BEEPOLEEN Tetragonula testaceitarsis ANTIBACTERIA (Propionibacterium acnes) TEST

by Enos Tangke Arung

Submission date: 25-Oct-2021 09:00AM (UTC+0700)
Submission ID: 1682988992
File name: Enos_Tangke_Arung-MCTrops_Paper_2020.pdf (317.18K)
Word count: 2720
Character count: 14828

Available online at http://ij.unmul.ac.id

MCTROPS

Vol 01 No 01
Mei 2020
ISSN:2746-1459

Short communication

BEEPOLEEN *Tetragonula testaceitarsis* ANTIBACTERIA (*Propionibacterium acnes*) TEST

Dinda Tiara Andini[1], R.R Harlinda Kuspradini[1,2], Irawan Wijaya Kusuma[1,2], Enih Rosamah[1], Rudy Agung Nugroho[2], Enos Tangke Arung[1,2*].

[1]Laboratory of Forest Product Chemistry, Forestry Faculty, Mulawarman University, JL. KH.Dewantara, Kamus Gn. Kelua (75123), Samarinda, Kalimantan Timur, Indonesia
[2]Research Center for Medicine and Cosmetics from Tropical Rainforest Resources, Mulawarman University, JL. KH.Dewantara, Kamus Gn. Kelua (75123)Samarinda, Kalimantan Timur, Indonesia
*Email Korespondensi : tangkearung@yahoo.com

ABSTRACT

Acne is a skin disease which is often found on the skin of most people in the world. Acne generally comes from a variety of bacteria, one of them is *Propionibacterium acnes*. Bee pollen from *Tetragonula testaceitarsis* contains alkaloids, flavonoids and tannins which has antibacterial potency. The purpose of this study was to determine the potential of beepollen from *T. testaceitarsis* which has a good antibacterial content as an anti-acne preparation. The method used in this research was the agar diffusion method. The results showed that the percentage of inhibition of beepollen samples from *T. testaceitarsis* was 47.77% at 500 ppm, 45.36% at 250 ppm and 40.21% at 125 ppm. Meanwhile, the average diameter of the resistance obtained value was15.44 mm

Keywords: Propionibacterium acnes, *Tetragonula testaceitarsis*, antibacteria

INTRODUCTION

Acne is a skin problem that is often found in the community is chronic and recurrent. Acne is not a life-threatening disease, but acne can cause psychological problems, ranging from feelings of inferiority to stress, besides that it is not uncommon that permanent scars can occur on the face (Sutanto, 2013). Acne appears when the skin oil glands are too active, so that the skin pores will be blocked by excessive fat deposits (Sawarkar et al., 2010). According to Witarsa (2011) these fatty acids can cause tissue inflammation when related to the immune system and support the occurrence of acne.

Currently, many special treatments have been carried out to treat or prevent the onset of acne, including the prevention of bacteria in the hair follicle channel and bacterial growth by using antibacterial. Antibacterial derives from various origin which can come from synthetic compounds such as clindamycin, erithomycin, benzoyl peroxide, azelaic acid, sulfur and can come from nature (Baumann and Keri, 2009). The use of natural ingredients as traditional medicines in Indonesia is increasing. Some natural materials have been also fabricated on a large scale. Considerably, the use of natural medicines has fewer side effects than drugs which derive from chemicals. In addition, the price of natural medicines is also more affordable than chemical drugs. One of natural medicines that derives from animals, is a product from bees (beepollen, honey and propolis).

Utilization of bee pollen as a herbal medicine has long been used, both direct use by traditional means and other forms such as for cosmetics and other body care products. One type of bee pollen that produces good pollen in Indonesia is the type of *Tetragonula testaceitarsis*. The bee pollen or

bee pollen itself is a very functional food because it contains carbohydrates, proteins, amino acids, fiber sugars, vitamins and mineral salts (LeBlanc et al., 2009). Previous studies found that beepollen from *Trigona* bee species contains flavonoids, tannins and alkaloids. Substances that are believed has a good content in bacterial deterrence.

Based on the background content of beepollen type *T. testaceitarsis*, it is expected that in the future it will be able to become one of the herbal choices as a good preparation for anti-acne ingredients. Thus, present study aimed to evaluate the potential of bee pollen from *T. testaceitarsis* as an antibacterial agent which can be prepared as an anti-acne.

MATERIALS AND METHODS

Research and sampling site

The study was conducted at the Laboratorium Kimia Hasil Hutan dan Energi Terbarukan, Forestry Faculty, Mulawarman University, Samarinda, Indonesia. Meanwhile, bee pollen from *T. testaceitarsis* bees was obtained from Penajam Paser Utara Regency, East Kalimantan Province, Indonesia.

Simplicia preparation and extraction

The bee pollen sample was weighed using analytical scales and immersed using 96% ethanol solution. The immersion was carried out for 3 x 24 hours with three times of filtering to separate the pure bee pollen extract solution. The extraction results were concentrated using a rotary evaporator with a temperature of 38-40˚C to obtained crude extract. The crude extract was oven for 5 to 7 days until the extract was completely concentrated.

Phytochemical Analysis

Phytochemical analysis was performed by using a colour change test referring to Harbone (1996) and Kokate et al. (2001) to detect the presence of primary metabolites (carbohydrates) and secondary metabolites (alkaloids, flavonoids, saponins, tannins, steroids, triterpenoids, coumarin , carotenoids). a) alkaloids: 5 mL extract + 2 mL HCl + 1 mL Dragendroff. Positive result if the colour of the solution turns orange or red. b) Flavonoids: 1 mL extract + 10 drops of 1% NaOH, the colour of the solution turns yellow, then in drops of HCl 1% as much as 10 drops of the solution turns into colourless, then positively contains flavonoids. C) Saponins: 5 mL extract + 2 mL acetone + 3 mL hot water, shake for 10 seconds. Positives which contain saponins if frothing for 10 minutes and did not disappear if 2N of HCl. d) Triterpenoids and Steroids: 1 mL extract + CH_3COOH 10 drops + H_2SO_4 10 drops. Positive steroids if the solution turns green or blue. The triterpenoid is positive when the solution turns red or purple. e) Carotenoids: 5 mL extract + 5 mL chloroform + H_2SO_4 85% 10 drops. Positive was indicates if the solution turns blue. f) Kumarin: 1 mL of extract + 10 drops of NaOH. Positive if the solution turns yellow. g) Tannins: 5 mL extract + (CH_3COO) 10 drops of 1% $_2$Pb, then precipitated. Positive if there is a yellow precipitate in the extract solution. h) Carbohydrates: 1 mL extract + Mollisch solution 2 drops + concentrated 10 drops H_2SO_4 Positive if there is a purple ring in the extract solution.

Antibacterial analysis preparation

The main ingredient used for making bacterial growth media is nutrient agar (NA). Bacterial growth media was made by mixing 10 g of agar powder, 6.5 g of nutrient broth (Merck, Darmstadt, Germany), 5 g of glucose and 500 mL of distilled water into a 500 mL erlenmeyer, The solution was boiled until completely dissolved and sterilized with an autoclave at

Available online at http://ij.unmul.ac.id

MCTROPS

Vol 01 No 01
Mei 2020
ISSN:2746-1459

121ºC for 15 minutes. Meanwhile, bee pollen extract was prepared. For stock solution, the 25 mg extract of bee pollen extract was weighed and dissolved into 1 mL of acetone.

Antibacterial activity assay

Antibacterial activity assay was carried out using the diffusion method referring to the Cappucino (2001) method with modification. In this assay, a 20 mL of NA and SDA were poured into sterilized Petri dish for 15 minutes at 121ºC in the autoclave. The sterile media was placed in aseptic state (in laminar flow) to let the media harden, then drops of 100 μL of bacteria were placed using a swap cotton and allowed to dry for ± 30 minutes. The wells were made using cork borer in each medium containing 20 μL samples with acetone as negative control, chloramphenicol as positive control, and samples test with final concentration of 125, 250 and 500 µg per well.

Data analysis

The relative inhibitory activity in antibacterial analysis was calculated using following equation:

Relative inhibitory activity (%) = 100 [x / y]

Where x is the diameter of inhibition (mm) in the test sample, containing extracts. Meanwhile, y is the diameter of positive control inhibition (mm) (Jones et al., 1991).

All tests were carried out with three replications. The average of three replications and standard deviation were tabulated and shown in a graph, followed by the percentage inhibition calculation. All data were performed in Microsoft Excel 2010 (Microsoft, Inc, USA). The percentage value of inhibiting bacterial growth can be seen based on Greenwood et al. (1995) (Table 1).

Table 1. Classification response of bacterial growth inhibition

Response classification Inhibition zone diameter (mm)	Response to inhibition
≥ 20	Very strong
16 – 20	Strong
10 – 15	Moderate
≤ 10	Weak

RESULTS AND DISCUSSION

Phytochemical screening test is carried out to identify the active compounds which contain in the plants. Present study showed that the ethanol extract of *T. testaceitarsis* bee pollen which obtained from Penajam Pasir Utara regency had several bioactive compounds (see Table 2).

Table 2. Phytochemical *screening* test of beepollen *Tetragonula testaceitarsis* samples

Phytochemicals	Indicator
Alkaloid	+
Flavonoid	+
Saponin	-
Tannin	+
Triterpenoid	-
Steroid	-
Carbohydrate	-
Carotenoid	-
Kumarin	-

Note : (+) = Present, (-) = Absent

The mechanism of action of the alkaloid as an antibacterial is predicted by inhibiting bacteria cell wall synthesis which caused cell lysis and die (González-Lamothe et al., 2009). The content of flavonoids can inhibit energy metabolism by inhibiting the use of oxygen by bacteria. Energy is needed by bacteria for macromolecular biosynthesis, so that if metabolism is inhibited then the bacterial molecule cannot

develop into complex molecules (Cushnie and Lamb, 2005). In addition, there were also phenolic compounds which can interfere with the growth of the *Porphyromonas gingivalis* bacteria in flavonoids. Phenol is an acidic alcohol that has the ability to denature proteins and damage bacterial cell membranes (Dwyana and Johannes, 2012). The mechanism of action of tannin as an antibacterial is by causing *Porphyromonas gingivalis* cells to become lysis. This because tannin has a target on the bacterial cell wall polypeptide wall, so that the formation of the cell wall becomes less the bacterial cell will die. Tannins also have the ability to activate bacterial enzymes and disrupt the course of proteins in the inner layer of cells (Ngajow et al., 2013). The compound that has been identified by phytochemical testing forms was the basis of further testing of the *Tetragonula testaceitarsis* ethanol beepollen sample as a good antibacterial preparation.

Antimicrobial or antibacterial activity consists of various kinds of microorganisms such as fungi or other bacteria, as well as other man-made substances. Antimicrobial or antibacterial activity is determined based on the percentage of inhibition relative to positive control. The presence of antibacterial activity is aimed at the formation of inhibitory zones. The formation of inhibitory zones shows the indication of antimicrobial or antibacterial activity (Muharni et al., 2017). Present results of the average diameter of inhibition are shown in Table 3, while the percentage of inhibition was in the Figure 2.

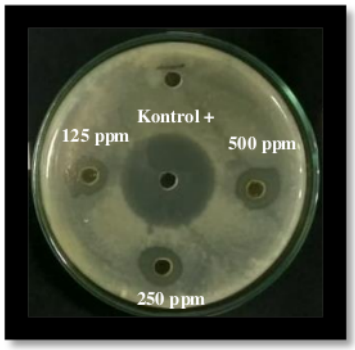

Figure 1. Antibakteri assay of bee pollen *Tetragonula testaceitarsis* against *Propionibacterium acnes*

Table 3. Mean diameter (mm) of beepollen *Tetragonula testaceitarsis* inhibition against *Propionibacterium acnes*

Sample name	Concentration (mg/20 μL)	Inhibition diameter (mm)	Inhibition response
Tetragonula testaceitarsis	Control (+)	32.33	Very strong
	500 ppm	15.45	Strong
	250 ppm	14.67 mm	Strong
	125 ppm	13.00 mm	Strong

The measurement of inhibition diameter with 3 replications obtained values for positive control was 32.33 mm (Figure 1), whereas for inhibition of beepollen samples of 3 concentrations namely 500 ppm, 250 ppm and 125 ppm, were obtained for 15.45 mm, 14.67 mm and 13 mm. These results indicated that a strong inhibitory response was obtained from the average diameter of beepollen inhibition of *P. acnes* from three different concentrations. The inhibition response refers to the scale of Greenwood et al. (1995) regarding the classification of inhibitory responses to

bacterial growth. In the inhibition with an average diameter above 20 mm is a very strong scale, while for the inhibition of the average diameter of 16-20 mm is a strong scale. The inhibition of the average diameter of 10-15 mm is a medium scale, and for the inhibition of the average diameter below 10 mm is categorized in the weak scale.

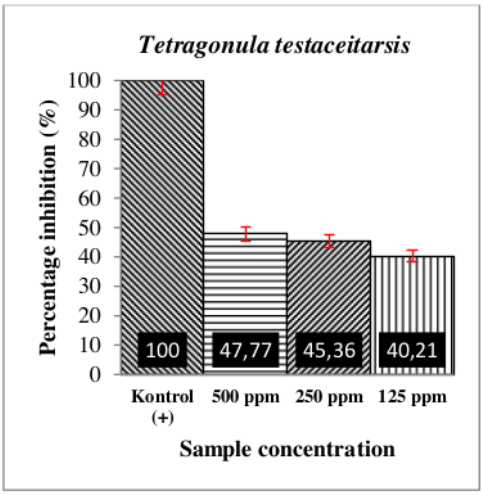

Figure 2. Inhibition percentage (%) of bee pollen *Tetragonula testaceitarsis* against P. acnes.

The percentage of *P. acnes* bacterial growth inhibition against beepollen from *T. testaceitarsis* bee species was compared to positive control (Chloramphenicol), acting in inhibiting bacteria. Positive control was considered as a reference in calculating the percentage of the sample's ability to inhibit the growth of the tested bacteria. In beepollen of *T. testaceitarsis* samples, a percentage of 47.77% was obtained at a concentration of 500 ppm, 45.36% at a concentration of 250 ppm and 40.21% at a concentration of 125 ppm. Calculation of the size of the percentage of inhibition is also influenced by the size of the average diameter of the bacterial growth. The closer to the diameter of the positive control, the greater the percentage the percentage of bacterial inhibition (Jones et al., 1991). Present result has never been reported that bee pollen extract from *T. testaceitarsis* can inhibit the growth of *P. acnes* bacteria, although there have been reports of Chuttong et al. (2018) that this bee pollen contained fatty acids and protein, but no biological activity has been reported.

Conclusion

It can be concluded that beepollen from *T. testaceitarsis* type contains alkaloids, flavonoids and tannins. The three compounds is the main ingredients in the inhibition of bacterial growth. The results of tests on the inhibition of *P. acnes* bacterial growth are also found to have a good result in inhibiting the growth of these bacteria. It is expected that beepollen from the type of *T. testaceitarsis* can be a good preparation product in inhibiting the growth of *P. acnes* which can cause acne in humans.

REFERENCES

Baumann, L., and J. Keri. 2009. Acne (Type 1 sensitive skin). Cosmetic dermatology principles and practice 2: 121-127.

Cappucino, J. G. a. S., N. The Benjamin Cummings Publishing Company. Rockland Community College.State University of New York. 2001. Microbiology: A Laboratory Manual. 2nd Edition.

Chuttong, B., R. Phongphisutthinant, K. Sringarm, M. Burgett, and O. M. Barth. 2018. Nutritional Composition of Pot-Pollen from Four Species of Stingless Bees (Meliponini) in Southeast Asia Pot-Pollen in Stingless Bee Melittology. p 313-324. Springer.

Cushnie, T. T., and A. J. Lamb. 2005. Antimicrobial activity of flavonoids. International journal of antimicrobial agents 26: 343-356.

Dwyana, Z., and E. Johannes. 2012. Uji Efektivitas Ekstrak Kasar Alga Merah *Eucheuma Cottonii* Sebagai Antibakteri Terhadap Bakteri Patogen. Jurnal Ilmu Kefarmasian 7: 1.

González-Lamothe, R. et al. 2009. Plant antimicrobial agents and their effects on plant and human pathogens. International journal of molecular sciences 10: 3400-3419.

Greenwood, D., R. Finch, P. Davey, and M. Wilcox. 1995. Antibiotics, Susceptibility (sensitivity) Test Antimicrobial and Chemoterapy. United State of America: Mc Graw Hill Company.

Harbone, J. 1996. Metode Fitokimia Terbitan ke II. Bandung: ITB.

Jones, D., S. Gorman, D. McCafferty, and A. Woolfson. 1991. The effects of three non-antibiotic, antimicrobial agents on the surface hydrophobicity of certain micro-organisms evaluated by different methods. Journal of applied bacteriology 71: 218-227.

Kokate, C., A. Purohit, and S. Gokhale. 2001. Carbohydrate and derived Products, drugs containing glycosides, drugs containing tannins, lipids and protein alkaloids. Text book of Pharmacognosy 7: 133-166.

LeBlanc, B. W., O. K. Davis, S. Boue, A. DeLucca, and T. Deeby. 2009. Antioxidant activity of Sonoran Desert bee pollen. Food Chemistry 115: 1299-1305.

Muharni, M., F. Fitrya, and F. Sofa. 2017. Uji Aktivitas Antibakteri Ekstrak Etanol Tanaman Obat Suku Musi di Kabupaten Musi Banyuasin, Sumatera Selatan. Jurnal Kefarmasian Indonesia 7: 127-135.

Ngajow, M., J. Abidjulu, and V. S. Kamu. 2013. Pengaruh antibakteri ekstrak kulit batang matoa (*Pometia pinnata*) terhadap bakteri *Staphylococcus aureus* secara in vitro. Jurnal Mipa 2: 128-132.

Sawarkar, H., S. Khadabadi, D. Mankar, I. Farooqui, and N. Jagtap. 2010. Development and biological evaluation of herbal anti-acne gel. Int J PharmTech Res 2: 2028-2031.

Sutanto, R. 2013. Derajat Penyakit Acne Vulgaris Berhubungan Positif Dengan Kadar MDA. Denpasar: Ilmu Biomedik Udayana.

Witarsa, W. 2011. Karakterisasi dan Skrining Fitokimia serta Uji Aktivitas Antibakteri Ekstrak Etanol Kulit Buah Tanaman Jengkol (*Pithecellobium lobatum* Benth.) Terhadap Beberapa Bakteri.

BEEPOLEEN Tetragonula testaceitarsis ANTIBACTERIA (Propionibacterium acnes) TEST

ORIGINALITY REPORT

5%	4%	1%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCHED SOURCE

3	Theo Audi Yanto, Mochammad Hatta, Agussalim Bukhari, Rosdiana Natzir. "Molecular and Immunological Mechanisms of Miana Leaf (Coleus Scutellariodes [L] Benth) in Infectious Diseases", Biomedical and Pharmacology Journal, 2020 Publication	1%

1%

★ Theo Audi Yanto, Mochammad Hatta, Agussalim Bukhari, Rosdiana Natzir. "Molecular and Immunological Mechanisms of Miana Leaf (Coleus Scutellariodes [L] Benth) in Infectious Diseases", Biomedical and Pharmacology Journal, 2020

Publication

Exclude quotes	On	Exclude matches	< 10 words
Exclude bibliography	On		