

# Epigallocatechingallate (EGCG) Antifungal Properties for *Candida* Isolates from HIV/AIDS Patients with Oral Candidiasis in Compare with Fluconazole

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## Abstract

**Background:** Oral Candidiasis (OC) still mainly oportunistic infection problem in HIV/AIDS Patients. Due to increasing report of fluconazole resistant as common antifungal drugs nowadays, there have been many studies focusing on natural substances and its antifungal properties. In this study, a form of green tea extract, named Epigallocatechingallate (EGCG) 1,25% were examined for their *in vitro* antifungal activity against *Candida* sp in comparison to fluconazole (2 mg/ml) as standard antifungal agents. **Objective:** To evaluate the antifungal activity of EGCG in compare with fluconazole against *Candida* isolates taken from HIV / AIDS patients with OC. **Methods:** Fourty *Candida* sp. isolates taken from HIV / AIDS patients with OC in the Outpatient Unit and Inpatient Installation of the Infectious Disease Intermediate Care Unit (UPIPI) Dr. Soetomo, Surabaya. Antifungal activity were evaluated by using microdilution tests. **Results:** The microdilution test revealed the MIC of EGCG for all *Candida* sp. was 0.625%, while the MIC of fluconazole was 100% for all *Candida* sp. There was significant difference ( $p < 0.05$ ) between the MIC values for *Candida* sp. by fluconazole and EGCG. The MFC values of EGCG was 50%, while value of fluconazole MFC was 100%. **Conclusion:** Antifungal activity of EGCG with fungistatic and fungicidal effect is better than fluconazole.

**Keywords:** Antifungal activity, *Candida albicans*, *Candida non-albicans*, EGCG, Fluconazole, HIV/AIDS, Oral candidiasis

## Introduction

Oral candidiasis (OC) develops in 80%–95% of the patients with Human Immunodeficiency Virus (HIV)/ Acquired Immunodeficiency Syndrome (AIDS). It is described as an oportunistic infection, often involved in the alteration of oral microflora, systemic diseases and reduced immunity of the host. Opportunistic infectious diseases in HIV/AIDS patients can decrease the quality of life in these patients<sup>(1,2)</sup>.

*Candida albicans* is the most prevalent and pathogenic species, but other *Candida species*, such as *C. tropicalis*, *C. krusei*, *C. glabrata*, *C. dubliniensis*, *C. guilliermondii*, *C. parapsilosis*, *C. kefyr*, and *C. pelliculosa*, has become a significant cause of infection in patients with HIV/AIDS<sup>(3,4)</sup>. Majority of the clinically used antifungals suffer from various drawbacks in terms of toxicity, drug-drug interactions, and lack of fungicidal efficacy, and emergence of resistant strains resulting from frequent usage. For example, fluconazole limited because of the high rate of primary and secondary resistance<sup>(5)</sup>. To identify substances that might be alternatives to traditional medicines, studies were conducted on the antimicrobial activity essential oils for the control of OC<sup>(6)</sup>.

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Research on alternative therapies using natural ingredients is currently on the rise. The use of natural extracts to treat *Candida* is becoming popular, one of which is green tea (*Camellia sinensis*) extract, which is found to have beneficial effects on health, due to its high its low toxicity with antioxidant and immunomodulatory effects. Research conducted by Rahayu and colleagues in 2018 on the immunomodulatory effect of green tea extract in immunocompromised mice with *Candida* using polyphenols and EGCG. After evaluation, it was found that EGCG 1.25% had an immunomodulating effect against *C. albicans* infection in immunocompromised mice by increasing the expression of IL-8, IL-17A and HBD-2. It was also found that the minimum inhibitor concentration of green tea extract on the growth of *Candida albicans* was 12.5% and the minimum bactericidal concentration was 25%. Therefore, administering EGCG can provide an immunomodulating effect against oral candidiasis in immunocompromised patients (7). Therefore, this study wanted to test the sensitivity of green tea extract and nystatin in oral candidiasis patients with HIV. The use of Epigallocatechingallate (EGCG) is expected to have a better sensitivity than fluconazole.

## Methods

### Strains and Growth Conditions

This research used 40 isolates divided in two groups 20 isolates of *Candida albicans* and 20 isolates *Candida non-albicans*, were obtained from forty patients Candidiasis oral with HIV/AIDS infection. *Candida non-albicans* species included in this study consisted of 7 species of *Candida krusei*, 6 species of *Candida glabrata*, 2 species of *Candida dubliniensis*, 2 species of *Candida parapsilosis*, 1 species of *Candida tropicalis*, 1 species of *Candida norvegensis* and 1 species of *Candida lypoitica*. All of the strains were grown on Sabouraud dextrose Agar under aerobic conditions at 37°C for 24 h before the antifungal assays. The yeast (107 cells/mL) suspensions used in the assays were prepared in sterile phosphate-buffered saline (PBS) at pH 7.2.

### Antifungal Tests

The minimum inhibitory concentrations (MIC) were obtained using the serial microdilution method in culture of 96-well cell plates based on the Clinical and Laboratory Standards Institute (CLSI). Prepare a 96

well microtiter plate that has been filled with Mueller Hinton Broth (MHB) and fluconazole with 10 levels of concentration using a multilevel dilution technique, which is 100%; 50%; 2.5%; 1.25%; 0.0625%; 0.312%; 0.156%; 0.078%; 0.0039%; and 0.0195%. Then enter the EGCG with the highest concentration of 100% in the first row and then do the multilevel dilution along the Y axis to the lowest concentration of 0.19%. *Candida* species were inoculated into the plate by leaving row one as a negative control. MIC observations are determined by observing at what concentration in the well begins to clear and there is no sediment that indicates the growth of *Candida* is inhibited. Minimal Fungicidal Concentration (MFC) is tested by taking 10 µL from the well with a predetermined MIC each well is inoculated on a Petri dish containing Sabouraud dextrose agar and incubated at 37 °C for five days. MFC is defined as the lowest concentration without growth that is seen to be used as the end point for the fungicidal effect.

## Statistical Analysis

The statistical differences were evaluated using using SPSS 17 version The data for microdilution methods were analyzed by *Chi square* on normal data distribution and *MannWhitney* on abnormal data distribution in statistic. Statistical significance was determined at  $p < 0.05$ .

## Results

Forty isolates taken from HIV / AIDS patients with OC. The male patients were 31 patients (77.5%) and female patients were 9 patients (22.5%). The age of patients from isolates in this study varied between the age group 17-25 years to 56-65 years. The most age range is 20 subjects (50%) in the 26-36 years old group. The isolates most were taken from HIV / AIDS patients with OC who had an absolute CD4 count <100 cells/L for 28 patients (70%).

Table 1 shows the results of microdilution, there is a statistically significant difference with the chi square test method at a concentration of 50% between fluconazole and EGCG ( $p < 0.05$ ). At a concentration of 25% there was no significant difference ( $p > 0.05$ ) and the rest could not be analyzed because all the results were still visible turbidity. MIC was produced by EGCG statistically through chi square test for *Candida albicans* species

at a concentration of 50%, while MIC fluconazole at a concentration of 100%.

Table 2 shows with the results of microdilution, there is a statistically significant difference with the chi square test method at a concentration of 50% between fluconazole and EGCG ( $p < 0.05$ ). From the concentration of 25% until 0,38% concentration there were no significant differences between fluconazole and EGCG ( $p > 0.05$ ). The MIC produced by EGCG was statistically through the chi square test for *Candida non-albicans* species at concentrations of 50% while MIC fluconazole at 100% concentration.

Shapiro Wilk test confirmed that the data were not normally distributed ( $p < 0.05$ ); therefore, nonparametric statistical tests, the Mann Whitney test, was performed. Based on data from Table 3 shows the results of microdilution method research, the MFC values EGCG statistically through the Mann Whitney test is 50%, with this concentration can kill the *Candida albicans* and *Candida non-albicans*. The value of fluconazole MFC was 100%. Mann Whitney test results showed there were significant differences between MFC fluconazole with EGCG on the growth of *Candida albicans* and *Candida non-albicans* ( $p < 0.05$ ).

**Table 1. Results Determination of Minimum Inhibition Concentration (MIC) of Fluconazole and EGCG 1.25% against *Candida albicans*.**

Concentration (%)		Treatment	Values	p
		Fluconazole	EGCG	
Concentration 100	(-)	20 (100%)	20 (100%)	-
Concentration 50	(+)	19 (95%)	0 (0%)	< 0.001
	(-)	1 (5%)	20 (100%)	
Concentration 25	(+)	20 (100%)	19 (95%)	1.000
	(-)	0 (0%)	1 (5%)	
Concentration 12.5	(+)	20 (100%)	20 (100%)	-
Concentration 6.25	(+)	20 (100%)	20 (100%)	-
Concentration 3.125	(+)	20 (100%)	20 (100%)	-
Concentration 1.56	(+)	20 (100%)	20 (100%)	-
Concentration 0.78	(+)	20 (100%)	20 (100%)	-
Concentration 0.38	(+)	20 (100%)	20 (100%)	-
Concentration 0.19	(+)	20 (100%)	20 (100%)	-

**Table 2. Results Determination of Minimum Inhibition Concentration (MIC) of Fluconazole and EGCG 1.25% against *Candida non-albicans*.**

Concentration (%)		Treatment	Values	p
		Fluconazole	EGCG	
Concentration 100	(-)	20 (100%)	20 (100%)	-
Concentration 50	(+)	11 (55%)	0 (0%)	< 0.001
	(-)	9 (45%)	20 (100%)	
Concentration 25	(+)	14 (70%)	20 (100%)	1.000
	(-)	6 (30%)	0 (0%)	
Concentration 12.5	(+)	17 (85%)	20 (100%)	1.000
	(-)	3 (15%)	0 (0%)	

**Cont... Table 2. Results Determination of Minimum Inhibition Concentration (MIC) of Fluconazole and EGCG 1.25% against *Candida non-albicans*.**

Concentration 6.25	(+)	19 (95%)	20 (100%)	1.000
	(-)	1 (5%)	0 (0%)	
Concentration 3.125	(+)	19 (95%)	20 (100%)	1.000
	(-)	1 (5%)	0 (0%)	
Concentration 1.56	(+)	19 (95%)	20 (100%)	1.000
	(-)	1 (5%)	0 (0%)	
Concentration 0.78	(+)	19 (95%)	20 (100%)	1.000
	(-)	1 (5%)	0 (0%)	
Concentration 0.38	(+)	19 (95%)	20 (100%)	1.000
	(-)	1 (5%)	0 (0%)	
Concentration 0.19	(+)	20 (100%)	20 (100%)	-

**Table 3. MFC Fluconazole and EGCG for growth *Candida albicans* and *Candida non-albicans***

Spesies	Treatment	Mean ± SD	p
C. albicans	Flukonazole	25.0 ± 44.42	0.002
	EGCG	27.5 ± 44.35	
C. non-albicans	Flukonazole	27.5 ± 44.35	0.021
	EGCG	58.0 ± 28.61	

## Discussions

Basic data of this study showing isolates mostly taken from male than female (77.5% and 22.5%). The most age group is productive age groups in the age range of 26-35 years with 20 subjects (50%). In 2017 report of the Directorate General of Disease Control and Environmental Health, Ministry of Health Republic of Indonesia, found HIV/AIDS patients more male than women, and related more common in young adult which makes it more likely to engage in unsafe sexual behavior that is at risk of HIV transmission (8). The domicile of HIV / AIDS patients with OC mostly came from Surabaya (90%). This is because most patients have to seek help from the nearest health center with most patients from within the city. The isolates most were taken from HIV / AIDS patients with OC who had an absolute CD4 count <100 cells / L for 28 patients (70%). This data is supported by a 2015 Indian study by Kumar that showed 71.4% of patients with a CD4 cell

count <200 cells /  $\mu$ L obtained by the fungus growth of *Candida* species from OC lesions (9).

The evaluation of the antifungal activity of EGCG against all strains of *Candida* isolates, showed MIC values ranging from 50%-100% and MIC values fluconazole were varying. MFC produced by EGCG for *Candida* sp. through the Mann Whitney test is 50%, in which concentration is able to kill the *Candida albicans* and *Candida non-albicans*. The value of fluconazole MFC was 100%, which was higher than EGCG. Previous study also found that EGCG perform better antifungal activity based on its MIC and MFC in compare to azole drugs (ketoconazole and fluconazole). EGCG showed a significant inhibitory effect in the growth of *Candida* sp especially through its ability to destroy the *Candida* biofilm and inhibit mature biofilm maintenance on its MIC. In an in vitro studies, it was shown that EGCG, EGC and ECG caused metabolic instability of *C. albicans* cultures even at the physiological polyphenol

concentrations found in green tea. Of the three catechins, EGCG was found to be the strongest in slowing down the formation and maintenance of *Candida* biofilms and interfering with the formation of biofilms. EGCG was also found to be able to bind strongly with ergosterol. This activity might result in pores creation on fungal cell membranes which eventually leading to fungal cells death. It was also shown that higher EGCG concentrations inhibited the chymotrypsin-like activity of *C. albicans* in vivo which suggests that the impaired proteasol activity contributes to the metabolic and cellular structural disorders of this fungus (10, 11).

Another study with murine model of oral candidiasis showed that EGCG increased the neutrophil count and decreased the amount of infected cells by *C. albicans*. The increasing concentration of EGCG leads to the the increase of neutrophil count. This might be due to immunomodulatory effect performed by EGCG itself which also beneficial for oral candidiasis therapy, especially in immunocompromised patient as in HIV/AIDS patient (12).

EGCG also produced synergistic effect when used together with fluconazole or ketoconazole resulting in higher fungicidal activity. The results in 4 species with EGCG (MFC) alone resulted in a reduction of 95.13%, while in synergistic combination resulted in a decrease of 92.27% for fluconazole and 97.51% for ketoconazole, compared to controls. The MIC value of fluconazole/EGCG or ketoconazole/EGCG decreased 3 to 4-fold in compare to the inhibitory effect of those drugs alone. Another study also found that the mechanisms of EGCG inhibitory effect on *C. albicans* is obtained via key enzymes in the biosynthesis of purines, pyrimidines and some amino acids, and independent of pH (10, 11).

### Conclusions

These results highlight the potential of EGCG as an antifungal drug candidate. Based on the data showed antifungal activity with fungistatic and fungicidal effect better than fluconazole. We acknowledge the need to determinate the active compounds that inhibit germ tube formation and their mechanisms of action. However, if these substance is planned to be used in medicinal purposes, issues of safety and toxicity will need to be addressed in the next research.

**Ethical Approval:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the Ethics Committee in Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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