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Research Article

Antifungal Activity Test of the Ethanol Extract of Kaembu-embu (*Blumea balsamifera*) Leaves Against *Candida albicans* Growth

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ABSTRACT

Research on the antifungal activity test of ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves against *Candida albicans* growth was carried out. This study aimed to examine the antifungal activity of ethanol extracts of Kaembu-embu (*Blumea balsamifera*) leaves on the growth of *Candida albicans*. The ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves was obtained through the maceration method. The ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves was tested for antifungal activity by liquid and solid dilution methods in determining minimum inhibitory concentration (MIC) and minimum kill concentration (MKC). The results of the antifungal activity of the ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves on the growth of the *Candida albicans* by the liquid dilution method showed the MIC value of 25 ppm and MKC value of 500 ppm. While the results of the antifungal activity of the ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves on the growth of the *Candida albicans* by solid dilution method showed the MIC value of 5 ppm with an inhibitory ability of 50.13% of media control and an MKC value of 250 ppm.

Keywords: ethanol extract, Kaembu-embu leaf, *C. albicans*, MIC, MKC

1. Introduction

The raw materials for traditional medicine are natural ingredients. This is because natural ingredients contain chemical compounds that have medicinal properties [1]. This is one of the bases for the exploration of natural ingredients by researchers. The more scientific information regarding the chemical components contained in natural ingredients, the more benefits natural ingredients have as raw materials for traditional medicines. One of the natural ingredients that have the potential as raw material for traditional medicine is the Kaembu-embu (*Blumea balsamifera*) plant.

The Kaembu-embu (*Blumea balsamifera*) is a type of herbaceous plant found in the forests of the island of Muna, Southeast Sulawesi. Communities in Kontumere Village, Kabawo District, Muna Regency have used Kaembu-embu leaves to cure various diseases such as menstrual pain, fever, aches, heart disease, mouth sores, and diabetes [2]. Kaembu-embu leaf boiled water is usually used as the main raw material for medicinal herbs for mothers who give birth. In addition, Kaembu-embu leaves are also used as medicine to treat candidiasis. Candidiasis is a disease caused by the *Candida* fungus which can cause infections of the mucous membranes, subcutaneous, vaginal, oral, and oral thrush. Candidiasis

has become an important health problem due to the increasing number of patients experiencing immunodeficiency disease (weakened immune system). More than 150 *Candida* species have been identified and about 70% of candidiasis is caused by *Candida albicans* [3].

Initial information regarding the content of secondary metabolites of Kaembu-embu leaves can be obtained through phytochemical screening methods. The results of phytochemical screening show that the ethanol extract of *Blumea balsamifera* leaves contains secondary metabolites of alkaloids, flavonoids, polyphenols, tannins, saponins, glycosides, terpenoids, and quinones [4] - [6]. The n-hexane extract of *Blumea balsamifera* leaves contains terpenoid secondary metabolites [7]. Ethyl acetate extract of *Blumea balsamifera* leaves contains secondary metabolites of flavonoids 204.58 mg Quinones Equivalent (QE)/g sample dry weight, phenol 225.33 mg Galic Acid Equivalent (GAE)/g sample dry weight, vitamin C 451.92 mg Ascorbic Acid Equivalent (AAE)/g dry weight of the sample. GC-MS results indicate that ethyl acetate extract is thought to contain compounds of the benzene, monoterpenes (camphor, L-Borneol), sesquiterpenes (α-guaiene, caryophyllene, humulene), and diterpenes compounds (neophytadiene) [8].

The ethanol extract of *Blumea balsamifera* leaves has antioxidant activity with an IC_{50} of 12.401 ppm [4] and 0.978 mg/mL [6]. The n-hexane extract of *Blumea balsamifera* leaves has antioxidant activity with an IC_{50} of 110.592 ppm [5]. The ethyl acetate extract of *Blumea balsamifera* leaves has an antioxidant activity with an IC_{50} of 37.09 ppm and an antioxidant capacity of 501.97 mg Galic Acid Equivalent Antioxidant Capacity (GAEAC)/g sample dry weight [8]. The ethanol extract of *Blumea balsamifera* leaves at a dose (of 200 mg/kg body weight) can lower blood glucose levels and improve the damaged pancreas better than other doses [9]. The ethanol extract of *Blumea balsamifera* leaves has antiacne activity against *Propionibacterium acnes* with the largest concentration of 75% with an inhibitory diameter of 2.26 cm [10]. The ethanol extract of *Blumea balsamifera* leaves can improve the testicular histology of wistar rats induced by high fat feed [11]. The extracts of n-hexane, ethyl acetate, and ethanol 70% of *Blumea balsamifera* leaves had heme polymerization inhibitory activity at a concentration of 1 mg/mL, each of 11.28; 26.26; and 56.88% [6]. Infusum of *Blumea balsamifera* leaves can inhibit the growth of *Escherichia coli* bacteria at a concentration of 90% of 4.2 mm [12]. The results of this study indicate that Kaembu-embu leaves have the potential to be used as raw materials for medicine. Therefore, it is necessary to test the antifungal activity of the ethanol extract of Kaembu-embu leaves.

2. Materials and Methods

2.1. Materials

The leaves of Kaembu-embu (*Blumea balsamifera*) were taken from Kabawo District, Muna Regency, Southeast Sulawesi Province. The chemicals used are ethanol (Technical), methanol (Technical), aquabidest (Onelab), aluminum foil (Diamond), tissue (Nice), filter paper (Whatman), H_2SO_4, p.a. (E. Merck), $BaCl_2$ p.a. (E. Merck), NaCl p.a. (E. Merck), cotton (Selection), gauze (Onemed), Potato Dextrose Agar (PDA) (BAM Media M127), Potato Dextrose Broth (PDB) (Merck KGaA), ketoconazole (Formyco) (positive control), and pure cultures of *Candida albicans* were obtained from the Laboratory of Microbiology, FMIPA UHO Kendari.

2.2. Methods

2.2.1. Extraction

The leaves of Kaembu-embu (*Blumea balsamifera*) are rinsed with water until clean. Then the Kaembu-embu leaves are aerated at room temperature until dry. The dried leaves of the Kaembu-embu are mashed in a blender until they form a powder. The leaf powder of Kaembu-embu (500 g) was extracted by maceration using ethanol (1500 mL) for 1 x 24 hours. After that, the macerate is filtered until the filtrate and residue are obtained. The filtrate is collected and the residue is macerated again with ethanol (1500 mL) for 1 x 24 hours. This maceration procedure is carried out up to 3 x 24 hours. The filtrate was combined and concentrated with a rotary vacuum evaporator at 78 ^oC to obtain concentrated ethanol extract (crude) of the leaves of Kaembu-embu (*Blumea balsamifera*).

2.2.2. Antifungal Activity Test

The antifungal activity of the ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves tested using the liquid and solid dilution method to determine the minimum inhibitory concentration (MIC) and the minimum killing concentration (MKC) [4], [7], [13] - [16].

2.2.3. MIC Testing

The MIC testing of the ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves was carried out using the liquid dilution method. The inhibition process that occurs is observed through the presence of turbidity in the liquid media which is generated during the dilution process after incubation and confirmed in the growth in solid media.

The liquid dilution method begins by preparing 11 sterilized test tubes, PDB media, test extract, test fungus or *C. albicans* culture, and control solution. The eleven test tubes were filled with PDB media, test extract, *C. albicans* culture, and control solution with the volume as shown in **Table 1**. Then all tubes were incubated for 24 hours at 37°C. After incubation, each tube was observed and recorded on the tube to which turbidity occurred. The cloudy tube shows the growth of *C. albicans* and the clear tube shows the absence of *C. albicans* growth [17]. MIC and MKC values were determined by means of a streak plate from the solid dilution antifungal test results. The test results used were all media that provided visual clarity. MIC is the smallest concentration that can inhibit microbial growth, indicated by *C. albicans* can still grow on the streak plate results. Meanwhile, MKC is the smallest concentration that can kill microbes, indicated by the fact that *C. albicans* is no longer able to grow on the streak plate results. This indicates that the test microbes died because of the test solution with this concentration [18], [19].

Table 1 The Composition of The Media in The MIC Test Using The Liquid Dilution Method

Test Tube Number	Concentration (ppm)	PDB Liquid Media (mL)	Ethanol Extract (mL)	*C. albicans* culture (mL)
1	5	3,97	0,03	1
2	25	3,84	0,16	1
3	50	3,67	0,33	1
4	75	3,50	0,50	1
5	100	3,33	0,67	1
6	250	2,33	1,67	1
7	500	0,67	3,33	1
8	750	0,25	3,75	1
9	Positive control[1]	3+1 (antibiotic)	-	1
10	Media control[2]	4	-	1
11	Negative control[3]	5	-	-

Information:

[1] Positive control = PDB liquid media + ketocenazol (antibiotic) + test fungus

[2] Control media = PDB liquid media + test fungus

[3] Negative control = PDB liquid medium

2.2.4. MKC Testing

The MKC test was carried out by growing the test results on PDB liquid media into sterile PDA solid media using the pour plate method. The solution of culture (1 mL) from the test results in PDB liquid media (**Table 1**) was poured into PDA sterile solid media in a petri dish. Furthermore, PDA sterile solid media was incubated for 24 hours at 37°C. The number of *C. albicans* colonies that grew on PDA sterile solid media on each plate was counted by colony counter. The lowest number of *C. albicans* colonies on PDA sterile solid media on each plate was called MIC. If the number of colonies of *C. albicans* growing on sterile PDA solid media on each plate < 0.1% of the total number of media control colonies, it is called MKC [20], [21].

3. Results and Discussion

3.1. Extraction Results

The extraction method used is maceration. The green powder of the leaves of Kaembu-embu (*Blumea balsamifera*) (500 g) was immersed in ethanol (1500 mL) for 3 x 24 hours. The macerate was filtered to obtain the filtrate (brownish green) and residue (dark brown). The filtrate is evaporated with a vacuum rotary evaporator at a temperature of 78°C until a crude (150 g) is formed with a blackish brown color. So, the percentage of maceration extraction yield is 30%.

3.2. MIC and MKC test results for the ethanol extract of Kaembu-embu (Blumea balsamifera) leaves

MIC and MKC were determined using the liquid and solid dilution methods. The liquid dilution method is a method to determine the minimum concentration of an antifungal that can inhibit or kill *C. albicans*. This method is carried out using liquid media that has been added with the test substance or the ethanol extract of the leaves of Kaembu-embu (*Blumea balsamifera*). The test substance can inhibit the growth of fungus with a certain dilution and then inoculate the fungal culture in the same amount. The response of the test substance was characterized by clarity or turbidity in the tube after incubation for 24 hours at 37°C.

Qualitative data of the results of the MIC and MKC tests of the ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves are presented in **Figure 1**.

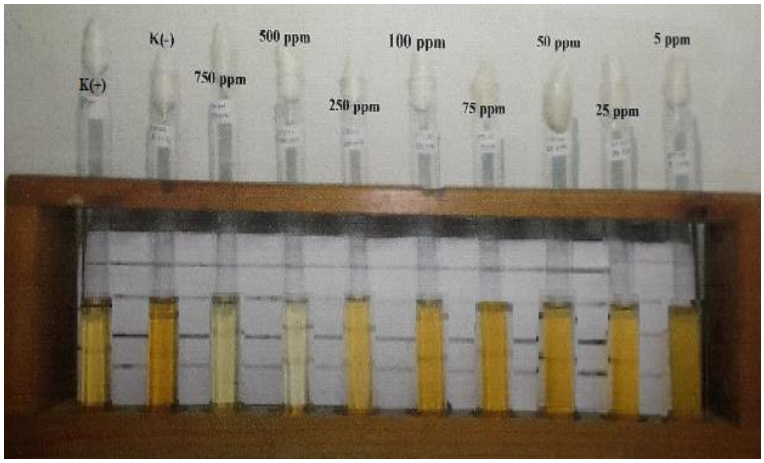

Figure 1 The results of the MIC and MKC test results of the ethanol extract of Kaembu-embu (Blumea balsamifera) leaves using the liquid dilution method

Figure 1 shows that the ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves has begun to clear at a concentration of 25 ppm. This shows that the MIC of the ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves is 25 ppm. **Figure 1** also shows that the ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves changes color from yellow (5 - 250 ppm) to clear at a concentration of 500 ppm. This indicates that there are no more *C. albicans* colonies growing at a concentration of 500 ppm. So, the MKC of the ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves was 500 ppm. However, the results of this test can still change because the turbidity level of each concentration cannot be directly observed visually properly. This is because all the colors of the tubes are cloudy when compared to the fungal control. Therefore, the MIC and MKC test results using the tube dilution method still need to be confirmed again by observing the growth of *C. albicans* on PDA solid media.

The determination of MIC and MKC using the solid dilution method was carried out by growing *C. albicans* on PDA solid media. The solid dilution method is a method for determining the minimum concentration of an antifungal that can inhibit or kill of *C. albicans*. The response of the test substance was characterized by no growth of *C. albicans* on the PDA solid medium or *C. albicans* colonies growing on the PDA solid medium with less than 0.1% of the number of initial inoculum colonies [22].

MIC and MKC were determined by observing the number of *C. albicans* colonies on PDA solid media that had been suspended with 1 mL of liquid dilution of each concentration. Determination of the number of *C. albicans* colonies growing on PDA solid media was calculated by colony counter. Data from the calculation of the number of colonies and the percentage of *C. albicans* growth on PDA solid media are presented in **Table 2**.

Table 2 The Number of Colonies and The Percentage of C. Albicans on PDA Solid Media

Treatment	Ethanol extract concentration (ppm)	*C. albicans* colony numbers	*C. albicans* colony numbers (CFU/mL)	Percentage of growth *C. Albicans* (%)
Control (-)[1]	0	0	0	0
Media control[2]	0	1.875	$1,875 \times 10^5$	100
Ethanol extract	5	935	$9,35 \times 10^4$	50,13
	25	683	$6,83 \times 10^4$	63,57
	50	359	$3,59 \times 10^4$	80,85
	75	137	$1,37 \times 10^4$	92,69
	100	45	$4,5 \times 10^3$	97,60
	250	0	0	0
	500	0	0	0
	750	0	0	0
Control (+)[3]	20000	43	$4,3 \times 10^3$	97,71

Information:
[1] Negative control = PDA solid media
[2] Control media = PDA solid media + fungus test
[3] Positive control = PDA solid media + ketocenazol (antibiotic) + fungus test

The data in **Table 2** shows that the inhibitory ability of the ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves against the growth of *C. albicans* increases with increasing concentrations used. In other words, the growth of *C. albicans* decreased with increasing concentration of ethanol extract used. This can be seen from the growth of *C. albicans* colonies of 9.35 x 10^4 CFU/mL at a concentration of 5 ppm of ethanol extract decreased to 4.5 x 10^3 CFU/mL at a concentration of 100 ppm of ethanol extract. This is also indicated by the absence of *C. albicans* colonies that grow at concentrations of 250, 500, and 750 ppm of ethanol extract. So, the higher the ethanol extract concentration, the higher the ability of the ethanol extract to inhibit the growth of *C. albicans*. This is because the higher the concentration of ethanol extract, the more active antifungal compounds contained in it so the ability of the active compounds to inhibit the growth of *C. albicans* is also higher [23].

The data in **Table 2** shows that the MIC of the ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves was 5 ppm with the ability to inhibit the growth of *C. albicans* by 50.13% from the control media. Meanwhile, the MKC of the ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves was 250 ppm. This is indicated by the absence of *C. albicans* colonies that are able to grow and multiply on PDA solid media during the incubation period. Desmara et.al [24] also reported that the ethanol extract of *Blumea balsamifera* leaves had MIC and MKC of 25% and 50%. Meanwhile, Muchtaromah [25] reported the MIC and MKC values of the ethanol extract of *Blumea balsamifera* leaves were 0.78% and 1.56%. These results indicate that the ethanol extract of *Blumea balsamifera* leaves can inhibit the growth of *C. albicans* during the incubation period. This is because the ethanol extract of the leaves of Kaembu-embu (*Blumea balsamifera*) used contains secondary metabolites which can inhibit the growth of *C. albicans*.

The content of secondary metabolites found in the ethanol extract of *Blumea balsamifera* leaves are alkaloids, flavonoids, terpenoids, polyphenols, tannins, and essential oils [4] - [6], [26], [27]. These secondary metabolites have antifungal activity. The main target of these antifungal compounds is the cell wall. The cell wall of *C. albicans* is composed of polysaccharides (mannan, glucans, chitin), proteins, and lipids with a cell membrane underneath that contains sterols [28]. If the cell wall protein of *C. albicans* is denatured, it will cause brittleness of the fungal cell wall so that it is easily penetrated by substances that are fungistatic [29].

One of the secondary metabolites contained in the ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves is polyphenols. Polyphenols are phenol polymers with high molecular weight. Phenols are compounds that have a hydroxyl group attached directly to the benzene ring. The way phenol compounds work is by causing the coagulation or clumping of proteins. Proteins that have clotted can undergo denaturation so that the protein does not function anymore [30]. Phenol can also cause protein denaturation by damaging the cell membrane [31]. The mechanism of action of phenol as an antifungal is by inhibiting protein synthesis so that the protein from the cell membrane of *C. albicans* is denatured. The presence of phenol OH groups from the ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves can interact through hydrogen bonds with the OH group of the side-chain of carboxylic acids from amino acid residues, so that protein synthesis can be inhibited (Figure 2) [32]. This is what causes a toxic effect on the growth of *C. albicans* during the incubation period because the nutrient transport route is cut off.

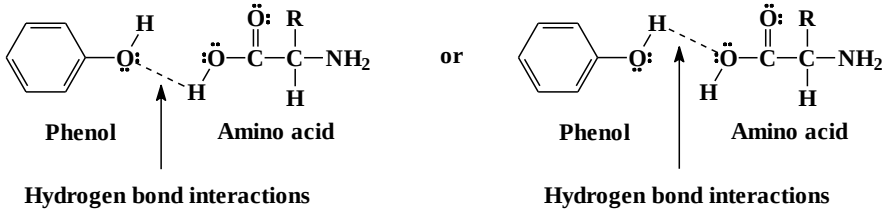

Figure 2. OH group of phenol interacting by hydrogen bonding with OH group of the side chain of carboxylic acid from amino acid residues of protein [32]

Flavonoids are compounds that have pharmacological effects as an antifungal. The effect of flavonoids on organisms is very diverse so plants containing flavonoids are used in traditional medicine [33]. Flavonoids can form complexes with proteins and damage cell membranes by denaturing proteins so that the cell membrane becomes lysed and flavonoids can penetrate the cell nucleus which causes fungi to not develop [34].

Triterpenoid compounds are nonpolar, so they more easily penetrate the fungal cell walls which are composed of lipids. Triterpenoids are included in the terpenoid class of compounds that have antifungal properties [35]. The mechanism of action of terpenoids as an antifungal is because these terpenoid compounds dissolve in fat so that they can penetrate the fungal cell membrane and affect its permeability and cause disruption in the structure and function of the cell membrane.

The results of the above study indicated that the decrease in the number of colonies and even the death of *C. albicans* was in line with the increase in the concentration of the ethanol extract of *Blumea balsamifera* leaves. This is reinforced by the existence of research data showing that the ethanol extract of *Blumea balsamifera* leaves contains active ingredients that can inhibit the growth of *C. albicans*. Therefore, the ethanol extract of *Blumea balsamifera* leaves has been shown to act as an antifungal compound against *C. albicans*.

4. Conclusion

The results of testing the antifungal activity of the ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves against *C. albicans* growth using the liquid dilution method showed MIC values of 25 ppm and MKC of 500 ppm. Meanwhile, the results of testing the antifungal activity of the ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves against *C. albicans* growth using the solid dilution method showed MIC value of 5 ppm with an inhibitory ability of 50.13% of the control media and an MKC value of 250 ppm.

This research needs to be continued until the isolation and structure elucidation stages so that secondary metabolites from the ethanol extract of Kaembu-embu (*Blumea balsamifera*) leaves which have the antifungal activity of *C. albicans* can be identified.

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