

# The prospects of complementary treatment using Red Piper betle (*Piper crocatum*) against *leucorrhoea*: an empirical study

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## ABSTRACT

One of the main issues for women in the reproductive age range is *leucorrhoea*, which causes a woman ongoing distress, and occasionally be the first sign of several of the main gynecological disorders. The widespread use of fluconazole antibiotics has caused *Candida sp* to become resistant. Alternative non-pharmacological treatments based on natural products are needed, especially *P. crocatum* which has antioxidant, antifungal, and anti-inflammatory activities. This study analyzed the empirical study of *P. crocatum* used against *leucorrhoea* due to *C. albicans*. The empirical study presented that vaginal discharge was reduced after using *P. crocatum* formulations. The infection parameter of vaginal pH level, the intensity of pain, and itching were significantly recovered with p values of 0.008, 0.000, and 0.001, respectively, while the reducing *C. albicans* colonies was 0.001. *P. crocatum* decoction formulation is effective in reducing vaginal yeast infections in women, close to the results of the positive control, ketoconazole.

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## 1. Introduction

One of the main issues for women in the reproductive age range is *leucorrhoea*, which becomes significant because, in addition to causing a woman ongoing distress, it may occasionally be the first sign of several of the main gynecological disorders (Ban Ki-moon & Organization, 2016). A white vaginal discharge known as *leucorrhoea* may be pathogenic or physiological, while pathogenic *leucorrhoea* shows white vaginal discharge without accompanying pain, burning, or discomfort is a symptom of the illness (Dhiman & Roga, 2014). The incidence of vaginal discharge in the world ranges from 75% with as many as 10 million visits to health facilities every year, with *leucorrhoea* caused by *Candida sp* of 90% (Sherrard et al., 2018; Venugopal et al., 2017).

Normal flora will not cause clinical symptoms if it only amounts to 20-50% in the vagina (Kyu et al., 2018). The normal flora functions to prevent infection by maintaining vaginal pH in normal conditions ranging from 3.8 to 4.4 (Rao & Mahmood, 2020). Changes in vaginal pH indicate an imbalance in the vaginal environmental ecosystem (acid conditions if  $H^+$  is greater than  $OH^-$  or alkaline conditions if  $H^+$  is less than  $OH^-$ ) thereby increasing the number of pathogens in the vagina which will cause pathological *leucorrhoea* (Chayachinda et al., 2021; Rice et al., 2016). *Leucorrhoea* can be caused by fungi such as *Candida albicans*; bacteria such as *Neisseria gonorrhoea*, *Chlamydia trachomatis*, *Gardnerella sp*, *Treponema pallidum*; parasites such as *Trichomonas vaginalis*; and viruses such as Human Papilloma Virus (HPV) types 16 and 18, and herpes simplex (Mirończuk-

Chodakowska et al., 2018). *Candida sp* is a normal microbiota in the human body that can be found in the mucosa of the oral cavity, gastrointestinal tract, and vagina (Sardi et al., 2013).

Currently, the pharmacological treatment for *leucorrhoea* uses antifungals that inhibit cell wall synthesis such as echinocandins; antifungal nucleic acid synthesis inhibitors such as flucytosine; antifungals destroying fungal microtubules such as griseofulvin and antifungals destroying cell membranes permeability such as azoles, allylamines, polyenes, morpholines, substituted pyridines, and thiocarbamates (Kathiravan et al., 2014; Lewis, 2013). The widespread use of fluconazole antibiotics has caused *Candida sp* to become resistant or slightly susceptible, which azoles should have to inhibit lanosterol 14 $\alpha$  demethylase as a target enzyme in ergosterol by accumulating 14 $\alpha$ -Methyl-3,6-diol, so that the content of this sterol enzyme becomes reduced, the structure and function of the fungal cell membrane changes and the growth of the fungus becomes inhibited (Sanguinetti et al., 2015). Therefore, alternative non-pharmacological treatments based on natural products are needed. *P. crocatum* as a natural product has analgesic, antimicrobial, antioxidant, antifungal, and anti-inflammatory activities according to ethnopharmacology data with 677 known compounds (Alfiana et al., 2022; Saputra et al., 2016; Siswina et al., 2023). *P. crocatum* contains secondary metabolites such as flavonoids (1), alkaloids (2), triterpenoids or steroids (3), hydroxychavicol (4), tannin (5), saponins (6), terpenes, and phenolics (Fig 1) (Ervidiana, 2011; Gutierrez et al., 2013). This study focused on the empirical study of *P. crocatum* used against *leucorrhoea* due to *C. albicans*.

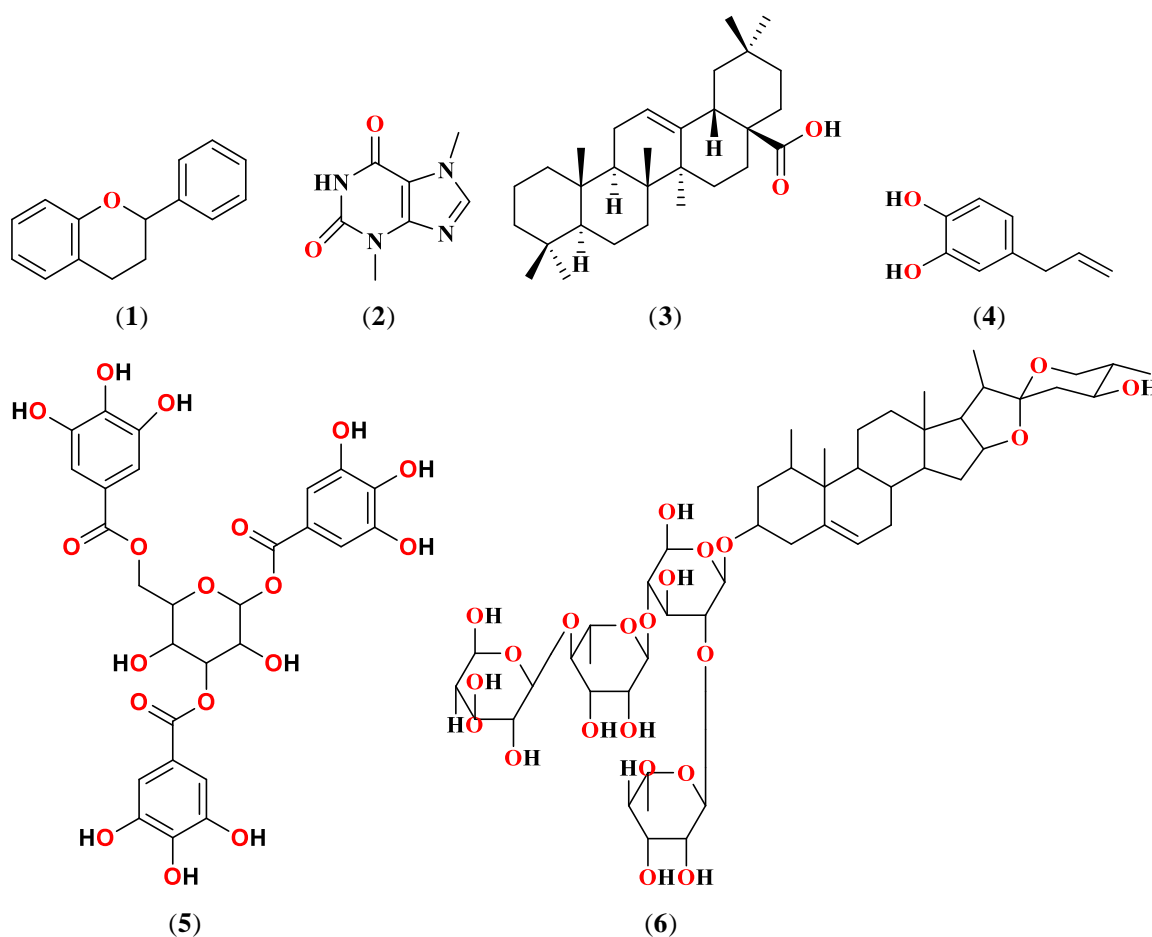


Fig. 1. Structure of *P. crocatum* leaf compounds: flavonoids (1), alkaloids (2), steroids (3), hydroxychavicol (4), tannins (5), saponins (6)

## 2. Methods

### 2.1. Ethical Guidelines

The ethics of this research have complied with the ethical principles stated in the 2008 Declaration of Helsinki through the Health Research Ethics Commission of Poltekkes Kemenkes Pontianak (Pontianak Ministry of Health Polytechnic), West Kalimantan, Indonesia. This research has met the

ethical principles through the Ethics Commission with the Ethical Approval Letter from No 166/KEPK-PK.PKP/VII/2022. This ethical clearance principles consist of ethical commitment, research justification, ethical issues that may be encountered, research methods, sampling techniques especially in vulnerable groups (guidelines 3), interventions provided, plans and justification for continuing or stopping standard therapy during research (guidelines 4 and 5), treatment or other that may be given or permitted or be contraindicated during the study (guideline 6), monitor results, discontinuation of the study and the reasons, adverse events and complications (guidelines 4 and 23), benefits (guidelines 1 and 4), informed consent (guideline 9), guardians (guidelines 16 and 17), benefits for participants (guideline 9), maintaining confidentiality (guidelines 3, 4, 11, 12, and 24), planning analysis and monitoring security (guideline 4), conflicts of interest (guideline 25), social benefits (guideline 8), rights to data and publication (guideline 24).

## 2.2. Empirical Study

The research takes place in Sambas, West Kalimantan, Indonesia. The respondents in this study were women of childbearing age who experienced vaginal discharge due to *C. albicans*. Determination of the sample using purposive sampling technique with double-blind randomized controlled trial (RCT). The double-blind RCT technique in this research was carried out so that the researchers and respondents did not know about the treatment or intervention being provided. The treatment in the form of using boiled and soaked *P. crocatum* leaves was prepared and carried out by midwives and cadres to the respondents, so that the respondents and researchers did not know whether the treatment received was in the form of boiled or soaked *P. crocatum* leaves. Determination of the sample size using the Federer formula as follows (Siswina et al., 2023):

$$(t-1)(r-1) \geq 15$$

This research was carried out by quantitative research using the true experimental design method and the pretest-posttest design with the control group. After being given the intervention, a posttest was carried out in each group. The group in this study was divided into four which selected through randomization by drawing lots. Group 1 received intervention in the form of using boiled *P. crocatum* leaves; Group 2 received intervention in the form of soaking *P. crocatum* leaves, while group 3 as a negative control and group 4 as a positive control. The research design in this study is as follows:

- Group 1: A – X<sub>1</sub> – B
- Group 2: C – X<sub>2</sub> – D
- Group 3: E – X<sub>3</sub> – F
- Group 4: G – X<sub>4</sub> – H

While A pretest in group 1; X<sub>1</sub> intervention in the form of giving seven *P. crocatum* medium leaves stew (boiled with 500 mL of distilled water for 30 minutes, then cooled to  $\pm 70^{\circ}\text{C}$  and wipe into the vagina, two times a day, for seven days); B posttest in group 1; C pretest in group 2; X<sub>2</sub> intervention in the form of giving seven *P. crocatum* medium leaves soak (soaked in 500 mL of distilled water for 30 minutes at  $\pm 70^{\circ}\text{C}$  and wipe into the vagina, two times a day, for seven days); D posttest in group 2; E pretest in group 3; X<sub>3</sub> intervention in the form of ketoconazole 200 mg drug orally as a positive control (two times a day, for seven days); F posttest in group 3; G pretest in group 4; X<sub>4</sub> intervention in the form of a washcloth using distilled water as a negative control (wipe into the vagina, two times a day, for seven days); H posttest in group 4.

First, respondents were asked to come to the Community Health Center Sambas District, West Kalimantan. Before the intervention, the respondents signed the informed consent as evidence of agreeing to be a respondent in the study and get an explanation about the research treatment. They perform a visual examination of the vagina using a sterile speculum and take a vaginal swab for examination of vaginal discharge by cotton swab and put the swab in object glass, then analyzed using a microscope and examination of vaginal pH levels using MQuant-pH indicator strips. After getting the results of a microscope examination with a magnification of 40x, the respondents who experienced vaginal discharge due to *C. albicans* continued to be research respondents and were given an observation sheet on the intensity of pain and itching in the vagina due to vaginal discharge. The observation sheet to measure the intensity of pain and itching in the vagina using a pain level scale of 1 to 10, where 1 represents no pain, 5 represents moderate pain and 10 represents very severe pain,

which is observed every day for 7 days. Then each group was given treatment according to the research design above. After seven days, the respondents were asked to come to the Community Health Center Sambas District for final inspection (re-examination with vaginal swabs and pH vagina levels).

Data were analyzed using univariate analysis to determine the pretest and post-test values of the four variables (vaginal pH level, intensity of vaginal pain, intensity of vaginal itching, number of *C. albicans* colonies), and bivariate analysis using the Levene statistic normality test to test the homogeneity of variance more than two group as well as ANOVA test with multiple comparison-post hoc Bonferroni to find out significant differences between one group and another group.

### 3. Results/Findings

#### 3.1. Plant Identification

The sample identified at the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia in the Laboratory of Biosystematics and Molecular with No 10/LBM/IT/11/2022, as follows:

Scientific Name	: <i>Piper crocatum</i> Ruiz & Pav
Synonym	: <i>Sterrensia crocata</i> (Ruiz & Pav.) Kunth
Local Name	: Red Piper
Tribe/Family	: <i>Piperaceae</i> Giseke

#### 3.2. Empirical Antifungal Activity

The number of respondents per group consists of six respondents, so a total of four groups is twenty-four respondents by random selection. The univariate analysis of vaginal pH level, the intensity of vaginal pain and itching, and the number of *C. albicans* colonies in the vagina as seen in [Table 1](#), and the bivariate analysis in [Table 2](#).

**Table 1.** Univariate Analysis of Empirical Antifungal Activity of *P. crocatum*

Group	N	Vaginal pH Level			Intensity of Pain			Intensity of Itching			Number of <i>C. albicans</i> colonies		
		Mean±SD		Std.	Mean±SD		Std.	Mean±SD		Std.	Mean±SD		Std.
		Pre-test	Post-test		Pre-test	Post-test		Pre-test	Post-test		Pre-test	Post-test	
1	6	5.6±0.8	3.6±0.8	3.8-4.4	3.8±1.1	0.17±0.4	0	4.1±0.7	0.3±0.5	0	21.3±16.3	2.0±4.8	
2	6	6.0±1.4	5.0±1.2		3.1±0.7	0.50±0.8		3.1±0.7	0.5±0.8		20.3±14.6	3.8±6.2	
3	6	5.1±0.7	4.1±0.7		4.0±1.0	0.00±0.0		4.3±1.0	0.0±0.0		27.5 16.3	0.0±0.0	
4	6	5.6±0.5	5.8±1.1		2.6±0.8	2.00±0.8		3.7±0.9	1.8±0.9		23.8±14.7	21.1±13.9	
<b>TOTAL</b>	24												

Note: Group 1 (*P. crocatum* decoction group), group 2 (*P. crocatum* soaked group), group 3 (positive control-ketoconazole), and group 4 (negative control-water)

**Table 2.** Bivariate Analysis of Empirical Antifungal Activity-ANOVA Test with Multiple Comparison-Post Hoc Bonferroni

Type of Treatment		Vaginal pH Level		Intensity of Pain		Intensity of Itching		Number of <i>C. albicans</i> colonies	
		Mean Diff	Sig	Mean Diff	Sig	Mean Diff	Sig	Mean Diff	Sig
1	2	-1.333	0.008	-0.333	0.000	-0.167	0.001	-1.833	0.001
	3	-0.500		0.167		0.333		2.000	
	4	-2.167*		-1.833*		-1.500*		-19.167*	
2	1	1.333	0.008	0.333	0.000	0.167	0.001	1.833	0.001
	3	0.833		0.500		0.500		3.833	
	4	-0.833		-1.500*		-1.333*		-17.333*	
3	1	0.500	0.008	-0.167	0.000	-0.333	0.001	-2.000	0.001
	2	-0.833		-0.500		-0.500		-3.833	
	4	-1.667		-2.000*		-1.833*		-21.167*	
4	1	2.167*	0.008	1.833*	0.000	1.500*	0.001	19.167*	0.001
	2	0.833		1.500*		1.333*		17.333*	
	3	1.667		2.000*		1.833*		21.167*	

\* Note: The relationship between the two variables is stated to be significant if the p-value <0.05

The data in **Table 1** showed that there was the biggest difference in vaginal pH levels was in group 1 where there was a decrease in vaginal pH levels by 2 when the vaginal pH level was  $5.6 \pm 0.8$  in the pre-test to  $3.6 \pm 0.8$  in the post-test. This pH vaginal level in group 1 reaches the normal vaginal pH level between 3.8-4.4 (Mustofa, 2012). Changes in vaginal pH to acid or alkaline will cause an imbalance in the vaginal environment ecosystem that causes pathological vaginal discharge (Morry et al., 2017). It also showed that there is the biggest difference in the intensity of pain in the vagina in group 3 because there was a decrease in the intensity of pain in the vagina up to 0 meaning no more pain in the vagina after using the ketoconazole. The result closest to group 3 is group 1 and *P. crocatum* stewed almost relieves the pain in the vagina after being used as a vaginal discharge treatment. There was a decrease in the intensity of itching in the vagina in group 3 up to 0 meaning no more itching in the vagina. The closest result was in group 1 means that *P. crocatum* decoction using can relieve vaginal itching almost similar to the results of treatment with ketoconazole. Then, the biggest difference in the number of *C. albicans* colonies was in group 3 because there was a decrease in the number of colonies up to 0 meaning no more the number of *C. albicans* colonies. The result that is closest to group 3 results in group 1 means the use of *P. crocatum* stewed as a vaginal discharge treatment can be nearly eliminated the total number of *Candida* colonies, similar to the benefits of ketoconazole. The use of *P. crocatum* leaf decoction can eliminate the number of *C. albicans* colonies close to 0 (no *C. albicans*).

According to data in **Table 2**, it was shown that there is a difference using ANOVA, then continued by using multiple comparisons-post hoc Bonferroni test, showed there's a meaningful difference between groups 1 and 4 of 2.167 with p value 0.008 ( $<0.05$ ) which means there is a significant difference in lowering vaginal pH levels by using *P. crocatum* stew as an antiseptic liquid for alternative treatment of vaginal discharge and water. There were differences between groups 1 and 4 of 1.833, groups 2 and 4 of 1.500, and groups 3 and 4 of 2.000 with p value 0.000 ( $<0.05$ ), and also between groups 1 and 4 of 1.500, groups 2 and 4 of 1.333, and group 3 and 4 of 1.833 with p value 0.001 ( $<0.05$ ) which means there are significant differences in a lower intensity of pain and itching in vagina because of *P. crocatum* stewed, *P. crocatum* soaked, and ketoconazole using to treat vagina discharge than water. There was also a significant difference in eliminating the number of *C. albicans* colonies in the vagina after using ketoconazole, *P. crocatum* stewed and soaked than water respectively 21.167, 19.167, and 17.333 with p value 0.001 ( $<0.05$ ). *P. crocatum* decoction is effective in reducing vaginal yeast infections in women to empirical study.

#### 4. Discussion

Proving the ethnobotanical and ethnopharmacological information in the community on the efficacy of *P. crocatum* herbal medicine with empirical studies that have been carried out indicated that *P. crocatum* has antifungal activity against *C. albicans* by using it as an antiseptic liquid for the treatment of vaginal discharge. Vaginal discharge consists of normal, which is clear or white, harmless, the amount varies over time, increases in pregnancy, contraceptive use and sexual stimulation, decreases during menopause; and abnormal, changes in color, odor, volume, consistency, accompanied by symptoms of itching, pain, pelvic pain, dysuria, postcoital or intermenstrual bleeding (Budinurdjaja et al., 2019; Calugi et al., 2013). Physiological discharge occurs when the number of normal flora (*Candida* spp) is 20-50% so that it does not cause clinical symptoms, because normal vaginal flora maintains a normal vaginal pH between 3.8-4.4 (Cassone, 2015), while abnormal vaginal discharge is caused by fungi, bacteria, parasites, and viruses (Sardi et al., 2013). *C. albicans* is a normal vaginal flora that only causes symptoms if there is a significant increase in the pathogen and is not considered a sexually transmitted disease (Erviana, 2013), a monomorphic yeast and yeast-like organism, that grows at temperatures of 25-30 and 35-37°C, breeds at pH 4.5-6.5 and grows under aerobic conditions as well as anaerobes, and under anaerobic conditions, had a longer generation time of 248 minutes, whereas under aerobic conditions it was only 98 minutes (Farrokhnia, 2020; Gutierrez et al., 2013).

Vaginal pH examination is carried out by wiping the vagina on the lateral wall and then placing it on spectrum litmus paper because vaginal pH examination is a sensitive (but not specific) predictor (Hay, 2018; Linhares et al., 2013). The use of *P. crocatum* leaves as an antifungal treatment has been carried out in vitro study with the results that the compounds in *P. crocatum* have antifungal activity with an inhibitory zone diameter of 14.5; 11.9; and 13.0 mm at a concentration of 10% w/v with an

MIC/MFC value of 0.31/1.2% w/v, with a time period of seven days of antibiotic use for treatment as the control positive for antifungal.

The use of *P. crocatum* stew can lower the vaginal pH level so that it becomes a normal vaginal pH as seen in [Tables 1](#) and [2](#). In the pre-menarche, vaginal pH is neutral, vaginal epithelial cells are only cuboidal in shape, and skin commensal epithelium is colonized; at puberty, squamous epithelial cells develop and lactobacilli become the predominant organisms so that the vaginal pH drops to 3.5-4.5; meanwhile, during menopause, atrophic changes occur in the vagina and the pH increases again to 7.0 ([Hay, 2018](#)). Changes in the vaginal pH value occur because glycogen derived from lactic acid is stored in the vaginal intermediate and superficial epithelial cells and lactobacilli proliferate, which causes enzymatic damage to the glycogen cells and produces more and more lactic acid and hydrogen peroxide.

The most common symptoms in women who experience vaginal discharge due to *C. albicans* are itching (pruritus), pain, and burning in the vagina, as shown in [Table 1](#) ([Wang et al., 2018](#)). Vaginal discharge caused by infection is usually accompanied by itching in the vagina and around the outer vaginal lips, can be spread and cause inflammation of the urinary tract, causing pain when the sufferer urinates. This inflammation can be caused by reactive oxygen species (ROS) which affect proinflammatory cytokines such as TNF- $\alpha$  and interleukin-6 (IL-6). With the activation of proinflammatory cytokines in the mitochondria, endoplasmic reticulum, and NO synthase, it will cause a hexose monophosphate shunt (HMP shunt) which converts NADP<sup>+</sup> into nicotinamide adenine dinucleotide phosphate (NADPH) via NADPH oxidase, resulting in a change in superoxide radicals into hydrogen peroxide due to reaction catalyzed by superoxide dismutase (Cu/Zn SOD or Mn-SOD) and through the Fenton reaction is converted to hydroxyl radicals, and these changes lead to vaginal inflammation in *C. albicans* ([Morry et al., 2017](#)). Antioxidants are needed to inhibit the occurrence of free radicals from various enzymatic processes so as not to cause oxidative stress ([Farrokhnia, 2020](#)). Antioxidants can activate anti-inflammatory cytokines such as TGF  $\beta$ 1 thereby increasing the differentiation and proliferation of naive B cells, increasing plasmablast immunoglobulin A (IgG class switching), activating CXCR5 receptor 5 (CXCR5) and CCR10 receptor 10 (CCR10), thus causing migration from the germinal center to the vaginal mucosa, so that *C. albicans* colonies decrease, inflammation decreases and the incidence of recurrent vaginal discharge also decreases. By giving *P. crocatum* leaf boiled water to vaginal discharge, it can reduce symptoms of pain to an average of 0.17 and itching to 0.33, approaching the effect of treatment using ketoconazole with an average pain and itching score of 0.

Diagnosis of vaginal discharge due to *C. albicans* can be confirmed by microscopy or swab culture ([Rice et al., 2016](#)). The discharge of the vagina was collected using the sterile vaginal speculum and swab by standard microbiological procedures ([Charan & Biswas, 2013](#)). Wet mount microscopy, easy to use diagnostic technique, is better and simple than the Gram stain to detect vaginal lactobacilli, with 50-80% sensitivity, showing distinct fungal spores or pseudo mycelia. The diagnostic test show pseudohyphae (40-60% cases), and blastopores (with KOH addition to the wet smear lyses epithelial cells to make apparent hyphae), and the criteria for diagnosis of vaginal candidosis using European (IUSTI/WHO) guideline on the management of vaginal discharge, 2011 (evidence level III, grade B) that pseudohyphae or yeast on vaginal discharge wet preparation (40-60% sensitivity), diagnostic supportive of smell absence, the positive vaginal culture of *Candida* species, and pseudohyphae or yeast on vaginal discharge gram stain (up to 65% sensitivity).

## 5. Conclusion

The study using empirical study show that *P. crocatum* decoction formulation is effective in reducing vaginal yeast infections in women. These findings provide important data and information that *P. crocatum* herbal formulation is potential to be used as a raw material for new antifungal drugs and can be developed as a complementary treatment solution to treat pathological *leucorrhoea* due to *Candida albicans*.

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