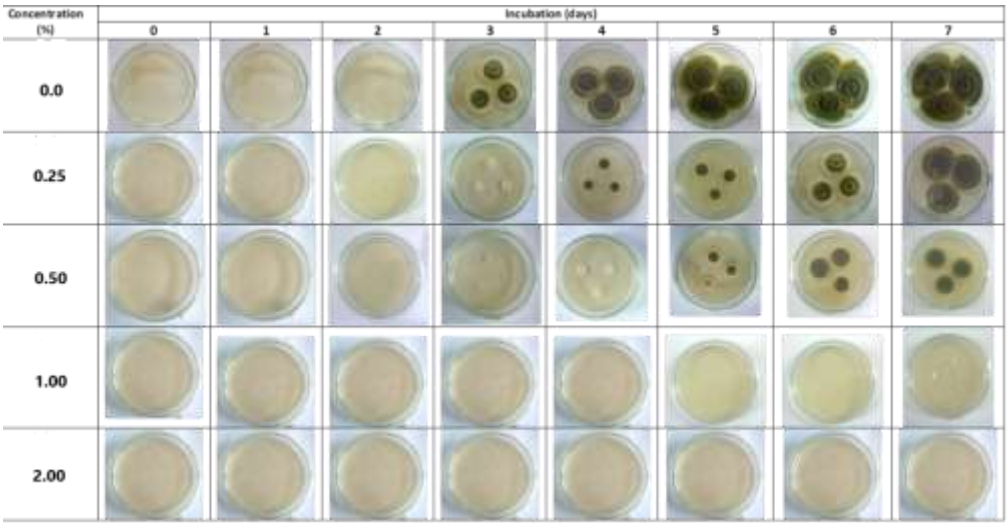

Figure 3. Antifungal activity of essential oil from turmeric leaves against *A. parasiticus*

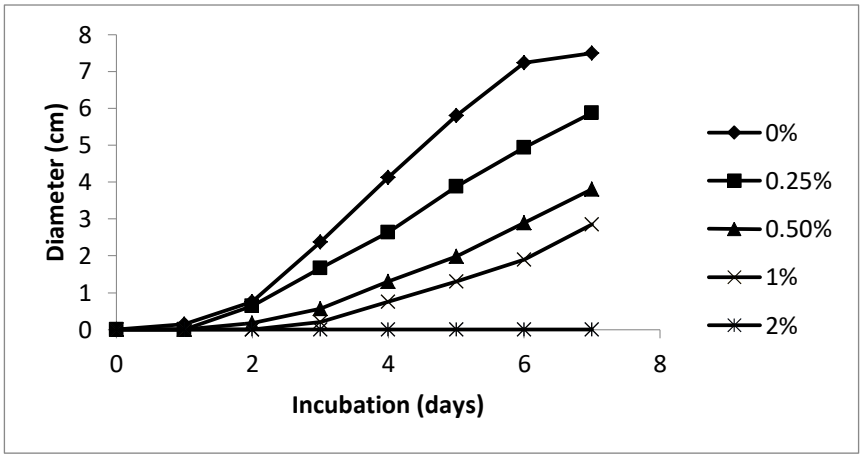

Figure 4. The effect of turmeric leaves oil concentration on mycelia diameter of *A. flavus* during 7 days of incubation

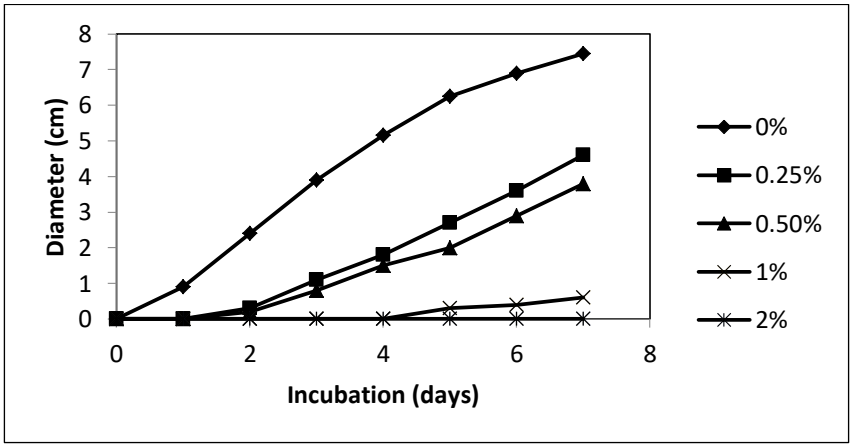

Figure 5. The effect of turmeric leaves oil concentration on mycelia diameter of *A. parasiticus* during 7 days of incubation

The growth patterns of *A. flavus* and *A. parasiticus* in growth media containing various concentrations of turmeric leaf oil were observed for seven days. The antifungal activity of turmeric leaf oil was demonstrated by its ability to inhibit the growth of *A. flavus* and A. *parasiticus* during seven days of observation. When essential oil was added to the media, mycelia formation was delayed even at concentration of as low as 0.25%. On the first day of incubation, the media without turmeric leaf oil were colonized by *A. flavus* and *A. parasiticus* mycelia. Inhibitions of *A. flavus* and *A. parasiticus* mycelium were demonstrated in media containing 0.25%, 0.50%, 1.00%, or 2.00% turmeric leaves oil. On the second day, mycelia began to grow on media treated with 0.25% and 0.50% turmeric leaf oil, but no growths were observed on the media with 1.00% or 2.00% turmeric leaf oil. At the 1.00% concentration, *A. flavus* mycelia began to grow on the third day, whereas *A. parasiticus* mycelia began to grow on the fifth day. After seven days of incubation, mycelia of the fungi did not appear in the media containing 2% turmeric leaf oil. The percentages of the inhibition by turmeric leave oil are shown in Table 2.

Table 2. Growth inbition by turmeric leave oil on *A. flavus* and *A. parasiticus for 7 days*

Concentration of oil (%)	*A. flavus* Inhibition (%)	*A. parasiticus* Inhibition (%)
0	0 [a]	0 [a]
0.25	21.67 [b]	38.26 [b]
0.50	49.33 [c]	48.99 [c]
1.00	62.00 [d]	91.95 [d]
2.00	100.00 [e]	100.00 [e]

Different superscript [a,b,c,d] in the same column indicates significant difference at α 5%

A. flavus and *A. parasiticus* growths were inhibited by about 21.67% and 38.26%, respectively, at the lowest concentration tested (0.25%). The higher the concentration of turmeric oil used, the higher the inhibition. *A. flavus* and *A. parasiticus* growth could be completely inhibited for 7 days at the 2% concentration. This inhibition was due to α-phellandrene, the main components of turmeric leaves oil.

Various phytochemicals such as α-Phellandrene, β-Phellandrene, ocimene, limonene, myrcene, and α-caryophyllene have been shown to have in vitro activity against *Bacillus* sp., *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *S. aureus* (18,19). Similarly, Zhang et al. (20) reported that α-Phellandrene and Nonanal significantly inhibit the mycelia growth of *P. cyclopium*. These chemicals can disturb the integrity of the fungal cell membrane, leading to the leakage of cell constituent and potassium ions, and causing an intensification of the total lipid content, extracellular pH and membrane permeability. This inhibitory effect was a result of α-phellandrene, one of the primary components of turmeric leaves oil.

The second largest compound in turmeric leaves oil is α-Terpinolene. The fraction of Eucalyptus oil with α-Terpinolene 88.4% had a fairly strong activity against *P. fragi, E. coli, S. typhimurium, S. aureus, S. cerevisiae* at concentrations of 0.1-0.4% (21).

α-Terpinolene is the second most abundant compound in turmeric leaf oil. At concentrations of 0.1-0.4%, the fraction of *Eucalyptus* oil containing 88.4% α-Terpinolene demonstrated moderate activity against *P. fragi*, *E. coli*, *S. typhimurium*, *S. aureus*, and *S. cerevisiae* (21).

α-Pinene is one of the minor components that has the ability to act as an antimicrobial. Essential oil with α-pinene (17.7%) as the predominant component, exhibits antifungal activity against Fusarium poae at a concentration of 2% (22). With minimal inhibitory concentrations of 0.02 to 0.15%, coriander oil fraction containing 89.4% α-pinene can inhibit the growth of *E. coli*, *S. typhimurium*, *L. monocytogenes*, *S. aureus*, and *S. cerevisiae* (21).

4. Conclusions

The component of essential oil from turmeric oil were mainly α-phelandrene followed by α-Terpinolene, 1,8-Cineole, benzene, 2- β Pinene : (17.39%), (8.78%), (4.24%), (3.64%), respectively.

Turmeric leaves oil indicated to have antifungal activity to *A. flavus* and *A. parasiticus*. The use of various concentrations of essential oil of turmeric leaves oil indicate a differences in the level of inhibition on the growth of fungi tested. In addition, the delay in spore

germination were different in both Aspergillus. In this investigation, the oil showed a better antifungal activity *to A. parasiticus*

The major constituents of turmeric essential oil were α-phelandrene, followed by α-terpinolene, 1,8-cineole, benzene, and 2-β pinene (17.39%, 8.78%, 4.24%, and 3.64%, respectively). Turmeric leaf oil has been shown to have antifungal activity against *Aspergillus flavus* and *Aspergillus parasiticus*. The use of different concentrations of turmeric leaves essential oil indicates a difference in the level of inhibition of the fungi tested. Additionally, the spore germination delay was different in both *Aspergillus* species. The oil demonstrated great antifungal activity against *A. parasiticus* in this study.

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Author Contributions

Rini Yanti, Yudi Pranoto, and Muhammad Nur Cahyanto, conceived and designed the experiments; Hermina Nurdiawati and Puji Wulandari performed the experiments; Rini Yanti contributed reagents/material/analysis tools; Rini Yanti wrote the paper.

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