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The Effect of Tablets Effervescent Cocoa Pod Husk Extract (*Theobroma Cacao L.*) as a Denture Cleanser Against the Growth of *Candida Albicans* on Thermoplastic Nylon Plate

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ABSTRACT

Denture stomatitis is a common oral health problem in denture users. It can occur due to fungal infections caused by *C. albicans*. An effervescent tablet is a chemical denture cleanser that can reduce plaque and microorganism accumulation, including *C. albicans*. The addition of natural ingredients such as cocoa pod husk extract, which contains chemical compounds like flavonoids, saponins, and tannins, has shown antifungal activity against microorganisms such as *C. albicans*. This research aims to determine the effect of cocoa pod husk (*Theobroma cacao L.*) effervescent tablets in inhibiting the growth of *C. albicans* after a 15-minute soaking period. The treatment groups were effervescent tablets containing cocoa pod husk extract at concentrations of 20% and 25%, polident and aquadest. The samples were thermoplastic nylon discs with a diameter of 10 mm and a thickness of 2 mm. The discs were immersed in the treatment solutions for 15 minutes and vibrated for 30 seconds using vortex, spreading 0,1ml Saboraud Dextrose Broth (SDB) on Saboraud Dextrose Agar (SDA) and incubated for 48 hours at 37°C. The number of *C. albicans* colonies grown on the SDA medium was counted using a colony counter. The results showed that the cocoa pod husk effervescent tablets with 25% concentration were more effective than the 20% concentration, with average colony counts of 231.8 and 372.6, respectively. The highest average *C. albicans* colony count was observed in the negative control group (694.8), while the lowest was in the positive control group (0). Based on the results effervescent tablets containing cocoa pod husk extract as a denture cleanser are effective in inhibiting the growth of *C. albicans.*

KEYWORDS: *Candida albicans*, cocoa pod husk, denture cleanser, denture stomatitis, effervescent tablets

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INTRODUCTION

Thermoplastic nylon is a material that is often used as a denture base besides acrylic resin. Thermoplastic nylon also has the disadvantage of being hydrophilic or easily absorbing water so in long-term use it causes damage to the polymer chains which has an impact on surface roughness.[1] Surface roughness can lead to plaque accumulation and microorganism colonization.[2]

Denture stomatitis is a disease of the oral cavity that occurs due to fungal infection *C. albicans* or the mechanical influence of the denture on the mucosal surface under the denture. Denture bases that are in direct contact with the oral mucosa can become a place for plaque accumulation which becomes a place to develop *C. albicans*.[3] Denture cleaning can reduce the formation of plaque accumulation and microorganisms such as *C. albicans*.[4]

The chemical denture cleaning agent that is often used is tablets effervescent.[5] Tablet effervescent is available in powder and tablet form which can dissolve quickly in water. Soaking time is categorized into long-term soaking 6-8 hours at night, and short-term soaking 15-45 minutes after eating.[6] Currently, many natural ingredients for tooth cleaning agents have been developed, the ingredients that can be used as denture cleansers namely cocoa pod husk (*Theobroma cacao*

L.). Based on previous research by Subaryanti *et al*. (2023) the concentration of cocoa pod husk extract (CPHE) contains active compounds in the form of flavonoids, saponins, tannins, and alkaloids which can show antifungal activity against *Trichophyton mentagrophytes* with a minimum inhibitory concentration of 10%.[7] Based on the description above, researchers will study the effect of CPHE in tablet effervescent at concentrations of 20% and 25% as a cleaning agent for thermoplastic nylon dentures with a soaking time of 15 minutes. A combination of denture cleaning tablets effervescent and CPHE is expected to inhibit the growth of *C. albicans* more effectively in a shorter soaking time.

METHODS

The type of research is a laboratory experimental design post-test only control group design. This research was carried out at the Abadi Dental Laboratory to make thermoplastic nylon plates, at the Laboratory Bioscience Faculty of Dentistry, University of Jember to make cocoa pod husk extract, at the Pharmaceutical Laboratory, Faculty of Pharmacy, University of Jember to make tablets effervescent cocoa pod husk extract (TECPHE) and the Microbiology Laboratory of the Faculty of Dentistry, Jember University for calculating the number of colonies C. albicans. The research was conducted from August 2024 to December 2024.

The sample used was a disc-shaped thermoplastic nylon plate (Valpast, China). Making thermoplastic nylon begins with preparing a master model which has been formed disc-shaped with a diameter of 10 mm and a thickness of 2 mm. After that, the master model was placed horizontally in the cuvette filled with gypsum mixture. The implanted master model has paired sprue which is made from red wax, the cuvette is closed and pressed with press begel for ± 30 minutes then boiled for 5 minutes at 100℃ to remove the red wax to form mould space. Thermoplastic nylon is melted to a temperature of 274-293 ℃ with a furnace for 15 minutes. Thermoplastic nylon material is injected into the mould space and emphasized 6-8 bars for 5 minutes with press hydraulic. The thermoplastic nylon disc that has been formed is trimmed and its thickness is measured again.[8]

CPHE is done by preparing cocoa fruit from PTPN Kertosari, Jember Regency, the criteria for cocoa used were 180 days after planting. The stages of making CPHE begin with drying 2.5 kilograms of cocoa husk under sunlight. Next, dry it in the oven at 60 ℃. After that, grind the cocoa pods using a blender until smooth and sift with a sieve mesh number 60. CPHE is made using 96% ethanol solvent for 48 hours using the maceration method. Maserate is separated using the filtration method with filter paper. Then evaporate the filtrate with a vacuum rotary evaporator at a temperature of ± 50℃ to produce a thick extract.[7]

TECPHE is made by making effervescent granules and mixing the acid and alkaline granules. Acid granules are made by mixing extract granules, citric acid, tartaric acid, and some polyvinylpyrrolidone (PVP). Base granules are prepared by mixing sodium bicarbonate with the remaining PVP. The acid and base granules are mixed and then dried in an oven at a temperature of 40-60℃ until dry. Tablet effervescent is made by pressing granules using a tablet pressing machine.[9]

Suspension manufacturer *C. albicans* by taking 1 ose C. *albicans* and inserting it into the medium sabouraud dextrose broth, a volume of 5 ml was incubated at 37°C for 48 hours.[10] Next, the suspension C. albicans adjusted the turbidity to the standard Mc Farland no. 0,5 (10^8 CFU/ ml).[11] Sterilized thermoplastic nylon is soaked in artificial saliva for 1 hour, then rinsed with Phosphate Buffer Saline (PBS) solution 2 times. Thermoplastic nylon is then inserted on Saboroud Dextrose Broth (SDB) (Himedia, India) which contains suspension *C. albicans* then incubated for 48 hours at 37ºC. The thermoplastic nylon discs were rinsed again using PBS 2 times. Thermoplastic nylon is then inserted into test tubes containing a TECPHE concentration of 20%, 25%, distilled water, and Polident with a soaking time of 15 minutes. The disc was placed in 10 ml of SDB and vibrated with a vortex for 30 seconds to release *C. albicans* attached to each treatment group. Suspension *C .albicans* on SDB were then cultured on media Saboraud dextrose agar (SDA) (Himedia, India) and incubated for 48 hours at 37ºC. C. *albicans* which grows on SDA media was counted with colony counter.[12]

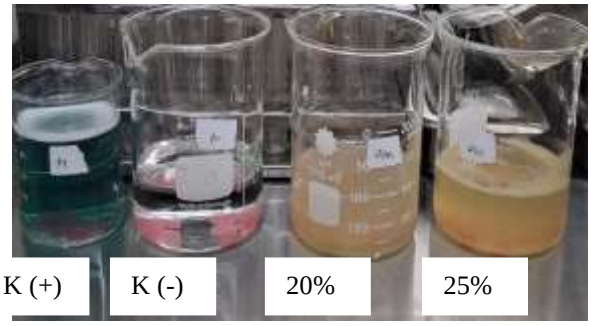

Figure 1. Nylon thermoplastic plate immersion (from left: K(+) (Polident), K(-) (Aquadest), TECPH 20% and 25%)

RESULTS

Table 1. The average number of *C. albicans* colonies on thermoplastic nylon plates after immersion in each group

Sample group	n	Mean	Standard Deviation
K (+)	5	0,00	0,00
K (-)	5	693,2	69,55
20%	5	372,6	68,11
25%	5	231,8	69,04

Based on Table 1, it can be seen that the average number of C. albicans colonies on thermoplastic nylon plates with the highest average being in the negative control group at 693.2 and the lowest average number of colonies being in the positive control group at 0 colonies. Meanwhile, the average number of colonies in the treatment group with 25%

TECPHE effervescent tablets was 231.8 and in the 20% TECPHE group was 372.6.

The results of the normality test using Shapiro-Wilk show the data is normally distributed ($p<0.05$) and the results of the homogeneity test using the Levene Test show homogeneous data ($p>0.05$). The data is normally distributed and homogeneous so it can be continued with parametric tests using One Way Anova. The results in One Way Anova, the whole group have a significance result of < 0.001 ($P<0.05$). After that, the Least Significant Differences (LSD) test was carried out to see the significance of each sample group.

Table 2. LSD test results

Sample group	K(+)	K(-)	20%	25%
K(+)	-	0,001*	0,001*	0,001*
K(-)	-	-	0,001*	0,001*
20%	-	-	-	0,002*
25%	-	-		-

*: The were significant differences ($p<0,05$)

Based on Table 5 the LSD test shows that there are significant differences in the number of *C. albicans* colonies between all treatment groups. This shows that there is a significant influence between all pairs of treatment groups on the number of *C. albicans* colonies on thermoplastic nylon plates. The results indicate that the average number of colonies of *C. albicans* after immersion between the treatment and control groups was a significant difference.

DISCUSSIONS

Based on the research results in Table 1 TCPHE 20% and 25% had an average number of colonies of *C. albicans* which is lower than the control group, which means it influences inhibiting the growth of the number of colonies of *C. albicans*. TECPHE 25% had an average number of colonies *C. albicans* which is lower than the average TECPHE 20%. This is in line with research by Astuti *et al.,* (2023) regarding the effect of natural ingredients biduri leaf extract on the number of *C. albicans* on acrylic resin plates, the higher the concentration of the extract, the antifungal potential also increases.[13]

The antifungal effect of cocoa pod skin is obtained from several bioactive compounds, based on phytochemical screening tests in research by Agwupuye *et al.* (2022) cocoa husk extract contains active compounds such as saponins, tannins, and flavonoids.[14] Flavonoid compounds are active compounds that have an antifungal mechanism that damages fungal cell membranes by interfering with ergosterol biosynthesis.[15] Meanwhile, tannin has antifungal activity by disrupting the formation of fungal cell walls by inhibiting chitin biosynthesis.[16] Saponin compounds are also the main bioactive compounds in CPHE which have antifungal activity by disrupting the stability of cell membranes, causing fungal cells to lyse.[16]

Table 4 shows the results of data analysis using tests One Way Anova with a significance value of 0.001 ($p<0.05$) which means that each treatment group has a significant difference in the average number of colonies of *C. albicans* after soaking. From these results, it can be seen that there is a significant difference between TECPHE 20% and 25% in inhibiting *C. albicans*. This is because the tablet effervescent Cocoa pods contain bioactive compounds in the form of alkaloids, saponins, tannins, and flavonoids which have antifungal mechanisms.[7] In line with previous research by Aji *et al*., (2020) regarding the effect of soaking tablets of effervescent celery leaf extract on a thermoplastic nylon plate where celery leaf extract contains the same phytochemical components as CPHE such as flavonoids, saponins, and tannins which can inhibit the growth of the number of colonies *C. albicans* on thermoplastic nylon.[17]

In Table 5 the LSD statistical test shows a significance value ($p<0.05$) which means there is a difference in the number of colonies of *C. albicans* significant between all treatment groups. These results are in line with research by Kristiana *et al.,* (2021) on tablets of effervescent tobacco leaf extract as a denture cleaner. There are significant differences between tablets effervescent Tobacco leaf extract concentrations of 25%, 50%, and 75% and the control group inhibited growth of *C. albicans*.[8] This significant difference between the control group and the treatment group was due to the TECPHE contains bioactive compounds in the form of alkaloids, saponins, tannins, and flavonoids which have antifungal mechanisms, while sterile distilled water does not contain bioactive compounds so it does not have an inhibitory effect on growth of *C. albicans*.[8]

The composition contained in the tablet effervescent also affects the inhibition of the colony *C. albicans*. Contents of tablets effervescent preparations such as citric acid can remove adhesions biofilm through the mechanism of calcium ion absorption, this process allows citric acid to break down calcium bridge thus disrupting biofilm formation.[18] Sodium bicarbonate in tablet composition effervescent can produce carbon dioxide gas when it reacts in water.[19]This process will produce foam that can clean biofilm from the surface of the denture base.[20] So the combination of tablets effervescent and cocoa pod husk extract is expected to increase the inhibitory effect on *C. albicans.*

CONCLUSIONS

Effervescent tablets containing cocoa pod husk extract as a denture cleanser are effective in inhibiting the growth of *C. albicans.*

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