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ORIGINAL ARTICLE

Comparison of the effectiveness of robusta coffee bean extract and arabica coffee bean extract on the growth of *Candida albicans*

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ABSTRACT

Oral thrush, a fungal infection often associated with inadequate oral hygiene, necessitates treatment with antimicrobial agents. Extracts from *Coffea canephora* (Robusta) and *Coffea arabica* L. (Arabica) coffee beans contain bioactive compounds that demonstrate inhibitory activity against *Candida albicans*. This study investigated the comparative inhibitory efficacy of Robusta and Arabica coffee bean extracts on *Candida albicans* growth. A randomised, post-test only control group design was employed in this experimental laboratory study. Twelve treatment groups were established, and the disc diffusion method was used to assess antibacterial activity. Inhibition diameters were measured using a vernier caliper. Statistical analysis was performed using the Kruskal-Wallis and Mann-Whitney tests. The mean inhibition diameters for Robusta coffee bean extract (*Coffea canephora*) were as follows: 9.28 ± 2.585 mm at 12.5% concentration, 12.35 ± 0.050 mm at 25%, 14.55 ± 0.050 mm at 50%, 16.50 ± 0.477 mm at 75%, and 18.55 ± 0.477 mm at 100%. For Arabica coffee bean extract (*Coffea arabica* L.), the mean inhibition diameters against *Candida albicans* were 8.10 ± 1.117 mm at 25% concentration, 10.91 ± 1.188 mm at 50%, 15.61 ± 2.115 mm at 75%, and 16.71 ± 1.980 mm at 100%. The positive control exhibited a mean inhibition diameter of 15.85 ± 0.180 mm. No inhibition was observed for Arabica coffee bean extract at 12.5% concentration or the negative control. The results indicate that Robusta coffee bean extract demonstrated a significantly greater inhibitory effect on *Candida albicans* growth compared to Arabica coffee bean extract. Liquid chromatography-mass spectrometry (LC-MS) analysis of both Robusta and Arabica coffee bean extracts revealed the presence of caffeine and chlorogenic acid, which likely contribute to their observed inhibitory activity.

Keywords: antibacterial, arabica coffee beans, *Candida albicans,* inhibition, robusta coffee beans

Introduction

Oral and dental diseases represent a significant global health challenge. A substantial portion of the population worldwide continues to suffer from oral health issues. According to the WHO's Global Oral Health Status Report, oral thrush, a fungal infection, affects approximately 5 to 10 percent of adults globally.[1] A lesion indicates structural or functional changes in body tissues due to disease. Oral cavity lesions are characterised by alterations in colour and size, as well as the loss of clinical features on the oral mucosa surface. These lesions commonly disrupt daily life, impacting mastication, swallowing, and speech. Soft tissue lesions in the oral cavity are caused by infections from bacteria, viruses, and fungi, systemic diseases,

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nutritional deficiencies, trauma or local irritation, and habits such as excessive tobacco, betel quid, and alcohol consumption.[2,3]

Candida albicans is a commensal organism and a component of the normal human microbiota, contributing to the microbial balance within the intestinal tract, skin, and genitourinary tract. The human oral cavity harbours a diverse microbiota, encompassing Eubacteria, Archaea, Protozoa, Mycoplasma, and Fungi. Commensal organisms residing within the oral cavity include *Candida albicans*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Streptococcus agalactiae*.[4] Macroscopically, *Candida albicans* appears round, oval, or round-oval.[5] Predisposing factors that promote the growth of *Candida albicans* include long-term antibiotic use, uncontrolled diabetes, continuous denture use, deficiencies in iron, vitamin B12, and phosphate, and inadequate immunosuppression.[6] Effective maintenance of oral cavity health is facilitated by oral hygiene products incorporating antimicrobial compounds, essential oils, and phytochemicals, including chlorhexidine, polyphenols, and flavonoids. These formulations contribute to the control of *Candida* levels within the oral cavity, thereby mitigating its toxicity.[7]

Oral candidiasis, commonly known as oral thrush, is an infection of the oral cavity caused by the fungus *Candida albicans*. It is characterised by the presence of soft, white plaques, reminiscent of milk curds, which can be removed, revealing underlying erythema.[8] Oral candidiasis can occur across all age groups. However, recent years have shown an increased frequency of infection in the elderly.[9] Oral candidiasis is prevalent in individuals with HIV and diabetes mellitus, those using unclean dentures, those undergoing radiotherapy, and those using medications such as corticosteroids, antiepileptic drugs, allopurinol, sulfonamides, and long-term broad-spectrum antibiotics, as well as in those with poor oral hygiene.[10] Chlorhexidine gluconate is a commonly used antifungal medication for treating various fungal infections. Chlorhexidine gluconate at concentrations of 0.12% to 2% is highly effective in dentistry, including oral surgery, periodontics, and general dentistry.[11] Natural substances are also believed to treat various diseases with minimal side effects, including coffee in this context.

Indonesia, with its tropical climate, is one of the world's leading coffee producers. Popular coffee varieties in Indonesia include Robusta (*Coffea canephora*) and Arabica (*Coffea arabica L.*). Robusta coffee thrives in lowland areas, while Arabica coffee grows well in tropical highlands, producing high-quality beans.[12] Ethanol extracts of Robusta and Arabica coffee beans contain caffeine, flavonoids, alkaloids, saponins, tannins, terpenoids, phenols, chlorogenic acid, and trigonelline, all of which are effective in inhibiting *Candida albicans* growth. This study aims to determine the difference in inhibitory power and optimal concentration of Robusta coffee bean extract and Arabica coffee bean extract on the growth of *Candida albicans* fungus.

Method

This research utilised a randomised post-test only control group design within a laboratory experiment. The extraction and dilution of Robusta and Arabica coffee bean extracts, across a range of concentrations, were performed at the Phytochemistry Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara. Antifungal activity assays were conducted at the Microbiology Laboratory, Faculty of Medicine, Universitas Sumatera Utara. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of the coffee bean extracts was carried out at the Forensic Laboratory Centre (Puslabfor), Indonesian National Police Criminal Investigation Agency (Bareskrim Polri), West Java. The study spanned the period from August 2024 to October 2024.

Pure cultures of *Candida albicans*, sourced from the Microbiology Laboratory, Faculty of Medicine, Universitas Sumatera Utara, constituted the research sample. The study incorporated twelve distinct groups: Robusta coffee bean extract at concentrations of 12.5%, 25%, 50%, 75%, and 100%; Arabica coffee bean extract at equivalent concentrations; dimethyl sulfoxide (DMSO) as a negative control; and 2% chlorhexidine digluconate as a positive control. Utilizing Federer's formula, the sample size for each group was determined to be two replicates. Consequently, the total sample size for this investigation was 24.

The equipment used in this research included incubators, ovens, analytical balances, autoclaves, spectrophotometers, hot plates, vortex mixers, callipers, centrifuges, pH meters, spatulas, agar media, distilled water, spirit lamps, alcohol, tissue paper, DMSO, vials, plastic wrap, bacterial isolates, and filter paper discs. The materials used were green Robusta and Arabica coffee beans, *Candida albicans*, 70% ethanol, spirit, DMSO (dimethyl sulfoxide), PDA (Potato Dextrose Agar) media, and 2% chlorhexidine gluconate.

Green Robusta and Arabica coffee beans were collected via purposive sampling from Suka Meriah (Siosar) Village, Karo Regency, North Sumatra. The materials were cleaned with running water, drained, weighed, and then dried in a drying cabinet until completely dry. Subsequently, they were re-weighed, placed in sealed containers, and stored. Before the initiation of the research, all equipment underwent sterilisation. Glassware was sterilised via dry heat in an oven at 170°C for approximately two hours, needles and forceps were subjected to flame sterilisation, and media were autoclaved at 121°C for 15 minutes.[13]

The research began with the preparation of the extracts. Robusta and Arabica coffee beans were roasted at 200°C for 20 minutes. After roasting, the coffee beans were cooled, weighed (225g), and ground using a blender. The ground coffee beans were transferred to a container, and 2.5 litres of 70% ethanol (ratio 1:10) were added gradually. The mixture was agitated for the initial six hours, followed by a 24-hour standing period with intermittent agitation. Subsequently, the mixture was filtered through filter paper, and the resulting filtrate (macerate I) was collected. The residual material underwent a second extraction using 2.5 litres of 70% ethanol, at a solid-to-solvent ratio of 1:10, yielding macerate II. The extraction was performed using maceration (FHI 2017). Both macerates were combined and evaporated using a rotary evaporator at 40°C to obtain a viscous extract. Various concentrations of the extract were prepared: 100%, 75%, 50%, 25%, and 12.5%, each at 2g. The required amount of extract for each concentration was weighed and diluted with DMSO to a final volume of 2g. Viscous extracts of Robusta and Arabica coffee beans (1.25g, 2.5g, 3.75g, and 5g) were diluted using 10% dimethyl sulfoxide (DMSO) solution to a final volume of 5ml. Each solution was then transferred to labelled vials.

A potato dextrose agar (PDA) solution was prepared by dissolving 9.75 g of PDA in 250 mL of distilled water within an Erlenmeyer flask. The mixture was heated until boiling to ensure complete dissolution of the medium. After cooling, it was sterilised in an autoclave at 121°C and 15 psi for 15 minutes. A single colony of pure *Candida albicans* culture was collected using a sterile loop and inoculated onto a slanted PDA medium by streaking. The inoculated medium was then incubated at 121°C for 48 hours.[14]

A 0.5 McFarland turbidity standard was generated by combining 9.95 mL of 1% sulfuric acid (H_2SO_4) with 0.05 mL of 1% barium chloride ($BaCl_2$). The turbidity of the test bacterial suspension was standardised using a turbidity standard.[15] Fungal colonies were aseptically collected from the stock culture with a sterile loop and suspended in 5 ml of 0.9% NaCl solution. The fungal suspension was adjusted to a turbidity equivalent to a 0.5 McFarland standard, corresponding to 1.5×10^8 colony-forming units per millilitre (CFU/ml).[16] Twenty millilitres of potato dextrose agar (PDA) were dispensed into Petri dishes and allowed to solidify. Subsequently, one millilitre of *Candida albicans* suspension was evenly spread across the agar surface using a sterile cotton swab. Sterile filter paper discs, 6 mm in diameter, pre-soaked for 10–15 minutes in either a positive control, a negative control, or varying concentrations of Robusta and Arabica coffee bean extracts, were then placed onto each Petri dish using sterile forceps. All plates were incubated at 37°C for 24 hours.[17]

The data were tested for normality using the Shapiro-Wilk test. Subsequent data analysis was performed using the Kruskal-Wallis and Mann-Whitney statistical tests. All data were processed using SPSS software.

Results

The research findings indicate that the mean inhibition zone diameters of *Candida albicans* for Robusta coffee bean extract at concentrations of 12.5%, 25%, 50%, 75%, and 100% were 9.28±2.585, 12.35±0.050, 14.55±0.050, 16.50±0.477, and 18.55±0.477, respectively. For Arabica coffee bean extract, the mean inhibition zone diameters were 8.10±1.117, 10.91±1.188, 15.61±2.115, and 16.71±1.980 at concentrations of 25%, 50%, 75%, and 100%, respectively. The positive control exhibited a mean inhibition zone diameter of 15.85±0.180, whereas no inhibition of *Candida albicans* was observed for Arabica coffee bean extract at a concentration of 12.5% or in the negative control.

The tests for normality and homogeneity indicated that the data violated the assumptions of normality and homogeneity of variance ($p<0.05$). Therefore, subsequent analyses were performed using the non-parametric Kruskal-Wallis and Mann-Whitney tests.

Table 1. Mean inhibition diameter of Robusta coffee and Arabica coffee bean extracts against the growth of *Candida albicans*

Group	Concentration	Inhibition diameter (mm)			
		Replicates			$\bar{x} \pm SD$
		1	2	3	
Robusta coffee seed extract	12,5%	6,3	10,85	10,7	9,28±2,585
	25%	12,3	12,35	12,4	12,35±0,050
	50%	14,55	14,5	14,6	14,55±0,050
	75%	16,45	17,0	16,05	16,50±0,477
	100%	19,0	18,05	16,05	18,55±0,477
Arabica coffee seed extract	12,5%	0	0	0	0
	25%	7,2	9,35	7,75	8,10±1,117
	50%	9,73	12,1	10,9	10,91±1,188
	75%	13,18	16,65	17,0	15,61±2,115
	100%	14,48	18,25	17,4	16,71±1,980
Negative control (DMSO)		0	0	0	0
Positive control (*Chlorhexidine digluconate* 2%)		15,65	15,9	16,0	15,85±0,180

The Kruskal-Wallis test revealed a statistically significant difference in the inhibitory effect of Robusta and Arabica coffee bean extracts on *Candida albicans* growth ($p = 0.001$, $p < 0.05$). Consequently, a comparative difference in efficacy against *Candida albicans* growth was observed between the two coffee bean varieties.

Table 2. Kruskal-Wallis test

Group	Concentration	$\bar{x} \pm \boldsymbol{SD}$	*p value*
Robusta coffee bean extract	12,5%	9,28±2,585	0,001*
	25%	12,35±0,050	
	50%	14,55±0,050	
	75%	16,50±0,477	
	100%	18,55±0,477	
Arabica coffee bean extract	12,5%	0	
	25%	8,10±1,117	
	50%	10,91±1,188	
	75%	15,61±2,115	
	100%	16,71±1,980	
Negative control (DMSO)		0	
Positive control (*Chlorhexidine digluconate* 2%)		15,85±0,180	

**Significant*

The Mann-Whitney U test results indicate a significant difference in inhibitory activity against *Candida albicans* between Robusta coffee seed extract at all concentrations and Arabica coffee seed extract at 12.5%, 25%, and 50% concentrations compared to the positive control ($p<0.05$). Furthermore, a significant difference was observed between Robusta coffee seed extract at all concentrations and Arabica coffee seed extract at 25%, 50%, 75%, and 100% concentrations compared to the negative control ($p<0.05$). Table 3 also reveals no significant difference in inhibitory activity against *Candida albicans* between Robusta coffee seed extract at 12.5% concentration and the negative control, nor between Arabica coffee seed extract at 75% and 100% concentrations and the positive control ($p>0.05$).

Table 3. Mann-Whitney test

Group		*p value*
Robusta coffee seed extract 12,5%	Robusta coffee seed extract 25%	0,050*
	Robusta coffee seed extract 50%	0,050*
	Robusta coffee seed extract 75%	0,050*
	Robusta coffee seed extract 100%	0,050*
	Arabica coffee seed extract 12,5%	0,037*
	Arabica coffee seed extract 25%	0,513
	Arabica coffee seed extract 50%	0,275
	Arabica coffee seed extract 75%	0,050*
	Arabica coffee seed extract 100%	0,050*
	K-	0,037*
	K+	0,050*

Robusta coffee seed extract 25%	Robusta coffee seed extract 50%	0,050*
	Robusta coffee seed extract 75%	0,050*
	Robusta coffee seed extract 100%	0,050*
	Arabica coffee seed extract 12,5%	0,037*
	Arabica coffee seed extract 25%	0,050*
	Arabica coffee seed extract 50%	0,050*
	Arabica coffee seed extract 75%	0,050*
	Arabica coffee seed extract 100%	0,050*
	K-	0,037*
	K+	0,050*
Robusta coffee seed extract 50%	Robusta coffee seed extract 75%	0,050*
	Robusta coffee seed extract 100%	0,050*
	Arabica coffee seed extract 12,5%	0,037*
	Arabica coffee seed extract 25%	0,050*
	Arabica coffee seed extract 50%	0,050*
	Arabica coffee seed extract 75%	0,513
	Arabica coffee seed extract 100%	0,513
	K-	0,037*
	K+	0,050*
Robusta coffee seed extract 75%	Robusta coffee seed extract 100%	0,050*
	Arabica coffee seed extract 12,5%	0,037*
	Arabica coffee seed extract 25%	0,050*
	Arabica coffee seed extract 50%	0,050*
	Arabica coffee seed extract 75%	1,000
	Arabica coffee seed extract 100%	0,513
	K-	0,037*
	K+	0,513
Robusta coffee seed extract 100%	Arabica coffee seed extract 12,5%	0,037*
	Arabica coffee seed extract 25%	0,050*
	Arabica coffee seed extract 50%	0,050*
	Arabica coffee seed extract 75%	0,050*
	Arabica coffee seed extract 100%	0,127
	K-	0,037*
	K+	0,050*
Arabica coffee seed extract 12,5%	Arabica coffee seed extract 25%	0,037*
	Arabica coffee seed extract 50%	0,037*
	Arabica coffee seed extract 75%	0,037*
	Arabica coffee seed extract 100%	0,037*
	K-	1,000
	K+	0,037*
Arabica coffee seed extract 25%	Arabica coffee seed extract 50%	0,050*
	Arabica coffee seed extract 75%	0,050*
	Arabica coffee seed extract 100%	0,050*
	K-	0,037*
	K+	0,050*
Arabica coffee seed extract 50%	Arabica coffee seed extract 75%	0,050*
	Arabica coffee seed extract 100%	0,050*
	K-	0,037*
	K+	0,050*
Arabica coffee seed extract 75%	Arabica coffee seed extract 100%	0,275
	K-	0,037*
	K+	0,513
Arabica coffee seed extract 100%	K-	0,037*
	K+	0,513
K-	K+	0,037*

Discussion

The antifungal activity of Robusta coffee bean extract and Arabica coffee bean extract, at concentrations of 12.5%, 25%, 50%, 75%, and 100%, against the fungus *Candida albicans* was observed over a 24-hour period. The parameter measured was the diameter of the inhibition zone formed around the edge of the paper disc. The inhibition zone was measured by determining the diameter of the clear zone using a vernier calliper.

The results of the study showed that the mean inhibition zone diameters for Robusta coffee bean extract (Coffea canephora) at concentrations of 12.5%, 25%, 50%, 75%, and 100% against *Candida albicans* were 9.28±2.585 mm, 12.35±0.050 mm, 14.55±0.050 mm, 16.50±0.477 mm, and 18.55±0.477 mm, respectively. Similarly, Arabica coffee bean extract (Coffea arabica L.) at concentrations of 25%, 50%, 75%, and 100% exhibited mean inhibition zone diameters against *Candida albicans* of 8.10±1.117 mm, 10.91±1.188 mm, 15.61±2.115 mm, and 16.71±1.980 mm, respectively. However, no inhibition was observed at a concentration of 12.5%.

According to Martsiningsih et al., one analytical factor that can influence inhibitory power is the concentration of the antimicrobial agent.[18] The larger the extract concentration, the wider the diameter of the inhibition zone, and thus the greater the inhibitory power.[19,20] The inhibitory power of extracts can be categorised as: weak (<5 mm), moderate (5 - <10 mm), strong (10 – 20 mm), and very strong (>20 mm).[20]

Based on the study results, the inhibitory power of Robusta coffee bean extract at concentrations of 25%, 50%, 75%, and 100% fell within the strong category, while the 12.5% concentration was classified as moderate. Arabica coffee bean extract at concentrations of 50%, 75%, and 100% exhibited strong inhibitory power, whereas the 25% concentration showed moderate inhibitory power. Previous research has indicated that Robusta coffee bean extract demonstrates the greatest inhibitory power against *S. epidermidis* and *S. aureus* bacteria starting at a concentration of 50%. In contrast, against *P. aeruginosa* bacteria, Robusta coffee bean extract exhibits the greatest inhibitory power at a concentration of 75%.[21]

The Kruskal-Wallis statistical test in this study revealed a significant difference in the effectiveness of Robusta coffee bean extract and Arabica coffee bean extract against the growth of *Candida albicans* ($p=0.001$; $p<0.05$). The antifungal and antibacterial capabilities of coffee bean extract have also been reported in previous research. Ranasatri et al.[19] observed that Robusta coffee bean extract exhibited antibacterial activity against *S. epidermidis* at concentrations ranging from 3.125% to 25%. Rakatama et al.[22] also noted that an ethanolic extract of Arabica coffee beans inhibited the growth of *Candida albicans* isolates from patients.

The antifungal effectiveness of Robusta coffee bean extract was found to be greater in inhibiting the growth of *Candida albicans* compared to Arabica coffee bean extract, with the optimal concentration being 100%. This is attributed to the higher caffeine and chlorogenic acid content in Robusta coffee bean extract compared to Arabica coffee bean extract. Specifically, the caffeine content was 68.85% in Robusta extract and 63.40% in Arabica extract, while the chlorogenic acid content was 7.46% in Robusta extract and 4.35% in Arabica extract.

Caffeine is the primary active compound found in coffee beans.[21] Caffeine is an alkaloid compound that exists as white crystals and is capable of inhibiting fungal growth. In addition to caffeine, coffee also contains antioxidant compounds from the phenol group, including chlorogenic acid. Chlorogenic acid is one of the main phenol groups found in high concentrations in coffee and possesses antifungal activity.[23] Each active compound exhibits a different antifungal mechanism. Tannins inhibit fungal growth by inactivating microbial adhesins (molecules that adhere to the host) on the cell surface and enzymes, as well as disrupting protein transport in the inner cell layer. Tannins also target cell wall polypeptides, leading to damage to the *Candida* cell wall.[24] Flavonoids disrupt fungal cells by damaging their cell walls due to differences in polarity between the lipids that make up DNA and the alcohol groups in flavonoid compounds. Chlorogenic acid, a short-chain fatty acid, enhances bacterial growth inhibition by more effectively penetrating fungal cell walls compared to long-chain fatty acids.[19]

The Mann-Whitney test indicated that 100% Robusta coffee bean extract exhibited greater effectiveness compared to the positive control against the growth of *Candida albicans* ($p>0.05$). In this study, the mean inhibition zone diameter for 2% chlorhexidine digluconate was 15.85±0.180 mm, while for 100% Robusta coffee bean extract, it was 18.55±0.477 mm. Chlorhexidine is a broad-spectrum antimicrobial antiseptic mouthwash. Its antifungal capability stems from its ability to coagulate nucleoproteins and alter fungal cell walls, resulting in the release of cytoplasmic components into the plasmalemma and subsequent cell damage. A key advantage of chlorhexidine is its ability to adhere to oral cavity tissues, providing a prolonged effect lasting 7-12 hours. This accounts for its high effectiveness in reducing *Candida albicans* counts.[25]

Almost all concentrations of Robusta and Arabica coffee bean extracts used in this study were effective in inhibiting the growth of *Candida albicans*, with the exception of 12.5% Arabica coffee bean extract, which showed no significant difference from the negative control in the Mann-Whitney test ($p>0.05$). The negative control used in this study was dimethyl sulfoxide (DMSO), as it was the same solvent used for diluting the

test substances. This was done to ensure that the solvent used for dilution did not affect the antifungal test results or that there was no antifungal activity for the compound being tested. The inhibition zone of DMSO on *Candida albicans* growth was 0 (zero), confirming that DMSO did not influence the antifungal test results.[26]

Differences in inhibitory power can also be influenced by the type of test fungus used, as microbes themselves develop resistance to sustain life. Additionally, microorganisms have varying preferences for specific substances. Besides the influence of fungal or bacterial species, differences in inhibitory power can also be attributed to varying sample concentrations. The active compounds present in Robusta and Arabica coffee bean extracts, as mentioned earlier, are likely responsible for the inhibitory power or antifungal activity against *Candida albicans* growth observed in this study. In general, the mechanism of action of antifungal compounds in coffee beans involves disrupting the outer membrane of the cell wall, leading to increased membrane permeability, cytoplasmic disruption, and subsequent fungal lysis.

Conclusion

The research findings demonstrate a statistically significant difference in the efficacy of Robusta coffee bean extract and Arabica coffee bean extract in inhibiting the growth of *Candida albicans*. Specifically, Robusta coffee bean extract exhibited a greater antifungal effect compared to Arabica coffee bean extract. This enhanced inhibitory capacity is attributed to the higher concentrations of caffeine and chlorogenic acid identified within the Robusta coffee bean extract through LC-LC-MS analysis.

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