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Effect of Cocoa Bean Extract Concentration on the Diameter of the Clear Zone Preparing Streptococcus Mutans Bacteria

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Abstract: Cocoa beans can inhibit the growth of pathogenic bacteria such as Streptococcus Mutans. Utilization of cocoa bean extract as an anti-bacterial inhibitor containing chemical compounds extracted in simplicia aims to increase the content in the form of alkaloids, flavonoids, tannins, saponins and polyphenols. Streptococcus Mutans is a gram-positive facultative anaerobic coccus that is usually found in the human oral cavity and one of the causes of tooth decay or cariogenic bacteria capable of creating an acidic atmosphere in the mouth and can metabolize carbohydrates. This paper describes the effect of the concentration of cocoa bean extract on the diameter of the clear zone of Streptococcus Mutans bacteria. The antibacterial test was carried out using the Mueller Hinton Agar (MHA) method, samples of cocoa bean extraction were prepared by maceration method with varying concentrations of 2.5% to 12.5% at 2.5% intervals. With an interval of three hours, measurements were carried out for 48 hours of observation. The results showed that the greater the concentration of cocoa bean extract, the larger the diameter of the clear zone, with the smallest diameter (8.44 ± 1.61) mm (for a concentration of 2.5%) and the largest diameter (9.70 ± 1.66) mm (for a concentration of 12.5%).

Keywords: Antibacterial; Cocoa bean; Well diffusion method; Streptococcus mutans; Clear zone

Introduction

Bacterial activity can attach to the surface of a solid coated matrix consisting of polysaccharides, extracellular DNA (eDNA) and proteins (Lakade et al., 2022). Certain types of ingredients will be successfully applied to deal with free radicals and prevent the formation of incoming bacteria, this is contained in the form of extracts of medicinal herbs (Abedi et al., 2023). One of them is that Cocoa has long played a role in the fields of both rehabilitative (Kalinovskii et al., 2023), preventive and curative or health needs which have the potential as an alternative to traditional medicine (Mardiah & Cut Aja Nuraskin, 2022), it is believed that it is not detrimental and causes side effects in it, besides that the compounds contained can be used as cosmetic ingredients and supplements, for such management it is necessary to be scientifically justifiable in terms of safety and benefits (Suparno & Simamora, 2023). The object of research continues to grow because it can be utilized by humans in general, one of which is herbal ingredients (Desvita et al., 2021; Yuanita, Ceson, et al., 2021; Yuanita, Wahjuningrum, et al., 2021).

Indonesia is known as an agricultural country that produces many natural materials known to be rich in biodiversity, so that it has business opportunities to develop to increase foreign exchange (Armi et al., 2023). In 2002, Indonesia as a country with abundant natural resources recorded cocoa plantations of 914,051 ha. 87.4% of the people who manage it, on average, are the main producing regions of South, Central and Southeast Sulawesi. A healthy lifestyle has been chosen by contemporary society by using natural ingredients for the efficacy of curing a disease (Binartha et al., 2021; Noviyanti et al., 2021; Rahman & Ariastuti, 2021).

Cacao bean production is almost the third largest in

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the world, seed content such as flavonoids, catechins and bioactive compounds can be used as potential antibacterial alternatives, by extracting the seeds can inhibit bacterial growth (Kenitasari et al., 2023), besides that the bioactive components of flavonoids, polyphenols, catechins, and saponins can prevent streptococcus mutans can be formed more slowly, in addition to the significant antibacterial effect on cocoa against Fusobacterium nucleatum, Porphyromonas gingivalis, and Prevotella intermedia (Ben Lagha et al., 2021; Cinar et al., 2021; Jean-Marie et al., 2021).

Streptococcus mutans occurs as an extracellular polymer of glucose (glucan) occurs due to the conversion of sucrose, glucan coagulates with other microorganisms, one of which is Candida albicans which is usually found in the oral cavity (Chaudhari et al., 2021), One of the efforts to find natural anti-glucosyltransferase substances is found in cocoa plants (Yumas et al., 2022), polyphenols in cocoa have strong anti-glucosyltransferase properties. Thus, cocoa bean husk extract has been shown to have antibacterial and antiglucosyltransferase activities (Prince et al., 2022). Flavonoids are a large class of heterocyclic organic compounds produced by the secondary metabolism of natural plants (Suripta et al., 2023), Widely found in fruits, vegetables and nuts in the form of flavonoid glycosides (Mueed et al., 2023), They play important probiotic effects on antioxidant, antibacterial and anti-inflammatory, antitumor, immune regulation, and gut health (Zhou et al., 2023).

In accordance with previous studies with the title concentration of cocoa bean extract as a natural material in inhibiting the growth of Streptococcus mutans, resulting in the inhibition of cocoa bean extract at certain concentrations (Gylan et al., 2022), In addition, it is supported by research on changes in the morphology of Staphylococcus aureus due to exposure to ethanol extract of cocoa beans in vitro, with the results of inhibition against Staphylococcus aureus unable to show any changes in morphology after being given cocoa bean extract (Chotimah et al., 2021; Utama et al., 2021).

In cocoa pods, there are ingredients that are proven to be antibacterial such as polyphenols, saponins and flavonoids (Sari, 2022). The results showed that cocoa beans contain many compounds as potential natural antibacterial and antioxidant substances, one of which is the polyphenols contained in it, which disrupts cell permeability so that the cell membrane or cell wall shrinks (Guan et al., 2023; Rana et al., 2023). As a result, the activity of living cells is inhibited by growth. As a result, the activity of living cells is inhibited by growth. Flavonoids, the most abundant type of secondary metabolite found in plants, are found in foods as glycosides, glucosyl, rutinosil, neohesperidinil, and rhamnolipidyl (Rodriguez et al., 2023).

Streptococcus mutans is an acid-producing and fermenting carbohydrate bacterial colony, as well as its cariogenic role in the oral cavity as an opportunist pathogen (Nittayananta et al., 2023), becoming dominant over other species due to the availability of nutrients, changing environment and increasing density will cause dysregulation of oral bacteria in homeostasis (Afni et al., 2015; Ben Lagha et al., 2021; Cinar et al., 2021).

The effect of cocoa extract is able to inhibit the presence of several types of bacteria, such as Streptococcus pyogenes, Streptococcus mutans and Staphylococcus epidermidis (Noviyanti et al., 2021), Several previous studies have been carried out on cocoa shells, it was stated that cocoa peel extract inhibited the antibacterial activity of Streptococcus aurens (Runa Soans et al., 2021), with the well method obtained inhibition zones in the concentration range of 15.6 to 1000 mg/ml. It is also able to inhibit the growth of Propionibacterium acne bacteria (Wahidah, 2017), and also Staphylococcus epidermidis (Adha & Ibrahim, 2021).

The content of these compounds includes flavonoids which are also proven as antioxidants and bacteria (Vásquez et al., 2019). It is known that cocoa beans have high polyphenolic compounds (Kongor et al., 2016), the content of phenolic compounds cinnamic acid, tannins, pyrogallol, resolsinol, catechins, each plant has a different ability to synthesize substances. Phenolic has secondary metabolite power, the content is in cocoa beans (teobrama cacao) (Pohan et al., 2020). In this study, researchers examined the effect of the effectiveness of cocoa bean extract in inhibiting the growth of Strepcoccus mutans on the diameter of the clear zone.

Method

Place and time of research

The research was carried out on 18-30 November 2021 at the Yogyakarta State University Microbiology Laboratory.

Ingredients

The tools and materials used in this study were cocoa bean extract (T. cacao) with a concentration of 2.5%; 5.5%; 7.5%; 10.5%; and 12.5%, Nutrient Agar (NA) Merck [1.05450.0500], Nutrient Broth (NB) Merck [1.05443.0500], 100% distilled water, chloramphenicol 200 mg/l, streptococcus mutans bacteria, OSE needle, 250 beaker ml and 500 ml, analytical balance, stir bar, Erlenmeyer 250 ml, vacuum pipette, test tube, room temperature in a shaker, blender, Whatman filter paper with a diameter of 90 mm, petri dish, water bath temperature 600 , centrifuge kec. 2000 rpm, caliper,

gloves and mask.

Method

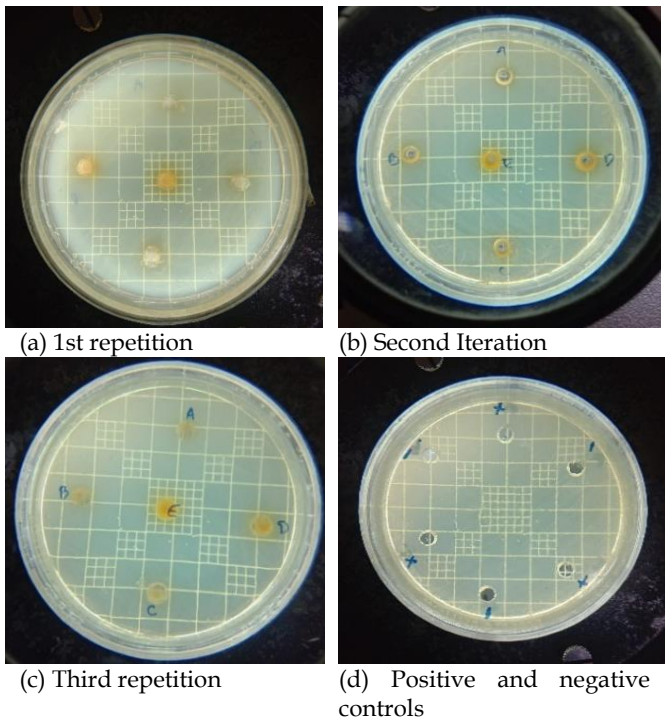
A sample of 500 grams of cocoa beans (Teobrama cacao) is dried until it is simplicia then in the oven, then in a blender at a certain speed until it is in powder form and filtered so that it is homogeneous. The cocoa bean powder is weighed using an analytical balance (Adha & Ibrahim, 2021) as much as 100 grams. The maceration process uses distilled water with a ratio of 1: 3 in a closed container for 24 hours. To obtain extracts in the form of a paste so that the active substances and solvents are separated (Afni et al., 2015) evaporated in a water bath for 20 hours. The concentration of the extract that has been obtained is 2.5%; 5.5%; 7.5%; 10.5% and 12.5% were used as samples with a volume of 50 ml.

Each concentration was tested 3 times with RAL (completely randomized design), observations of the clear zone were carried out during the 1st hour to the 41st hour for 5 days in each petri dish, the diameter of the clear zone was reduced by the diameter of the wells (Binartha et al., 2021), by using calipers in millimeters, then the data is interpreted in tabular form.

The method used in this study was complete randomized design (CRD) (Adha & Ibrahim, 2021). Data were analyzed using ANOVA statistics (one-way variant) with the aim of knowing an effect after the treatment was carried out, then Duncan's test was carried out to compare the results of each treatment (Haerussana et al., 2022).

Result and Discussion

The antibacterial test activity of cocoa bean extract against Streptococcus mutans can be concluded that it has an effect on the well method. This can be seen by the formation of a clear zone around the wells that have been made with a petri dish. forty one with positive control, and negative control formed a clear zone from the fourth to the eighth hour around the well area, it can be said that cocoa bean extract can inhibit the growth of Streptococcus mutans bacteria.



(a) 1st repetition (b) Second Iteration

(c) Third repetition (d) Positive and negative controls

Figure 1. Results of Antibacterial Activity of Cocoa Seed Extract

The results of the data obtained from the clear zone were analyzed using SPSS and tested with one way ANOVA, obtained a significance value (0.054) > α value (0.05), meaning that the data is normally distributed, with a calculated F value (3.653) > table F value (2.77) that the seed extract had an effect on the growth of Streptococcus mutans bacteria, then the Duncan test was carried out to determine the best concentration of cocoa bean extract in inhibiting the growth of these bacteria. The data is presented in Table 1.

Table 1. Average diameter of the clear zone formed from the activity of cocoa seed extract (T. cacao) against Streptococcus mutans (T. cacao) bacteria

Number.	Extract concentration	Mean clear zone (mm) ± SD
1.	Negative control (100% Aquades)	6,40 ± 0,58
2.	2,5%	8,44 ± 1,61
3.	5,5%	8,32 ± 1,82
4.	7,5%	9,48 ± 1,37
5.	10,5%	9,70 ± 1,66
6.	12,5%	9,70 ± 1,70
7.	Positive control (Cloramphenicol 200/ml)	9,80 ± 1,82

Note: Different notation indicates a significant difference based on Duncan's test with a significance level of 0.05

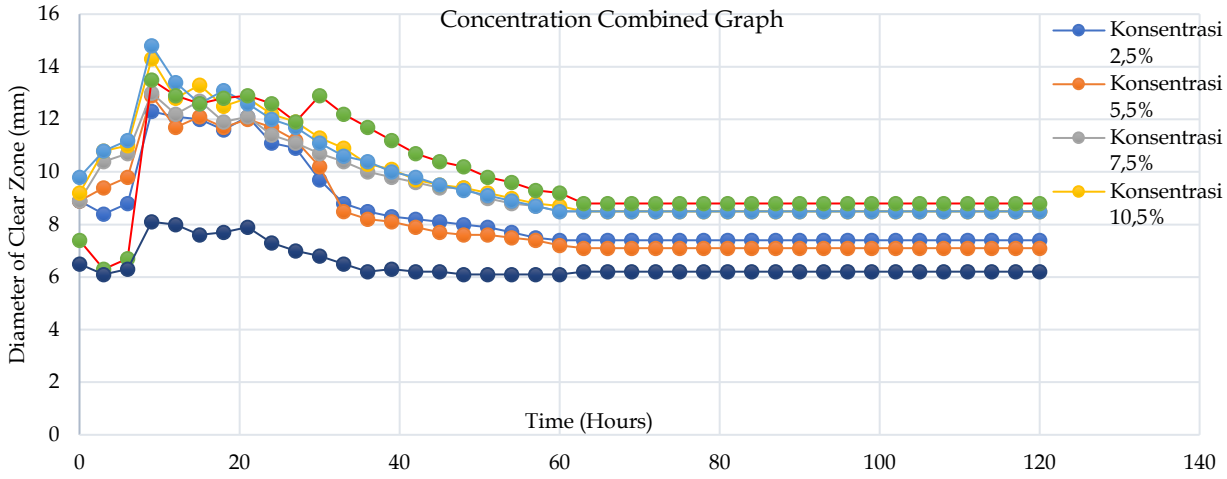


Figure 1. Combined Concentrations

Description: graph of the effect of several concentrations of cocoa extract on the diameter of the clear zone in Streptococcus mutans, at one to four one hours.

Duncan's test shows that the average clear zone value can be seen that the higher the concentration level the greater the resulting clear zone is formed, but from these data it shows that in the 10.5% and 12.5% concentration treatments there was a slight change in significance therefore the average was obtained the same, for concentrations of 5.5% and 7.5% there was a very significant change in the average yield. This shows that there is great antibacterial activity. The positive control showed a significant difference in the inhibition zone with the negative control which was not large in showing a significant change in inhibition of Streptococcus mutans bacteria. Polyphenols show good antibacterial activity in Streptococcus mutans, the content of these compounds includes flavonoids which are also proven as antioxidants and bacteria.

It is known that cocoa beans have high polyphenolic compounds, containing phenolic compounds of cinnamic acid, tannins, pyrogallol, resolsinol, catechins, each plant has a different ability to synthesize substances. Phenolic has secondary metabolite power, the content is found in cocoa beans (teobrama cacao). Catechins contained in flavonoids have antibacterial properties on gram-positive and negative, are involved in the interaction of the lipid bilayer and flavonoids which are proven to be able to break the bacterial membrane by deactivating or inhibiting enzyme synthesis and binding to the lipid bilayer. The ability of the concentration of cocoa bean extract as an antibacterial against Streptococcus mutans can be said to be antibacterial against a clear zone with a diameter of 6 mm. Based on the results of the research that has been done, it can be concluded that the concentration of cocoa bean extract has an effect on the formation of clear zones on Streptococcus mutans bacteria. As in the research, Streptococcus mutans has antibacterial properties (Kiros et al., 2022), and cocoa bean extract as a natural material in inhibiting the growth of Streptococcus mutans (Sanchez-Capa et al., 2022).

Conclusion

Based on research that has been done that the concentration is 2.5%; 5.5%; 7.5%; 10.5% and 12.5% of cocoa bean extract (teobroma cacao) clear zone diameter inhibited with increasing cocoa concentration, it can be concluded that cocoa beans have anti-bacterial abilities that increase with increasing concentration. The lowest value of the clear zone is 8.44 ± 1.61 mm and the highest is 9.70 ± 1.66 mm. has the effect of forming a clear zone on Streptococcus mutans bacteria with an average diameter of more than 6 mm.

Author Contributions

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Conflicts of Interest

The authors have no conflict of interest with any person/institution/organisation or the like associated with this research.

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