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**RESEARCH**

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## Effectiveness of Kemangi (*Ocimum basilicum*) Leaf Methanol Extract against *Candida albicans* Colonies

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### Abstract

*Candida* species, for women are the foremost common cause of parasitic contaminations. *Candida* species affect contamination in 75% of women and at slightest 6-9% of women involvement repetitive vulvovaginal candidiasis. *Candida albicans* (CA) accounts for 85-95% of yeast strains separated from the vagina. The treatment which has been administered for candida infections is antifungal drugs such as clotrimazole and fluconazole. When applied topically, synthetic antifungal drugs cause allergic reactions, resistance, and a burning sensation. It is necessary to conduct research on plant-based herbal medicine as an alternative treatment. Kemangi, also recognized as *Ocimum Basilicum* (OB), is a plant native to Indonesia which has medicinal properties. The objective of this study is to examine how effective OB methanol extract is against CA colonies. The study was performed at Brawijaya University's Microbiology Laboratory in Malang. The experimental laboratories with Posttest Only Control Group Design were employed in this study, with four repetitions of OB concentrations of 15%, 20%, 25%, 30%, and 35% against CA colonies. One-way ANOVA was utilized as the hypothesis test, with a significance level of 0.05. The results demonstrated that OB extract with a concentration of 15% was able to inhibit the growth of CA colonies. In the OB extract with a concentration of 35%, no CA colony growth was revealed. One-way ANOVA test obtained  $p < 0.000 < 0.05$ . Conclusion OB owns adequacy in restraining the development of CA organism with negligible murdering rate at a concentration of 35%. Research required to be performed to identify the antifungal potential of OB extract in vivo.

**Keywords:** Kemangi Leaf Methanol Extract (*Ocimum basilicum*), *Candida albicans*.

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## 1. INTRODUCTION

*Candida albicans* (CA) might be a pathogen causing contagious diseases. CA spreads broadly, affects skin, mucosal surfaces, and causes systemic diseases. As numerous as 400.000 systemic parasitic diseases are affected by candida species (Mukaremera et al., 2017). CA is the foremost general causative specialist of diseases of all species and is mindful for 70% of contagious diseases around the world (Morad et al., 2018). CA possesses several morphological forms, encompassing blastospores, pseudohyphae and hyphae (Talapko et al., 2021).

Candida species are the most frequent cause of yeast diseases in women. Candida species affect contamination in 75% of women, and 6-9% of women experience recurrent vulvovaginal candidiasis (Jang et al., 2019). The primary cause of candida infection in most countries is CA. CA accounts for 85-95% of yeast strains separated from the vagina (Sobel, 2007). The treatment performed for candida infections is antifungal drugs comprising of clotrimazole and fluconazole (Sobel, 2014). The antifungal inhibition mechanism of these medicinal compounds functions by inhibiting the formation of ergosterol which is a constituent of fungal cell walls, for instance in azole drugs such as ketoconazole. However, treatment by employing oral azoles illustrates drug interactions which leads to an allergic response. Resistance of candida strains to antifungal treatment is also frequently unveiled (Sobel, 2007). It is due to gene mutations, continuous inhibition of lanosterol 14-dimethylase (14C -demethylase) by antifungal drugs will generate 14C-reductase which then became a wall barrier of fungal cells (Fitria, 2020). Another treatment administered is the utilization of topical azoles, although it is safer but some patients experience a burning sensation (Wang et al., 2010). Due to the resistance and effects of synthetic antifungal drugs, it is necessary to possess a novelty within the treatment of parasitic diseases. Along with the development of plant-based herbal medicine as a community-based alternative treatment, research on plants with antifungal properties is required. Because, aside from being inexpensive and employing traditional medicine, it rarely possesses side effects (Adiyasa & Meiyanti, 2021).

Indonesia may be a tropical nation incorporating differences of plants which possess the potential to be administered in the health sector. Previous research has been conducted to examine the antifungal potential of several types of plants in Indonesia. Plants examined against CA encompass cinnamon bark (*Cinnamomum burmanii* Blume) (Aini & Mardiyarningsih, 2018; Rangel et al., 2018; Apriliyani, Priani & Hidayat, 2021), moringa (*Moringa oleifera* Lamk) (Verma et al., 2020; Santos et al., 2021), turmeric (*Curcuma longa* Linn)(Murugesha et al., 2019), *Sansevieria trifasciata* Prain (Seniwati, et al., 2021), white frangipani (*Plumeria acuminata*)(Sari et al., 2020), noni (*Morinda citrifolia* Linn) (Hardani et al., 2020; Ene, Enweani-Nwokelo & Obaji, 2021), green tea (*Camelia sinensis*) (Akroum, 2018; Rahayu et al., 2018), alamanda (*Allamanda cathartica* L)(Tun et al., 2020), cashew nuts (*Anacardium occidentale* L)(Costa et al., 2021) and *Acorus calamus* (Febrianti, Khairina & Alisa, 2018).

Kemangi, recognized as *Ocimum basilicum* (OB), is a plant which is generally discovered in Indonesia. Kemangi leaves are frequently utilized by the community as a vegetable consumed. OB in the world of health can be employed as an antipyretic, antifungal, analgesic, antiseptic, antibacterial, hepatoprotector, immunomodulator, antirepellant and anti-expectorant (Kumalasari & Andiarna, 2020). Kemangi leaves are widely available, relatively inexpensive, and have numerous health benefits. As a result, kemangi leaves merit further investigation as an alternative herb for treating candidiasis. Although kemangi leaves have been extensively researched as an antifungal, there has been no recent research on the effectiveness of basil leaf extract against candida albicans, the cause of vaginal discharge. The objective of this study is to determine how effective OB methanol extract is against CA colonies.

## 2. RESEARCH METHOD

The research was conducted at the Microbiology Laboratory, Brawijaya University, Malang in 2021. The method administered in this study was experimental laboratories with Posttest Only Control Group Design by applying four repetitions of OB concentrations of 15%, 20%, 25%, 30% and 35% against CA colonies. The hypothesis test utilized is One-way ANOVA with a significance level of 0.05. An ethical test from the Health Research Ethics Committee of the Malang State Polytechnic of Health with number 206/KEPK-POLKESMA/2021 was administered to qualify this research.

This research employed a sample of kemangi plants acquired from the Malang Regency, East Java. The simplicia of the kemangi plant applied is a group of dried fresh kemangi leaves. The manufacture of kemangi leaf extract (OB) was performed at the Pharmacology Laboratory, Faculty of Medicine, Universitas Brawijaya Malang. Vaginal CA ATCC 1023 colonies were obtained from the Microbiology laboratory of Brawijaya University, Malang, cultured in petri dishes with Sabouraud Dektrose Agar (SDA) medium. The concentration test of kemangi leaf extract (OB) against CA was performed at the Microbiology Laboratory of Brawijaya University, Malang. This study utilized the Tube Dilution Test to examine the antifungal effect of kemangi leaf extract (OB) against CA Colonies.

Kemangi leaf extract (OB) is manufactured using the maceration method, which involves providing 1000 g of fresh kemangi leaf petals and drying them at room temperature for 10 days. The dried kemangi leaves are then blended to generate 200 grams of kemangi powder. The powder was placed in a 1-liter Erlenmeyer glass and soaked in methanol to a volume of 900 ml (3 times). It was shaken until completely mixed (+30 minutes), set aside for 1 night to settle, then was placed the top layer of the ethanol mixture with the active substance which has been administered. Then, it was placed in the 1-liter evaporation flask, and on the evaporator and filled the water bath with water until it was full. All series of tools incorporating rotary evaporator, water bath heater was installed (set to 70 C), connect to electricity. The methanol solution was applied to separate from the active substance in the flask. Waiting was required until the methanol flow stops dripping on the reservoir flask ( $\pm$  1.5 to 2 hours for 1 flask). The results obtained are approximately 1/3 of the dry matter (31 g) then the extract was placed into a plastic bottle and stored in the freezer (Kumalasari & Andiarna, 2020).

Test Phase of Kemangi Leaf Methanol Extract (OB) against CA colonies. The test in this study was conducted to determine the ability to eliminate microorganisms at each concentration of kemangi leaf extract. The test employed a concentration of 15%, 20%, 25%, 30%, 35%. SDA powder in the amount of 65 grams was mixed with 70 ml of distilled water, stirred, then covered with aluminum foil and sterilized in an autoclave for 15 minutes at 121o C. The first layer of SDA liquid was poured into a sterilized petri dish and allowed to solidify. A 1 ml culture of CA ATCC 1023 was administered in an osche heated over a spirit lamp until it smoldered and allowed to cool. Then, 0.1 mL of kemangi leaf extract (OB) was applied at 15%, 20%, 25%, 30%, and 35% concentrations. On 10  $\mu$ l SDA media, full streaking was performed. Petri dishes were incubated in an incubator at 37o C for 48-72 hours (Mutawati, 2016). Calculating growth (Colony Counter) by employing the Total Plate Count (TPC) method (Lay, 1994). The experiment was repeated 4 times. Then, the results were examined statistically by administering one way ANOVA.

## 3. RESULTS AND DISCUSSION

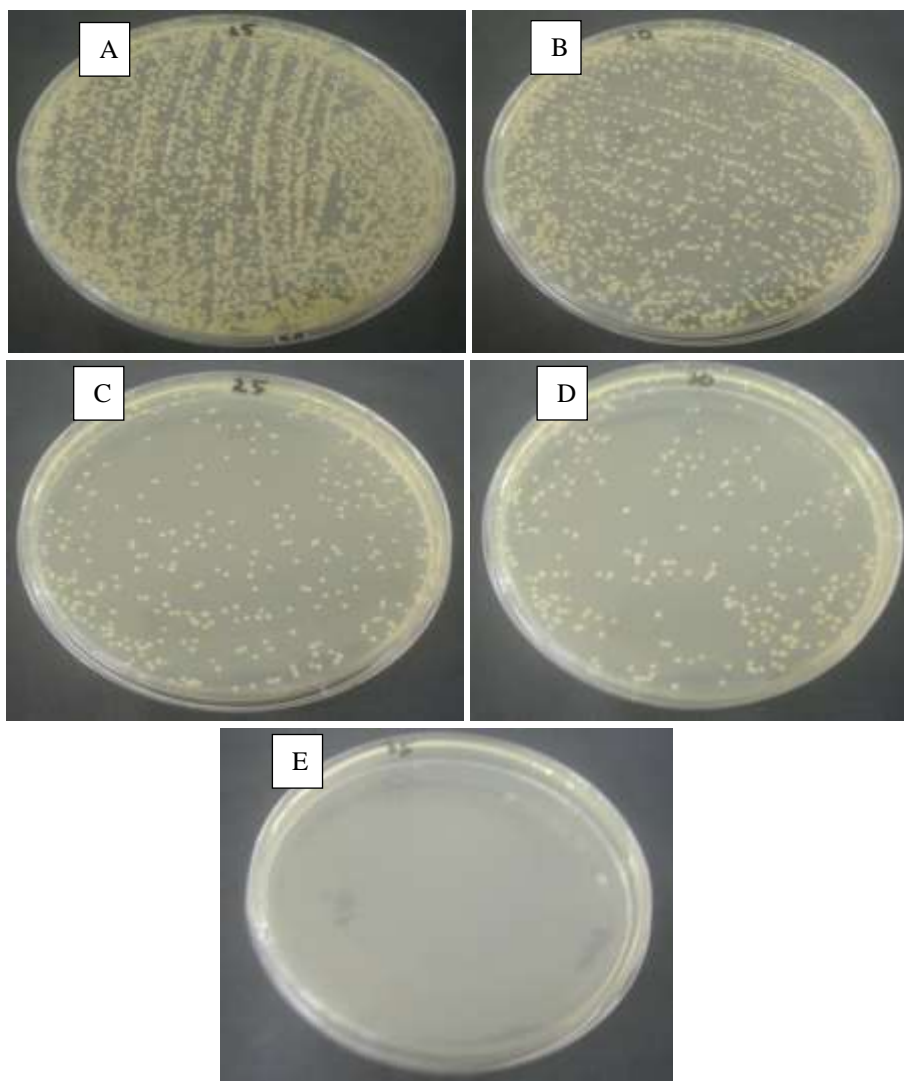
OB is one of the species in the class *Ocimum* spread in tropical and sub-tropical regions (Asia, Africa and America). *Ocimum basilicum* in Indonesia is well-recognized by various names, which are lampes or surawung in Sunda, kemangi or kemangen in Java, kemangih in

Madura, uku-uku in Bali, and lufe-lufe in Ternate. OB are extensively utilized by the people of Indonesia as food and medicine. Kemangi leaf extract is evident to embody chemical compounds of flavonoids, alkaloids, saponins and tannins. Therefore, kemangi leaves can be utilized as an alternative to herbal medicine (Kumalasari & Andiarna, 2020).

**Table 1.** The results of the calculation of the CA colonies number at each concentration of kemangi leaf extract.

Repetition	The number of CA colonies growing at each concentration				
	15%	20%	25%	30%	35%
I	1043	601	260	119	0
II	1270	691	317	134	0
III	1077	748	226	107	0
IV	1145	567	215	95	0
Average	1134,4	652,2	255,2	113	0

The results of the calculation of the number of colonies in table 1 reveal that as the concentration of OB leaf extract increased, the number of colonies growing on SDA decreased. The largest and most dense CA colony growth was 1134 CFU/ml at a concentration of 15%. There was no progress of CA colonies at 35% concentration. It is because the more active compounds contained in the OB leaf extract, the higher the concentration of OB extract. Figure 1 depicts the growth of CA colonies after treatment with OB leaf extract.



**Figure 1.** CA colony growth after being applied OB leaf extract, (A) CA colony growth at 15% concentration, (B) CA colony growth at 20% concentration, (C) CA colony growth at 25% concentration, (D) growth CA colonies at a concentration of 30%, (E) growth of CA colonies at a concentration of 35%.

**Table 2.** ANOVA Test Results Number of CA Colonies at Each Concentration.

Concentration	N	(X)	SD	F	p-value
15%	4	1134,4	2.062	270.439	0,000
20%	4	652,2	6.000		
25%	4	255,2	15.351		
30%	4	113	2.754		
35%	4	.00	.000		

Statistical test by employing One-way ANOVA illustrated  $p = 0.000$  with alpha 0.05, thus, it can be indicated that there are significant differences in each concentration of OB leaf extract. The higher the concentration of OB leaf extract, the lower the number of CA colonies which grow. It demonstrates that OB leaf extract possesses antifungal potential against CA with a minimal killing rate at a concentration of 35%.

Phytochemical tests were administered on fresh dried OB leaves and then extracted by employing ethanol as a solvent, illustrated that the OB leaf extract embodied flavonoids, alkaloids, saponins and tannins. These compounds function as antifungal, antipyretic, analgesic, antiseptic, antibacterial, hepatoprotector, immunomodulatory, antirepellant and anti-expectorant which hinder the development and slaughter CA (Kumalasari & Andiarna, 2020). The mechanism of antifungal activity on alkaloids is by inhibiting the proliferation of protein formation, and respiration in cells which causes fungal death (De Ornay, Prehananto & Dewi, 2017). Alkaloid compounds are alkaline with a  $pH > 7$ . This alkaline nature suppresses the development of the organism CA as fungus grows at a pH of 4.5 – 6.5 (Sari, Gunadi & Kristiana, 2019).

Furthermore, OB leaf extract encompasses flavonoids. Flavonoids own a mechanism of action as antifungals by interferometer with the penetrability of parasitic cell membranes. The hydroxyl group located in flavonoid compounds affects changes in organic components and nutrient transport which ultimately results in fungal cell lysis (Astutik, Yuswantina & Vifta, 2021). Flavonoids inhibit the growth of fungi by generating complex compounds with extracellular proteins, and flavonoids possess soluble properties which damage fungal cell membranes and are applied after by the discharge of intracellular compounds (Sari & Sumadewi, 2019).

One of the active substances in OB leaves playing a pivotal role in anti-fungal activity is essential oil. The essential oils produced in kemangi encompass methyl chavicol and linalool (Fitria, 2020). The result of another study discovered that the largest component of OB leaf essential oil is citral (Guntur et al., 2021). The essential oil in OB was able to restrain the development of CA cells by 35%. These results indicated a twofold effect when compared to ketoconazole (Bona et al., 2016). OB oil contains MIC80 inhibition against CA NYCY 1363 and CA NYCY 135BM2/94 at a concentration of 0.1% (Serra et al., 2018). The mechanism which plays a significant role in the antifungal activity of OB basic oil is terpene compounds, which are E-citral, Z-citral and linalool (Fitria, 2020). The mechanism of inhibition occurs as terpene compounds such as citral in OB oil are able to damage cell walls and cell membranes of fungi comprising of chitin, mannan (a type of polymer), -1, 6-glucan and -1, 3-glucan proteins. The fungal cell membrane is a polysaccharide connected with glycosidic bonds. Damage to the cell wall implies the occurrence of cytotoxicity then structural damage occurs.

Hence, the cell becomes lysed and causes the release of cell organelles and the death of fungal cells (Leite et al., 2014).

Tannins are dynamic compounds functioning as antifungals (Safrida, Mardiana & Husna, 2021). These antifungal compounds work by neutralizing enzymes involved in fungal invasion, damaging fungal cell membranes, inhibiting fungal enzyme systems, hence, they interfere with hyphae tip arrangement, and affecting nucleic acid and protein synthesis. Tannins possess ability to inhibit fungal development by inhibiting the synthesis of chitin, which is responsible for the arrangement of cell dividers in fungi.

Research conducted by Pasaribu et al., (2018) demonstrates that OB leaf extract owns antifungal action against CA. The concentration of kemangi leaf extract at a volume of 60  $\mu$ L to 80  $\mu$ L illustrates more extensive inhibition zone than ketoconazole (Pasaribu, Sudrajat & Buarlele, 2018). The results of this study revealed that kemangi leaf extract can inhibit the growth of CA. Kemangi leaf extract has numerous health benefits, particularly its antifungal properties. Unfortunately, the development of herbal ingredients for the treatment of CA-related vaginal discharge has not been prevalently pursued. This study is an initial study that can be utilized as a foundation for future research to develop herbal medicine as an alternative treatment for vaginal discharge exacerbated by CA in women.

#### 4. CONCLUSION

OB leaf extract possesses antifungal potential against CA with minimal killing rate (MBC) at a concentration of 35%. The antifungal potential of OB leaves warrants further investigation as an alternative treatment for CA-caused vaginal discharge. In vitro research has demonstrated that OB leaf extract possesses antifungal properties against CA. More research is required to determine the antifungal potential of OB leaf extract in vivo.

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