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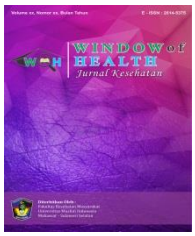

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Formulation of Body Odor Bacteria Inhibiting Deodorant Spray from Ethyl Acetate Extract of Klika Kesambi

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ABSTRACT

Acne is one of the skin damages caused by acne-causing bacteria. Flavonoids, saponins, and tannins in taro tubers can potentially have activity against acne-causing bacteria. This study aims to determine the formulation of extract transparent solid soap preparation that meets the characteristics and assess the activity of soap preparation as anti-acne against acne-causing bacteria. Taro tubers were extracted by maceration method. The extract was formulated as transparent solid soap preparations with variations in extract concentration, namely F0 0%, F1 2%, F2 4%, and F3 8%. The soap was evaluated for characteristics including organoleptic test, pH, moisture content, free fatty acids and alkali, mineral oil test, and foam stability test. Antibacterial activity testing of transparent soap extracts was carried out against acne-causing bacteria. *The* extraction results obtained an extract yield of 10.86%, the three extract formulas have transparent solid soap characteristics that meet the requirements. Soap preparation formula 1 has an inhibition zone of 21.66 ± 0.31 mm against P. *acnes* bacteria, 19.11±0.53 mm against S. *epidermidis*, 21.74±0.34 mm against S. *aureus*. Soap preparation formula 2 has an inhibition zone of 18.53±0.26 mm against P. *acnes* bacteria, 18.71±0.49 mm against S. *epidermidis*, 20.16±0.34 mm against S.*aureus* and soap preparation formula 3 has an inhibition zone of 19.03±0.68 mm against P. *acnes* bacteria, 19.93±0.86 mm against S. *epidermidis*, 22.75±0.59 mm against S.*aureus*. In conclusion, formula 2 with 2% extract concentration has the best soap characteristics and has the most optimal inhibition zone.

Keywords: Deodorant spray; klika kesambi; *Staphylococcus aureus*; *Staphylococcus epidermidis*.

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INTRODUCTION

Indonesia is a tropical country that has high sun intensity, so excessive sweat production is inevitable. Excessive sweating can cause problems, such as body odor.[1] Unpleasant body odor often makes a person insecure and can interfere with daily life, especially for socializing and appearance.

Body odor occurs due to a lack of body hygiene and the presence of bacteria that can break down sweat into substances that smell bad. Usually, human body odor comes from the apocrine glands. Apocrine glands can secrete most of the chemical compounds needed by the skin flora to produce odor.[2] The resulting odor is caused by the activity of bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis, Corynebacterium acne, Pseudomonas aeruginosa,* and *Streptococcus pyogenes*.[3] One solution to prevent body odor is by using cosmetics.

The use of soap as a body wash is not enough to prevent body odor, therefore many prefer to use an additional alternative, namely deodorant. Deodorant is a cosmetic product used to treat body odor caused by sweat mixed with bacteria, deodorant reduces body odor by suppressing the growth of bacteria, bacteria that cause body odor [4]. One of the most commonly used types of deodorant is liquid deodorant.

Deodorants in liquid form can usually be packaged in *spray* bottles so that they are easier and more practical to use and easy to carry around. Deodorant spray is a cosmetic preparation used to absorb sweat, cover body odor, and reduce body odor by spraying on certain body parts. The main advantage of *spray* deodorant when compared to other forms of deodorant is that the *spray* deodorant *delivery* system does not involve contact between the deodorant and the user's skin so hygiene is high.[5]

Deodorants generally contain antibacterial active substances that can be of natural or synthetic origin. The use of synthetic ingredients can cause effects such as skin irritation, Alzheimer's disease, prostate cancer, and breast cancer.[6] Deodorants containing herbal ingredients are relatively safer to use for cosmetics than synthetic ingredients. One of the natural ingredients that has antibacterial activity to overcome body odor bacteria is klika kesambi *(Schleichera oleosa* Lour. Oken).

Empirically, the kesambi plant is believed to be a medicinal plant, klika kesambi can be used as a tanning material, a very potent skin medicine, especially against scurvy and other skin diseases. Clika kesambi extract contains triterpenoid, flavonoid, steroid, and phenolic compounds.[7] Triterpenoids from kesambi bark, namely *teraxerone* and *tricadenic acid A,* have antibacterial activity against *Staphylococcus aureus*.[8]

Antibacterial activity research on kesambi bark extract with a concentration of 5000 µg/ml has an inhibition zone against *Staphylococcus aureus* and *Eschericia coli* bacteria. Ethyl acetate extract of kesambi bark has a high inhibition zone against *S.aureus* bacteria (8.9 mm) and *E.coli* (7.9 mm) compared to methanol extract and n-hexan extract.[7] Based on the background that has been described that kesambi can have an effect as an antibacterial and is widely used empirically by the community, it is necessary to formulate and test the effectiveness of kesambi clica extract *(Schleichera oleosa* Lour. Oken) against *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria. There is no report on

the formulation of deodorant spray preparations and the effectiveness test of the preparation, so it is important to conduct research.

The purpose of the study was to obtain the inhibition zone of klika kesambi extract (*Schleichera oleosa* Lour. Oken) against *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria. *To* obtain a deodorant formula of ethyl acetate extract of klika kesambi that is physically stable and to determine the effectiveness of deodorant of ethyl acetate extract of klika kesambi in inhibiting bacteria that cause body odor.

METHODS

In conducting this research, an experimental method was used with a *post-test with a control group* design. This research was conducted at the Pharmaceutical Biology Laboratory, Pharmaceutics and Microbiology Laboratory of the College of Pharmacy Makasar in June - August 2020. The tools used in this study are an autoclave (Onemed®), maceration vessel, sterile petri dish (Andromax®), climatic chamber (Climacell®), incubator (Memmert®) and caliper (Krisbow® **).** The materials used in this study were distilled water, bacterial culture of *Staphylococcus aureus* and *Staphylococcus epidermidis*, DMDM Hydantoin, 10% DMSO (Emsure®), ethyl acetate (Onemed®), klika kesambi, *Mueller Hinton Agar (*MHA) medium, Tetracycline paper disk, (Oxoid®), propylenglikol and tween 80. Sample collection and processing, namely the sample used is klika kesambi (*Schleichera oleosa* Lour. Oken) obtained from Bajeng Village, Patallassang District, Takalar Regency, South Sulawesi. Samples were taken, collected then wet sorted and washed with running water until clean, then drained. The samples were then cut into pieces and dried, after which dry sorting was carried out. The making of klika kesambi extract was carried out by maceration method, namely, simplistic weighed as much as 500 g and then soaked using ethyl acetate solvent as much as 5000 ml for 3 x 24 hours. The extract was then filtered, obtained filtrate and residue. The residue was remacerated with the same solvent and maceration time. Then, the filtrate obtained is evaporated until a dry extract is obtained. [7] Chemical content of the extract is tested, namely (1) Alkaloid test[9] (2) Flavonoid test[9] (3) Tanin test[10] (4) Saponin test[10] (5) Steroid/Terpenoid test[9]**.** Testing of antibacterial activity is carried out by the diffusion method using a paper disk. The test bacterial suspension was scratched on the MHA medium that had solidified in a Petri dish. A 6 mm diameter paper disk that has been soaked with a test solution of ethyl acetate extract of klika kesambi with concentrations (1%, 3% and 5%), 10% DMSO as a negative control, and Tetracycline as a positive control, is placed in a Petri dish. Incubated at 37°C for 1 x 24 hours and then measured the inhibition zone with a caliper.

Table 1. Deodorant Spray Formulation

Composition	Concentration (%b/v)			Usability
	Formula 1	Formula 2	Formula 3	
Ethyl acetate extract klika kesambi	1	3	5	Active substance
Propylenglycol	10	10	10	Humektan
Tween 80	1,5	1,5	1,5	Dispersers
DMDM hydantoin	0,1	0,1	0,1	Preservatives
Aquadest	ad 100	ad 100	ad 100	Solvent

Preparation of Deodorant *spray* was made by mixing ethyl acetate extract of klika kesambi with tween 80, then adding propylenglycol, crushed ad homogeneous added DMDM Hydantoin and distilled water, and then homogenized. The evaluation of deodorant spray preparation includes (1) Accelerated Storage Test: an accelerated test was carried out (storage with accelerated conditions) using a climatic chamber at 40 ° C with 70% Rh for 4 weeks[11] (2) Organoleptic Test: observed changes in color, aroma, and shape that occur in deodorant preparations.[11] Performed before and after accelerated storage, (3) Homogeneity Test**:** the deodorant preparation is placed on a glass object then leveled and observed visually. Homogeneity is indicated by the absence of coarse grains.[11] Performed before and after accelerated storage. (4) pH test: the deodorant preparation is dipped in a pH meter to see the pH of the preparation.[12] Performed before and after accelerated storage conditions. (5) Specific gravity test: Performed using a pycnometer that is dried and weighed first. Water was put into the pycnometer and allowed to stand at 25°C for 10 minutes. The pycnometer is removed and weighed.[11] Repeated using deodorant preparation instead of water. Performed before and after accelerated storage conditions. (6) Transferred Volume Test: A calibrated 30 ml bottle. The deodorant preparation is put into a 30 ml bottle until the limit mark. Poured the preparation back into a 100 ml measuring cup to know the volume moved and the accuracy of calibration.[12] The deodorant *spray* effectiveness test was carried out using the pitting method. The inoculated *Staphylococcus aureus* and *Staphylococcus epidermidis* bacterial suspensions were poured into the solidified MHA medium. Then pits were made on the MHA medium using iron plugs. The test was carried out by inserting a stable formula, the negative control was formula 4 in the form of deodorant preparations without extracts and the positive control was herbal deodorant product brand X into the wells as much as 30 µl. Petri dishes were incubated for 1 x 24 hours at 37°C. Measurements were made on the clear zone formed around the wells, which showed the area of bacterial growth inhibition.[11] The data obtained were analyzed descriptively based on the results of the physical stability test of the deodorant *spray* compared to the standard testing parameters and the results of the deodorant effectiveness test against *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria.

RESULTS

Table 2. Extraction results of Klika Kesambi

Sample	Fresh sampel (gram)	Extarct (gram)	Extract yield (%)
Klika kesambi	500	6,22	1,244%

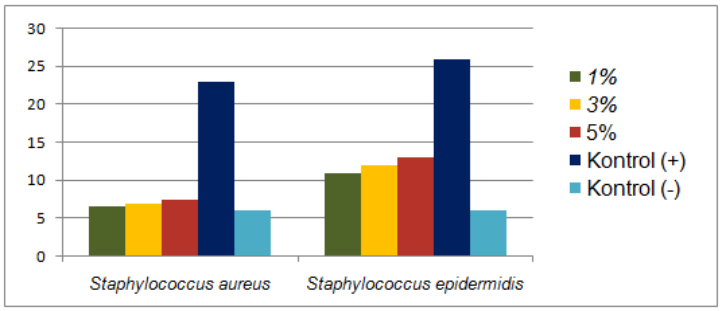
Based on table 2, the extract yield is 1.244%. Extract yield shows the percentage of raw materials that can be used or utilized with total raw materials. The higher yield value indicates that the raw material has the opportunity to be utilized.

Table 3. Phytochemical Screening Test Results of Extracts

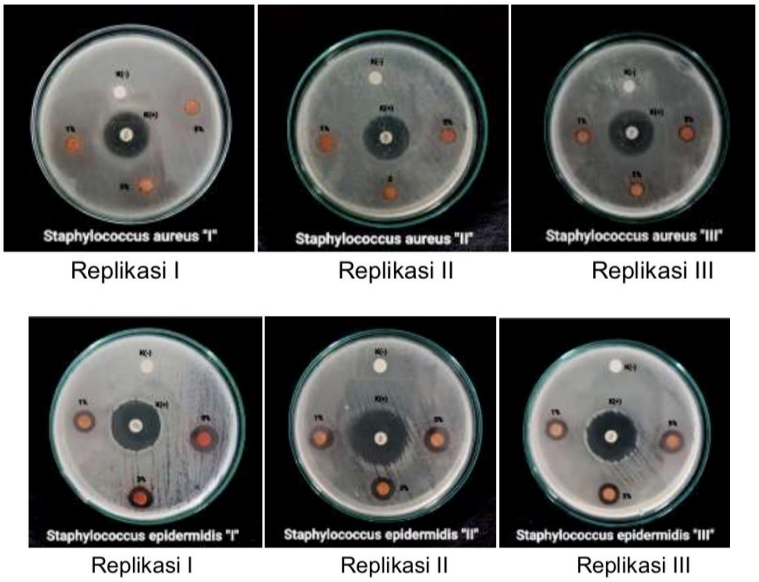
Test	Reagents	Theory	Results	Documentation	Information
Alkaloid	Mayer	white/yellow precipitate	Dark red Precipitate		-
	Dragen dorf	Red/orange precipitate	Brown precipitate		
	Wagner	Brown precipitate	Orange solution		
Flavonoid	Mg powder, HCl	Red/Orange/purple	Red		+
Tanin	Hot water, $FeCl_3$	Blackish/ Blackish blue	Blackish green		+
Saponin	Hot water, shake for (foam), HCl	1-10 cm foam, 10 minutes not disappear	Orange solution does not foam		-
Steroid/terpenoid	n-hexane, anhydrous acetic acid, concentrated sulfuric	Steroid : green/blue Terpenoid : red/orange/purple	Red		+

Description: (+) : Contains chemical compounds
(-) : Does not contain chemical compounds

The results of phytochemical screening of klika kesambi extract in table 3 show the content of secondary metabolites including flavonoids, tannins and terpenoids that have the potential as antibacterial causes of body odor.



Figh 1. Graph of antibacterial activity of ethyl acetate extract of klika kesambi against *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria with concentrations of 1%, 3% and 5%. As well as using control (-) DMSO 10% and control (+) Tetracycline.



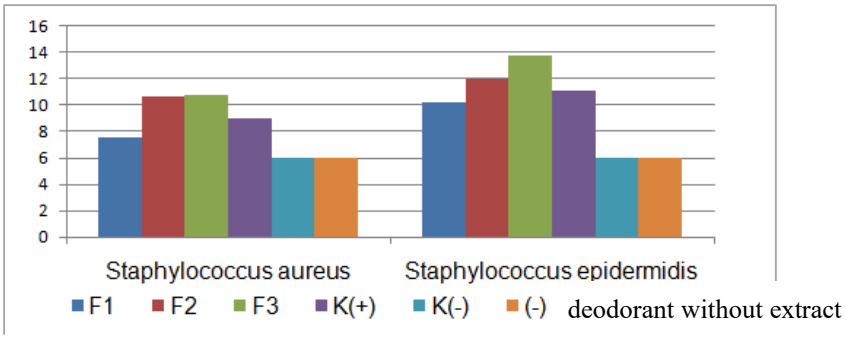
Figh 2. The results of the antibacterial activity test of ethyl acetate extract of klika kesambi against *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria with concentrations of 1%, 3% and 5%. As well as using control (-) DMSO 10% and control (+) Tetracycline in Petri dishes.

The results of the antibacterial activity test of klika kesambi extract against bacteria in Figure 1 and Figure 2 show activity against *Staphylococcus aureus* and *Staphylococcus epidermidis bacteria*, the higher the concentration of the extract, the greater the inhibition zone.

Tabel 4. Deodorant Spray Preparation Evaluation Results

Parameters	Formula	Before accelerated storage conditions	After accelerated storage conditions
Organoleptic :			
Color	F1	Orange	Orange
	F2	Brown	Brown
	F3	Dark brown	Dark brown
Aroma	F1	Characteristic aroma of the extract	Characteristic aroma of the extract
	F2	Characteristic aroma of the extract	Characteristic aroma of the extract
	F3	Characteristic aroma of the extract	Characteristic aroma of the extract
Shape	F1	Liquid	Liquid
	F1	Liquid	Liquid
	F2	Liquid	Liquid
Homogenieity	F1	Homogeneous	Homogeneous
	F2	Homogeneous	Homogeneous
	F3	Homogeneous	Homogeneous
pH	F1	4,86	4,82
	F2	4,77	4,72
	F3	4,69	4,61
Specific gravity	F1	1,012	1,011
	F2	1,011	1,010
	F3	1,018	1,010
Displaced volume	F1	30 ml	30 ml
	F2	30 ml	30 ml
	F3	30 ml	30 ml

The results of the evaluation of the deodorant *spray of* klika kesambi extract in table 4, show the stability of the preparation during the shelf life after accelerated storage conditions and all three formulas meet the preparation requirements according to compedial.



Figh 3. The results of the effectiveness test of deodorant *spray* preparations in F1 (1% extract); F2 (3% extract); F3 (5% extract); K(+) herbal deodorant preparation brand X; K(-) deodorant without extract and K(-) deodorant without extract and preservative

The results of the effectiveness test of the deodorant *spray* preparation in Figure 3 show the effectiveness of the preparation against *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria.

DISCUSSION

The extracted klika kesambi obtained a yield of 1.244%. The extract yield obtained is higher than the yield results in the research of Situmeang et al, where the yield obtained was 0.85%.[7] Extract yield is the percentage of raw materials that can be used or utilized with total raw materials. The higher the yield value indicates that the raw material has greater efficacious substances.[13] The results of phytochemical screening of ethyl acetate extract of klika kesambi contain secondary metabolite compounds including flavonoids, tannins and terpenoids that are thought to function as antibacterials. The results obtained are in accordance with Situmeang's research, where kesambi extract contains flavonoids, tannins and terpenoids.

Furthermore, the antibacterial activity of klika kesambi extract was tested with a concentration of 1%, 3% and 5%, using 10% DMSO as a negative control and Tetracycline paper disk as a positive control. Testing the antibacterial activity of ethyl acetate extract of klika kesambi against *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria using agar diffusion method using paperdisk. This method is used because it is quite simple and effective to determine the antibacterial inhibition zone activity of an extract using paperdisk. The average diameter of the inhibition zone of ethyl acetate extract of klika kesambi concentrations of 1%, 3% and 5% against *Staphylococcus aureus* and *Staphylococcus epidermidis is* categorized as moderate to strong in inhibiting bacterial growth. Based on the test results contained in the graph above, it can be concluded that the higher the concentration of the extract, the greater the inhibition zone formed. The concentration of ethyl acetate extract of klika kesambi 1%, 3% and 5% is then formulated in a deodorant *spray* preparation. The physical stability test before and after accelerated storage conditions using *climatic chamber* includes organoleptic, homogeneity, pH, specific gravity, and volume moved tests.

The organoleptic test aims to determine the physical properties of the deodorant and observe any changes in shape, color and aroma that may occur during storage. Table 4 shows that the higher the concentration of extract, the more intense the color produced in the preparation. The results of observations before and after accelerated storage conditions did not change in the three formulas. It can be concluded that there is no chemical reaction between the ethyl acetate extract of klika kesambi and additional ingredients in the deodorant formula.

The purpose of this preparation homogeneity test is to see the uniformity of the preparation particles so as to produce maximum effect. Homogeneity is an important factor in preparation because it can affect drug distribution. The preparation is said to be homogeneous when there is a color similarity and the absence of particles or coarse material that can be felt. The results of testing the

homogeneity of the preparations of the four formulas before and after accelerated storage conditions are homogeneous, remain stable, there is no separation or sediment between the components in the preparation.

Ph stability is one of the important parameters to determine whether a preparation is stable or not. The pH value measurement is carried out using a pH meter, the pH value test is carried out to ensure that the pH of the kesambi bark extract deodorant preparation made does not irritate the skin, the pH value of the preparation should not be too acidic because it will cause irritation to the skin and should not be too alkaline because it will cause scaly skin. Underarm skin has a pH that is different from physiological skin in general where the physiological pH of the skin is 4.5-6.5 while the pH of underarm skin is 4-6.8.[14] pH testing was observed both before and after accelerated storage conditions. Testing the pH before accelerated storage conditions has decreased pH, it can be concluded that the higher the concentration of extract used, the more acidic the pH of the preparation deodorant. The results of the pH values obtained in formulas 1, 2, and 3 also decreased after accelerated storage conditions. Although there is a decrease in pH, the three formulas remain in the pH range that is by the physiological pH of the skin 4.5-6.5, and the pH of the underarm skin 4-6.8. The decrease in pH can be caused by environmental factors such as temperature and poor storage, but the decrease is not much different. One of the things that can affect the decrease in pH is the hydrolysis that can occur in the preparation. The component that can undergo hydrolysis is tween 80. Storage of tween 80 at room temperature results in hydrolysis of fatty acid esters. The temperature between (25-40°C) and interaction with water supports hydrolysis so that long-chain fatty acids are formed. This supports a decrease in pH in preparations containing tween 80.[15]

A specific gravity test was conducted to determine the specific gravity of the deodorant preparation. The specific gravity of water based on the Indonesian National Standard is 1.01-1.1 g/ml. The value of the specific gravity of this deodorant preparation is influenced by its constituent ingredients and physical properties. Based on the results of the specific gravity test, the value is between 1.00-1.02 g/ml, which means that the formulated deodorant preparation still meets the standards set by SNI. Thus, the specific gravity of this deodorant can be easily cleaned with running water because it has a specific gravity that is close to the specific gravity of water.[14] The volume moved test was carried out using a measuring cup, which aims to determine whether the preparation has a fixed volume after being transferred from the bottle to the measuring cup.[14] The results of the displaced volume test of the three spray deodorant preparations produced a fixed volume before and after accelerated storage conditions.

Testing the effectiveness of the deodorant *spray* preparation of ethyl acetate extract of kesambi bark was carried out by the solid diffusion method, namely making wells on the media. The well method was chosen because the process is relatively easy and allows the preparation test material to be directly in contact with the walls of the agar media, so that the inhibition zone will be more easily observed visually by measuring the inhibition zone around the wells where the bacteria are inhibited by

antibacterials. Testing against *Staphylococcus aureus* bacteria found that the average inhibition zone formed in formulas 1, 2 and 3 included the category of moderate to strong inhibition zone, the positive control which is a deodorant spray product on the market has a moderate inhibition zone category and in the negative control, no inhibition area is formed.

The inhibition zone formed is due to the presence of antibacterial compounds in kesambi bark. Antibacterial compounds found in kesambi clica are flavonoids, tannins and terpenoids. Flavonoids, tannins and terpenoids are compounds in plants that have antibacterial activity. The mechanism of action of flavonoids provides bacteriolytic effects, inhibits protein synthesis, DNA synthesis, RNA and damages membrane permeability. Flavonoids have antibacterial activity due to the ability of flavonoids to interact with cell membranes and affect the bioactivity of cell membranes and it has been reported that flavonoids are able to reduce the fluidity of bacterial cell membranes. which is directly related to damage to the cytoplasmic membrane or indirect damage through autolysis or weakening of the cell wall and consequent osmotic lysis.[16]

The mechanism of tannins as antibacterial is by wrinkling the cell wall or cell membrane so that it disrupts the permeability of the cell itself. Due to the disruption of permeability, the cell cannot carry out life activities so that its growth is inhibited or even dies.[17] While the mechanism of action of terpenoids is to have broad antimicrobial activity against bacteria, yeast and filamentous fungi. Terpenoids are antimicrobial because they can damage yeast cell membranes or damage lipid membrane synthesis which results in membrane permeability resulting in leakage of cell components.[18]

CONCLUSIONS AND SUGGESTIONS

Based on the results of the research conducted, it can be concluded that klika kesambi extract has the potential to be formulated in the form of deodorant spray preparations that meet stability requirements after accelerated storage conditions. The results of the effectiveness test of the three formulas against *Staphylococcus aureus were* 7.60 ± 0.36 mm, 10.65 ± 0.35 mm and 10.77 ± 0.09 mm and *Staphylococcus epidermidis* were 10.22 ± 0.06 mm, 12.03 ± 0.11 mm and 13.81 ± 0.11 mm respectively with a strong category. In further research it is recommended to conduct safety and liking tests on the preparation of deodorant *spray* ethyl acetate extract of klika kesambi.

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