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WIDYA BIOLOGI

POTENTIAL MIXTURE OF PEGAGAN (Centella asiatica) AND PASPASAN (Coccinia grandis) EXTRACT WITH GREEN TEA AROMA AS ACNE MEDICINE

(POTENSI CAMPURAN EKSTRAK ETANOL PEGAGAN (*Centella asiatica)* DAN PASPASAN (*Coccinia grandis*) DENGAN AROMA TEH HIJAU SEBAGAI OBAT JERAWAT)

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ABSTRAK

Salah satu faktor penyebab jerawat adalah bakteri Propionibacterium acnes yang merupakan bakteri Gram positif dan anaerob yang hidup normal dalam saluran philosebaseae. Antibiotik sering digunakan untuk mengatasi jerawat dan penyakit lain yang disebabkan oleh bakteri. Padahal, selain mahal, pemakaian antibiotik terus menerus dapat memicu resistensi. Di Bali, banyak tumbuhan lokal yang bersifat antibakteri yang digunakan secara tradisional dan sudah tercatat dengan baik dalam lontar usadha Taru Premana. Tujuan penelitian ini adalah untuk mengetahui potensi antibakteri campuran ekstrak daun pegagan, daun paspasan, dan daun teh hijau sehingga dapat digunakan sebagai kandidat obat jerawat. Percobaan yang dilakukan adalah persiapan simplisia dengan pengeringan dan penepungan, ekstraksi dengan etanol, evaporasi untuk mendapatkan ekstrak kental etanol. Pengujian aktivitas antibakteri dilakukan dengan metode agar tuang dengan menggunakan bakteri uji Escherichia coli (Gram negatif) dan Micrococcus luteus (Gram positif). Didapatkan hasil bahwa paspasan dan pegagan bersifat bakteriostatik terhadap E.coli dan bakterisidal terhadap M.luteus dengan diameter daerah hambat terbesar adalah 2,5 cm. Ditemukan juga bahwa penambahan ekstrak teh hijau tidak mempengaruhi aktivitas antibakteri kedua simplisia. Disimpulkan bahwa ekstrak etanol pegagan dan paspasan berpotensi digunakan sebagai bahan obat jerawat.

Kata kunci: *pegagan, paspasan, teh hijau, obat jerawat, ekstrak etanol*

ABSTRACT

Propionibacterium acnes is one of the factors that cause acne. It is a Gram positive anaerobic bacterium that normally live in the philosebaseae canal. Antibiotics are usually used to eradicate the bacteria. In fact, besides being expensive, continuous use of antibiotics can trigger bacterial resistance. In Bali, many local plants that have antibacterial properties are used traditionally and have been well documented in the papyrus medicinal manuscript Taru Premana. The aim of this study is to determine the antibacterial potential of a mixture of gotu kola leaf extract, wild ivy leaves and green tea leaves as acne medication. The experiment was carried out by preparing simplicia by drying and flouring, extracting using ethanol and evaporation to obtain an ethanol

thick extract. Antibacterial activity testing was carried out by using diffusion agar method and Escherichia coli (Gram negative) and Micrococcus luteus (Gram positive) as the test organisms. The results show that wild ivy and gotu kola are bacteriostatic against E. coli and bactericidal against M. luteus with the largest diameter inhibition area is 2.5 cm. It was also found that the addition of the aroma substance of green tea did not affect the antibacterial activity of both simplicia. It was concluded that the ethanol extract of Centella asiatica and Coccinia grandis had the potency as acne medication.

Keywords: *gotu kola, wild ivy, teh hijau, obat jerawat, ekstrak etanol*

INTRODUCTION

Acne is a skin problem characterized by inflammation that is often experienced by teenagers. Acne will usually appear as the first sign of puberty and one year before the first menstruation for women. The process of acne formation is characterized by four factors, namely blockage of the philobasae duct, excessive sebum production, inflammation, and the activity of the bacteria *Propionibacterium acnes*. The initial formation of zits begins with a blockage of the oil and hair ducts (the philosebaseae channel) caused by androgen hormones which stimulate excessive formation of sebum (facial oil). Clogged philosebase canal causes sebum, keratin, and bacteria to accumulate in the skin and form blackheads. Blackheads containing sebum, keratin, and these bacteria will then expand. The contents of blackheads over time will come out and cause an inflammatory reaction (Movita, 2013).

The next factor that causes acne is the bacteria *Propionibacterium acnes*, which is a Gram positive and anaerobic bacteria that live normally in the philosebaseae canal. *P. acnes* in the infected skin break down the sebum component into free fatty acids which cause colonization of *P.acnes* which triggers inflammation (Movita, 2013). Understanding of acne formation factors (pathogenesis of zits) can help finding out how to treat and cure acne. Treatments include reducing the activity of the philosebaseae channel, reducing the population of *P.acnes*, and suppressing inflammation (Harper, 2010). Reducing the population of *P.acnes* can be done using antibiotics, such as erythromycin, clindamycin, and tetracycline (Harahap, 2000 in Rahmi, et al., 2015).

However, excessive use of antibiotics can lead to increased bacterial resistance to these antibiotics (Roslizawaty, 2013). Resistant bacterial organisms will use their genes to protect themselves from the bactericidal effects of antibiotics. In Madelina and Sulistyaningsih (2018), more than 50 percent of the *P. acnes* were resistant to clindamycin, making it less effective in the treatment of acne. Therefore, it is necessary to investigate other acne treatment methods, e.g. using medication made of natural ingredients.

Gotu kola (*Centella asiatica*) is a plant that has a myriad of benefits. In Southeast Asia, gotu kola is widely used as a medication for skin diseases, anti-inflammation, rheumatism, diarrhea, and dehydration. Prakasih et al. (2017) stated that gotu kola has an effective antibacterial and anti-inflammatory properties. In vitro experiments on *Centella asiatica* leaf extract activity against Gram positive bacteria, *Streptococcus aureus,* showed that the zone covered by leaf extract was quite extensive, namely 5-7 mm. In addition, the anti-inflammatory content of gotu kola leaves is also traditionally believed by the community to reduce inflammation. At concentrations of 2 mg/kg, the extracts showed moderate anti-inflammatory properties in prostaglandin E2-induction inflammation based on each dose. Gotu kola plants are also found in the traditional Balinese medicine stated in Taru Pramana papyrus medicinal manuscript which has been used since ancient times as a medicine for low blood pressure and chest tightness (Tengah et al., 1995).

In addition to gotu kola leaves, there are many types of medicinal plants recorded in the Usada Taru Pramana which are often used as traditional medicine. One of them is wild ivy leaves. This creeper was used in the treatment of Usada Bali as a medicine for fever and malaria (Tengah et al., 1995). However, research conducted by Farrukh et al. (2008) showed significant antibacterial activity against *Pseudomonas aeruginosa* and *Shigella boydii* bacteria. Sivaraj's et al. (2011) also showed high antibacterial activity in postpartum plants against *E. coli, B. sereus, S. aureus, S. phyogens*, and *K. pneumoniae*.

In the presence of antibacterial activity on the two plants, this research aimed to examine the potential of combination of gotu kola leaf extract and wild ivy leaves coupled with green tea

leaf extract as an aroma and antioxidant as an antibacterial agents.

Apparatuses

The apparatuses used in this study include blenders, sieves, a set of laboratory glassware (petri dishes, erlenmeyer, drop pipettes, sample bottles, methanol lamps), filter paper, micro pipettes, corkborers, hotplates, autoclave, laminar flow cabinet equipped with UV lights.

Materials

The materials used in this study included 95 percent ethanol, aquades, gotu kola leaves, wild ivy leaves, green tea extract, nutrient agar, nutrient broth, *Micrococcus luteus*, and *Escherichia coli.*

Variabels

The independent variables used in this study were extract concentrations namely A, B, C, D, and E. The control/fixed variable was ethanol solvent. While the dependent variable is the area of inhibition.

Methods

Samples Preparations

Gotu kola leaves and wild ivy leaves, each washed with clean water and drained. Then it is dried with a fan to produce wind dried material. The fan was used instead of sun drying in order that the aktive material contained in the simplicia is not damaged by heat.

Metabolite Extraction

Wind dried leaves were mixed with a blender and macerated with 200 ml of 95% ethanol for 48 hours and to speed up extraction, the marinade and solvent are re-blended and filtered with a wire filter. The filter was concentrated by evaporating the solvent using a fan. The concentrated extract is weighed.

Bioassays

Bioassays for the ethanol concentrated extract was carried out in to stages:

Preparation of Bacterial Suspension

One loop of test bacteria was inoculated into 50 ml *nutrient broth* (NB) in erlenmeyer which then incubated at 30 ^{0}C for 12 hours.

Bioassays

This stage was carried out in a laminar flow cabinet that has been sterilized with UV lights. 0.5 ml of bacterial suspension were transferred using micropipettes to each 25 ml of nutrient agar (NA) liquid with a temperature of about 50 ° C and poured aseptically into a sterile petri dishes and allowed to set at room temperature. After being set, holes was punched using a sterile cork borer. Samples were put into

each hole as much as 60 μL, then the agar were incubated for 12 hours at 30^{o}C. 95% ethanol is used as a control.

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RESULTS

Preparation of samples, extraction and evaporation

Of the 120 grams of fresh gotu kola leaves, 15 grams of air dried samples were obtained with 30% moisture content. While from 110 grams of wild ivy leaves, 50 grams of air dried material with 50% moisture content. Whereas the extraction and evaporation resulted in 2 ml of gotu kola extract and 4 ml of wild ivy thick extract.

Bioassays

Bioassays of gotukola thick extract and wild ivy thick extract against Gram negative test organism *Escherichia coli*

Antibacterial activity of gotu kola thick extract and wild ivy thick extract against Gram negative *Escherichia coli* bacteria were given in Table 1 and Table 2.

Table 1. Antibacterial activity of gotukola thick extract against Gram negative *Escherichia coli*

No.	Volume of gotukola thick extract (μl)	Diameter of inhibition zone (cm)	Volume of wild ivy thick extract (μl)	Diameter of inhibition zone (cm)
1	60	1.2	60	1.2
2	30	1	30	0.8
3	15	1	15	0.8
4	5	1	5	0.8

Table 2. Antibacterial activity of wild ivy thick extract against Gram negative *Escherichia coli*

No.	Volume of gotukola thick extract (μl)	Volume of wild ivy thick extract (μl)	Diameter of inhibition zone (cm)
1	60	0	1
2	30	30	1
3	15	45	1
4	5	55	1
5	0	60	1.2
6	30	30	1.0
7	45	15	1.0
8	55	5	1.0

From Table 4.1 and Table 4.2, namely the results of the antibacterial activity of thick extract of gotukola and thick extract of wild ivy against *E. coli* test bacteria, the results showed that gotu kola extract and wild ivy thick extract inhibited bacteriostatic growth of *E. coli*. The inhibition zone is formed which then after 24 hours is overgrown by the same test bacteria which is characterized by homogeneous turbidity in the agar medium but is more turbid in areas outside the inhibitory zone. Bacteriostatic is the ability of antibacterial materials to inhibit bacterial growth but does not kill the bacteria, while bactericidal is the ability of antibacterial materials to inhibit and kill bacteria.

Bioassays of gotukola thick extract and wild ivy thick extract against Gram positive test organism *Micrococcus luteus*

Table 3 Antibacterial activity of gotukola thick extract and wild ivy thick extract against Gram positive test organism *Micrococcus luteus*

No.	Volume of gotukola thick extract (µl)	Diameter of inhibition zone (cm)	Volume of wild ivy thick extract (µl)	Diameter of inhibition zone (cm)	Volume of ethanol 95% (µl)	Diameter of inhibition zone (cm)
1	60	0	1	60	0	2
2	30	30	1	30	30	1
3	15	45	1	15	45	1
4	5	55	1	5	55	1
5	0	60	0	0	60	0

Tabel 4.4 Antibacterial activity of mixture of gotukola thick extract and wild ivy thick extract against Gram positive test organism *Micrococcus luteus*

No.	Volume of gotukola thick extract (µl)	Volume of wild ivy thick extract (µl)	Volume of ethanol 95% (µl)	Diameter of inhibition zone (cm)
1	60	0	0	2
2	30	30	0	2
3	15	45	0	2
4	5	55	0	2
5	0	60	0	2.5
6	30	30	0	2
7	45	15	0	2
8	55	5	0	2
9	0	0	60	0

From Table 4.3 and Table 4.4, namely the results of antibacterial activity of gotu kola thick extract and wild ivy thick extract against *M. luteus* test bacteria, the results showed that gotu kola extract and wild ivy extract inhibited the growth of bacterial bacterial M. luteus test, which was characterized by the formation of inhibitory zones transparent. The inhibition zone produced by the wild ivy extract against the Micrococcus luteus test bacteria is very large compared to the inhibition zone formed in E. coli bacteria. Sivaraj et al. (2011) also found that wild ivy leaves were potentially antibacterial against Gram negative E. coli bacteria and some Gram positive bacteria *B.sereus, S.aureus, S.phyogens, and K.pneumoniae*. Whereas Prakasih, et al. (2017) states that gotu kola has an effective antibacterial effect. In vitro experiments of gotu kola leaf extract activity against Gram positive bacteria, Streptococcus aureus produced a large inhibition zone (5-7 mm).

Interaction of Gotu kola extract and wild ivy thick extract as antibacterial agents against Gram negative *Escherichia coli* test bacteria and Gram positive bacterial test *Micrococcus luteus*

The interaction of the active ingredients of the drug can be complementary (mutually adding effects), synergistic (mutually reinforcing effects) or agonists (canceling out effects) (Pharmacology Section FK UI, 2017). The results of this study indicate that gotu kola leaf extract and wild ivy leaf extract work complementary as bactericidal against Gram positive bacteria but bacteriostatic against Gram negative test bacteria. This is indicated by the lack of or increase in the area of inhibitory zones in different volume combinations of the thick extracts of the two simplicia (Table 3 and Table 4).

The results of the antibacterial activity of thick extract of green tea leaves against Gram negative Escherichia coli and Gram positive test bacteria Micrococcus luteus

Table 5 shows that green tea leaf extract does not produce inhibitory zones for the two test bacteria and also does not reduce the area of inhibitory zone when added to the thick extract of gotu kola and wild ivy. Thus, the aroma of green tea can be added to the mixture of gotu kola extract and wild ivy extract as an anti-acne drug preparation without affecting the antibacterial properties of the two ingredients.

Table 5. Antibacterial activity of mixture of gotukola thick extract, wild ivy thick extract and green tea extract against Gram positive test organism *Micrococcus luteus*

No.	Volume of green tea thick extract (µl)	Volume of gotukola thick extract (µl)	Volume of wild ivy thick extract (µl)	Volume of ethanol 95% (µl)	Diameter of inhibition zone (cm)
1	60	0	0	0	2
2	6	60	0	0	2
3	6	30	30	0	2
4	6	5	60	0	2
5	0	0	0	60	0

The results of this study indicate that the thick extract of both simplicia (gotu kola and wild ivy) has the potential to be used as an acne medication because *Propionibacterium acnes* which is an acne-causing bacterium is a Gram positive bacterium (Movita, 2013). The mixture of the three ingredients in 95% ethanol is also an added value if used in the preparation of acne medication because the polar nature of ethanol is the same as water. This can increase the hydration of the skin (Naibaho et al., 2013).

The antibacterial activity of wild ivy was also reported by Sukmarianti (2007) who conducted antibacterial tests against the same test bacteria. In thick ethanol extract, the area of inhibition of *M. luteus* was much greater than that of *E. coli* as test organisms. But in further purification using n-hexane, ethyl acetate, and chloroform (the order of polarity decreases), the area of the inhibition zone increases both aginst *E. coli* and *M. luteus*. In addition, it was also found that in the thick extracts of the three solvents above, the zone of inhibition of *E. coli* was greater than that of *M.luteus*. This shows that the anti-bacterial potential of wild ivy may be even higher if a drug preparation is in the form of an ointment (oil soluble).

DISCUSSIONS

Sukmarianti (2007) found that the antibacterial active ingredients contained in the extract of wild ivy was 1.5-dimethylbicyclo (3,3,1) -non-en-2,9-dione and hexadioic dioctyl ester. In her research, the results of the phytochemical test showed that the antibacterial active fraction did not contain compounds from flavonoids, alkaloids, triterpenoids, steroids, and saponins.

The antibacterial active ingredients contained in *Centella asiatica* are phenol and terpenoid compounds. According to Rachmawati and Nuria (tt), phenol in low concentrations can cause damage to the cytoplasmic membrane and cause cell nucleus leakage, whereas at high concentrations, phenol coagulate cellular proteins. This activity is very effective when bacterial cells are in very thin conditions during cell division which facilitate the phenol molecules to damage the cell nucleus. While the mechanism of terpenoid compounds as antibacterial is to react to the outer membrane of the bacterial cell wall which results in damage to the porin. Porin damage will reduce the permeability of the cell wall and become the entrance for terpenoid compounds so that bacteria lack nutrients until lysis occurs.

CONCLUSION

Antibacterial activity of gotu kola extract and wild ivy leaf extract was found in the active compounds contained in each simplicia. Gotu kola contains terpenoids and phenols which play a role in damaging bacterial cells. Whereas the mixture containing 1,5-dimethylbicyclo (3,3,1) -non-en-2,9-dione and hexadioic dioctyl ester act as antibacterial active compounds. In addition, the activity of gotu kola as an antibacterial against Gram positive bacteria *Micrococcus luteus* and Gram negative *Escherichia coli* showed significant results, namely the inhibition area of 1 cm and 1.2 cm with a concentration of 60 ml. While the wild ivy extract showed significant results, namely the inhibition area of 1.2 cm and 2 cm respectively against Gram positive bacteria and Gram negative bacteria at the same concentration.

The mixture of gotu kola extract, wild ivy leaves, and green tea leaves showed significant antibacterial effectiveness with a inhibition area of 2 cm at a fixed concentration of green tea extract and gotu kola extract and wild ivy leaf extract varied.

Gotu kola extract, wild ivy leaves, and green tea leaves work synergistically or mutually reinforcing which is shown in a consistent inhibition area of 2 cm with a fixed concentration of green tea leaf extract, and wild ivy extract and gotu kola extract vary.

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