Antifungal Activity of Curcuma.....

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Submission date: 16-Nov-2022 10:37PM (UTC+0700)
Submission ID: 1955849773
File name: Diastuti_2019_IOP_Conf._Ser.__Mater._Sci._Eng._509_012047.pdf (392.44K)
Word count: 2945
Character count: 16437

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IOP Conf. Series: Materials Science and Engineering **509** (2019) 012047 doi:10.1088/1757-899X/509/1/012047

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 *Staphyllococcus aureus*, *Bacillus subtillis*, *C. albicans* and *Aspergillus flavus* [13]. The essential oils of *C. soloensis* 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. soloensis* 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]. Curcumine 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. soloensis* 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. soloensis* (collected from Solo, Indonesia), redestillated 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. soloensis rhizomes

The fresh rhizome of *C. xanthorrhiza* (10 kg) and *C. soloensis* (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. Xanthorrhixa* (1.2 kg) and *C. soloensis* 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. xanthorriza* (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	MIC (μg/mL)								
	Kzl	Cx-A	Cx-H	Cx-C	Cx-E	Cs-A	Cs-H	Cs-C	Cs-E
Fumigatus	6.25	50	25	25	100	100	50	50	200
Albicans	12.5	50	50	25	50	100	50	50	100
Epidermophyton sp	6.25	12.5	12.5	12.5	50	200	50	50	50
Penicillium sp	6.25	25	100	12.5	100	200	200	100	200
T. rubrum	6.25	12.5	50	25	100	100	50	50	100

Kzl=ketoconazole; Cx= *C. xanthorriza*; 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. xanthorriza* 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. xanthorriza* 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 [5, 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 curcuminoids 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, curcuminoids 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.

Acknowledgement

We thanks to Universitas Jenderal Soedirman for financial support through BLU research grant in 2018.

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