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Minimum Inhibitory And Fungicidal Concentrations For Combined Extracts Of Papaya Leaves And Garlic

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Abstract

Introduction: Oral Candidiasis is a common fungal infection caused by *Candida albicans*-a predominant cause of fungal infections in the oral cavity. Prolonged use of antifungal agents for oral candidiasis can be associated with side effects, indicating a need to consider alternative therapies. Natural resources such as papaya leaves and garlic have the effect of antifungal against *Candida albicans* that may serve as alternative therapies. In vitro study of the minimum inhibition and fungicidal concentration of combined extracts, papaya leaves and garlic against *Candida albicans* need to be examined. **Purpose:** To determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of combined extracts of papaya leaves and garlic against *Candida albicans*. **Methods:** A laboratory experimental study with a post-test control group design was established. The minimum inhibitory concentration was tested using Kirby-bauer method and the minimum fungicidal concentration was tested with agar dilution method. The ratio of each extract in each concentration is 1: 1 which made into five concentrations; 5%, 10%, 15%, 20% and 25%. **Results:** This study showed the 5%, 10%, 15%, 20%, and 25% extracted of papaya leaves and garlic combination could not inhibit and eliminate *Candida albicans* colonies. **Conclusion:** No inhibitory and fungicidal effect of 5%, 10%, 15%, 20% and 25% papaya leaves and garlic extract combination. Higher concentration need to be considered.

Keywords : Antifungal agents; *Candida albicans*; fungicidal properties; garlic; minimum inhibitory concentration

Introduction

Oral candidiasis is a common infection of the oral mucosa caused by *Candida albicans*. The infection predisposed by local factors such as poor oral hygiene or systemic factors including immunocompromised states.[1] Oral candidiasis is treated with antifungals that are classified into several groups named polyenes, echinocandins, and azoles. Nystatin is a common topical therapy for oral candidiasis.[2] A long term use of nystatin could precipitate side effects.[2] Alternative medication was being studied using natural agents such as papaya and garlic.[3]

Medicinal plants have long been recognized for their therapeutic properties because of the bioactive compounds they contain. Among these plants, papaya leaves from *Carica papaya* and garlic from *Allium sativum* are widely known for their antimicrobial activities.[4,5] Papaya

leaves contain phytochemicals such as flavonoids, alkaloids, tannins, and saponins, which have been reported to possess antifungal and antibacterial properties.[4,6] Garlic, on the other hand, is rich in sulfur-containing compounds, particularly allicin, which exhibits strong antimicrobial effects against various microorganisms, including fungi.[7]

Several studies have independently demonstrated the inhibitory effects of papaya leaf extract and garlic extract against fungal species.[5] However, limited research has focused on the combined effects of these two natural extracts. Combining plant extracts may enhance antifungal activity through synergistic interactions between their active compounds, potentially resulting in improved effectiveness at lower concentrations.[8]

The determination of the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) is essential in evaluating the antifungal potency of plant extracts. Therefore, this study aims to determine the minimum inhibitory concentration and minimum fungicidal concentration of combined extracts of papaya leaves and garlic against *Candida albicans*. The findings of this research may contribute to the development of natural and cost-effective antifungal treatments derived from medicinal plants.

Methods

This research was approved by Ethical Committee for Medical and Health Ethical Research Committee of Medical Faculty Universitas Sriwijaya, Palembang-Indonesia (No. Protocol: 055-2021).

Papaya leaves and garlic extract

Green and fresh papaya leaves (*Carica papaya* L) aged between 5-6 months were taken from local farmer at Tanjung Lago, Banyuasin Regency-South Sumatera, Indonesia, whilst fresh and clean garlic (*Allium sativum*) cloves aged between 3.5 – 4 months were taken from local farmer at Kebun Balai Penelitian Rempah dan Obat (Balitro) in Lembang, Bandung-West Java, Indonesia.

The papaya stems were removed from the leaves, whereas garlic peels were removed also. Both papaya leaves and garlic were washed and cut into pieces. After that, papaya leaves and garlic were dried in the oven at 40ºC for 3 to 5 days. After it dried, the papaya leaves and garlic were ground separately.

The ground papaya leaves and garlic were filtered separately using 80 mesh sieve until their become simplicia. Each simplicia (350 gms of papaya leaves simplicia and 400 grams of garlic simplicia) was soaked into a 96% ethanol with 1:10 powder-liquid ratio and left for 3 days. The submersion at times stirred. After 3 days, each submersion was filtered with Whatmann filter paper #1 to get papaya leaves and garlic filtrate. Each filtrate then put into 50 ºC rotary evaporator to get 100% extract.

Synthesis of papaya leaves and garlic combination extract

Each papaya leaves and garlic extract was weight 1:1 in ratio and mixed to get 100% papaya leaves and garlic extract combination. The pure combination extract then diluted to get 5 different concentrations, named 5%, 10%, 15%, 20% and 25%.

Candida albicans

Candida albicans ATCC 60193 were taken from Balai Besar Laboratorium Kesehatan Palembang, Indonesia. The *Candida albicans* were diluted in normal saline and follow the McFarland Turbidity Standard of $1{,}5x10^8$ CFU/ml to make *Candida* suspension. *Sabouraud Dextrose Agar* (SDA) and *Sabouraud Dextrose Broth* (SDB) were prepared. *Candida albicans* ATCC 60193 were cultured in both SDA and SDB, incubated in 37ºC for 48 hours.

Minimum Fungicidal Concentration (MFC)

Sabouraud Dextrose Broth (SDB) were used in MFC with agar dilution method. *Candida albicans* ATCC 60193 were diluted in SDB and vortexed. After homogenized, SDB were incubated in 37ºC for 48 hours.

Seven sterile tubes were prepared, named negative control with aquadest (A); positive control with nystatin (B); 5µL 100% combination of extract of papaya leaves and garlic with 95 µL SDB (C); 10µL 100% combination of extract of papaya leaves and garlic with 90 µL SDB (D); 15µL 100% combination of extract of papaya leaves and garlic with 85 µL SDB (E); 20 µL 100% combination of extract of papaya leaves and garlic with 80 µL SDB (F); 25µL 100% combination of extract of papaya leaves and garlic with 75 µL SDB (G). 10 µL *Candida albicans* suspension were added to each tube and incubated in 37ºC for 24 hours.

After 24 hours, *Candida albicans* from each tube were taken, streaked onto SDA medium and incubated in 37ºC for 24 hours (Figure 1). *Candida albicans* colonies were counted using colony counter.

Minimum Inhibitory Concentration (MIC)

Candida albicans, taken from Candida suspension, were streaked into Sabouraud Dextrose Agar (SDA) petri dishes and incubated in 37ºC for 48 hours. Kirby-bauer method or paper disk diffusion method was used to analyse the MIC. Paper disks were prepared and each of them were dipped into a 5%, 10%, 15%, 20% and 25% combination of extract of papaya leaves and garlic. Paper disks as a negative control with aquadest and positive control with nystatin were also prepared. Group of paper disks then put into SDA petri dish and incubated in 37ºC for 24 hours. Four times repetitions were done in this study.

Results

From our study, all tested concentrations failed to eliminated *Candida albicans* colonies. Minimum fungicidal concentration result was summarized in Table 1. The combinations extraction also did not exhibit *Candida albicans* growth in all the tested concentrations, as summarized in Table 2.

Table 1. Minimum fungicidal concentration result

Repetition	Total Plate Count (CFU/ml)						
	Negative control (A)*	Positive control (B)**	Combined extract of papaya leaves and garlic Concentration				
			5%	10%	15%	20%	25%
1	TNTC	0	TNTC	TNTC	TNTC	TNTC	TNTC
2	TNTC	0	TNTC	TNTC	TNTC	TNTC	TNTC
3	TNTC	0	TNTC	TNTC	TNTC	TNTC	TNTC
4	TNTC	0	TNTC	TNTC	TNTC	TNTC	TNTC

*Aquadest, **Nystatin 1:100.000 IU
[!]TNTC: Too Numerous To Count (>300CFU/ml)

Table 2. Minimum inhibitory test results

Repetition	Inhibitory Diameter (mm)						
	Negative control (A)*	Positive control (B)**	Combined extract of papaya leaves and garlic Concentration				
			5%	**10%**	**15%**	**20%**	**25%**
1	0	11.2	0	0	0	0	0
2	0	11.2	0	0	0	0	0
3	0	9.2	0	0	0	0	0
4	0	9.2	0	0	0	0	0
Total	**0**	**40.8**	**0**	**0**	**0**	**0**	**0**
Mean	**0**	**10.2**	**0**	**0**	**0**	**0**	**0**

*Aquadest, **Nystatin 1:100.000 IU

Discussion

From our study above, 5%, 10%, 15%, 20%, and 25% extracted of papaya leaves and garlic combination could not inhibit and eliminate *Candida albicans* colonies. Only the positive control can inhibit and eliminate *Candida albicans.* Compare to the previous study that has found direct crude extracts of papaya leaves and garlic combination could inhibit and eliminate Grams negative and positive bacteria strains, it needs further investigation whether this combination have the antifungal effect.[3] The tested microorganism may possess intrinsic resistance mechanisms that reduce its susceptibility to plant-derived antimicrobial agents.[9] Variations in cell wall structure, biofilm-forming capacity, efflux pump activity, and enzymatic degradation mechanisms can influence microbial sensitivity to natural products.[4] Consequently, higher extract concentrations or alternative extraction techniques may be required to achieve inhibitory effects. The colonies were also overcrowded and need to be diluted before undergoing Total Plate Count.

The validity of the experimental procedure is supported by the positive control, which consistently produced inhibition zones ranging from 9.2 to 11.2 mm, while the negative control showed no inhibition. This confirms that the test organism was capable of responding to antimicrobial agents and that the absence of inhibition in the treatment groups was attributable to the limited efficacy of the combined extract rather than methodological failure.

The differences observed in the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the combined papaya leaf and garlic extracts may be attributed to variations in the types and amounts of bioactive compounds present in the extracts.[14,15] Plant extracts contain numerous phytochemical constituents whose antimicrobial

activities may differ depending on the extraction process, concentration, solvent used, and interaction between compounds.[9] In this study, the combined extracts may have exhibited varying antifungal effects due to synergistic, additive, or antagonistic interactions between the active substances found in papaya leaves and garlic.

Papaya leaves contain compounds such as flavonoids, tannins, alkaloids, and saponins, while garlic is known to contain sulfur-containing compounds, particularly allicin, which is responsible for its strong antimicrobial activity.[4] Differences in the stability and concentration of these compounds during extraction may influence the effectiveness of the extracts against fungal organisms.[10] In addition, fungal species may respond differently to the extracts because of variations in their cell wall composition, resistance mechanisms, and susceptibility to phytochemicals.

Another possible reason for the observed differences is the extraction method itself. Factors such as extraction time, temperature, solvent polarity, and plant maturity can significantly affect the yield and potency of active compounds. Incomplete extraction or degradation of phytochemicals during preparation may reduce antifungal activity, leading to variations in MIC and MFC values.[10]

Therefore, it is recommended that future studies include phytochemical screening tests to identify and analyze the active components present in the combined extracts.[3] Phytochemical analysis would help determine which compounds are primarily responsible for the antifungal activity and whether synergistic interactions occur between the constituents of papaya leaves and garlic. Furthermore, quantitative analysis of these phytochemicals could provide a better understanding of the relationship between compound concentration and antifungal effectiveness.[6] Such investigations may contribute to the development of more standardized and effective plant-based antifungal agents.[11]

Combining 2 extracts even though each extraction itself has antifungal effect, not always have the synergistic effect but also could have antagonist effect.[12,13] In some cases, the interaction between bioactive compounds may produce an antagonistic effect, where the combined activity becomes less effective than the activity of each extract alone.[14] Although both plants have individually demonstrated antimicrobial properties in previous studies, combining different extracts may alter the activity of their bioactive compounds. Interactions among phytochemicals can reduce the availability, stability, or biological activity of active ingredients, resulting in lower antimicrobial effectiveness than expected. This antagonistic

interaction may occur because certain phytochemicals can interfere with the mechanism of action, stability, or absorption of other active compounds.[14] As a result, the antifungal activity of the combined extracts may decrease, leading to higher MIC and MFC values.[12] Therefore, further phytochemical screening and analysis are recommended to identify the active components and evaluate the nature of the interaction between the compounds present in papaya leaves and garlic extracts. Further investigation needs to be done to develop synergistic effects from extract combinations.

Conclusion

The tested concentration of 5%, 10%, 15%, 20%, and 25% extracted of papaya leaves and garlic combination could not inhibit and eliminate *Candida albicans* colonies.

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