Fractionation of bioactive materials *temulawak* rhizome (*Curcuma xanthorrhiza*) on fungal *Candida albicans* in search of phytopharmaca

D Novianti* and T Kartika
Biology Department, Faculty of Mathematics and Natural Sciences, Universitas PGRI Palembang, Indonesia

* dewinovianti1980@gmail.com

Abstract. *Candida albicans* fungus is a microbiota in the human body that is opportunistic. In general, infections caused by *C. albicans* are called candidiasis. Some studies have shown that *C. albicans* is resistant to antifungal agents, so an alternative antifungal medication is needed at affordable and safe prices. One alternative is to increase the use of medicinal herbs. The purpose of the research was to study the possibility of utilizing the *Temulawak* rhizome as a phytopharmaca material to treat candidiasis disease by *C. albicans* infection. The specific objective is to isolate the bioactive material and to determine the class of antifungal active fraction of *Temulawak* rhizome against *C. albicans*. Isolation of *temulawak* rhizome by way of extracted by stratified using soxhlet, then done fractionation using the method of vacuum chromatography, then bioautographic test using thin plate chromatography. Testing of bioactive fraction *temulawak* rhizome using thin plate chromatography obtained purple color indicating the class of terpenoids compounds and yellow color indicating the class of phenols compounds. The fraction of methanol extract from *Temulawak* rhizome makes it possible to be used as raw material of phytopharmaca because it has antifungal effectiveness against *C. albicans*.

1. Introduction
The Indonesian state has a tropical climate with humid and hot air. These conditions if environmental sanitation is low, fungal infections will easily occur. Fungal disease is closely related to the habits and level of personal hygiene. One of the fungi that cause infection is *Candida albicans*. Candidiasis, caused by *Candida albicans* is the most common opportunistic fungal infection and a serious medical problem that causes significant morbidity and mortality, particularly in AIDS patients, transplant recipients, and other immunocompromised people. In spite of the continuous expansion of the arsenal of antifungal drugs, the available drugs cannot meet the ever-increasing requirements to combat *Candida* infections in Patients [1]. Treatment of infections caused by fungi can be done symptomatic and causative. Candidiasis on the skin can be prevented with a simple way, namely by maintaining personal hygiene and healthy lifestyle habit. Causative treatment, by the way, the germs cause it to be turned off using anti-fungal substances. Treatment is usually in the form of cream-based topical antifungal drugs to be applied to the skin. Especially for candidiasis infections that have developed into systemic infections (attacking the entire body), oral antifungal drugs can be given. Management of oral candidiasis is typically drug based but the extensive use of azoles has resulted in resistance in *Candida* species and...
subsequent treatment failures. Problems associated to common antifungal drugs has led to new therapeutic methods to be thought [2].

However, in the developing countries (such as Indonesia) have a long tradition of using herbal medicine. However, the development of herbal medicine in an Appropriate scientific procedure using only began in the past decade. One of the nine herbal plants that are intensively studied by the Indonesian Ministry of Health is temulawak. The use of temulawak, also known as Java turmeric, with scientific name Curcuma xanthorriza as the raw material in herbal medicines and the food is increased 5.4% annually, which makes the high demand on this rhizome in herbal industries. The extraordinary properties possess curcumin are those of anti-inflammatory, antioxidant, immune-modulation, antimicrobial, anti-cancer and many more. Various researches on over time have confirmed the potential therapeutic applications in a number of diseases ranging from infections, diabetes, cardiovascular problems to cancer, Alzheimer's and so on. Curcumin is long known for its antimicrobial activity. Studies have highlighted its anti-fungal action against many common fungal pathogens, including Candida. Javanese turmeric, including volatile oil, curcumin, starch, protein, cellulose fat, and minerals. Chemical analysis has shown that the main substances in Javanese turmeric are starch, fibers, volatile oil as phellandrene, camphor, tumoral, cineol, borneol, xanthorrhizol, and curcuminoid as curcumin, and desmethoxycurcumin [3].

Scientific research on the antifungal ability of rhizomes temulawak against C. albicans has not been widely reported. Based on the description above, it is necessary to conduct further research to determine the effectiveness of temulawak rhizome on C. albicans fungi causing candidiasis, by isolating and fractionating bioactive ingredients from the temulawak rhizome and determining antifungal active bioactive material as one of the phytopharmaca search efforts.

2. Method
The research stages are as follows:

2.1. Making simplicia temulawak rhizome
The temulawak rhizome samples were cleaned from the sticking, thinly sliced and then dried until the rhizomes dried up. Curcuma rhizome was mashed by using a blender so that it was obtained simplicia of the temulawak rhizome.

2.2. Isolation of antifungal compounds using multilevel simplicia extraction
Solvents used for the extraction of simplicia are based on polarity, namely n-hexane, ethyl acetate, and ethanol. Simplicia powder as much as 100 grams is wrapped with filter paper and then put into soxhlet then given 1 liter of n-hexane solution and heated on an electric stove. Extraction is carried out for 6 days. In one day it is heated for 5 hours (until the solution is clear) so that it is obtained dregs and extracts of liquid n-hexane. Dregs are removed and dried. The dried pulp was then extracted with ethyl acetate solvent for 6 days to obtain liquid pulp and acetate extract. The dried pulp has been extracted with methanol for 6 days so that later it is obtained the liquid methanol pulp and extract. Liquid n-hexane extract, liquid ethyl acetate, and liquid methanol were evaporated with a water bath until a thick extract was obtained. The thick extract was dried to obtain n-hexane extract, ethyl acetate extract, and methanol extract in the form of paste paste [4].

2.3. Antifungal test of temulawak rhizome extract
The antifungal test was carried out on C. albicans by agar diffusion method (Kirby Bauer) in the following manner: the test fungus was inoculated into PDA media as much as 3 minutes then incubated for 24 hours at 37 °C. 1 ml of mushroom suspension was put into the cup petri and added 10 ml PDA media with a temperature of ± 40 °C and homogenized to ensure the cells are evenly distributed and allowed to freeze. On top of the media containing the fungus, a disc paper with a diameter of 6 mm was added and then a 20 μl of a solution of the extract was extracted at a concentration of 2%. This treatment was repeated twice. The media was incubated for 24 hours at 37°C. Testing of the antifungal ability was
declared active if around the paper disc there was a clear zone that was free of mold growth. Furthermore, the diameter of the clear zone or the inhibition zone that is formed is measured [5].

2.4. Bioautography test and determination of active antifungal fraction groups
The active fraction was tested by bioautographically to determine the Rf value of the antibacterial compound using Thin Layer Chromatography (TLC). The active fraction with a concentration of 2% was sprayed on a silica gel 60 CF 254 plate, the active fraction was doubled on the chromatogram. Both of these plates are inserted in a vessel containing eluent to separate active compounds. The first chromatogram was used to detect active compounds by chromatogram placed in a petri dish containing mushroom culture, the active fraction in the chromatogram was allowed to stick to the media so that for 1 hour so that the bioactive material was attached and diffused into the media, then carefully removed. The petri dish containing the mushroom culture was then incubated for 24 hours and then observed the clear area which showed the inhibitory growth of fungi and was the active fraction area and calculated the Rf value. The second chromatogram used to detect chemical compounds by spraying a solution of H2SO4 on silica gel plates, then dried by heating over a water bath so it will look bioactive materials based on the color formed [6].

3. Results and discussion

3.1. Extraction rhizome temulawak
Based on the results of multilevel extraction with Soxhlet using n-hexane, ethyl acetate, and methanol solvents, that from 100 grams of temulawak rhizome simplicia three extracts were obtained, in Table 1.

| No. | Solvent     | Extract Weight (grams) | Percent Weight (%) |
|-----|-------------|------------------------|--------------------|
| 1   | n-hexane    | 6.2                    | 6.2                |
| 2   | ethyl acetate | 12.7                   | 12.7               |
| 3   | methanol    | 30.2                   | 30.2               |

Table 1. Simplicia rhizome temulawak extraction results.

From the extraction results as shown in Table 1, it can be seen that there is a difference in the weight of the extract produced from each solvent. The solvents used in the extraction process have the ability to attract different compounds found in the simplicia of ginger rhizomes. N-hexane solvents will dissolve non-polar compounds (eg flavonoids), ethyl acetate solvents will dissolve semi-polar compounds (eg terpenoids), and methanol solvents will dissolve polar compounds (eg phenols and alkaloids). In extracts, there may be compounds from different chemical compounds according to their polarity. In this research, the extraction process was carried out in a hot way. Continuous extraction method with soxhlet tool was chosen because the extract obtained by this method was more than the maceration method [7].

![Figure 1. Inhibitory zone of temulawak rhizome extract against C. albicans.](image-url)
Based on Figure 1 in above, methanol extract produced 12.5 mm inhibition zone diameter for the growth of *C. albicans* greater than ethyl acetate extract, while n-hexane extract did not produce inhibitory zones meaning that n-hexane did not have the ability to inhibit or not have antifungal activity against *C. albicans*. The biggest diameter of methol inhibition zone shows that the extract is the most active compared to other extracts.

3.2. *Fractionation of temulawak rhizome methanol extract*

Fractionation was carried out using Vacuum Liquid Chromatography (KCV) method. Fractionated extracts are extracts that have the greatest antifungal effectiveness, namely methanol extract. Fractionation results obtained 11 fractions called fractions F1 to F11. The fractions with numbers F1, F2, F3, F4, F5, and F6 not shows diameter of the inhibitory zone which means that the six fractions have no inhibitory effect on *C. albicans*. The fractions of F7, F8, F9, F10, and F11 are active fractions against *C. albicans* as indicated by the formation of the inhibition zone diameter around the paper dish. The diameter of the inhibitory zone in the F7 fraction is 9.1 mm, F8 is 11.6 mm, F9 is 12.3 mm, F10 is 22 mm, and the largest diameter of the inhibition zone is F11 which is 34.5 mm.

3.3. *Bioautography*

Determination of the active compound class contained in the extract was carried out with color reagents. The results show that curcumin extract contains groups of phenolic and terpenoid compounds. Analysis using TLC is the separation of chemical components based on the principle of adsorption and partitioning which is determined by the stationary phase (adsorbent) and the mobile phase (eluent). The chemical component moves up following the mobile phase because the adsorbent's absorption of chemical components is not the same so that the chemical components can move at different distances based on the polarity level [8].

| No | Fraction | Factor retardance (mm) | Color of Spots | Faction group |
|----|----------|------------------------|----------------|---------------|
| 1  | F7       | 0.42                   | Purple         | Terpenoids    |
| 2  | F8       | 0.42                   | Purple         | Terpenoids    |
| 3  | F9       | 0.42                   | Purple         | Terpenoids    |
| 4  | F10      | 0.38                   | Yellow         | Phenols       |
| 5  | F11      | 0.38                   | Yellow         | Phenols       |

Figure 2. Bioautography test results.
Table 2 and Figure 2 shows fractions F7, F8, and F9 produce the same color spots, namely purple and have the same retardance factor of 0.42 mm. The three fractions are terpenoids. Fraction F10 and F11 also produce the same spot color yellow and has the same factor retardance value are 0.38 mm. Both of these fractions are phenols. There are many different types of curcumin against this fungus. One of the mechanisms is found out of the disruption of the plasma membrane of the fungus by curcumin, which prevents the growth. It has also been found that curcumin regulates the expression of proteins such as calcineurin that is involved in the synthesis and maintenance of the cell wall of the fungus. This leads to the destruction of cell walls and thus kills the fungal cells. Another anti-fungal mechanism of curcumin is mediated by its ability to induce reactive oxygen species generation in the fungal cells. This leads to elevation of oxidative stress which in turn induces apoptosis (cell death) in the cells. The TUP-1 (involved in DNA synthesis) gene which prevents the division of the cells and thus, prevents the fungal growth. In the case of vaginal candidiasis, a curcumin formulation has been found to be effective in the treatment of the infection [9].

In a study by Garia Gomes AS et al in 2012 concluded that curcumin has a great capability to inhibit Fluconazole resistance by the isolates of C. albicans [10]. Due to the increasing incidence of Fungal infections, combined with the lack of effective vaccines and drug therapies, fungal diseases have a clear cause of morbidity and mortality, suggesting the need for more effective anti-fungal therapies [11]. Curcumin (diferuloylmethane) is a natural compound obtained from the spice, turmeric. The rhizome of the plant Curcuma Longa gets its yellow-orange color due to this compound. It is the most bioactive component of turmeric, by virtue of which it has numerous medicinal benefits. Curcumin modulates the metabolic and molecular pathways in a cell to exhibit its medicinal properties [9]. The mechanism of its antifungal activity has been suggested that its fungicidal mechanism may be involved in accumulation on the cell wall outer layer. However, curcumin has been suggested as an irresistible antiviral, bacterial and antifungal agent [12]. The results of the research by Puspitasari et al [13], Javanese turmeric extract inhibitory effect against C. albicans both in planktonic forms and in the early phase of biofilm formation. The antifungal effect of Javanese turmeric extract against planktonic C. albicans is stronger than its eradication effect against C. albicans biofilm. At a concentration of 35%, Javanese turmeric extract reduced 90% of the viability of C. albicans in the adhesion and proliferation stages of the early stage of biofilm formation. No differences were found in the eradication effects of Javanese turmeric extract between the adhesion and proliferation stages of the early phase the formation of C. albicans biofilm.

4. Conclusion

Temulawak rhizome contains the most terpenoids and phenol fractions. Methanol extract fraction of temulawak rhizome allows it to be used as a raw material for phytopharmaca because it has antifungal effectiveness against Candida albicans.

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