COMBINATION OPTIMUM INHIBITOR POWER EICHHORNIA ETHANOL EXTRACT CRASSIPES AND PISTIA STRATIOTES TO CANDIDA ALBICANS ATCC 10231 IN VITRO

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ABSTRACT
Candida albicans (C.albicans) merupakan fungi patogen oportunistik penyebab kandidiasis. Tumbuhan Eichhornia crassipes dan Pistia stratiotes diketahui mengandung senyawa antifungi. Tujuan penelitian ini untuk meganalisis daya hambat optimum kombinasi ekstrak Eichhornia crassipes (EC) dan Pistia stratiotes (EP) terhadap C.albicans ATCC 1023. Penelitian ekspermental secara in vitro ini menggunakan metode difusi kertas cakram. Data zona hambat dari ekstrak EC dan PE 25%, 50%, 75%, dan 100% dan kontrol ketokonazol dinalisis menggunakan uji One-way Anava dan post-hoc Duncan. Hasil penelitian menunjukkan zona hambat dari perlakuan kombinasi EC+PS tergolong sedang (8,3±0,50mm) sampai sangat kuat (21,12±002mm). Kombinasi EC100%+PS100% menghasilkan efek yang setara dengan ketokonazol ($p<0.05$). Simpulannya, daya hambat optimum dari kombinasi ekstrak etanol E.crassipes dan P.stratiotes terhadap C.albicans adalah pada kadar 100% (1:1).

KEYWORDS
Candida albicans; Eichhornia crassipes; Pistia stratiotes; Ekstrak etanol; Uji difusi

INTRODUCTION

Candidiasis is still a disease problem that occurs in people in areas with tropical climates such as Indonesia, with abnormalities affecting the folds of the body, namely parts of the body that are moist and warm, such as the axillary folds, groin, and other skin folds[1,2]. It is estimated that as many as 75% of women can suffer, at least once in a lifetime. 3 The causative agent most often associated with candidiasis cases in Indonesia is the type of Candida albicans, which is around 56% [1]. Most (70%) *Candida albicans* are commensal fungi in the digestive and genitourinary tracts of humans and will only become pathogenic fungi under certain conditions (opportunistic pathogenic fungi)[3].

The most common form of candidiasis in Indonesian society is vulvovaginitis which is often characterized by abnormal vaginal discharge. It is estimated that around 90% of women in Indonesia have the potential to experience vaginal discharge caused by *Candida albicans*. Leucorrhoea can arise physiologically due to hormonal or pathological influences. Characteristics of abnormal (pathological) vaginal discharge include discoloration, an unpleasant odor, and associated symptoms such as vaginal itching, burning, or soreness[4].

Treatment of infections by *Candida albicans* generally uses antifungals belonging to the Azole class which work by inhibiting egoserol biosynthesis in *Candida albicans cell membranes*. However, several *Candida sp*. cause candidiasis to become resistant to the azole group. This is influenced by the chemical composition of the biofilm matrix formed by Candida sp[4]. On the other hand, in the current conditions, people are more interested in using traditional medicines because they are believed to have lower side effects and side effects compared to synthetic drugs[5]. This encourages researchers to find herbal/alternative drugs that are effective for treating an infectious disease, including candidiasis due to *Candida albicans.*

The potential of various herbal plants in Indonesia as antimicrobials (antibacterial, antifungal, antiviral) has been widely proven through various studies and has been developed as a source of antifungal agents[6,7]. Among the plants that have been studied to have pharmacological activity as

antimicrobials are plants that live in wetlands or waters, namely *Eichhornia crassipes* and *Pistia stratiotes* . Several previous studies have proven the antifungal activity of *Eichhornia crassipes* and *Pistia stratiotes extracts* against *Candida albicans*[8.9]. The minimum inhibition level for the treatment of methanol extract of *Eichhornia crassipes* against *Candida albicans* was 15.06 mg/ml with an inhibition zone of 14.9 mm; while the minimum inhibition level of the *Pistia stratiotes treatment was* 17.06 mg/ml which had an inhibition zone of 14.23mm[8]. In the 64 mg/ml *Eichhornia crassipes ethanol extract treatment, an* 8.5 mm inhibition zone was formed against *Candida albicans*, while the minimum inhibition level of the *Pistia stratiotes ethanol extract treatment* against *Candida albicans* was 300 mg/ml. The inhibitory activity produced by the extracts of *Eichhornia crassipes* and *Pistia stratiotes* against *Candida albicans* was still below the effect of the positive control treatment of ketoconazole on Candida albicans, which was 21 mm[10]. An effort to increase antimicrobial activity is to make herbal combination treatment preparations. Dulger's study (2014) stated that the combination of the ethanol extract of the leaves and roots of *Hypericum havvae* against *Candida albicans* also showed a greater antifungal effect compared to the single preparation[14]. Budiarti *et al* (2021) stated that the combined infusion of *Stenochlaena palustris* and *Sauropus androgynus* obtained results in the form of an inhibitory effect. better against *Candida albicans* than the single preparation treatment and the optimum inhibition zone size was obtained which was equivalent to the ketoconazole positive control[11].

RESEARCH METHOD

This research is study *true experimental* with design *posttest-only with control group design,* ie composed plan from treatment preparations combination extract *Eichhornia crassipes* and *Pistia stratiotes* (on concentrations of 25%, 50%, 75%, and 100%), ketoconazole (control positive) and DMSO 10% (control negative). Amount repeats for each group treatment is 3 times.

Research materials to be used is *Eichhornia crassipes* and *Pistia stratified* taken from the Anyar Continent River, Banjarmasin City. *Eichhornia* plant *crassipes* and *Pistia stratiotes* taken for this research material is whole from each plant viz start from leaf until roots, selected colored plants green still brown fresh and not withered. Isolate material *Candida albicans* ATCC 10231 is pure fungal isolates obtained from Faculty of Microbiology Laboratory Medical Lambung Mangkurat University. Research materials the other medium used in this study is Sabouraud's media *Dextrose Agar* (SDA), *Brain Heart Infusion* (BHI) media, *cotton swabs* sterile, 96% ethanol, 10% DMSO, paper filter, distilled water sterile, sterile paper disk, ketoconazole disk, and solution standard *McFarland* I (equivalent total bacteria 3 x 108 CFU/ml).

Determination and identification of the test plants studied carried out in the laboratory Biology Faculty of Mathematics and Natural Sciences, University of Lambung Mangkurat. Simplisia *Eichhornia Crassipes* and *Pistia stratiotes* used as much as 4 kilograms (kg). Extraction simplicity *Eichhornia crassipes* and *Pistia stratiotes* conducted with method maceration for 3 days use solvent 96% ethanol (1:10) Test carried out in *laminar air flow.* Isolate *Candida albicans* ATCC 10231 which has been standardized corresponding *Mc Farland* I (3 x 108 CFU/ml), with help sterile cotton swab smeared (swab) on *Sabouroud Dextrose Agar* (SDA) media. After used, the cotton stick is placed in a filled glass container solution antiseptic . Then each paper disk that has been containing treatment concentration extract *Eichhornia Crassipes* and *Pistia stratiotes* (25%, 50%, 75%, 100%) were placed on top of SDA media previously has containing test fungus isolates. Next all test media conducted incubation for 24 hours on temperature 37°C. Results growth of the test fungus on every treatment the be marked with exists magnitude zone inhibit (zone clear). Then on each zone diameter resistor conducted measurement using a ruler caliper (unit mm).

Data form zone resistor treatment extract combination *Eichhornia crassipes* and *Pistia stratiotes* against the test function as measured in units millimeters and evaluated in a manner statistics. Research data analyzed use test *One-way Anova* and test *Post-Hoc Duncan*, respectively on level 95% confidence.

RESULTS

This research tests inhibition activity combination extract *Eichhornia crassipes* and *Pistia*

stratiotes in inhibiting growth *Candida albicans* ATCC 10231, with parameters namely magnitude zone inhibit. Results measurement magnitude average zone resistor from each treatment on this research can see on Table 1.

Table 1. Average Zone Resistor Combination of EC and PS

No	Extract Combination		Inhibition Zone(mm)	
	EC (%)	*PS (%)*	Average	SD
1	25	25	8 , 63	0.50
2	25	50	9.58	0.45
3	25	75	11 , 90	0.14
4	25	100	13.85	0.31
5	50	25	10.46	0.49
6	50	50	11.40	0.23
7	50	75	13.36	0.64
8	50	100	15,37	0.07
9	75	25	12.58	0.31
10	75	50	13.75	0.61
11	75	75	15,49	0.47
12	75	100	17,19	0.46
13	100	25	15.75	0.08
14	100	50	17.59	0.44
15	100	75	19,43	0.57
16	100	100	21,12	0.42
17	Ketoconazole		21.03	0.02
18	DMSO 1%		0.00	0.00

Treatment ketoconazole give effect inhibition against Candida albicans with magnitude average zone highest inhibition, ie of 21.03±0.02mm. According to standard *Clinical and Laboratory Standards Institute* (CLSI) 2011, size zone resistor ketoconazole about 21-27 mm, classified has intermediate anti-fungal power to *Candida albicans*[12].

Analysis next to find out ratio equality or no equivalent Among the resulting effect treatment with control positive, then conducted test advanced use Test *Post Hoc Duncan*. Results test *Post Hoc Duncan* could seen on Table 2.

Table 2. *Duncan's Post-hoc* Test

Treatment	Extract Combination		Magnitude effect on *C . albicans*
	EC (%)	*PS (%)*	
1	25	25	8.63h *
2	25	50	9.58i *
3	25	75	11.89g *
4	25	100	13.85e *
5	50	25	10.46h *
6	50	50	11.43g *
7	50	75	13.63e *
8	50	100	15.36s *
9	75	25	12.57f *
10	75	50	13.75e*

11	75	75	15.49s *
12	75	100	17.19c *
13	100	25	15.74s *
14	100	50	17.58c *
15	100	75	19.43b *
16	100	100	21,12a
17	Ketoconazole		21.03a
18	DMSO 1%		0.000

* Different letter notifications indicate that there are significant differences in the effects of each treatment being compared

Duncan's Post Hoc Test (Table 5.5) obtained a value of p> 0.05, indicating a comparison of the effect (in the form of an inhibition zone) produced between the extract treatment groups and the comparison with the positive controls, some were significantly different, and some were not significantly different. The comparison of the effects produced between the EC+SP combinations which differed significantly was from the CE100% + PS100% treatment with the EC+SP combination treatment at variations below 100% concentration. The effect comparison, which was not significantly different, was from the CE100% + PS100% treatment with a positive control. The results that were not significantly different indicated that the effects of the two treatments being compared had equal inhibition in inhibiting the growth of *Candida albicans.*

DISCUSSIONS

Ketoconazole work on cytochrome P-450 enzyme for 14α -dimethylase by interacting with C-4. This antifungal inhibits dimethylated lanosterol into ergosterol which is an essential sterol for fungal membranes. The resulting inhibition is annoying function membrane and increase permeability membrane *C.albicans* fungal cells[13].

Treatment combination extract *Eichhornia crassipes* (EC) and *Pistia stratiotes* (PS) on various variation concentration produce average zone different inhibition to growth *Candida albicans*. In general, on this research found enhancement area zone the resulting inhibition treatment combination extract EC and PC against growth of Candida albicans, concomitantly with enhancement concentration combination given extract. Results this research is not different with study test combination extract *Mangifera Foetida* and *Ziziphus Mauritiana* to *C. albicans* on second test plant extracts contain relatively active compound (flavonoids, saponins and tannins) were obtained the more big extract so the more big zone inhibition formed[3,8]. These results prove that concentration extract could influence activity as antimicrobial. Effect form zone the resulting inhibition on *Candida albicans* influenced by concentration from combination EC and PS extracts. Increase in active ingredients in comparison straight with increasing concentration big. On concentration more extract high, then solubility content active substance in it the more too high, so activity antifungal will increase big. Otherwise, the lower concentration extract so the lesser content active substance in it so that activity antifungal will increase reduce[14].

Formed zone resistor on each treatment combination of EC and PS, because exists active ingredients or compound metabolites secondary which has antifungal power so that could hinder growth *C. albicans.* Contained compounds on *Eichhornia crassipes* that is phenols, flavonoids, tannins, alkaloids, terpenoids, sterols and glycosides[15,16,17]. *Pistia stratiotes* own content phytochemicals in the form of flavonoids, phenols, saponins, tannins, steroids and alkaloids activity as antifungal[18]. Flavonoid compounds have activity as an antifungal by way of work damage plasma membrane, induction dysfunction mitochondria, inhibition formation Wall cells, inhibition division cells, inhibition RNA and protein synthesis, and inhibition system mediated pumping efflux[19]. Mechanism work from alkaloids are with bother metabolic pumping and necrosis cell. Mechanism saponins work by increasing permeability membrane fungal cells.28 Mechanism work tannins that is with binds to ergosterol so damage membrane fungal cell[20].

Based on results analysis test *Duncan* 's Post Hoc then hypothesis on this research is accepted that is treatment combination extract *Eichhornia Crassipes* and *Pistia stratiotes* to *Candida albicans*

ATCC 10231, produces optimum inhibition. Optimum inhibition of EC100 %+PC100% treatment of *C. albicans*, able produce equivalent effect with ketoconazole. It can explained based on a number of study previously used extract combination and produce effect synergistic. The resulting optimum inhibition power treatment preparations combination, because exists effect synergistic from the active ingredients contained on second plant extracts[21]. Activity combination extract could characteristic hinder fungal growth (static) and kill growth of fungi (sida). This is because content metabolites secondary both plants can work synergies, namely flavonoids cause damage Wall cells, alkaloids that can bother peptidoglycan in fungal cells, damaging saponins membrane fungal cells, and tannins interfere with metabolic processes pathogen the until cell dead[22,23,24]. Results this research is in line with results study drag test on *C.albicans* is amazing treatment combination from two different test plant preparations (*Stenochlaena palustris* and *Sauropus androgynus*) produce more effect big than control suspected on the two test plants contain class compounds of flavonoids, saponins and tannins[11]. Study others produce effect synergistic that is extract leaf betel (*Piper betle*) and extract citrus peel (Citrus *reticulata*), on both of these plants contain the same active compound namely alkaloids, flavonoids, tannins, and saponins[25]. Based on results this research proved that treatment combination extract *Eichhornia crassipes* and *Pistia stratiotes* produce optimal inhibition against *Candida albicans* ATCC 10231.

CONCLUSION

Based on results this research, in general it is proven that the optimal inhibition power was obtained from treatment combination extract *Eichhornia crassipes* (EC) and *Pistia stratiotes* (PS) against *Candida albicans* ATCC 10231. Based on zone diameter the resulting inhibition treatment combination extract EC and PS on various variation concentration test to *C. albicans,* aimed medium inhibition category very strong (8.63±0.50mm -21.12±0.42mm). Based on results analysis statistic, obtained the optimum inhibition of treatment extract EC and PS against *C. albicans,* that is on treatment EC100%+PS100% combination and its effects equivalent with control ketoconazole ($p<0.05$).

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