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Antimicrobial Activities of Some Indonesian Medicinal Plants against *Propionibacterium acnes* (ATCC 27853) and *Staphylococcus epidermis* (ATCC 12228) causing Acne

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Secondary metabolites investigation of local plants, such as *Psidium guajava* L, *Curcuma longa rhizome*, *Citrus hystrix* and *Lawsonia intracellularis* shown that they have antibacterial activities against *Propionibacterium acnes* (ATCC 27853) and *Staphylococcus epidermis* (ATCC 12228) with chloramphenicol as antibiotic controlled. The phytoscreening method used to find out compounds in the plants and inhibition tests carried out by diffuse test with a concentration variation of 10, 5, 2.5 and 1.25 %. It was found that *Lawsonia intracellularis* plants has the highest inhibitory growth of 17; 12, 15, 14, 12 mm at 10 % concentration.

Keywords: Indian medicinal plants, Phytoscreening, *Propionibacterium acnes*, *Staphylococcus epidermis*.

INTRODUCTION

Secondary metabolite compounds are chemical compounds which have bioactive ability such as antibacterial [1,2] and function as plant protectors from pests and their environment. In general, secondary metabolites in biological materials consist of the nature and typical reaction of the secondary metabolite with specific reagents, which are alkaloids, terpenoids, flavonoids, phenolics, saponins, coumarins, quinone and carotenoids [3].

In general, *Propionibacterium acnes* and *Staphylococcus epidermis* are known as Gram-positive bacteria present in human skin causing inflammation and acne, which marked by the growth of blackheads, papules, pustules and nodules found on the face, chest and back and considered as a chronic inflammation [4,5]. Acne treatment is better carried out by sebum inflammation which is part of the oil found on the skin surface [6]. Medicinal plant extracts which have antibacterial activity against Gram-positive and Gram-negative bacteria are known as anti-inflammatory agents [7]. The plants can be used as antibacterial agents especially for killing vulgaris bacteria causing acne [8-12].

Several Indian medicinal plant extracts can be used to inhibit the growth of the *P. acnes* and *S. epidermis* bacteria on human skin [13]. Many tropical plants such as *Psidium guajava* L, *Citrus hystrix*, *Curcuma longa rhizome* and *Lawsonia intracellularis* have been intensely studied and shown antibacterial and inflammatory activities [14-17]. The plant's environment influences the activity strength of the secondary metabolite of the plants where they grow [18]. Scientifically it is necessary to explore traditional plants that can be used for acne treatment so that clinical test can be used to determine active compounds that serve as anti-acne [19]. Plant biodiversity found in Indonesia is about 28,000 species as a tropical region with an area of around 143 million hectares. Generally, 80 % of the world's medicinal plants grown in the region [20]. Recently investigated the activity of ethanol extract of *Psidium guajava* L, *Curcuma longa rhizome, Citrus hystrix* and *Lawsonia intracellularis* toward the growth inhibition of *Propionibacterium acnes* and *Staphylococcus epidermis*.

EXPERIMENTAL

The apparatus used in this study were measuring glass 1000 mL, Beaker glass 200, 100, 50 mL (Pyrex), spatula, analytical scales (Type Nbl 254, capacity 250 g × 0.0001 g), autoclave (TOMY, high-pressure steam stelizier ES-315), petri dish (Iwaky), test tubes (Pyrex), Buchner funnel (vacuum filtration), scissors, topless plastic, aluminium sieves (100 mesh), vacum rotary evaporator (Heidolph), glass tops, Erlen-

meyer flask (two necks, Pyrex), funnels, blender (Maspion), fun (Maspion), micropipette/syringe, incubator (Memmert), refrigerator (sharp), cotton bud, preparation glass, pinset, vortex (Tubes Mixer-Agitateurde TubeS), impulse bottle, cuvet, micro tube, micro tip, laminar flow (B-ONE V 915 S).

Collection materials: The plant leaves (*Psidium guajava* L, *Curcuma longa rhizome*, *Citrus hystrix* and *Lawsonia intracellularis*) got from the farmers of Deli Serdang, North Sumatra, Indonesia.

Chemicals used for the research were: Citric acid (Merck), ethanol (Merck), Mueller-Hilton media (MHA) (oxsoid, CM0337), MHB (HIMEDIA: GM 391), aquadest, *Propionibacterium acnes* bacteria (ATCC 27853) and *Staphylococcus epidermis* bacteria (ATCC12228), DMSO (Sigma), disc paper blank (oxoid), chloramphenicol standard (Oxoid), NaCl powder (Merck), chloramphenicol powder (Sigma), ammonia (Merck), petroleum ether (Merck), chloroform (Merck), HCl (Merck), dragendroff reagent (Merck), Mayer reagent (Merck), CH_3COOH anhydrate (Merck), H_2SO_4 98 % p.a. (Merck), Lieberman-Buchardreagent (Sigma), magnesium plate (Merck), amyl alcohol (Merck), $FeCl_3$ (Merck), stiassny reagent (Sigma), natrium acetate and NaOH (Merck).

Sample preparation: The investigation was started by sampling preparation. A total of 5 kg of *Psidium guajava* L, *Curcuma longa rhizome, Citrus hystrix* and *Lawsonia intracellularis* leaves clean washed with 2500 mL of distilled water. The samples were blanched in boiled water at 100 °C for 5 min in 0.05 % citric acid solutions then drained and dried at room temperature while being blown once for a while and then reversed. The drying process was carried out in a sun-free room to protect from the sunlight and to avoid damaging the samples metabolites due to the direct contact with the sunbeam. During the drying process, the samples were blown with a fan to avoid fungus and caterpillar growth. In this condition, it is expected that the secondary metabolites contained in the sample conserved, after drying the leaves were mashed into powder to expand the surface area so that the leaves extracted optimally.

Extract preparation: The sample powder was macerated three-fold with ethanol solvent for 3 × 24 h and then filtered with Buchner funnel to separate the filtrate and residue. The obtained filtrate was concentrated in a vacuum rotary evaporator to get ethanol extract. Then, the antibacterial test was carried out towards the bacteria *Propionibacterium acnes* and *Staphylococcus epidermis*.

Antibacterial test with diffusion method [21]

Sterilization of equipment and media: The apparatus was sterilized in an autoclave at 121 °C and pressure of 2 atm for 15 min.

Media preparation and sterilization: Bacterial growth was prepared in a laminar flow and a heat-resistant closed bottle using Mueller-Hilton media (MHA). Then, weighed 38 g of the MHA flour and dissolved in 1 L of distilled water in the heat-resistant bottle and stirred homogenously, afterward heated in an autoclave at 121 °C and pressure 2 atm for 15 min. Then poured it into a petri dish glass and left the media solidified.

Rejuvenation of microbial culture: The microbial culture was rejuvenated before being used for antibacterial testing. The bacteria were cultured on sterilized MHA agar then incubated at 37 °C for 24 h in an incubator (Memmert).

Preparation of the bacterial suspension: The bacteria cultured were taken one strike using a cotton bud then suspended into 2 mL of 0.9 % NaCl in impulse tube. Suspended the bacteria in 0.9 % NaCl, then vortexed and compared its turbidity level with McFarland standard 0.5 (0.05 mL of barium chloride in 9.95 mL of sulfuric acid, 1.5×10^{-8}/mL) [22].

Antimicrobial activity testing: Paper disc diffusion test is a quantitative test was carried out three-fold to determine the antibacterial power of the samples against *P. acnes* and *S. epidermis* bacteria. Before the diffusion test carried out, the sample extract solution was prepared at concentrations of 1.25, 2.5, 5 and 10 %. Then, weighed 100 mg of the sample extracts and dissolved in 1 mL DMSO and dissolved again in a 10 mL of distilled water (0.025 w/v). Poured 100 µL of the bacterial suspension into the selective gelatin medium and platen with a spider, then a paper disc was inserted into the gelatin bacterial suspension and saturate the paper disc by dripping 20 µL of the sample extract with a micropipette. The paper disc contained chloramphenicol (Oxoid) used as controlled and a blank paper saturated with DMSO, then the sample incubated at 37 °C for 24 h. The clear area around the disc paper showed no bacterial growth (resistance zone) measured with a digital micrometer.

Phytochemical screening [3,23]**:** The crude ethanol extracts of leaves were tested for the presence of alkaloids, flavonoid, steroids, tannins and saponins. The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals.

Alkaloids test: 45 mg of each extract was separately stirred with 1 % HCl (6 mL) on a water bath for 5 min and filtered. These filtrates were divided into three equal parts:

Dragendorff's test: To one portion of the filtrate, Dragendorff's reagent (potassium bismuth iodide solution) (1 mL) was added; an orange red precipitate shows the presence of alkaloids.

Mayer's test: To one portion of filtrate, Mayer's reagent (potassium mercuric iodide solution) (1 mL) was added. Formation of cream coloured precipitate gives an indication of the presence of alkaloids.

Wagner's test: Potassium iodide (2 g) and iodine (1.27 g) were dissolved in distilled water (5 mL) and the solution was diluted to 100 mL with distilled water. Few drops of this solution were added to the filtrate; a brown coloured precipitate indicates the presence of alkaloids.

Flavonoid test

Identification of the flavonoid group: Identification of flavonoid was carried out by dissolving 0.5 g of the concentrated sample extract and shaken in a hot methanol solution and added 0.1 g of Mg powder and five drops of concentrated HCl. A yellow coloured precipitate indicates the presence of flavonoids.

Identification of terpenoids and steroids: Identification of terpenoids and steroids were carried out by dissolving 0.5 g of the concentrated extract samples and shaken with 0.5 mL chloroform then added 0.5 mL of acetic anhydride and dripped the mixture with 2 mL concentrated H_2SO_4 through the tube wall. A blue coloured precipitate indicates the presence of terpenoid and a red colour showed the presence of steroids.

Identification of saponins: Identification of the saponin was carried out by dissolving 0.5 g of the concentrated sample extract and mixed with 10 mL of hot water and shaken vigorously for 10 seconds. The occurrence of foam that does not immediately disappear indicates the presence of saponins.

Identification of the tannin group: Identification of tannin was carried out by dissolving 0.5 g of concentrated extract samples and shaken with 10 mL of distilled water and filtered and then added three drops of 1 % $FeCl_3$ into the filtrate. A green coloured precipitate indicates the presence of tannin.

RESULTS AND DISCUSSION

Phytochemical screening: The phytochemical screening of crude ethanol extracts of leaf samples of *Psidium guajava* L, *Curcuma longa rhizome, Citrus hystrix* and *Lawsonia intracellularis* revealed the presence of some secondary metabolites such as alkaloids, flavonoids, steroids and tannins (Table-1).

Secondary metabolites from various plants have a variety of bioactivity such as antibacterial or antioxidant [24,25], the same with plants that have antibacterial activity. The samples have the potential to use as an inhibitor agent for the growth of acne bacteria. *P. acnes* is known as a type of bacteria that cause acne on human skin which results in chronic inflammation [26]. The inhibitory of the ethanol extract sample against the bacteria *P. acnes* compared to chloramphenicol as control is shown in Table-2.

As shown in Table-3, it can be seen that the sample leaves (*P. guajava* L, *Curcuma longa rhizome, Citrus hystrix* and *Lawsonia intracellularis*) have a promising inhibitory power for limiting *S. epidermis* bacteria and *P. acnes* growth on human skin.

The inhibitory power of the ethanol extract was higher at a concentration of 10 % against the bacteria *P. acnes* compared to chloramphenicol as shown in Fig. 1.

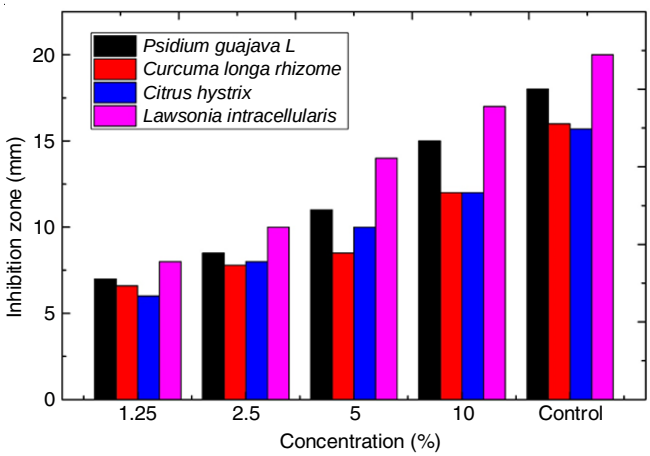

Fig. 1. Inhibitory ethanolic extract from the plant leaves with chloramphenicol 0.02 % as control

The inhibitory power of the ethanol extract was higher at a concentration of 10 % against the bacteria *S. epidermis* compared to chloramphenicol (Fig. 2).

Although the inhibitory power of the samples was different against both bacteria; however, the figures showed that they could be used as a source of medicinal plants for healing acne. It is because of the use of herbal medicines for acne can reduce side effect and combinations of herbal plants and drugs in the treatment of the skin acne can be used to increase the treatment of acne-induced infections [8]. However the skin moisture level can be used to measure how severe the skin has been damaged and it can be used in handling its effects [27].

TABLE-1
PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACTS TYPE OF PLANT LEAVES

Type of plant leaves	Alkaloids	Flavonoids	Saponins	Steroids	Tannins
Psidium guajava L	–	++	++	+	+
Curcuma longa rhizome	–	+++	++	++	–
Citrus hystrix	–	++	+	++	–
Lawsonia intracellularis	++	+++	++	++	+

TABLE-2
INHIBITORY ZONE OF ETHANOLIC EXTRACT OF THE PLANT LEAVES (mm) AGAINST *P. acnes*

Type of plant leaves	Concentration various of sample (%)/Zone inhibitory (mm)				Control
	10 %	5 %	2.5 %	1.25 %	Chloramphenicol (0.02 %)
Psidium guajava L	15	11	8.5	7	18
Curcuma longa rhizome	12	8.5	7.8	6.6	16
Citrus hystrix	12	10	8	6	15.7
Lawsonia intracellularis	17	14	10	8	20

Inhibitory of the ethanol extract sample against bacteria *S. epidermis*

TABLE-3
INHIBITORY ZONE OF ETHANOLIC EXTRACT OF THE PLANT LEAVES (mm) AGAINST *S. epidermis*

Type of plant leaves	Concentration various of sample (%)/Zone inhibitory (mm)				Control
	10 %	5 %	2.5 %	1.25 %	Chloramphenicol (0.02 %)
Psidium guajava L	10	8	7	7	16
Curcuma longa rhizome	11	8.5	7.8	6.6	14
Citrus hystrix	12	10	8	6	15.7
Lawsonia intracellularis	12	10	8	8	20

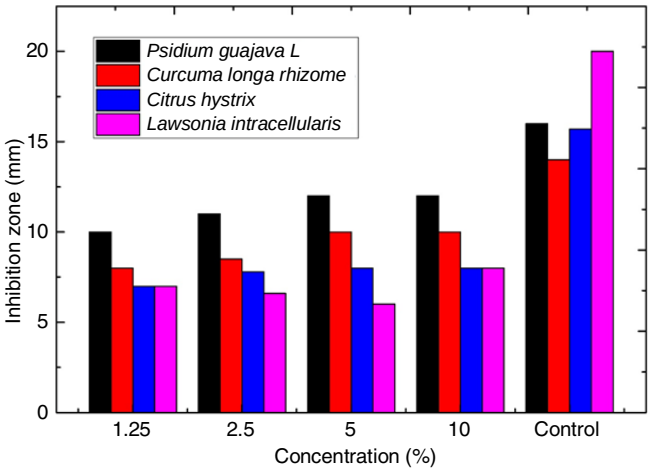

Fig. 2. Inhibitory ethyl acetate extract from the plants leaves with chloramphenicol 0.02 % as control

Conclusion

The phytochemical screening has shown that flavonoid, saponin and steroid found in the *P. guajava* L, *Curcuma longa rhizome, Citrus hystrix* and *Lawsonia intracellularis*. An alkaloid found in *Lawsonia intracellularis* and Tanin founded in *P. guajava* L and *Lawsonia intracellularis*.

The antibacterial activity of ethanolic extract of the sample has a higher inhibitory power on bacteria *P. acnes* and *S. epidermis* at a concentration of 10 % than chloramphenicol. Acne treatment can be carried out by using skin care antibiotics, but the use of antibiotics for a long time resulting *P. acnes* resistance and makes it difficult to cure [28]. The usage of the medicinal plants to cure skin acne can overcome the effects of the antibiotics resistance because it has multifunctional such as anti-inflammatory and antioxidants [29], through scientific studies have been done to get new antibiotics from plants [30].

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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