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Original article

The beneficial effect of Indonesian propolis wax from *Tetragonula* sp. as a therapy in limited vaginal candidiasis patients

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ABSTRACT

Vaginal candidiasis characterized by abnormal vaginal discharge and itching usually treated by azole's drug or nystatin; however, some results of treatment are unsatisfied and become recurrent. Propolis containing polyphenols and flavonoids is known to have anti-inflammatory and antimicrobial activity. This study investigated the effect of Indonesian propolis wax from *Tetragonula* sp. as a therapy in limited vaginal candidiasis patients. The subjects were women who came to the Tasik Community Health Centre met the inclusion criteria such as clinical complaint and laboratory evaluation (positive hyphae/pseudohyphae and culture on Sabouraud Dextrose Agar (SDA) medium) from a vaginal swab. Evaluation of anti-candida effect of propolis was determined by clinical remission and the absence of Candida's growth on SDA medium. Forty subjects were randomly assigned to those receiving treatment by ovule propolis (n = 20) and that treatment by nystatin (n = 20) as a control, once daily, for seven days, respectively. All methods have been approved by the Ethics Commission of the Faculty of Medicine, Universitas Indonesia. Our results indicated no significant difference in the laboratory evaluation of patients who have treated ovule propolis compared to standard therapy. This study suggests that propolis wax has a beneficial effect to develop as an anti-candida agent for vaginal candidiasis therapy.
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1. Introduction

One of the reproductive health concern in women that need to be observed is reproductive tract infections frequent characterized by abnormal vaginal discharge. In women, vaginal discharge can arise physiologically due to hormonal or pathological influences caused by infection by bacteria or fungi (Mitchell, 2004). Abnormal (pathological) vaginal discharge is a vulnerable problem for women because it can cause psychological disturbance or as a symptom of an infection of genital organs such as vulvitis, vaginitis, cervicitis, and pelvic inflammatory disease. The characteristics of abnormal (pathological) vaginal discharge include a change of color, unpleasant odors, and related symptoms such as itching, burning, or pain during sexual intercourse (Bitew and Abebaw, 2018). In Indonesia, about 90% of women have the potential to experience vaginal discharge caused by fungal infection because Indonesia is a tropical country, so the fungus is easy to grow (Nurul and Qomariyah, 2001). Also, 75% of women are thought at least once suffered from vaginal candidiasis (Mitchell, 2004). *Candida albicans* was the most common cause of vaginal candidiasis (Sobel et al., 2004; Sobel, 2016).

C. albicans is classified as a normal flora colonized in mucosal tissue (oral, vaginal and gatrointestinal), nail and skin, where circumstances changes allow *C. albicans* transition from yeast-like to hyphal morphologies growth leads to cause candidal infection (Haghdoost et al., 2016). Risk factors that can increase the incidence of *C. albicans* infection include inadequate immune system,

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hormone imbalance, pregnancy, and poor personal hygiene. In general, the treatment of candidiasis infection is treated by Azole medication which works by inhibiting the ergosterol biosynthesis required in the generation of *C. albicans* cell membrane. Nowadays, however, it has been found that some of the *Candida* species caused vaginal candidiasis are becoming resistant to the Azole group (Dota et al., 2011; Lemos, 2009) that influenced by the chemical composition of biofilm matrix of *Candida* species (Al-Fattani and Douglas, 2006; Uppuluri et al., 2009). Therefore, it is necessary to find an effective alternative drug for the treatment of vaginal candidiasis.

Propolis is a sap (resin) substance produced/secreted by leaf buds and also the stem part, which seeps out through the skin of plants collected by bees and mixed with beeswax and wax. Bees use propolis not only as a nest-building material but also as a means of maintaining low levels of bacterial and fungal concentrations in bee hives. Propolis is a bioactive compound in a honeycomb containing polyphenols, terpenes and flavonoids (Bankova et al., 2004, 2014, that have anti-inflammatory, antimicrobial, antioxidant and antiviral activities (Lotfy, 2006, Farnesi, 2009, Cardoso et al., 2010, Kim et al., 2011, Ordonez et al., 2011, Trusheva et al., 2011, Bittencourt et al., 2015, Nina et al., 2016). These compound make propolis have potential anti-fungal therapy. Furthermore, an in vitro study conducted by Siqueira et al. (2015), showed that the anti-fungal ability of red propolis against most samples of Candida sp. In utilization, propolis obtained must be purified before further processing. The by-product of the propolis purification process is the propolis wax which has so far not been utilized further because the polyphenolic content in the propolis wax is less than that of pure propolis. However, in such a small amount, polyphenols in wax propolis were proven to be effective in inhibiting *C. albicans*' growth reaching 76% in concentrations of 79.43 mg/ml (Hudnall, 2007). Furthermore, (Velikova et al., 2000) reported that Brazilian propolis from native stingless bee had antibacterial effect.

Based on the location of the formation, propolis is distinguished over two types, i.e., fine propolis (inside honeycomb) and rough propolis (outside of the honeycomb). The compounds of rough and fine propolis may be different which will have an impact on the activity of each. Antifungal activity possessed by propolis can be used to treat abnormal vaginal discharge.

This study examined the beneficial effect of the innovation drug containing an effective type and concentration of propolis wax material could inhibit *Candida* sp growth as a treatment of vaginal candidiasis. The novelty of this study is selecting the kind of propolis among fine propolis, rough or fine-rough mixtures based on their chemical compounds and sufficient concentrations that can inhibit the growth of *C. albicans* in ovule preparations.

2. Material and methods

The research design was a study of the benefits of propolis wax implemented to a limited number of vaginal candidiasis patients. The wax propolis was extracted from Tetragonula sp. propolis from Sulawesi island. The research subjects were women who came to the Tasik Community Health Centre with complained about abnormal vaginal discharge and itching which fulfilled the inclusion criteria and signed the informed consent. The inclusion criteria include (1) there is a clinical complaint of abnormal vaginal discharge (2) married women, aged 20–50 years old; (3) positive hyphae/pseudo hyphae and culture of vaginal swab; (4) not pregnant; (5) not received treatment. Forty subjects were randomly divided into two groups: the group receiving treatment with ovule propolis (n = 20) and the control group receiving standard treatment with nystatin (n = 20) intra vagina, once a day before sleep, for seven days, respectively.

2.1. Ovule preparations

Propolis wax ectract 5% from *Tetragonula* sp. as an active ingredient was mixed with Oleum Cacao and other added ingredients that have been melted on a hot plate 60 °C. The mixture was then poured into the ovule mold. After 15 min left at room temperature, the ovules were put into the refrigerator 4 °C for 2 h. Then, the ovule propolis was removed from the refrigerator and put at room temperature for 30 min. Furthermore, ovula propolis was then removed from the mold and packaged into aluminum foil which has been sprayed with alcohol before. The methods of the ovule preparations used are the result of the previous study from the research team who has obtained the patent of Intellectual Property Rights.

2.2. Wet examination of samples

We collected a vaginal secret using a sterile swab. Then a sterile swab was inserted into a sterile tube contained 0.9% sterile saline fluid. After mixing, the swab containing the sample was applied to the sterile object glass until the sample was shed and then covered with a glass lid. Furthermore, the sample was examined under a microscope with a 40× magnification. Positive *Candida* sp. was determined by finding the hyphae/pseudohyphae.

2.3. Culture examination of samples

The sterile sample from vaginal swab was inserted into a sterile tube contained 0.9% sterile physiological fluid. To identify the species of *Candida*, all samples were then inoculated onto to culture medium, Sabouraud Dextrose Agar (SDA) medium and chromogenic agar medium parallel, respectively. Inoculation on SDA and the chromogenic medium was carried out in Biosafety cabinets (BSC) II. SDA medium does not need to be wrapped in anything, while the chromogenic medium must be package in aluminum foil to avoid light. Furthermore, the samples were then incubated at room temperature for SDA medium and to be at 37 °C for chromogenic media. Chromogenic medium readings were carried out over 48 h while in SDA medium after 72–120 h. Positive of *Candida*'s growth was determined by the presence of more than 10 Candida colonies on SDA medium.

3. Results and discussion

Drug preparations in the form of vaginal suppositories (ovules) was selected because the propolis as an active ingredient can directly reach the vagina as the target area for treatment to be faster and more effective. Ovule propolis with dimension size 3 × 1.1 × 1.1 cm and its specific form are suitable for adult women (Fig. 1). Compared to using swab of water soluble propolis in vaginal track, ovule propolis preparation is easier to use by themselves and propolis get longer contact with vaginal mucosa. Moreover, the administration before sleep at night make propolis was kept in vaginal tract around 6 h and it can be more effective to control candidiasis. Furthermore, the vaginal wall is very well suited for the absorption of drugs, since it contains a vast network of blood vessels. But the disadvantage of ovule preparation is make discomfort during intercourse because of molten ovule propolis. Therefore, it is recommended to apply ovules before intercourse or to use a condom during intercourse.

The results of the positive laboratory evaluation included positive hyphae/pseudohyphae and culture on Sabouraud Dextrose Agar medium from a vaginal swab were shown in Fig. 2. The results of identifying of the *Candida* species isolated on Sabouraud Dextrose Agar medium and chromogenic agar medium from 40

Fig 1. Vaginal suppository (ovule) containing propolis wax 5%.

patients are shown in Table 1. Those isolates showed that 38 (95%) were identified as *C. albicans*, 1 (2.5%) as *C. krusei*, and 1 (2.5%) as *Candida glabrata*. The results were similar to a multicenter study by Sobel et al. (2004), Sobel (2016) who reported that *C. albicans* was the most common cause of vaginal candidiasis (>90%), followed by 3% as *C. glabrata*, 0.2% as *C. krusei*, although the 5 most common pathogens causing of superficial candidiasis were *C. albicans*, *C. glabrata*, *C. krusei, C. tropicalis*, and *C. parapsilosis* (Berkow and Lockhart, 2017). However, the distribution patterns of Candida species was different from the study on Ethiopia's women by Bitew and Abebaw (2018) who reported that the most frequent isolate was *C. albicans* for 58.6%, followed by *C. krusei* 17.2%, and *C. glabrata* 3.4%.

The number of dropped out subject during treatment observation was shown in Table 2. Five subjects dropped out and loss to follow up because they did not come for laboratory evaluation (culture and vaginal swab) after seven days of treatment, two subjects from the propolis group and three subjects from the nystatin group. One dropped out subject from group therapy of propolis was caused by infection of *C. crusei* isolate, while all dropped out subject from nystatin group were caused by infection of *C. albicans* isolate.

The evaluation of seven days of treatment by ovule containing propolis wax 5% compared to standard therapy in inhibiting *C. albicans*' growth are shown in Table 3. The results of this study showed no significant difference in culture evaluation (culture of *C. albicans's* growth on Sabouraud Dextrose Agar medium) from vaginal candidiasis patients who have treated ovule propolis wax 5% compared to standard therapy nystatin, respectively.

The results of this study indicated the effectivity of ovule propolis wax 5% from *Tetragonula* sp. to inhibit the growth of *C. albicans*, but the effect of propolis on *C. krusei* did not evaluate due to drop

Table 1
Identification of *Candida* species isolated from vaginal swab.

Organism	Number of patients (percent)	Group therapy Propolis	Group therapy Nystatin
C. albicans	38 (95%)	19 (95%)	19 (95%)
C. glabrata	1 (2.5%)	–	1 (5%)
C. crusei	1 (2.5%)	1 (5%)	–

Table 2
The number of dropped out subjects between groups.

Group therapy	Number of subjects	Number of dropped out (DO) subjects
Propolis	20	2 (one DO subject was caused by *C. crusei*)
Nystatin	20	3 (all DO subjects were caused by *C. albicans*)

Table 3
Laboratory evaluation (culture of vaginal swab) before and after therapy.

Group therapy	Culture test before therapy	Culture test after therapy
Propolis (n = 18)	(+)	(−)
Nystatin (n = 17)	(+)	(−)

out. The finding of this study was supported by Dota et al., 2011, Berkow et al., 2015, Gavanji et al., 2011; Gavanji and Larki, 2017; Soekanto et al., 2018 who showed the antifungal activity of Propolis. This finding was also supported by Al-Waili et al., 2012 who reported the propolis from Saudi and Egypt that combined with honey had the synergistic effect toward drug multi-resistant of *C. albicans*. This study also demonstrated that 100% *C. albicans* was susceptible to Indonesian Propolis wax of stingless bee *Tetragonula* sp. from Sulawesi-Indonesia, while the study by Bitew and Abebaw (2018) demonstrated that 2% of *C albicans* was resistant to fluconazole and 33.3% of *C. krusei* was resistant to fluconazole. This anticandida effect of propolis wax due to polyphenols compounds in propolis wax which has proven effective in inhibiting *C. albicans* reaching 76% in concentrations of 79.43 mg/ml (Hudnall, 2007). Moreover, in such a small amount, polyphenols in wax propolis of *Tetragonula* sp. have antioxidant activity (Pratami et al., 2018, Christina et al., 2018). Our results also showed that Tetragonula sp. propolis from Sulawesi has different properties compared with others propolis (Miyata et al., 2019, Alqarni et al., 2019). Several new compounds was found in the propolis, such as Sulawesin A and B that not found in other propolis (Miyata et al., 2019).

The finding of this study was supported by Mahadewi et al. (2017) that showed the level of flavonoid compounds of the *Tetragonula* propolis wax from Sulawesi was higher than propolis from Rumania, Taiwan, Brazil, China (Huang, 2002). The bioactive compound in propolis containing polyphenols, terpenes and flavonoids

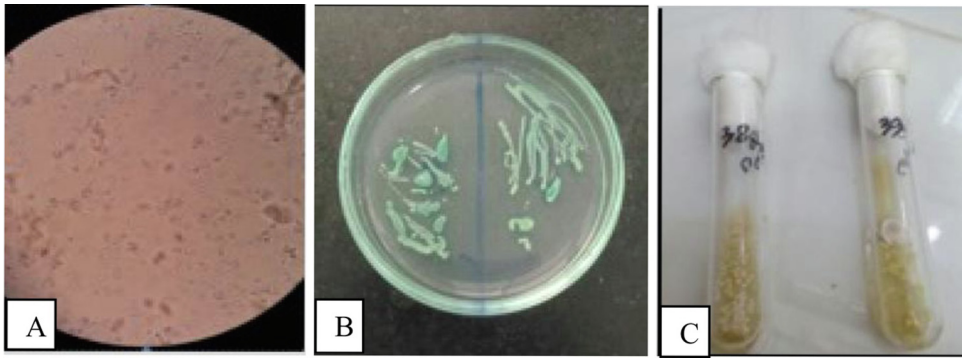

Fig 2. Positive hyphae/pseudohyphae (A); positive of *Candida*'s growth on Chrom Agar medium (B); positive of *Candida*'s growth on SDA medium (C).

have anti-inflammatory, antimicrobial, antioxidant and antiviral activities (Cardoso et al., 2010, Kim et al., 2011, Odonez et al., 2011). Furthermore, Mahadewi et al., also reported that Indonesian propolis wax contained anti-fungal marker compounds such as thymol, curcumene, tetraline, and E-p-coumaric acid that had potential as anti-candida. This result was also supported by Da Silva et al. 2015 that showed terpenes compound in thymol had MIC to *C. albicans* 64 mg/ml. Unfortunately, we cannot evaluate the effectivity of ovule propolis to inhibit the growth of *C. krusei* because the subject did not come to laboratory evaluation (culture and vaginal swab) after seven days of treatment. The recent studies reported that the mechanism of action of propolis wax as an anti-candida by inhibition the production of biofilm matrix (Capoci et al., 2018) and germ tube formation as virulence attribute (Haghdoost et al., 2016) of *C. albicans*. Moreover, Alves de Lima et al., 2018 showed that propolis can improve the immune response againts *C. albicans* by increasing the microbial activity of neutrophils. To ensure the effectivity of anti-candida effect of propolis wax in vaginal candidiasis patients, we will continue the clinical study with enough sample size.

4. Conclusion

Based on these results, the ovule propolis wax from *Tetragonula* sp. at concentration 5% has a beneficial effect of being implemented and developed as an anti-candida agent for vaginal candidiasis therapy. The anti-candida effect of propolis wax due to their phytochemical compounds.

Ethical approval

All research methods used have been approved by the Ethics Commission of the Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia, with approval number No. 810/UN.2.FI/ETIK/2017.

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