BIOTIK
Jurnal Ilmiah Biologi Teknologi dan Kependidikan
P-ISSN: 2337-9812, E-ISSN: 2549-1768
Vol.14, No.2, 2026. Page 202-216

Comparative Evaluation of Kratok Bean (*Phaseolus lunatus* L.) Flour and Boiled Extract as Alternative Media for *Candida albicans* Growth

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Accepted : July 1, 2026
Published: September 6, 2026

Abstrak: *Candida albicans* adalah jamur patogen oportunistik penyebab utama kandidiasis, memerlukan media kultur selektif untuk identifikasi di laboratorium sebagai pengganti media sintesis yang harga relatif mahal seperti *Potato Dextrose Agar* (PDA). Penelitian ini menguji potensi biji kacang kratok (*Phaseolus lunatus* L.) dalam bentuk tepung (FBKA) dan air rebusan (BEKA) sebagai media alternatif pertumbuhan jamur. Penelitian ini menggunakan desain eksperimental laboratorium dengan membandingkan karakteristik makroskopis dan mikroskopis antara media PDA sebagai kontrol dan media alternatif kacang kratok setelah inkubasi 3 hari pada suhu 37°C dengan tiga kali pengulangan (n=3). Hasil penelitian menunjukkan kedua media alternatif dapat mendukung pertumbuhan *C. albicans* dengan ciri koloni berwarna putih kekuningan, berbau khas ragi, dan berbentuk cembung pada hari ke-3. Pengamatan mikroskopis melalui uji germ tube pada kedua media alternatif menunjukkan terbentuknya *pseudohifa*. Uji Kruskal-Wallis menunjukkan perbedaan signifikan ukuran koloni antar ketiga media pada hari ke-3 (H=7,200; p=0,027). Dan analisis post-hoc Dunn test menunjukkan bahwa BEKA menghasilkan koloni berdiameter ≤3 mm pada hari ke-3, tidak berbeda signifikan dengan PDA (z=1,342; p=0,179, ns), sedangkan FBKA menghasilkan koloni ≤2 mm dan berbeda signifikan dari PDA (z=2,683; p=0,007). Media air rebusan biji kacang kratok terbukti lebih baik dibandingkan media tepung dan berpotensi sebagai media substitusi kultur *C. albicans*.

Kata kunci: *Candida albicans*; Media alternatif; *Phaseolus lunatus L.; Potato Dextrose Agar* (PDA); Pertumbuhan Jamur.

Abstract: *Candida albicans* is an opportunistic pathogenic fungus that causes candidiasis and requires selective culture media for laboratory identification as a substitute for relatively expensive synthetic media such as *Potato Dextrose Agar* (PDA). This study tested the potential of kratok bean seeds (*Phaseolus lunatus* L.) in the form of flour (FBKA) and boiled extract (BEKA) as alternative media for fungal growth. This study used a laboratory experimental design by comparing the macroscopic and microscopic characteristics between PDA media as a control and the alternative kratok bean media after 3 days of incubation at 37°C with three replications (n=3). The results showed that both alternative media could support the growth of *C. albicans* with yellowish-white colonies, a distinctive yeast odor, and a convex shape on the 3rd day. Microscopic observation through the germ tube test on both alternative media showed the formation of *pseudohyphae*. The

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Kruskal-Wallis test showed a significant difference in colony size between the three media on the 3rd day (H=7.200; p=0.027). And the post-hoc Dunn test analysis showed that BEKA produced colonies with a diameter of ≤3 mm on day 3, not significantly different from PDA (z=1.342; p=0.179, ns), while FBKA produced colonies with a diameter of ≤2 mm and was significantly different from PDA (z=2.683; p=0.007). The boiled extract media of kratok bean seeds was proven to be better than the flour media and has the potential as a substitute medium for *C. albicans* culture.
Keyword: *Alternative media; Candida albicans*; *Potato Dextrose Agar* (PDA); *Phaseolus lunatus* L.

1. Introduction

Candida albicans is an opportunistic pathogen causing infections ranging from superficial mucosal disease to life threatening systemic candidiasis [1][2][3]. It poses a major clinical challenge for immunocompromised patients, including those with HIV/AIDS, chemotherapy recipients, and solid-organ transplant recipients [4]. *C. albicans* cause 50-90% of candidiasis cases and 39-70% of invasive candidiasis cases, depending on clinical context and region [5][6]. Globally, invasive bloodstream candidiasis causes an estimated 1.565.000 cases and 995.000 deaths (63.6%) annually [7].

C. albicans was the most prevalent species (39.0%) among 10.109 isolates from low-middle-income countries (LMICs), with an 18,7% case fatality rate [8]. Diagnostic capacity in these settings remains limited: blood culture sensitivity for invasive candidemia is only 40%, so roughly 20% of patients with negative cultures miss appropriate antifungal therapy [9]. Pathogens in its 2022 Fungal Priority Pathogens List, calling for the urgent reinforcement of mycology laboratory capacity [10].

Reliable culture media are essential for diagnosing fungal infections, particularly those caused by *C. albicans*. *Potato Dextrose Agar* (PDA) is the gold standard medium for sustaining microbial, especially fungal, growth, combining dextrose as a carbon/energy source with patato extract as a nutritional substrate [11][12]. Its low pH (4.5–5.6) favors fungal proliferation [11]. Timely fungal culture is essential for precise diagnosis and antifungal therapy selection [13]. Commercial PDA, however, has practical limitations: high cost, hygroscopic storage requirement, and limited distribution [11].

Rising media costs have driven interest in economical, effective alternative substrates for routine laboratory culture.

Legume Fabaceae, owing to the high protein and carbohydrate content, are promising candidates fungal culture substrates. Crude French bean (*Phaseolus vulgaris*) supported growth of multiple fungal species [14]; a red kidney bean medium supported *C. albicans* growth with a mean colony diameter of 1.19 mm [15]; boiled soybean sustained *C. albicans* growth in culture [16]; cowpea (*Vigna unguiculata*) media sustained *C. albicans* growth in culture[17]; and boiled red kidney bean extract used as a *Sabouraud Dextrose Agar* (SDA) substitute supported growth with characteristics consistent with established standards, despite slightly smaller colony sizes than SDA [18].

The kratok bean (*Phaseolus lunatus* L.) known internationally as the lima bean, is a dietary staple in Indonesia, especially Aceh and Java. Each 100 g of dried seeds contains 70.3 g carbohydrate, 25 g protein, 1.6 g fat, and various minerals [19]. Beyond its nutritional profile, *P. lunatus* is also rich in phenolics (gallic acid, catechin, rutin, and quercetin), flavonoids, and antioxidant activity. Processing increases polyphenol and flavonoid content, while antinutritional factors including tannins, phytates, oxalic acid, and cyanide are markedly reduced following boiling [20]. This supports the potential of *P. lunatus* as a fungal growth substrate: carbohydrates supply carbon and energy, proteins supply nitrogen and amino acids essential for biomass biosynthesis, and reduced antinutrients improve bioavailability [21].

No prior study has compared flour-based versus boiled-extract media derived from kratok bean seeds as alternative culture substrates for *C. albicans*. This study therefore evaluated the effectiveness of kratok bean (*Phaseolus lunatus* L.) seed flour and boiled extract as alternative culture media for *C. albicans* growth relative to PDA. Findings are expected to support development of cost-effective alternative culture media for resource limited conditions laboratories.

2. Research Method

2.1 Study Design

This study constituted a laboratory based experimental study, using a Completely Randomized Design (CRD). All experimental procedures were carried out at the Microbiology Laboratory of the Diploma III Program in Medical Laboratory Technology, Poltekkes Kemenkes Aceh (Health Polytechnic of the Indonesian Ministry of Health, Aceh Province). Groups comprised a positive control (PDA), Medium A Flour-Based Kratok Agar (FBKA) and Medium B Boiled-Extract Kratok Agar (BEKA). A negative control was prepared for each medium without *C. albicans* inoculation. All experiments were performed with three independent replicates (n = 3) and incubated for 72 hours at 37°C. Negative controls were prepared and incubated in parallel under identical conditions to validate medium sterility and monitor for contamination.

2.2 Materials and Fungal Isolate

Commercial PDA, a *C. albicans* fungal isolate (a local clinical isolate from the Microbiology Laboratory stock culture collection), and kratok bean (*Phaseolus lunatus* L.) seeds were sourced from Aceh Tengah (Central Aceh), Aceh Province, Indonesia. Glassware, Petri dishes, and equipment were sterilized separately by autoclaving at 121°C for 15 minutes, inoculation loops were flame sterilized before and after each streaking maintain aseptic.

2.3 Preparation of Alternative Culture Media

Flour-Based Kratok Agar (FBKA)

Dried kratok bean seeds were cleaned, ground and sieved to flour. 200 g flour was dissolved in 1,000 mL distilled water and boiled for 30 minutes. The filtrate was filtered through cheesecloth, powdered agar (15 g/L) added, and reheated with stirring until homogeneous. The medium was autoclaved (121°C for 15 minutes), dispensed into Petri dishes (approximately 20 mL per plate) and solidified at room temperature.

Boiled-Extract Kratok Agar (BEKA)

A total of 200 g of dried kratok bean seeds were washed and boiled in 1,000 mL of distilled water for 30 minutes. The filtrate was filtered through cheesecloth and adjusted to 1,000 mL with distilled water. Powdered agar (15 g/L) was incorporated and the mixture was reheated with continuous stirring until fully homogeneous, sterilized by autoclaving at 121°C for 15 minutes, aseptically poured into Petri dishes (approximately 20 mL per plate), and allowed to solidify at room temperature [17].

2.4 Inoculation and Incubation

A pure culture of *C. albicans* was suspended in 0.9% NaCl and adjusted to 0.5 McFarland standard. Medium pH was measured post autoclave (FBKA 5.8–6.2, BEKA 5.5–5.9, and PDA 4.5–5.6 (standard). Each medium was inoculated by streaking 10 µL of the suspension with a sterile loop and incubated at 37°C [22]. Macroscopic growth was assessed every 24 hour for 3 days, recording diameter and morphology (form, color, surface topography, and odor) [17].

2.5 Macroscopic and Microscopic Examination

Macroscopic examination conducted every 24 hours, assessing colony morphology (surface form), color, size, and odor [23]. Microscopic examination on day 3, used a 40× objective to assess *C. albicans* cellular morphology, including yeast cells, budding cells, and pseudohyphal formation [22][24].

2.6 Statistical Analysis

Growth data for *C. albicans* were analyzed using both descriptively (Colonial morphology and viability across replicates) and inferentially (colony diameter (mm) at day 3 of incubation, ($\alpha = 0.05$). The analytical framework comprised two test. First, the Kruskal-Wallis test was used as non-parametric comparison across the three media, given the ordinal, small sample data (n=3). Second, Dunn's post-hoc test with Bonferroni correction determined pairwise differences where the Kruskal-Wallis test yielded a significant result ($p < 0.05$). The Bonferroni correction controlled the type I error rate in multiple comparisons ($\alpha = 0.05/3 = 0.017$).

3. Results and Discussion

The macroscopic growth characteristics of *C. albicans* colonies across three culture media over three days of incubation, with three independent replicates (n = 3), are presented in Table 1.

Tabel 1. Macroscopic Observations of *C. albicans* Colony Growth on PDA, FBKA, and BEKA after 72 Hours of Incubation (n = 3)

Hari ke-	Media	Replicate I (mm)	Replicate II (mm)	Replicate III (mm)	Colonial Morphological Characteristics
1	Positive Control (PDA)	–	–	–	No growth (lag phase)
	FBKA	–	–	–	No growth (lag phase)
	BEKA	–	–	–	No growth (lag phase)
	Negative Control (all media)	–	–	–	No growth (sterile)
2	Positive Control (PDA)	2.0	2.1	1.9	Yeast; flat surface; cream-yellowish; yeast odor (+)
	FBKA	1.0	1.1	0.9	Yeast; flat surface; cream-yellowish; yeast odor (+)
	BEKA	1.9	2.0	2.1	Yeast; flat surface; cream-yellowish; yeast odor (+)
3	Positive Control (PDA)	3.0	3.1	2.9	Yeast; convex surface; cream-yellowish; yeast odor (+)
	FBKA	2.0	1.9	2.1	Yeast; convex surface; cream-yellowish; yeast odor (+)
	BEKA	2.5	2.4	2.6	Yeast; convex surface; cream-yellowish; yeast odor (+)

Note: FBKA = Flour-Based Kratok Agar; BEKA = Boiled-Extract Kratok Agar; PDA = Potato Dextrose Agar; (–) = no growth; (+) = positive result; mm = millimeter. Colony diameter measured per replicate (n = 3). Negative controls remained sterile across all media, with no turbidity, discoloration, or microbial growth observed at any of the three 24-hour observation points during incubation, confirming the absence of contamination and validating the sterility of each culture medium. Cream-to-yellowish coloration and characteristic yeast odor were observed across all growth-positive media.

The sterility of each culture medium was verified prior to fungal inoculation, following standard microbiological quality-control practice [23]. After autoclaving, one uninoculated plate per medium per batch was pre-incubated at 37°C for 24 hours to confirm no pre-existing contamination. Negative controls were then monitored throughout the 72 hour incubation, no turbidity growth, or discoloration appeared on any control plate, confirming the media remained sterile that observed growth was attributable solely to the inoculated *C. albicans* culture.

a. Macroscopic Observation of *C. albicans* Colonies on Alternative Media

Macroscopic examination of both kratok bean–based alternative media confirmed their capacity to support *C. albicans* growth, evidenced by colonial morphology. No growth occurred at day 1, consistent with lag phase. By day 2 (Figure 1, D–F), PDA and BEKA reached of ≤2.0 mm, while FBKA was smaller (≤1.0 mm). By day 3 (Figure 1, G–I), PDA reached 3.0 mm, BEKA 2.5 mm, and FBKA 2.0 mm. All colonies displayed characteristic *C. albicans* morphology: a cream-to-yellowish coloration, a typical yeast odor, with surface progression from flat (day 2) to convex (day 3), consistent with Talapko et al. (2021), who reported that *C. albicans* colonies are generally white to cream-yellowish, yeast-scented, smooth, and soft in texture [25].

This growth differential indicates BEKA more effectively sustains *C. albicans* (proliferation than the flour-based formulation, consistent with studies on boiled soybean (*Glycine max*) extract as an alternative medium for *C. albicans*, yielding colonies with a cream-to-yellowish color, characteristic yeast odor, and smooth surface after 72 hours of incubation at 37°C [16].

Similarly, cowpea (*Vigna unguiculata*) media have been shown to support *C. albicans* growth, with round, smooth, cream-yellowish, yeast-scented colonies consistent across tested media [17]. A red kidney bean (*Phaseolus vulgaris*) alternative medium supports *C. albicans* growth with a mean colony diameter of 1.19 mm, albeit smaller than *Sabouraud Dextrose Agar* (SDA), while retaining similar macroscopic morphology [15]. Both kratok bean–based media

in sustained *C. albicans* growth via the nutritional composition of *P. lunatus* seeds.

Kratok bean (*Phaseolus lunatus* L.) seeds of Indonesian origin possess a rich nutritional profile, comprising 68.89% carbohydrate, 41.96% starch, 15.93% protein, and a spectrum of essential minerals including magnesium, phosphorus, potassium, calcium, and iron [26]. Carbohydrates and protein are key macronutrients; carbohydrates provide carbon/energy, proteins supply nitrogen and amino acids for biomass biosynthesis [21]. Boiling, used in preparing BEKA, appears to more efficiently solubilize water-soluble nutrients than direct use of seed flour, delivering higher dissolved nutrients concentration to the medium.

During boiling of *Phaseolus lunatus* L. seeds, the carbohydrate content increases (from 64.16% to 71.56%) due to starch gelatinization, while levels of tannins, phytates, oxalates, and cyanide are significantly reduced [27]. This improves nutrient bioavailability and fungal uptake, since tannins and phytates otherwise inhibit digestive enzymes and sequester minerals needed for fungal metabolism [27][28]. Incompletely dissolved flour particles in FBKA may physically impede nutrient absorption, slowing growth [26][27].

Medium pH, measured prior to fungal inoculation, Was FBKA 5.8–6.2, BEKA 5.5–5.9, and PDA 4.5–5.6 (standard). Although pH reduction occurred during autoclaving, final pH values of FBKA and BEKA remained weakly acidic, conducive to *C. albicans* growth. This is consistent with locally derived composite media (combining corn, soybean, and sweet potato), which also showed post autoclave pH reduction (final pH 5.15 compared with SDA (pH 5.05), yet still supported optimal fungal growth [29].

Day To	PDA (Control +)	Flour-Based Kratok Agar (FBKA)	Boiled-Extract Kratok Agar (BEKA)
1 Lag phase	A	B	C
2 (Yeast flat surface	D	E	F
3 Yeast convex surface	G	H	I

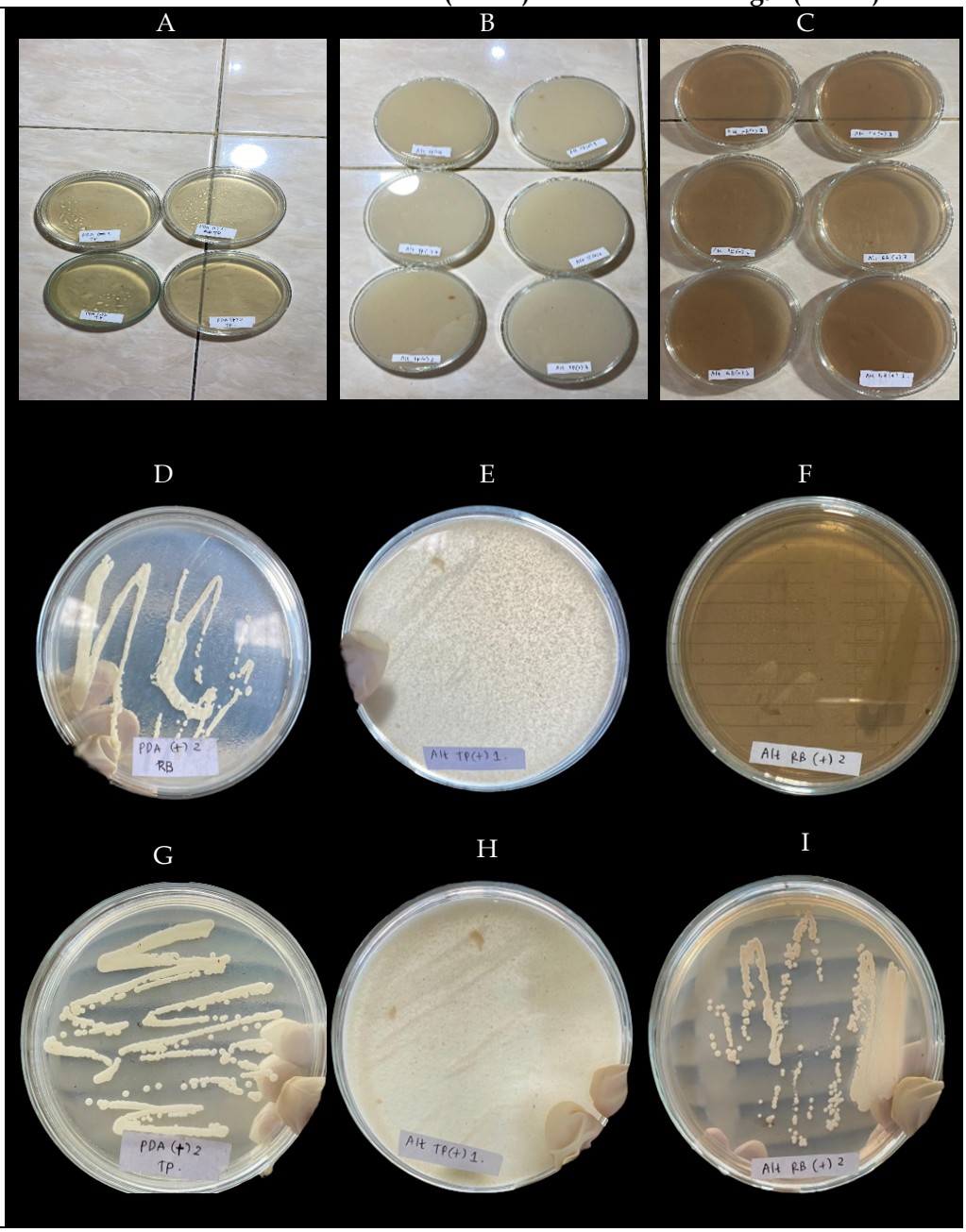


Figure 1. Macroscopic appearance of *C. albicans* colonies on three culture media following 72 hours of incubation at 37°C. Panels A–C: Day 1 (lag phase, no growth); Panels D–F: Day 2 (yeast colonies, flat surface, diameter ≤2 mm); Panels G–I: Day 3 (yeast colonies, convex surface, diameter ≤3 mm). Left column: PDA (positive control); center: FBKA; right: BEKA. Representative images from Replicate 2 (n = 3).

b. Microscopic Examination: Pseudohyphal Formation and *Germ Tube* Test

Microscopic examination at day 3 revealed pseudohyphal formation in both alternative media FBKA and BEKA indicative of *C. albicans*. Pseudohyphal formation is one of the key identifying features of this species, *C. albicans* undergoes morphological transitions between yeast, pseudohyphal, and true

hyphal forms, transitions central to its capacity to invade host tissue [24]. The ability of the kratok bean based media to induce pseudohyphal formation in the *germ tube* assay indicates these substrates can support the expression of the organism's dimorphic phenotype.

The dimorphic plasticity of *C. albicans* is a central virulence mechanism, facilitating host tissues adhesion to and invasion, enabling penetration of epithelial/endothelial barriers a critical step in the pathogenesis of systemic candidiasis [13][30]. Recent studies show hyphae can traverse epithelial layers via trans cellular tunnels that minimize tissue damage in the early infection [31]. This underscores that evaluating a medium's capacity to stimulate morphological transitions is not merely a descriptive exercise but a clinically meaningful validation criterion. Across various germ tube test media, the substrate's nutritional composition particularly protein and peptide content strongly influences germ tube formation. Media supplemented with peptides, such as 1% peptone, achieve a *germ tube* formation rate of 58.33%, whereas fetal bovine serum yields 100% formation [25].

Microscopic findings from the *germ tube* test at day 3 of incubation are illustrated in Figure 2. Pseudohyphal structures were clearly evident in both test media. Pseudohyphal formation is driven by cellular responses to nutrient signals, particularly nitrogen and carbon sources [20]. The abundant protein content of dried kratok bean seeds (25 g/100 g) provides a substantial nitrogen supply, and essential amino acids including lysine, isoleucine, and valine released during cooking act as nutrient inducers of the yeast-to-hypha transition [19][20]. The 37°C incubation temperature was selected as the optimal condition for inducing *C. albicans* dimorphism: at this physiological host temperature, the cAMP–PKA and MAPK signaling pathways are activated, upregulating filamentous genes including HWP1, ECE1, and ALS3 [24].

Both kratok bean–based media induced *germ tube* formation and pseudohyphal development across all replicates, demonstrating their suitability for the preliminary identification of *C. albicans*

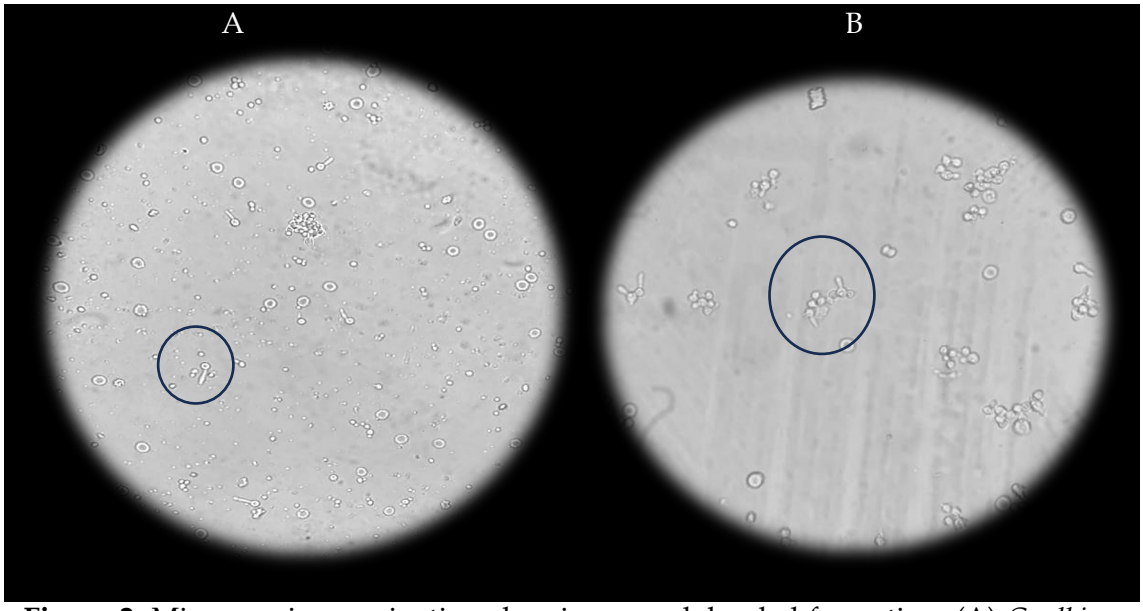


Figure 2. Microscopic examination showing pseudohyphal formation. (A) *C. albicans* pseudohyphae on *germ tube* test BEKA (40× objective); (B) *C. albicans* pseudohyphae on germ tube test FBKA (40× objective)

c. Statistical Analysis: Comparative Evaluation of Media Effectiveness

The Kruskal–Wallis test revealed a statistically significant difference in colony diameters among the three media at day 3 (H = 7.200; p = 0.027). Post-hoc Dunn's test with Bonferroni correction demonstrated that BEKA did not differ significantly from PDA (z = 1.342; p = 0.179), whereas FBKA differed significantly from PDA (z = 2.683; p = 0.007), as detailed in Table 2. The equivalence carries clinical significance. Non-parametric tests suited the ordinal, small sample data (n = 3), consistent with comparable studies [12][32][33].

Table 2. Statistical Analysis of the Comparative Effectiveness of Three Culture Media

Media Comparison	Statistical Test	Test Statistic	p-value	Conclusion
All three media (Day 3)	Kruskal-Wallis	H = 7.200	p = 0.027	Significantly different (*)
PDA vs BEKA	Dunn Test (Bonferroni)	z = 1.342	p = 0.179	Not significant (ns)
PDA vs FBKA	Dunn Test (Bonferroni)	z = 2.683	p = 0.007	Significantly different (*)
BEKA vs FBKA	Dunn Test (Bonferroni)	z = 1.342	p = 0.179	Not significant (ns)

*Note: *p < 0.05 (significant); ns = not significant. a = 0.05; n = 3 per group; post-hoc Dunn's test with Bonferroni correction (a' = 0.017)*

The absence of a significant difference between BEKA and PDA (z = 1.342; p = 0.179) carries important practical implications, given that Kruskal-Wallis test followed by Dunn's post-hoc test with Bonferroni correction suited this ordinal, small sample data (n = 3), consistent with prior research. BEKA outperformed the red kidney bean medium (mean colony diameter 1.19 mm) [15] approached boiled breadfruit (*Artocarpus communis*) seed extract medium, reported to be statistically equivalent to PDA [12], supporting BEKA as a viable substitute for *C. albicans* culture.

4. Conclusion

This study demonstrates a significant difference between the two processing methods applied to kratok bean seeds as alternative culture media for *C. albicans*. The boiled-extract formulation (BEKA) showed growth-supporting capacity that was statistically comparable to PDA (z = 1.342; p = 0.179, ns) for both colonial growth and pseudohyphal formation. Conversely, the flour-based formulation (FBKA) proved suboptimal and differed significantly from PDA (z = 2.683; p = 0.007). BEKA offers a cost-effective, implementable alternative for facilities with limited access to commercial media. A limitation is small sample size (n = 3), future studies (n ≥ 5), concentration standardization, shelf life evaluation, and testing against non-albicans *Candida* species and other pathogenic fungi, are recommended. Overall, this study in establishes boiled kratok bean extract (BEKA) as a statistically validated, low cost, and locally accessible alternative to PDA for *C. albicans* culture, offering practical value for laboratories with limited access to commercial media.

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