

Original Article

Comparison of tea tree oil 5%, tea tree oil 10%, and nystatin inhibition zones against vaginal *Candida* isolates in pregnancy

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Abstract

Introduction: Vulvovaginal candidiasis (VVC) in pregnancy frequently develops into recurrent infections. Clinical study suggests that conventional topical treatments for VVC are not always enough to eradicate *Candida* spp. from the vaginal microenvironment. This study aimed to evaluate the antifungal activity of *tea tree oil* (TTO) 5% and TTO 10% against *Candida* species causing VVC in pregnancy.

Methodology: *In vitro* experimental study was conducted in the Mycology Laboratory at Dermatovenereology Outpatient Clinic Dr. Soetomo General Hospital Surabaya. Eighteen isolates of *Candida* species were isolated from the vaginal thrush of 15 pregnant women diagnosed with VVC from March to May 2021. Antifungal susceptibility of TTO 5% and TTO 10% was evaluated by the disc diffusion method, with the inhibitory zone diameter as the main outcome.

Results: The mean inhibitory zone diameter of TTO 5%, TTO 10%, and nystatin against all *Candida* spp. was 7.26 mm, 8.64 mm, and 25.57 mm, respectively ($p < 0.001$). The mean inhibitory zone diameter of TTO 5%, TTO 10%, and nystatin tend to be larger in *C. albicans* compared to the non-*albicans*, but the difference is not significant. Nystatin displayed the largest mean inhibitory zone diameters compared to TTO 5% and TTO 10% ($p < 0.001$) in all *Candida* species. Increased concentration from TTO 5% to TTO 10% resulted in a slight increment in the mean inhibitory zone diameters in all-*Candida* species ($p = 0.001$).

Conclusions: *Tea Tree Oil* displayed antifungal activity against *Candida* species causing VVC in pregnancy. Further studies are required to investigate optimal TTO concentrations as a VVC treatment in pregnancy.

Key words: Vulvovaginal candidiasis; *tea tree oil*; nystatin; inhibition zone; sexual and reproductive health care

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Introduction

One of the most common gynecological problems is vulvovaginal candidiasis (VVC), and in pregnant women, the incidence of VVC is almost doubled [1]. Candidiasis in pregnancy often results in a less effective response to treatment [2]. The incidence of VVC in Dr. Soetomo General Academic Teaching Hospital was still relatively high, with 16 (53%) of the 30 smear samples collected from the posterior vaginal fornix of pregnant women testing positive for *Candida* species [3]. Complications of VVC in pregnancy have been linked to an increased risk of prematurity and low birth weight and significant discomfort to the mother, including

redness, increased discharge, burning sensation in the vulva area, and itching [1,4].

According to epidemiological and clinical statistics, topical therapy does not effectively remove *Candida* infection in the vaginal environment, and about 13% of patients develop recurrent VVC that develop into chronic infections [5]. As many as 5 of 16 women will relapse. Due to the uncomfortable nature, most women employ natural products or drugs that are not licensed for treatment or relapse prevention [6,7].

Essential oils from some plants, such as *Melaleuca alternifolia*, have been studied for their *in vitro* microbicidal actions and their ability to decontaminate the vaginal canal and prevent fungal colonization by

combining fungicidal action [6]. *Tea tree oil* (TTO) significantly inhibits de novo biofilm formation. In many countries, TTO-containing medications are used to treat vaginal infections and are specially manufactured to be administered intravaginally [8,9]. However, there were no studies on the effectiveness of TTO in treating VVC during pregnancy in Indonesia. Therefore, it is crucial to develop new antifungal agents that broaden their spectrum against *Candida* spp. and become an alternative combination option between conventional and standard antifungal agents in the treatment of VVC during pregnancy. This study aims to assess the antifungal activity of TTO 5%, and TTO 10% compared with nystatin against *Candida* spp. causing VVC in pregnancy.

Methodology

Subjects

This study was carried out *in vitro*. The study design has been approved by Ethics Committee of Dr. Soetomo General Academic Teaching Hospital Surabaya. The study protocol was explained to subjects wishing to participate in this study by providing study clarification. Patients gave their consent prior to the study. Samples were collected during a 3-month period between March 2021 to May 2021 using total sampling method, and 15 pregnant patients attending Obstetrics and Gynecology outpatient clinic were diagnosed with VVC. Inclusion criteria of subjects were patients diagnosed with VVC having at least one of the VVC symptoms (vaginal discharge like cheese or cracked milk, vaginal itching, erythema, dyspareunia) and fungal positivity (*pseudohyphae* and/or *blastospores*) by Gram staining smears. VVC pregnant patients treated with antifungal therapy, intravaginally or systemically, within the last 2 weeks and with no colony growth on *CHROMagar* media were excluded from the study.

Clinical isolate

Two swabs were taken simultaneously from each patient and immediately sent to Mycology Laboratory at the Clinical Microbiology Laboratory of Dr. Soetomo General Hospital Surabaya. The 1st swab was used to make thin smears on microscopic slides for Gram staining and the second swab was cultured on *CHROMagar* media. The cultures were cultivated for 36-48 hours before species identification. In this study, 18 isolates of *C. albicans* species and non-*albicans* were found. The susceptibility of antifungal was assessed by the disc diffusion method on *Mueller Hinton* agar with 2% glucose and methylene blue. *Candida* spp. isolates were plated on agar and paper discs containing TTO 5%, TTO 10%, and nystatin were placed on the media. The cultures were incubated for as long as 24-48 hours, and the inhibition zone was measured with a Vernier caliper. The diameter of zones of inhibition between TTO 5%, TTO 10%, and nystatin were then compared.

Results

A total of fifteen (15) subjects were assigned for isolation and identification of *Candida* species and antifungal susceptibility test. Subjects' characteristics are presented in Table 1. The majority of subjects were in the 3rd trimester of pregnancy (11; 73.3%), followed by the 2nd trimester of pregnancy (4; 26.7%). No subject was in the 1st trimester of pregnancy as shown in Figure 1.

Among the 18 vaginal isolates collected from pregnant women with vulvovaginal candidiasis (VVC), four *Candida* species were isolated and identified: *Candida albicans*, *Candida glabrata*, *Candida dubliniensis*, and *Candida parapsilosis*. Seven (38.8%) of the isolates were *C. albicans* while eleven (11) isolates were non-*albicans* (61.1%) species, consisting of *C. glabrata* (45.4%), *C. dubliniensis* (36.3%) and *C.*

Table 1. Demographic data of subjects.

No.	Category	Group	Basic Data	
			N	%
1	Age	Late teens (18 – 25 year)	2	13.3
		Early adult (26 – 35 year)	12	80
		Late adult (46 – 55 year)	1	6.7
		Early senior adult (46 – 55 year)	0	0
		Late senior adult (56 – 65 year)	0	0
2	Education	Elementary school	0	0
		Junior high school	3	20
		High school	5	33.3
		Diploma/bachelor	7	46.7
3	Occupation	Housewives	10	66.7
		Private sector employee	2	13.3
		Government employee	1	6.7
		Nurse	2	13.3

Figure 1. The distribution of patients’ gestational age.

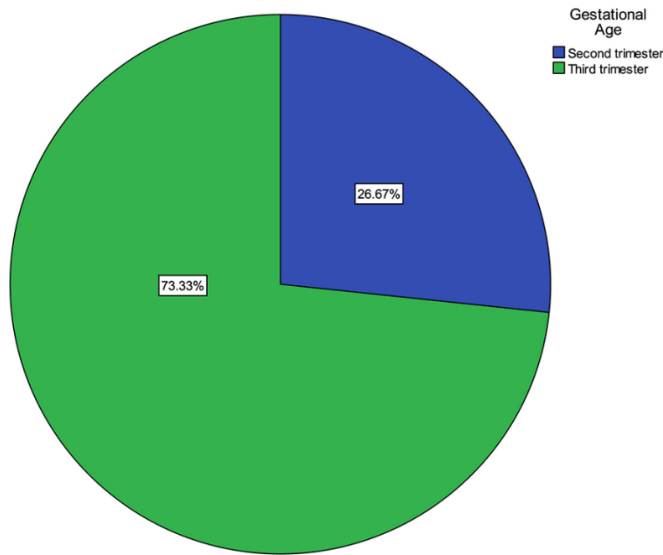
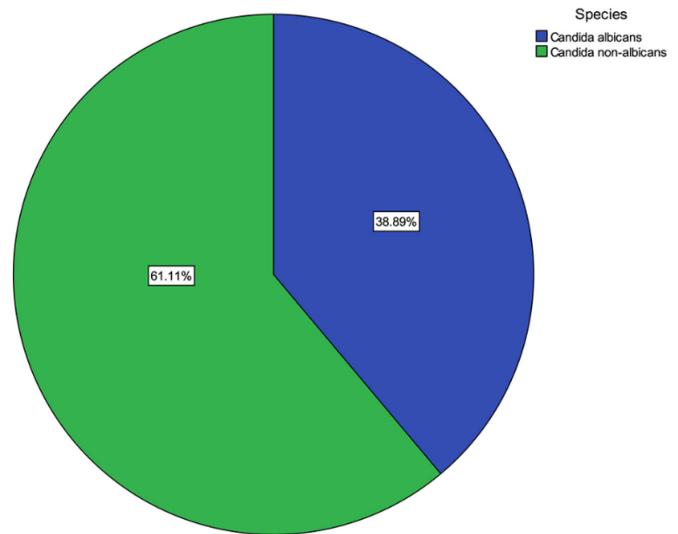


Figure 2. The distribution *Candida* species found in patients.



parapsilosis (18.1%) as shown in Figure 2 and Figure 3. More than one species of *Candida* was found in 3 subjects.

All *Candida* species showed growth inhibition in TTO 5%, TTO 10%, and nystatin. The mean inhibitory zone diameter of TTO 5% in all isolates was 7.26 mm, while *C. albicans* and non-*albicans* had 7.77 mm and 6.85 mm mean inhibitory zone diameters, respectively. The mean inhibitory zone diameter of TTO 10% in all isolates was 8.64 mm, while *C. albicans* and non-*albicans* had 9.01 mm and 8.36 mm mean inhibitory zone diameters, respectively. Compared to TTO 5%, TTO 10% displayed slightly larger mean inhibitory zone diameters in all-*Candida* species but was statistically significant ($p = 0.001$). However, we could not determine TTO sensitivity because there were no Clinical and Laboratory Standards Institutes (CLSI) criteria for TTO.

The mean inhibitory zone diameter of nystatin in all isolates was 25.57 mm, while the mean inhibitory zone diameter in *C. albicans* and non-*albicans* was 25.94 mm and 25.29 mm, respectively. Sensitivity criteria according to CLSI for nystatin were used to determine *Candida* sensitivity (Table 2). According to the criteria, all isolates were sensitive to nystatin, and no strains were resistant to it.

In all *Candida* species, the mean diameter of the nystatin inhibition zone was larger than TTO 5% and TTO 10%, which was 25.57 mm compared to 7.26 mm

and 8.64 mm, respectively (Figure 4). Kruskal-Wallis tests revealed a significant difference between the diameters of the inhibitory zone between TTO 5%, TTO 10%, and nystatin ($p < 0.001$), whereby nystatin was superior to both TTO 5% and TTO 10% in both *C. albicans* and non-*albicans*. In this study, we found that the mean inhibitory zone diameters of TTO 5%, TTO 10%, and nystatin was slightly larger in *C. albicans*

Figure 3. The distribution of *Candida non-albicans* species found in patients.

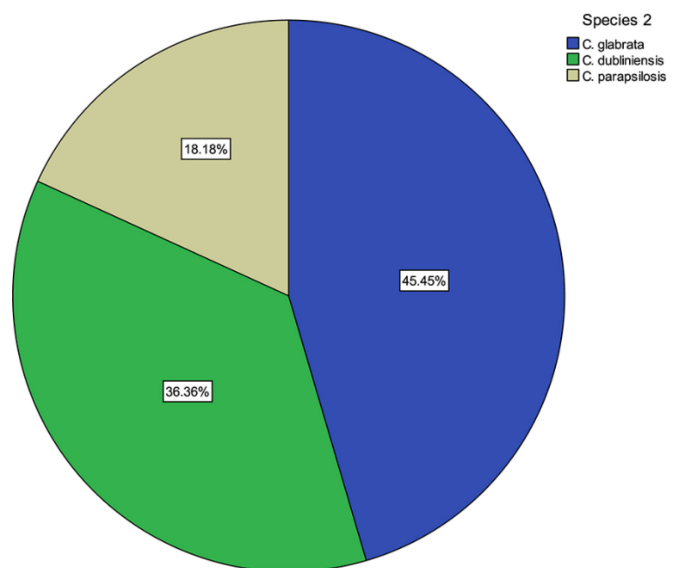


Table 2. Nystatin inhibition zone for *Candida* species (10).

Drug	Concentration	Inhibition Zone Diameter (mm)		
		Sensitive	Intermediate	Resistant
Nystatin	100 U/disc	≥ 15	10-14	≤ 10

compared to *C. non-albicans*, but the difference was not statistically significant (Figure 5).

Discussion

According to previous reports, one-quarter of all women are exposed to vaginal candidiasis during their lives [11]. This study revealed a total of 15 pregnant women with vulvovaginal candidiasis (VVC) during three months period between March 2021 and May 2021 at Dr. Soetomo General Hospital. The prevalence

of VVC in this study was at a higher frequency within the age range 26-35 years (80%) because women in this age range are younger and sexually active. Moreover, some women in this age range are becoming more desirous of having children. They also have a possible low vaginal defense mechanism against *Candida* species and have increased levels of estrogen and corticoids and, therefore, are more susceptible to *Candida* species infections [11]. This study found that the women in the 3rd trimester had the highest prevalence rate of VVC. In 3rd trimester pregnant women, symptomatic recurrences are more likely, and therapeutic response is diminished [12]. The immune system of pregnant women in the 3rd trimester of pregnancy is weakened compared to the 2nd and 1st trimesters, increasing the chance of *Candida* species becoming pathogenic. Vaginal colonization and symptomatic vaginitis are more common during pregnancy. These factors contributed to the highest prevalence of VVC in the 3rd trimester of pregnancy.

Candida albicans is the most common cause of fungal infections of the reproductive tract in women of childbearing age [13]. However, there have been reports in recent years of *Candida non-albicans* species being identified from VVC patients, especially *Candida glabrata*, which is increasingly being identified as the infection's source [14,15]. Our findings showed that the most common *Candida* species isolated from vaginal discharge were non-*albicans* there are *C. glabrata* (54.4%), followed by *C. dubliniensis* (36.3%), and *C. parapsilosis* (18.1%). A similar study by Nelson et al. reported that *C. glabrata* was the most common cause of VVC in pregnant women [16]. The shift towards *C. non-albicans* as the cause of VVC is concerning for pregnant women, as it has the potential to make VVC chronic, recurrent, and more resistant to antifungal drugs than *C. albicans*. The high resistance levels of *C. non-albicans* species to routinely used medications, together with an increase in their identification in women with VVC, emphasizes the need to identify *Candida* species in vaginal samples to give clinicians information about the best treatment for their patients.

Tea tree oil (TTO) at a concentration of up to 20% was considered safe and without major adverse effects [17]. This study used TTO with 5% and 10% concentrations. This concentration was chosen to prepare the medication for future human use by using the minimal concentration that was effective with low toxicity risk. TTO 5% and TTO 10% showed antifungal activity against *Candida* species in this study. This effect was proved by the TTO inhibition produced in the media with a diameter of 7.26 mm and 8.64 mm in

Figure 4. The mean inhibitory zone diameters of TTO 5%, TTO 10%, and nystatin in all *Candida* species.

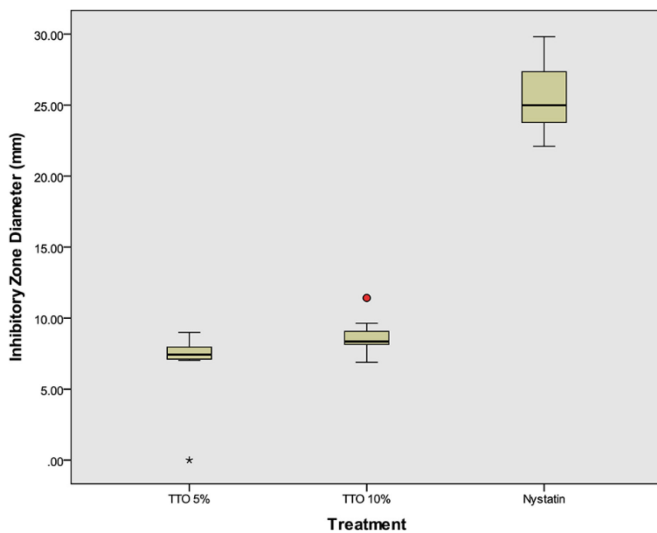
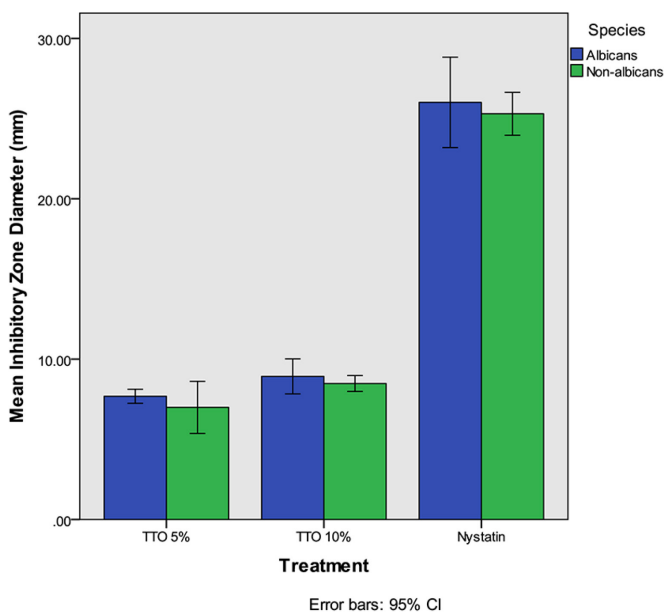


Figure 5. The comparison between mean inhibitory zone diameters of TTO 5%, TTO 10%, and nystatin in *C. albicans* and non-*albicans*.



all *Candida* species. According to Hammer *et al.*, TTO and their components improved membrane cell permeability, and this could inhibit the growth of *Candida* [18]. *Tea tree oil* could also produce membrane lipid bilayers that change the component keeping the membrane intact. These changes lead to the inhibition of the growth of *Candida* [19]. The mean diameter of TTO inhibition zone of *C. non-albicans* was slightly lower than *albicans* species meaning that the antifungal susceptibility of *C. albicans* was better than those of *C. non-albicans*.

Non-*albicans* candidiasis is more likely among those with diabetes mellitus, the elderly, prior antifungal drugs, or low socioeconomic status. The pathogenic mechanisms of *C. non-albicans* are less well understood than those of *C. albicans*, where more extensive research has been carried out [20]. Intrinsic resistance or low-dose susceptibility to azole antifungals, the first-line treatment, is a prominent feature of non-*albicans* species, which leads to treatment failure [21]. Identification and antifungal susceptibility testing are required for optimal treatment in pregnant women of these infections, especially in settings where the diagnosis is based on clinical presentation or limited laboratory testing.

The inhibitory zone of nystatin was significantly higher than TTO in diameter. This could be due to different drug mechanisms. Nystatin was discovered to bind ergosterol, which is the primary component of the fungal cell membrane. It created a pore-like structure, which allowed plasma to flow out, resulting in fungal cell death [22]. This was different from the TTO mechanism, which increased the permeability of the fungal cell membrane but did not form a pore-like structure as in nystatin. *Tea tree oil* was fungistatic, but only at higher concentrations did it become fungicidal [23]. *Tea tree oil* has also been shown to inhibit biofilm formation. The ability of *C. albicans* to adhere to and form a biofilm is very important in the incidence of VVC. The antifungal activity of TTO and *terpinen-4-ol* was able to control the proliferation of biofilms *in vitro* [24,25]. On the other hand, nystatin lacks this mechanism. Therefore, TTO can be considered as adjuvant therapy for VVC in pregnancy.

In both *C. albicans* and *C. non-albicans*, there was a statistically significant difference in the inhibitory zone diameters of TTO 5% and TTO 10% compared to nystatin ($p < 0.001$). The inhibitory zone diameters of *C. albicans* were slightly larger than other *Candida* species but were not statistically different. Based on this result, further study of TTO antifungal activity was needed to establish the optimal concentration of

antifungal treatment of VVC in pregnancy. *Tea tree oil* must be used in higher concentrations to prevent *C. albicans* from growing [26]. This could be due to the ability of *C. albicans* to form a germination tube and biofilm, which would protect the fungi from TTO-induced environmental changes [27]. *Tea tree oil* demonstrated fungistatic effects, but only at higher concentrations did it have a fungicidal effect (26). In our study, we found a significant difference in the inhibition zone diameter between TTO 5% and TTO 10% for each species of *C. albicans* and *C. non-albicans*, but the increment in antifungal activities with increasing TTO concentrations were minimum. Given the risks of administering TTO, especially during pregnancy, a concentration of TTO 5% may be sufficient to offer a therapeutic effect on *Candida* that causes VVC.

According to this study, our opinion is that combining TTO with standard medications such as nystatin might be helpful to treat chronic VVC or VVC in pregnancy. Further studies are required to determine the antifungal activity of natural medicinal components and to uncover synergistic interactions with routinely used antifungal drugs.

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