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Antibacterial nardus grass kombucha (cymbopogon nardus (l.) rendle) against staphylococcus epidermidis and propionibacterium acnes

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ABSTRACT

The bacteria Propionibacterium acnes (PA) and Staphylococcus epidermidis (SE) cause acne vulgaris, an infection marked by inflammation in the sebaceous glands, as well as obstruction and accumulation of keratinous components. Kombucha contains antimicrobial chemicals, among other components. The active components' antibacterial properties should be strengthened by the addition of Nardus grass. Finding the most ideal concentration and the efficiency of Nardus grass kombucha in suppressing PA and SE bacteria were the goals of this investigation. The Kirby-Bauer antibacterial testing method was used to test Nardus grass kombucha against PA and SE bacteria after it had fermented for 14 days at concentrations of 70%, 80%, and 90%. ANOVA and SPSS software were used to analyze the data. According to the test results, the development of clean zones demonstrated that Nardus grass kombucha at all concentrations may stop bacterial growth. At 70%, 80%, and 90% concentrations, the inhibition zones' average diameters against PA bacteria were 16.60 mm, 18.53 mm, and 20.96 mm, respectively. At the same doses, the inhibition zones for SE bacteria were 15.40 mm, 16.30 mm, and 17.53 mm. The results of the study showed that nardus grass kombucha can inhibit PA and SE bacteria, with 90% being the ideal concentration.

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1. Introduction

Acne is a common skin disease that affects 85% of the world's population aged 11-30 years. The prevalence of acne sufferers in Indonesia ranges from 80-85% in adolescents with a peak incidence at the age of 15-18 years, 12% in women aged > 25 years and 3% at the age of 35 - 44 years [1]Acne or acne vulgaris is an infection in the form of inflammation of the multi sebaceous layer, accompanied by blockage and accumulation of keratin material, and is triggered by the bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis* [2].

A probiotic beverage with East Asian origins is kombucha. This beverage is created by fermenting a tea and sugar mixture with kombucha culture, a bacterial and yeast symbiosis. Kombucha has been recognized for centuries for its numerous health benefits[3]. Kombucha can be produced by several groups of microorganisms, including Acetobacter, Acetobacter xylinum, and Saccharomyces sp. Kombucha contains Lactobacilli which are known as probiotic bacteria, which act as antibacterial agents through metabolites produced by these probiotics. The metabolites that kombucha produces can stop microorganisms from growing.

One plant that has many benefits is nardus grass(3). Citronella (*Cymbopogon nardus* (L.) Rendle) is a plant that contains essential oils which are biologically active as antifungal and antibacterial, so it can be used as a natural antimicrobial.

Citronella also contains saponin was compounds which have been proven to be effective in inhibiting the growth of gram-positive bacteria, as well as flavonoid compounds which have anti-inflammatory, antioxidant, anti-cancer and antibacterial properties [5]. In research conducted by [6]it was discovered that the essential oil content of citronella can inhibit Propionibacterium acnes bacteria. And in [7] research, it was discovered that essential oil compounds can inhibit Staphylococcus epidermidis bacteria.

2. Method

The Tools

Pyrex® beaker glass, stirring rod, vial bottle, petri dish, funnel, Erlenmeyer, incubator, vernier caliper, ose needle, flannel cloth, filter paper, disc paper, stove, label paper, laminar air flow, mortar and pestle, micropipette, analytical balance, oven, pH meter, test tube rack, alcohol, tea strainer, syringe, thermometer, glass jar, test tube.

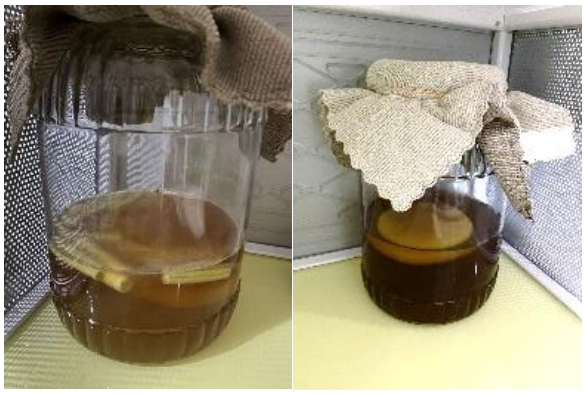
The materials

Water (Nestle Purelife), sterile distilled water, clindamycin antibiotic, acetic anhydride, FeCl3, granulated sugar, 2N HCL, concentrated H2SO4, *Propionibacterium acnes* and *Staphylococcus epidermidis* bacterial isolates, Nutrient Agar (NA) media, NaCl, reagents (Mayer, Dragendorf, and Wagner) SCOBY, Mg powder, nardus grass, green tea.

2.1. The Making of Nardus grass kombucha

To prepare Nardus grass kombucha (*Cymbopogon nardus* (L.) Rendle) using the modified [8] manufacturing method, first gather the tools and ingredients. Begin by boiling 1 liter of mineral water in a package, heating it to a temperature of 90-100°C. Once boiling, add 4 packets of black tea and stir for about 5 minutes, allowing the tea to infuse fully. Afterward, incorporate 100 grams of granulated sugar, and let the tea mixture cool to room temperature. Once cooled, strain the tea to remove the dregs and transfer the liquid into a glass jar. Next, add the SCOBY and starter solution to the jar.

Store the jar in a safe, clean, and dry location, away from direct sunlight. Cover it with a napkin and secure it with a string to ensure proper fermentation. Allow the kombucha to ferment for 7 days. On the seventh day of fermentation (after a full 7 days or 168 hours), the solution was retrieved and filtered once more for pH testing and analysis for phytochemicals compound, continuing the fermentation process, a stalk of chopped nardus grass weighing 25 grams was added.



(a) (b)

Figure 1. Nata kombucha from nardus grass kombucha

Description:

(a) = Fermented For 7 Day
(b) = Fermented For 14 Day

The mixture was left to ferment again for another week. On the fourteenth day, the Nardus grass kombucha tea was again filtered, and the resulting filtrate was tested for pH, as well as for its effects against *Propionibacterium acnes* and *Staphylococcus epidermidis*. Additionally, a phytochemical analysis was conducted, examining Alkaloids, Flavonoids, Tannins, Saponins,steroids and Phenols.

2.2. Phytochemicals screening

Kombucha extract underwent phytochemical screening by examining secondary metabolites like flavonoids, alkaloids, saponins, terpenoids, phenols and tannins using selective reagens.

2.2.1. Alkaloid Testing

A total of 5 mL of the sample was combined with 1 mL of 2N HCl and heated in a water bath for 2 minutes. The mixture was then cooled and filtered. The resulting filtrate was divided into three test tubes. Mayer's reagent was introduced to the first tube, Dragendorff's reagent to the second, and Wagner's reagent to the third. A positive result in the first tube is indicated by the formation of a white precipitate, while a red color in the second tube signifies a positive outcome, and a brown precipitate in the third tube indicates a positive result as well [9].

2.2.2. Flavonoid testing

To identify flavonoids, 5 ml of the sample is mixed with concentrated magnesium powder and hydrochloric acid. The presence of flavonoids is indicated by the formation of foam and a color change to yellow, orange, or red [10].

2.2.3. Saponin testing

A 5 ml sample was placed in a test tube, heated, and then shaken vigorously for 10 seconds. The presence of saponin is confirmed by the formation of stable foam that lasts for at least 10 minutes, reaching a height of 1 to 10 cm[11].

2.2.4. Phenols testing

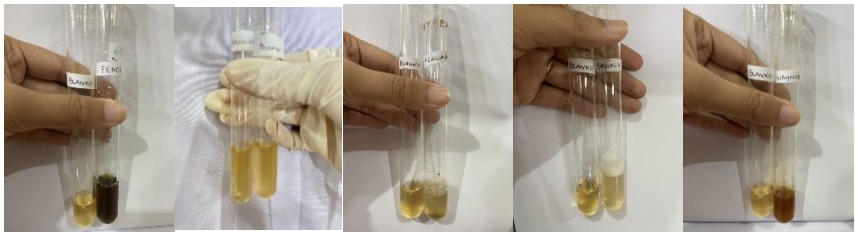
A 5 ml sample was placed in a test tube, heated, and then shaken vigorously for 10 seconds. The presence of saponin is confirmed by the formation of stable foam that lasts for at least 10 minutes, reaching a height of 1 to 10 cm [11].

2.2.5. Tannins testing

A total of 5 ml of the sample was treated with 1% FeCl3 solution. The presence of tannins is indicated by the emergence of a blackish-blue or brownish-green color [9].

2.2.6.Steroid/triterpenoids testing

A total of 5 ml of sample was combined with 3 drops of acetic anhydride and 1 drop of concentrated H2SO4 solution. The formation of a red color indicates the presence of triterpenoids, while a green color suggests the presence of steroids [11].



(a) (b) (c) (d) (e)

Figure 2. Phytocemicals screening from nardus grass kombucha

Description:
(a) = fenolik
(b) = steroid
(c) = alkaloid
(d) = saponin
(e) = flavonoid

2.3. Antimicrobial activity

2.3.1. The Nutrient Agar (NA) Media Preparation

The process of making Nutrient agar (NA) requires the initial step of weighing out 48 grams of agar and dissolving it in 160 mL of distilled water. The mixture is heated until it becomes liquid, and then subjected to sterilization in an autoclave at 121°C for 15 minutes. Once this process is complete, the NA medium will be ready for cultivating *Propionibacterium acnes* and *Staphylococcus epidermidis* [12].

2.3.2.Bacterial suspension procedures

A test tube was used to culture *Propionibacterium acnes* in a loop, which was then streaked onto 5 mL of solidified Nutrient Agar media. This was incubated at a temperature of 35°C to 37°C for 18 to 24 hours. Following incubation, a loop of the inoculum was taken and introduced into a test tube containing 10 mL of NaCl, where it was homogenized. The resulting bacterial suspension is now prepared and ready for antibacterial activity testing [12]. After being cultured in a single loop, Staphylococcus epidermidis was then streaked onto 5 mL of solidified media and then placed in an air-tight test tube. This was incubated at a temperature of 35°C to 37°C for a period of 18 to 24 hours. Subsequently, another loop of the inoculum was collected and added to a test tube containing 10 mL of NaCl solution, where it was homogenized to create a bacterial suspension. This suspension is now prepared for testing antibacterial activity [12].

2.3.3. Preparation of the solution

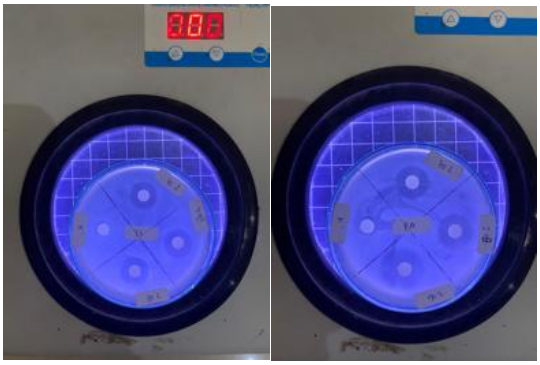
The preparation of positive control is by weighing 500 mg of Clindamycin powder as much as 0.01 g, then dissolved with aqua pro injection up to 1 ml. Likewise with the preparation of the fragrant nardus grass kombucha test preparation with a concentration of 70%, 80%, 90% then added with 1 mL of sterile aquadest and sterilized.

2.3.4.Antimicrobial testing

The sterilized NA media was put into a test tube as much as 15 mL; 3 drops of bacterial suspension were included, homogenized, at that point put into a petri dish and cleared out to cement. The paper disk was doused in each concentration of the nardus grass kombucha test arrangement, at that point the disk was joined to the agar surface. As a negative control, 3 mL of refined water was utilized and the positive control utilized was 1 mL of clindamycin. This treatment was rehashed 3 times. After that, the petri dish was incubated for 24 hours at a temperature of 24-27°C. Antibacterial activity was determined by measuring the diameter of the inhibition zone formed using a vernier caliper. Measurement of the diameter of the inhibition zone can be done with a vernier caliper using the formula [13].

$$R(\%) = \frac{(D1+D2)\ x\ 100\ \%}{2} \quad (1)$$

Note:
R = Inhibitory Power (mm)
D1 = Diameter of the longest inhibition zone (mm)
D2 = Diameter of the shortest inhibition zone (mm).



(a) (b)

Figure 3. Zone antibacterial nardus grass kombucha

Description:

(a) = nardus grass kombucha (Concentration 70 %,80 %, dan 90 %) with *Staphylococcus epidermidis*

(b) = nardus grass kombucha (Concentration 70 %,80 %, dan 90 %) with *Propionibacterium acnes*

3. Results and Discussion

3.1. pH Value of Nardus grass kombucha

Results of pH testing on Nardus grass kombucha (*Cymbopogon nardus* (L.) Rendle) which was fermented on the 7th and 14th days. after the kombucha tea was made, ph estimations were carried out on kombucha without the expansion of Nardus grass (*Cymbopogon nardus* L.) Rendle) on the 7th day, the comes about gotten were pH 4, whereas Kombucha with the expansion of nardus grass (*Cymbopogon nardus* L.) Rendle) on the 14th day gotten the same comes about, specifically pH 4 The pH value of Kombucha without the addition of nardus grass (*Cymbopogon nardus* L.) Rendle) and Kombucha also nardus grass (*Cymbopogon nardus* L.) Rendle) created is still inside secure limits for utilization and can be acknowledged by the body[14].

Table 1. pH Value of nardus grass kombucha

DAYS	pH
1	4,55
4	4,46
7	4,35
10	4,21

3.2. Phytocemicals Screening

The result of phytocemical screening (Table 2) have been shows that Kombucha Nardus grass (*Cymbopogon nardus* L.) Rendle) contains flavonoids, tannins, saponins, and phenols. Usually in agreement with Najmah's inquire at (2023) suspected that this compound gives antibacterial impacts [15].

Tabel 2. Phytochemical screening of nardus grass kombucha (*cymbopogon nardus* l.)rendle

Sample	Test	Reagent	Result	Information
Nardus grass kombucha (*Cymbopogon nardus* (L.) Rendle)	Fenol	$FeCl_3$ 10%	+	Blac
	Saponin	Hot Water	+	Forming Foam
	Flavonoid	Magnesium +HCl concentrated	+	yellow
	Tanin	$FeCl_3$ 1%	+	Blackhis green
	Steroid/ Triterpenoid	Acetic acid anhiyrade + H_2SO_4 concentrated	-	unformed red and green colour
	Alkaloid	Mayer	-	white sedimentation
		Wagner	-	Red sedimentation
		Dragendorff	-	Brown sedimentation

Tabel 3. Inhibitory test results of nardus grass kombucha (*cymbopogon nardus* (l.) rendle) against propionibacterium acnes and staphylococcus epidermidis bacteria

treatment	Bacteria testing	Average Inhibition Zone Diameter ± SD	remarks
Negatif Control	Propionibacterium acnes	0 ± 0b	-
Positif control		38,10 ± 2,71a	Very strong
Kombucha nardus grass 70%		16,60 ± 0,00ab	strong
Kombucha nardus grass 80%		18,53 ± 0,40ab	strong
Kombucha nardus grass 90%		20,96 ± 0,91ab	strong
Negatif Control	Staphylococcus epidermidis	0 ± 0b	-
Positif control (Klindamisin)		34,50 ± 1,25ab	Very strong
Kombucha nardus grass 70%		15,40 ± 0,17ab	strong
Kombucha nardus grass 80%		16,30 ± 0,30ab	strong
Kombucha nardus grass 90%		17,53 ± 0,40ab	strong

The antibacterial activity test of Nardus grass kombucha (*Cymbopogon nardus* (L.) Rendle) observed against *Propionibacterium acnes* bacteria and *Staphylococcus epidermidis* bacteria produced an inhibition zone which can be seen in Table 3, Propionibacterium acnes bacteria in successive concentrations of 70%, 80%, 90% Nardus grass kombucha (*Cymbopogon nardus* (L.) Rendle) showed an average inhibition zone diameter of 16.60 mm, 18.53 mm, and 20.96 mm, these samples showed a strong inhibition category.

Meanwhile, in Staphylococcus epidermidis bacteria, successively, concentrations of 70%, 80%, 90% of nardus grass kombucha (*Cymbopogon nardus* (L.) Rendle) showed an average diameter of the inhibition zone of 15.40 mm, 16.30 mm, and 17.53 mm, indicating a strong inhibition category. Based on the literature, the inhibition zone category is ≤ 5 mm (weak inhibition), inhibition zone 5-10 mm (moderate inhibition), inhibition zone 11-20 mm (strong inhibition), and inhibition zone >21 mm (very strong inhibition) (Susanto et al., 2012). So that the inhibition by Nardus grass kombucha (*Cymbopogon nardus* (L.) Rendle) shown against Propionibacterium acnes bacteria and Staphylococcus epidermidis bacteria is included in the strong category.

The results of pH testing on obtained the same results, namely pH 4. Based on these results, it can be assumed that the addition of nardus grass does not affect the pH of kombucha tea because the decrease in pH during the fermentation process is influenced by the breakdown of sugar into alcohol and lactic acid by lactic acid bacteria [16]. Black tea is the best substrate for making kombucha[17], When sugar was included, the expansion of sugar was done since the kombucha mushroom (SCOBY) which contains an advantageous

culture of bacteria and Yeast needs a carbon source to thrive, and in the maturation of kombucha, this carbon source is derived from the fermentation of sugar. [18]Furthermore, it is fermented by SCOBY for 7 days without the addition of Nardus grass (*Cymbopogon nardus* (L.) Rendle), after which it continues for 14 days with the inclusion of (*Cymbopogon nardus* (L.) Rendle), with fermentation lasting for 7-14 days to achieve the best results [8].

The kombucha fermentation process also produces several components including organic acids that have functional properties; these acids are lactic acid, acetic acid, malic acid, oxalic acid, gluconic acid, butyric acid, nucleic acid, amino acids, enzymes, vitamins, and polyphenols[18].The presence of secondary metabolite content produced by bacterial and yeast consortium in kombucha has more potential as an antibacterial in gram-positive bacteria compared to gram-negative bacteria. The cellular mechanism of secondary metabolites produced by the kombucha microbial consortium is by damaging the peptidoglycan components in the cell walls of gram-positive and negative bacteria [19]. Kombucha has antibacterial activity derived from fermentation metabolites that have the ability to inhibit microbial growth (Table 3). The active compound produced by lactic acid bacteria is called bacteriocin. Bacteriocin is an active compound produced by bacteria containing lactic acid that can act as a bacteriostatic agent, one of which is found in fermented drinks[8].

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The antibacterial activity test of Nardus grass kombucha (*Cymbopogon nardus* (L.) Rendle) observed against Propionibacterium acnes bacteria and Staphylococcus epidermidis bacteria produced an inhibition zone which can be seen in Table 3, Propionibacterium acnes bacteria in successive concentrations of 70%, 80%, 90% Nardus grass kombucha (*Cymbopogon nardus* (L.) Rendle) showed an average inhibition zone diameter of 16.60 mm, 18.53 mm, and 20.96 mm, these samples showed a strong inhibition category. Meanwhile, in *Staphylococcus epidermidis* bacteria, successive concentrations of 70%, 80%, and 90% of Nardus grass kombucha (*Cymbopogon nardus* (L.) Rendle) showed an average inhibition zone diameter of 15.40 mm, 16.30 mm, and 17.53 mm, indicating a strong inhibition category. The best concentration to inhibit the growth of *Propionibacterium acnes* and *Staphylococcus epidermidis* bacteria is a concentration of 90% as stated in (Table 3). These results are in accordance with the literature, namely that the greater the concentration of kombucha used, the greater the inhibition zone formed because the antibacterial substances in kombucha also increase. One of the organic chemical acids produced during the kombucha fermentation process in general is acetic acid. Acetic acid that has been formed through kombucha fermentation has great potential to inhibit the growth of gram-positive and negative bacteria. The results of research conducted by Kumar & Joshi, [20], concluded that acetic acid that has been formed during kombucha fermentation will decompose through the mechanism of releasing free protons, causing the pH of the media to be low. In (Table 3) can be seen that the results of the inhibition test of Nardus grass kombucha ((L.) Rendle) show that the inhibitory activity against *Propionibacterium acnes* bacteria is greater than that of *Staphylococcus*

epidermidis bacteria. According to research by Marbun et al, (2021), the difference in results between *Propionibacterium acnes* and *Staphylococcus epidermidis* bacteria can be because each bacteria has different properties and resistance to an antibacterial even though the bacteria are included in the same group, namely both are Gram-positive bacteria. Propionibacterium acnes bacteria have slow bacterial growth properties (lag phase), while Staphylococcus epidermidis bacteria are the opposite[21]. Several organisms associated with food borne illnesses were sensitive to a Kombucha solution which had reached a pH of 2.5. Additionally, the study noted that even when the pH was made neutral and the solution was thermally denatured there was still an antimicrobial affect exerted against the bacteria indicating the presence of other antimicrobial compounds [22].

4. Conclusion

The inhibition zones of Propionibacterium acnes in lemongrass kombucha (Cymbopogon nardus (L.) Rendle) at concentrations of 70%, 80%, and 90% showed average inhibition zone diameters of 16.60 mm, 18.53 mm, and 20.96 mm, respectively, categorized as strong inhibition. Meanwhile, Staphylococcus epidermidis bacteria at concentrations of 70%, 80%, and 90%, respectively, showed average inhibition zone diameters of 15.40 mm, 16.30 mm, and 17.53 mm, respectively, categorized as strong inhibition.

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