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PEANUT SUCROSE AGAR (PSA) OF TAKAR-2 PEANUT VARIETY As A SUBSTITUTE FOR ALTERNATIVE MEDIA FOR Trichophyton mentagrophytes By Retno Sasongkowati

PEANUT SUCROSE AGAR (PSA) OF TAKAR-2 PEANUT VARIETY As A SUBSTITUTE FOR ALTERNATIVE MEDIA FOR Trichophyton mentagrophytes Suliati1, Retno Sasongkowati1, Juliana Christyaningsih2 1 Health Analyst Department Health Polytechnic Surabaya 2 Nutrition Department Health Polytechnic Surabaya suli_ati@rocketmail.com ABSTRACT Background Trichophyton mentagrophytes caused dermatophytosis should be supported by laboratory tests using fungi culture. Method This experimental study was used a modified media Peanut Sucrose Agar (PSA) of local varieties of peanut in East Java: Hypoma-2, Talam-1, Jerapah, Bima, Takar-1, Tuban and Takar-2 with a concentration of 300 mg / 500 mL and cultivated Trichophyton mentagrophytes. Gold standard media was used PDA and to determine the nutritional composition then all media were analyzed PSA and PDA: lipid content, protein, reducing sugar and starch. Results The result indicated that the best growth Trichophyton mentagrophytes colony on PSA of Takar-2 peanut variety on the colony size, shape microconidium, macroconidium and hyphae, when its compared with the growth in PDA. Media PSA can be used as an alternative media that has economic value to the user community Mycology Laboratory. Conclusion The general conclusion of the research is a media PSA can be used as a replacement of PDA media with the best results of colony growth Trichophyton mentagrophytes and have economic value. Keywords: Trichophyton mentagrophytes, PSA, local peanut varieties in East Java INTRODUCTION Trichophyton mentagrophytes is one of a class of dermatophyte fungi that it causes dermatophytosis. Dermatophytosis is a disease caused by a fungal infection that it attacks the nails, hair and skin1. Trichophyton mentagrophytes has keratinase enzyme so as to damage the keratin in nails, hair, and skin2 . Dermatophytosis diagnosis should be supported by laboratory examination. One procedure in the laboratory to support the diagnosis of dermatophytosis is to use culture media3,4. In the culture media has a requirement for the adequacy of nutrients, temperature and pH as needed microorganisms will be bred. The different nutritional requirements depending on the type of microorganisms, but basically have the same basic needs : water, carbon, energy, minerals5. One of culture media for growing mushrooms was Potato Dextrose Agar (PDA)6 and a group of dermatophytes such as Trichophyton sp. can grow on PDA because the potato has a carbohydrate as nutrition and dextrose that becomes a carbon source for the growth of mold. Rehydrate medium PDA is relatively expensive, so it is necessary to study alternatives to dextrose in the medium mushrooms7. Sucrose is a sweet carbohydrates that are relatively inexpensive that it used as a carbon source other than dextrose. In addition

to sucrose, a culture medium for the fungus also need a source of other nutrients such as protein, lipid, phosphorus, and vitamins. The potatoes in PDA media is a source of protein, lipid and the nutritional content in accordance with the needs of the fungus. As alternatives, other nutrient sources to consider are peanut also contains carbohydrates, protein, lipid, phosphorus, and vitamin bigger than potatoes. The existence of phosphorus in the media used the fungus to develop a cellular component, and vitamins needed to accelerate the growth of mildew fungus8. From the content of the nutrients found in peanuts, so peanuts are believed to be used as a medium for the growth of fungi such as Trichophyton mentagrophytes9, 10. The Fifi's research (2015) indicates that the media modifications made from peanuts or peanut Sucrose Agar (PSA) can be used as a replacement PDA medium for mold growth as Trichophyton mentagrophytes. The concentration of peanuts in the media PSA on study Fifi, showing optimal colony growth was 300 mg / 500 mL. Peanuts used by Fifi, it is not clear if the analyst varieties so that other laboratories and institutions make the media PSA does not necessarily get the same results of growth of colony Trichophyton mentagrophytes11 . Based on data from the Ministry of Agriculture, Indonesia have 29 varieties of peanuts while in East Java there are seven varieties are spread in the area of agriculture. If the manufacture of PSA media was used different peanut varieties will possibly produce quality colonies of Trichophyton mentagrophytes, because the nutritional content of peanuts certain varieties will affect the nutritional profile media PSA is produced, ie the levels of carbohydrate, lipid and protein in the media PSA. Based on these descriptions, it is necessary to do a study to analyze the feasibility study and a nutrition profile as a media Peanut Sucrose order modification for Trichophyton mentagrophytes so that later can be used as an alternative media that has economic value to the user community laboratories RESEARCH METHOD This design of the research was the experimental study, with exploratory design research. The samples are peanuts several varieties found in East Java, was taken with random allocation. Some varieties of peanuts are obtained at the Research Institute of Plant Assorted beans and tubers, Malang, East Java. Peanut varieties are Takar 1, Takar 2, Tuban, Jerapah, Talam 1, Hypoma 2 dan Bima. Media were manufactured from Peanut Sucrose Agar media of the 7 varieties and Potato Dextrose Agar. Strain of Trichophyton mentagrophytes was purchase from Dr. Soetomo Hospital. The materials were used in this study is strain of Tricophyton mentagrophytes, peanuts of 7 varieties, sucrose or sugar, bacteriological agar, chloramphenicol, dyes Methylen Blue , Potato Dextrose Agar, Biuret reagent, Whatman filter paper, tube , amylase, Nelson A and Nelson B reagents, arsenomolybdat solution, vaseline, petroleum ether and distilled water . The instrument were used in this study : inoculum , autoclave, cotton lipidty, analytical balance, a petri dish, erlenmeyer flask, Bunsen, tripod, wire netting, funnel , soxhlet, spectrophotometers, cuvettes, centrifuge, test tubes, object glass , cover glass, microscope, analytical balance, pipette, vortex, water bath, mortar, desiccator, oven, condenser, soxhlet, hot plate and beaker glass . Manufacture Potato Dextrose Agar (PDA) as the Gold Standard (Neogen Corp , no. Cat . 7149) Among 19.5 grams of PDA powder media, dissolved in 500 mL of distilled water into the erlenmeyer. PDA solution was heated until homogeneous and boiling above the hot plate. pH media was adjusted to 5.6 and then sterilized in an autoclave at 121 °C , for 15 minutes. Chloramphenicol as much as 10 mg per 100 ml. Media was poured in petridish and cooled at room temperature . Manufacture of Modified Peanut Sucrose Agar About 300 grams of peanuts with certain varieties were refined manner by blending without water and then it boiled in 500 ml of distilled water for 15 minutes. Peanuts was filtered to using a gauze so that the filtrate of peanuts. At the filtrate was added 20 grams sugar, 10 grams of

agar and some distilled water to obtain a final volume of 1000 ml and heated. media pH is 5.6 and the medium were sterilized in an autoclave at 121 ° C, for 15 minutes. Chloramphenicol was added as much as 10 mg per 100 ml of the media poured in petridish and cooled at room temperature11 Growing of Trycophyton mentagrophytes Trichophyton mentagrophytes was inoculated in culture media modification of PSA and PDA, and then they were incubated at a temperature of 25? C for approximately 2 weeks. Identification of Trycophyton mentagrophytes Object glass was spilled one to two drops of dye Blue Methylen. Colony of each media was retrieved by using inoculum and mixed with dye Methylen Blue. It was closed with a cover glass and then fixed two to three times. It was viewed on a microscope to identify the structure of the morphological mold. The analysis of protein in Peanut Sucrose Agar and Potato Dextrose Agar media Peanut Sucrose Agar media and Potato Dextrose Agar sterile were crushed by using a mortar to reduce the size and weighed 3 grams by using an analytical balance. Samples distilled water and shaken out again. The sample were added 10 ml of distilled water to dissolve solution was measured absorbance at a the water-soluble protein. Samples were mixed wavelength of 540 nm. The absorbance value of and filtered using Whatman paper and filled into the sample - the absorbance value of the blank is test tube. Samples were centrifugated at 3000 then converted to mg / mL reducing sugar based rpm for 15 minutes to separate supernatant and compound standard regression equation. precipitate pellet, then added 2 ml of ethyl ether Reducing sugar is a reducing sugar levels which serves to dissolve the lipid in nuts and without enzyme. Starch = (reducing sugar levels centrifuge again with the speed of 3000 rpm for after a given enzyme amylase - reducing sugar 10 minutes to obtain the precipitate dried put in without enzymes) x 0.9 a test tube. Then take 1 ml of the supernatant is inserted into a test tube, and then all samples are The analysis of lipid in media Peanut Sucrose added 3 ml of distilled water. Add 6 ml biuret as Agar and Potato Dextrose Agar an indicator and wait for 30 minutes or The media of PSA and PDA incubation serves to allow time for the Biuret sterile crushed by using a mortar, then sample reagent to react with protein samples. Then weighed 5 grams by using an analytical balance. measuring the absorbance at a wavelength of The sample was entered into the extraction 520 nm with a spectrophotometer. The series of instrument. Petroleum ether as many as 35 ml standard protein solution were created and and put into the extraction instrument. After that added to distilled water until 4 mL. Biuret lit button on the Soxhlet and reflux for 5 hours. reagent was added 6 ml and wait for 30 After 5 hours, soxhlet apparatus turned off and minutes to allow time for the Biuret reagent to then the sample was inserted into the oven at react with the protein. Then the absorbance was 105 °C for 2 hours to reduce the water vapor measured using a spectrophotometer with a that still exist in the sample. Then cooled down wavelength of 520 nm and the results are in a desiccator for 15 minutes, then weighed and plotting to obtain the curve and linear regression the weight of the sample flask. Lipid was equation of standard . calculated based on a formula that has been determined, so that the results obtained lipid The analysis of carbohydrate on media levels in the media. Peanut Sucrose Agar and Potato Dextrose Agar Analysis feasibility study of PSA order as Carbohydrates were analyzed by the media modifications to Trichophyton method of Nelson-Samogyi. PSA and PDA mentagrophytes mediums sterile (5 g) were dissolved in 5 mL of 1. Define the quality of mold growth by distilled water added 143.75 mg of Amilase measuring the diameter of Trichophyton enzyme then shaken and allowed to stand for 6 mentagrophytes colonies on PSA media hours. Samples (1 mL) were added amilase and from certain peanut varieties, compared samples (1 mL) without amilase, each plus with media Gold Standard. distilled water until a final volume of 10 mL, 2. Analyze

the nutritional profile in the PSA then taken 1 mL added with 9 mL of distilled media for certain varieties by the best water and shaken with a vortex. The sample colony growth Trichophyton solution (1 mL) was added 1 mL Nelson mentagrophytes among all varieties of (Nelson A solution mixture and Nelson B; 25: 1 media compared to the Gold Standard v /v), then heated with a water bath at a 3. Analyzing the economic value of media temperature of 100 °C for 20 minutes. Sample producing modifications Peanut Sucrose solution was cooled to room temperature, then order with peanut varieties than the media added 1 mL of solution arsenomolybdat. The selected Gold Standard sample solution is shaken, then added 7 mL of RESULT Table 1. Results of observation of colony growth Trichophyton mentagrophytes in order Sucrose Peanut media with a variety of local peanut varieties No Peanuts varieties of PSA Colony size of Trichophyton mentagrophytes (cm) 1 Takar 1 2,5 2 Takar 2 3,5 3 Tuban 2,5 4 Jerapah 2,5 5 Talam 1 2,5 6 Hypoma 2 2,5 7 Bima 2,0 8 Potato Dextrose Agar (Gold 2,0 Standard media) From Table 1 showed that the growth of Trichophyton mentagrophytes with an incubation period at a temperature of 25?C for 2 weeks have the size of the colonies on media PSA made from local peanut types of Takar 2 (75 % greater) largest than colonies of mold growing on PDA as media gold standard. It showed that the composition of the nutrients found in PSA media Takar 2 is very suitable for the growth of the Trichophyton mentagrophytes indicated by the maximum size of the colony . Fig 1. The colony size of Trichophyton mentagrophytes on media. Table 2. The results of reduction sugar, starch, protein and lipid in a local variety of peanuts No Peanuts varieties Starch (%) Reduction Sugar Protein (%) Lipid (%) (%) 1 Takar 1 4,09 3,75 29,8 42,6 2 Takar 2 3,82 4,09 32,8 40,3 3 Tuban 3,07 2,77 21,4 42,5 4 Jerapah 3,85 4,67 21,5 43 5 Talam 1 3,65 3,95 26,3 45,4 6 Hypoma 2 5,01 5,28 23,08 47,97 7 Bima 4,23 4,18 25 46 Mean 3,96+0,59 4,10+0,78 25,70+4,30 43,97+2,60 From table 2 showed highest starch content contained in peanut varieties Hypoma 2 , the sugar content was highest reduction in peanut varieties Jerapah, the highest protein content of peanut varieties contained in Takar 2, the highest lipid content found in peanut varieties Hypoma 2. Test results nutrient content in peanuts by varieties somewhat different result but after statistical test, it was found that the research data was homogeneous (sig = 0.999) and furthermore do Anova statistical test to analyze differences in nutrient local peanuts. One Way ANOVA analysis results showed no significant difference in the nutritional peanut based varieties (sig = 1.00). Table 3. The result of nutritional content of Peanut media Sucrose Agar (PSA) with local variety of peanut and Potato Dextrose Agar (PDA) No Media Starch (%) Reduction Sugar (%) Protein (%) Lipid (%) 1 2 3 4 5 6 7 PSATakar 1 PSA Takar 2 PSA Tuban PSA Jerapah PSA Talam 1 PSA Hypoma 2 PSA Bima 3,72 2,59 2,96 2,80 3,20 3,98 3,44 3,96 2,99 3,08 3,18 4,04 4,04 3,87 3,62 2,98 3,53 2,88 3,02 3,42 3,08 1,08 1,02 1,19 1,25 1,28 1,58 1,62 Mean 3,24 3,59 3,22 1,29 8 PDA 3,67 4,02 3,68 1,69 From Table 3 showed the average nutrient of PSA made from a variety of local peanut varieties, have lower value than the PDA media as a medium gold standard , in terms of the starch content, reducing sugar, protein and lipid. The results of the examination content of nutrients in the media PSA by peanut varieties after a statistical test, it was found that the research data was homogeneous (sig = 0.992) and normal, so do Anova statistical test to analyze differences in the nutrient media PSA and PDA. One Way ANOVA analysis results showed no significant differences in nutrient media every peanut varieties PSA and PDA (sig = 0.912). Table 4. Calculation of the economic value of producing Peanut Sucrose order as a medium for Trichophyton mentagrophytes modifications compared with standard media PDA and SDA. Information Potato Dextrose Agar Sabaroud Dextrose Agar Peanut Sucrose Agar Price of media/ Rp 890,5 Rp 1.495,- Rp 430,- plate Trichophyton mentagrophytes is caused dermatophytosis12 and

dermatophytosis that include tinea capitis that affects many school children in a rural hospital Maharastra in India. Of the 79 patients with the skin disease with the test materials such as hair, skin, and skin scrapings (in the period of the study) was found 19 (24.05%) positive cultures. Highest cause of tinea capitis was Trichophyton mentagrophytes, followed by Microsporum gypseum, Microsporum canis and Trichophyton rubrum13. The similar results were found in patients attending Dermatology outpatient department of the SRM Medical College Hospital and Research centre kattankulathur during period from January 2013 to December 2013, caused skin disease were 42,46% dermatophytes and 57,53% non-dermatophytes. Trichophyton mentagrophyte 48.38% was the commonest dermatophyte followed by Trichophyton rubrum 32.25%, Microsporum gypseum 12.90% and Microsporum cannis 6.45% 14. The morphological of Trichophyton mentagrophytes is identificated with slide culture. Rosana (2014) said slide culture method is best for oblique slide by slide culture and the modification because it can visulized dermatophytes structure better and faster than the conventional one. Media Germination should have nutritional requirements according to the needs of microorganisms15. The media use is rehydrate PDA and PSA. In the known potato protein content of about 2%, but in peanuts around 25.70+4.3%. If seen with colony growth Trichophyton mentagrophytes in PSA, the mean diameter of the colony is the largest diameter of 2.57 cm and 3.5 cm in peanut varieties takar2. Protein content was on the variety Takar2 by 32.8% and is the highest protein content of 7 varieties of peanuts. While pd PDA colony of Trichophyton mentagrophytes grew to a diameter of 2 cm with protein content of only 2%. Potato lipid content is 0.1 g peanuts while at 43.97+2.6%, and if been associated with the growth of fungal colonies was indeed Trichophyton mentagrophytes colony diameter is greater than the growth in media PDA CONCLUSION AND RECOMMENDATION The general conclusion of the research is a media PSA can be used as a replacement of PDA media with the best results of colony growth Trichophyton mentagrophytes and have economic value. This study was received financial support from the Polytechnic Health Surabaya.

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