Antifungal activity of curcuma xanthorrhiza and curcuma soloensis extracts and fractions

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Abstract. In this research, the antifungal activity of acetone extracts, and fractions of n-hexane, chloroform and ethylacetate of C. xanthorrhiza and C. soloensis rhizomes have been conducted. The antifungal activity was carried out by using agar dilution method and evaluated against Aspergillus fumigatus, Candida albicans, Epidermophyton sp, Penicillium sp and Trichophyton rubrum. The result showed that acetone extract and chloroform fraction of C. xanthorrhiza exhibited significant activities against A. fumigatus, Epidermophyton sp, Penicillium sp and T. rubrum with MIC 12.5-25.0 µg/mL. The n-hexane fraction of C. xanthorrhiza showed significant activity on Epidermophyton sp with MIC 12.5 µg/mL. Meanwhile, the extract and fraction of C. soloensis showed moderate and weak activities against all tested fungal with MIC 50-200 µg/mL.

Keywords: C. xanthorriza, C soloensis, extract and fraction, antifungal.

1. Introduction

Fungus is one of the microbes that cause infection, especially in tropical countries. Tropical climate with high air humidity as in Indonesia was strongly supports the growth of fungus. The proliferation of fungus infections is also supported by low public awareness of environmental hygiene, sanitation, and healthy lifestyles. One attempt to suppress the spread of fungal infections is through the use of antibiotics or synthetic food preservatives. But within a certain time, the ability of antibiotics is gradually decreased, because the targeted microbes were developing its immunity. Development of microbial resistance has stimulated researchers to find new antibiotics either by synthesis or from natural compounds, of particularly from plants [1].

Curcuma is an important medicinal plant in Indonesia, because more than 50 recipes of herbs circulating in Indonesia using Curcuma rhizome. These herbs are used to treat various diseases, including gastrointestinal and liver disorders, kidney inflammation, gall stones, hemorrhoids, rheumatism, high cholesterol, menstruation, lack of breast milk and appetite [2, 3]. In addition, Curcuma rhizome is also widely used as a spice on a variety of cuisine, giving the yellow color on food, to keep the body fresh, and for cosmetic raw materials [3].

Previous study showed that C. xanthorrhiza rhizome extracts can lower cholesterol levels in patient with high cholesterol. C. xanthorrhiza is also known as hepatoprotector, the regular consumption of boiling of three slices of rhizome C. xanthorrhiza and one piece of papaya leaf can decrease serum glutamic pyruvic transaminase (SGPT) and SGOT (serum glutamic oxaloacetic transaminase) of hepatitis patients to normal, for a week [4]. Extract of C. xanthorrhiza rhizome has been scientifically proven to have hypothermic effects [5], analgesic and antidiuretic activity [6, 7], immunostimulant [8],...
anticarcinogenic [9] and antibacterial [10] Kertia et al. [11] reported that C. xanthorrhiza is commonly used to prevent arthritis (osteoarthritis) due to its anti-inflammatory effect [12]. The results of the research on four species of Curcuma namely C. longa, C. caesia, C. amada, and C. aromatica also known that water extract and alcohol extract C. longa and C. aromatica showed antimicrobial activity after tested against Staphylococcus aureus, Bacillus subtilis, C. albicans and Aspergillus flavus [13]. The essential oils of C. solensis was reported to inhibit the growth of Staphylococcus aureus, S. epidermis, and Streptococcus haemolyticus [14]. The extracts of n-hexane, methylene chloride and ethylacetate of C. solensis rhizome were also showed the antifungal activity against C. albicans [15].

The phytochemical studies of the Curcuma rhizomes indicate that they contain two mayor types of secondary metabolites, namely diarilheptanoid (curcuminoide) and terpenoid mainly sesquiterpenes [16]. Curcumen is the most widely studied diarylheptanoid compound, including hepatoprotector, antioxidant, antitumor, anticancer, anti-inflammatory, anti-HIV and antimicrobial [12, 16]. Santorizol as one of the main terpenoid compounds in the Curcuma rhizome is known to have high activity against some pathogenic bacteria [17-19] some Candida [20], Malassezia [21] and filamentous fungi [22]. Germacrone and furanodienon was known to also have antibacterial activity [23, 24].

Some of the results of this study showed the potential of Curcuma rhizome as antimicrobial, but has not been studied comprehensively, especially its activity as an antifungal. In this study will be conducted the antifungal properties of extract and fraction of C. xanthorrhiza and C. solensis rhizomes against A. fumigatus, C. albicans, Epidermophyton sp., Penicillium sp., and T. rubrum.

2. Material and Methods

2.1. Materials
We used rhizome of C. xanthorrhiza and C. solensis (collected from Solo, Indonesia), redistilled solvents of n-hexane, ethylacetate and methanol, chloroform (Merck), demineralized water, fungal strains: A. fumigatus, C. albicans, Epidermophyton sp., Penicillium sp., and Trichophyton rubrum, Sabaroud Dextrose Agar (Oxoid), Ketoconazole (Merck) and dimethyl sulfoxide (Merck).

2.2. Extraction and fractionation of C. xanthorrhiza and C. solensis rhizomes
The fresh rhizome of C. xanthorrhiza (10 kg) and C. solensis (10 kg) were washed with water to remove the impurities, then cut to small pieces and air dried for 5 days. The dry rhizomes were ground into powder. A dry powder of C. Xanthorrhiza (1.2 kg) and C. solensis rhizomes (1.0 kg) were extracted with acetone (three times) for three days, at room temperature. The each of acetone extract was filtered and concentrated using a rotary evaporator. Furthermore, the acetone extract was partitioned into n-hexane: methanol (1:1). Then n-hexane soluble extract (n-hexane fraction) was concentrated with a rotary evaporator. In other hand, the methanol soluble extract was partitioned into chloroform: water (1:1). The chloroform soluble extract (chloroform fraction) was concentrated with a rotary evaporator, then the water soluble was extracted into ethylacetate to give ethylacetate fraction.

2.3. Antifungal activity assays [25]
In vitro antifungal activity assays was carried out with agar dilution methods against five fungal i.e. A. fumigatus, C. albicans, Epidermophyton sp, Penicillium sp., and Trichophyton rubrum. The concentration of sample was 400, 200, 100, 50, 25, 12.5, 6.25 μg / mL. The sample was dissolved in 10% DMSO in distilled water.

Selected fungal were cultured for 48 hours at 27 °C under aerobic conditions on agar media (Sabaroud Dextrose Agar). Afterwards, the fungal were suspended in a 0.9% NaCl solution (w/v). The concentration of fungal suspension was adjusted to 10^7 fungal cells /mL.

A total of 1.0 ml of each test solution was put in a test tube, then each 3.0 mL of agar (SDA) was added which was still liquid. Place each tube in a tilted position and allow it to stand until the sample-media solution solidifies. The fungal suspension (10 μL) was then inoculated to the agar medium surface which containing the sample solution, then they were incubated for 48 hours at 27°C. Furthermore, the fungal growth was observed. The lowest concentration of solution where no fungal growth was stated
as the minimum inhibitory concentration (MIC). The same method was carried out to the negative control (without extract or fraction) and standard antibiotics ketoconazole (positive control). The assay was repeated three times.

3. Result and Discussions

Extraction of the dried powder of C. xanthorrhiza (1.2 kg) and C. soloensis (1.0 kg) rhizomes with acetone both yielded the brownish yellow paste 87.6 g and 58 g respectively. The liquid-liquid fractionation of acetone extract of C. xanthorrhiza with n-hexane, chloroform and ethyl acetate respectively, was yielded n-hexane fraction 55.6 g, chloroform fraction 10.5 g and ethyl acetate fraction 3.7 g, while the liquid-liquid fractionation of acetone extract of C. soloensis into n-hexane, chloroform and ethyl acetate was obtained n-hexane fraction 38.2 g, chloroform fraction 6.5 g and ethyl acetate fraction 2.1 g. The weight of n-hexane fractions of C. xanthorrhiza and C. soloensis were most than chloroform and ethyl acetate fractions, these showed that practically most of the mass acetone extract soluble in n-hexane fractions. The previous study reported that n-hexane fraction of Curcuma contain the essential oils with the major constituents was sesquiterpene, while chloroform fractions was contain curcuminoids as its main component [19].

The antifungal activities of the acetone extract, n-hexane, chloroform and ethylacetate fractions of C. xanthorrhiza and C. soloensis was presented in Table 1 as minimum inhibitory concentration (MIC) values.

### Table 1. MIC values of C. xanthorrhiza and C. soloensis extracts and fractions

| Fungal                  | Kzl | Cx-A | Cx-H | Cx-E | Cs-A | Cs-H | Cs-C | Cs-E |
|-------------------------|-----|------|------|------|------|------|------|------|
| *A. fumigatus*          | 6.25| 50   | 25   | 25   | 100  | 100  | 50   | 50   |
| *C. albicans*           | 12.5| 50   | 50   | 25   | 50   | 50   | 50   | 100  |
| *Epidermophyton sp*     | 6.25| 12.5 | 12.5 | 12.5 | 50   | 200  | 50   | 50   |
| *Penicillium sp*        | 6.25| 25   | 100  | 12.5 | 100  | 200  | 200  | 200  |
| *T. rubrum*             | 6.25| 12.5 | 50   | 25   | 100  | 50   | 50   | 100  |

Kzl=ketoconazole; Cx= C. xanthorrhiza; Cs=C. soloensis; A=acetone extract; H=n-hexane fraction; C= chloroform fraction; E= ethylacetate fraction.

As shown in Table 1, the extract and fractions of C. xanthorrhiza and C. soloensis were potential as antifungal agent due to both have the MIC values <1000 µg/mL [25]. The highest antifungal activity (MIC 12.5 µg/mL) was showed by acetone extract of C. xanthorrhiza against *Epidermophyton sp* and *T. rubrum*, n-hexane fraction of C. xanthorrhiza against *Epidermophyton sp*, and chloroform fraction of C. xanthorrhiza against *Epidermophyton sp* and *Penicillium sp*. While the ethylacetate fraction of C. xanthorrhiza showed moderate and weak activities with MIC values 50-100 µg/mL. The extract and fraction of C. soloensis also showed moderate and weak antifungal activity with MIC values 50 -200 µg/mL. The acetone extract of C. soloensis showed weak activity (≥ 100 µg/mL). against all the tested fungal with MIC 100-200 µg/mL, the n-hexane and chloroform fractions of C. soloensis showed moderate activities (< 100 µg/mL) against *A. fumigatus*, *C. albican*, *Epidermophyton sp*, and *T. rubrum* with MIC values 50 µg/mL.

The difference in antifungal activity level caused by each extract and fractions have different component. The n-hexane fraction of C. xanthorrhiza and C soloensis were containing essential oils (type sesquiterpenoids and monoterpenes) as the main component, while the chloroform fraction was containing curcuminoids as major component [19]. Previous research reported that the essential oils and curcuminoids of Curcuma have biological activities, one of them as antimicrobial activity [26, 27].

The antifungal mechanism of terpenoids and curcuminoid has been reported. The study have shown that the site action of cyclic hydrocarbon, including terpenoids and curcinoids was at cell membrane. Terpenoids was interfere permeability of cell membranes, which had a consequence a permeability increase and loss of cellular constitutes. These causes inhibition of enzyme, which are crucial to the
energy system in a cell [28]. Meanwhile, curcinoids was disturbs the membrane potential and disrupts membrane integrity. The previous study assumed that curcumin forms electrostatic and/or hydrophobic interaction with fungal cell membrane and cell wall causing membrane disruption [29].

4. Conclusions
The acetone extract and chloroform fraction of *C. xanthorrhiza* exhibited significant activities against *Epidermophyton sp*, *Penicillium sp* and *Trichophyton rubrum* with MIC (minimum inhibitory concentration) 12.5-25.0 µg/mL. The *n*-hexane fraction of *C. xanthorrhiza* showed significant activity on *Epidermophyton sp* with MIC 12.5 µg/mL. Meanwhile, the extracts and fractions of *C. soloensis* showed moderate and weak activities against all tested fungal with MIC 50-200 µg/mL.

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