Short Communication:  
Selected medicinal plants in East and North Kalimantan (Indonesia) against Propionibacterium acnes

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2Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Mulawarman. Jl. Kuaro, Gunung Kelua, Samarinda Ulu, Samarinda 75119, East Kalimantan, Indonesia  
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Abstract. Arung ET, Pasedan WF, Kusuma IW, Hendra M, Supriadi MB. 2017. Short Communication: Selected medicinal plants in East and North Kalimantan (Indonesia) against Propionibacterium acnes. Biodiversitas 18: 321-325. Based on community based research on ethnomedicine local knowledge and medicinal plant in Indonesia by Medicinal Plant and Traditional Medicine Research and Development Center of Indonesian Ministry of Health in 2012 and 2015, various data on medicinal plants including for cosmetic in all Indonesia region has been collected. There are 38 medicinal plants use for skin care/cosmetics. This study focused on some selected medicinal plants used by several Dayak ethnic groups in East and North Kalimantan, Indonesia for cosmetic, especially for anti-acne. Four plants were selected for evaluating its property on anti-acne, namely Crotalaria pallida, Lepisanthes amoena, Premna corymbosa, and Vitex pinnata by well diffusion and dilution methods. The zone inhibition (mm) against Propionibacterium acnes of these leaves extract on anti-acne at 1000 μg/mL was 9.33; 16.44; 13.78; 11.00 mm, respectively and chloramphenicol (positive control) was 29.44 mm at 500 μg/mL. These results were in line with traditional used by Dayak tribes in East and North Kalimantan, Indonesia.

Keywords: Anti acne, cosmetic, medicinal plants, traditional medicine

INTRODUCTION

Cosmetics are referring to all products used to treat and clean human skin and make it more beautiful. The desire to use cosmetic products is defending the body, protects from environmental effects, and the aging process, change appearance, and change for the better body odor. Cosmetic products are used by everyone to clean, perfume, protect and change the appearance of the skin (Amasa et al. 2012). Roosita et al. (2008) reported more than 1300 species of medicinal plants used as traditional medicine in Indonesia which well known as ‘jamu’. Concerning ‘jamu’, it has four functions of medicine such as health care, beauty care (cosmetics), tonics, and protection (Soedarsono and Harini 2002). The traditional medicines provide an interesting and largely unexplored source for development of potential new drugs or cosmetics. A traditional cosmetic product made by the Dayak tribe (Native people in Kalimantan island, Indonesia) called ‘bedak dingin’ which sold in traditional markets in Samarinda and other cities in Kalimantan island, Indonesia (Figure 1), contain some medicinal plants for skin-care. Based on survey and research in 2012 and 2015 on medicinal plants by Medicinal Plant and Traditional Medicine Research and Development Center, in various ethnic groups in Indonesia, particularly in the East and North Kalimantan which recorded 38 species of plants used specifically for skin care/cosmetics and 16 of them were scientifically identified (Wahyono et al. 2013, 2015). Unfortunately, the scientific evidences about those medicinal plants have not been known yet. Therefore, in the present study the selected medicinal plants were validated for their anti bacterial especially against Propionibacterium acnes.

Figure 1. ‘Bedak dingin’ a traditional cosmetic made by Dayak tribe contain some medicinal plants.
MATERIALS AND METHODS

Reagents
EtOH, acetone, dimethyl sulfoxide (DMSO), nutrient agar, nutrient broth; 2,3,5-triphenyl tetrazolium chloride (TTC), chloramphenicol, Dragendorff solution, HCl, NaOH, H₂SO₄, (CH₃COO)₂Pb, acetone, acetic acid, Liebermann-Burchard solution and other chemicals were of the highest grade commercially available.

Plant materials and extraction
Plant materials such as leaf of Crotalaria pallida, Lepisanthes amoena, Premna corymbosa, and Vitex pinnata were collected in July-September, 2016 from some areas in East and North Kalimantan, Indonesia (Table 1). Voucher specimens were identified in Dendrology Laboratory and deposited in the Forest Product Chemistry Laboratory, Department of Forest Science, Faculty of Forestry, Universitas Mulawarman, Samarinda, East Kalimantan, Indonesia. The Propionibacterium acnes bacteria was used and purchased from South Korea (Korean Culture Center of Microorganism, KCCM).

The leaves were dried at room temperature and powdered. The dried materials of L. amoena (25 g), V. pinnata (50 g), C. pallida (100 g), P. corymbosa (100 g) were extracted with EtOH at room temperature for 48 h. The extract solutions were filtered and concentrated in vacuo, to obtain the crude extracts 0.58, 2.85, 6.25, and 11.09 g, respectively.

Phytochemical analysis
Phytochemical analysis was determined for alkaloid, flavonoid, and tannin (Kokate 2001), as well as saponin, steroid, and terpenoid (Harbone 1984).

Fungal inoculum preparation
Propionibacterium acnes from KCCM 41747 (South Korea) was used as a test bacteria in agar diffusion (nutrient agar and broth) assay. Stock inoculum suspension of P. acnes was prepared from 7 to 15 days sold cultures grown on nutrient agar and broth at 28°C. Mature colonies were covered with approximately 10 mL of sterile saline (0.85%) by scraping the surface with the tip of a Pasteur pipette and adjusted to 10⁶ CFU/mL (Kusuma et al. 2010).

Agar diffusion assay
Ten milliliter aliquots of sterile nutrient agar and broth were transferred to petri dishes and allowed to solidify. The plates were inoculated with 10 μL of P. acnes spore suspension spread uniformly on the surface of the plates. The 20 μL of sample extracts were added to the plate containing P. acnes with concentration of 1000, 500, and 250 μg/well. The samples or chloramphenicol (positive control) at 1000, 500, and 250 μg/well were put on the surface of each inoculated plate. The plates were incubated in the dark at 37°C for 72 hours in anaerobe chamber. Zones of inhibition around the discs were measured in mm (Kusuma et al. 2014).

Dilution assay
The Minimum Inhibitory Concentration (MICs) and Minimum Bactericidal Concentration (MBCs) of the samples were determined by the broth microdilution method in 96-well micro-titre plates as described by Lunga et al. (2014) with minor modification. The 96-well plates were prepared by dispensing into each well 100 μL of nutrient broth for P. acnes. Samples and chloramphenicol (positive control) were prepared for the final concentration of 1250, 625, 312.5 and 156.25 ppm. Then, stock samples diluted into 96-well plates as much as 50 mL. Added 100 mL of nutrient broth and 50 mL of the bacterial suspension which has been suspended with distilled water. The bacteria were incubated for 24 hours in an incubator at a temperature of 37°C. One hour before the incubation process was completed, added 50 mL of a solution of 2,3,5-triphenyl tetrazolium chloride (TTC). The red color suspension was indicated the bacterium still alive and the clear indicated of bacterium inhibition. The clear suspension was used for MBC test. The MBCs were determined as follow: 50 μL from the clear suspension was taken and put in 96-well plates which contained 100 μL of nutrient broth. The bacteria were incubated for 24 hours in an incubator at a temperature of 37°C. One hour before the incubation process was completed, added 50 mL of a solution of 2,3,5-triphenyl tetrazolium chloride (TTC). All tests were performed in duplicates.

RESULTS AND DISCUSSIONS

Plant materials
Acne is a disease that most often affects the skin, although acne usually manifested attacking teenager and middle-aged, the study epidemiology mention that acne does not have a lifespan to attack humans. Acne is most common in between the time of puberty age (16-18 years) up to the age of 30 years (Sparavigna et al. 2015). Acne affects ~80% of teenagers and young adults and ~650 million people in global, thus making it one of the top ten most common diseases entire world. It is caused by Propionibacterium acnes that resides within the pilosebaceous follicles, adjacent to the glands of sebaceous. Although it is still unknown what triggers it, the overproduction of sebum by these glands, or the blockage of follicles, causes the overgrowth of P. acnes, which causes inflammation in the skin (Dinant and Boulos 2016). Several medicinal plants are traditionally used by Dayak people for skin care/cosmetics, including Crotalaria pallida, Lepisanthes amoena, Premna corymbosa, and Vitex pinnata (Wahyono et al. 2013, 2015).

The Lepisanthes is a widespread and variable genus in the family Sapindaceae, has about 24 species. The distribution ranges from tropical Africa, Madagascar, Sri Lanka, India, China (Hainan), through Malesia to New Guinea and north-western Australia (Boonsuk and Chantaranontai 2016). This plant has antimicrobial properties, as well as antifungal, antioxidant, and antityrosinase (Batubara et al. 2010; Harlinda et al. 2012).
The genus *Vitex* approximately includes 270 known species of trees and shrubs within tropical and sub-tropical regions, although few species may be found in temperate zones. Several *Vitex* species are used as folk remedies such as alleviate dysentery, analgesic, anti-inflammatory, the treatment of scorpion stings, diarrhea, stomach ache, gastrointestinal affections, antimalarial, antimicrobial, antifungal, antioxidant, and antityrosinase (Arung et al. 2009; Meena et al. 2010).

The *Crotalaria* belongs to Fabaceae and contains about 550 species. The habits are vary from shrubs to herbs and the genus is common in the tropics and subtropics, with the greatest number of species occurring in Africa. One of the important fiber and green manure crops is *Crotalaria juncea* L. Several other economically important species are *C. intermedia* Kotschy, *C. mucronata* Desv. (syn. *C. striata* DC.), and *C. retusa* L., which are grown as green manures, forages, and ornamentals. Many species of *Crotalaria* contain alkaloids toxic to animals but some are not (Wang et al. 2002). Govindappa et al. (2011) was reported this plant has function for antimicrobial, antioxidant, anti-inflammatory, anti-lipoxygenase, anti xanthine oxidase, and anti acetylcholinesterase.

The genus *Premna* (Verbenaceae) is widely distributed in tropical and subtropical regions of Africa, Asia, Australia and the Pacific islands and has about 10-20 species. The various medicinal uses reported for this genus are for treating diabetes, chyluria, gonorrhea, inflammation, swelling, bronchitis, dyspepsia, headache, liver disorder, piles, constipation and fever. Some pharmacological studies have revealed that the plant possess anti-coagulant, anti-inflammatory, anti-arthritic, antinoceptive, hypoglycemic, gastroprotective, antimicrobial properties and cardioactivity (Suresh et al. 2011; Bose et al. 2013).

**Phytochemical constituents**

Alkaloids, flavonoids, tannins, steroids, terpenoids and saponins are class of phytochemicals plant extracts tested in this research. In Table 2, *L. amoena* extract contained alkaloids, flavonoids and steroids. According to Herman (2013), the leaf of this plant contains alkaloids, phenols, tannins and saponins. In addition, Pramana and Saleh (2013) isolated the steroid compounds from these leaves.

Phytochemicals leaf extract of *V. pinnata* contained alkaloids, flavonoids, tannins, and triterpenoids. In connection with the results of phytochemical *V. pinnata* leaves, Meena et al. (2010) was reported that the *Vitex* genus containing iridoid compounds, flavonoids, diterpenoids, and steroids from various parts of the plant (leaves, bark and wood).

In this study, *C. pallida* leaf extract contains alkaloids, flavonoids, tannins and steroids. The content is in line with those reported by Govindappa et al. (2011), by stating that the ethanol extract of *C. pallida* all parts of the plant contain alkaloids, flavonoids, tannins, steroids, terpenoids, saponins, and phenol. In addition it was reported also by Cogni and Trigo (2016) that has been isolated alkaloid from the leaf and fruit of *C. pallida*.

The results of the phytochemical extracts of *P. corymbosa* leaf contained alkaloids, flavonoids, tannins, steroids and triterpenoids. Radhika et al. (2013) was reported that *P. corymbosa* leaf contain alkaloids, flavonoids, phenols, tannins, carbohydrates, terpenoids, coumarin and quinones. In addition, Otsuka et al. (1993) managed to isolate iridoid compounds (class monoterpenoid) from leaf extract. Differences that occur between the results of studies with some of the research that has been reported previously possible due to the different parts of the plant used, where samples were grown, and other factors. The inhibition both extracts *C. pallida* and *V. pinnata* on *P. acnes* might be related with their phytochemicals as explained above.

**Propionibacterium acnes inhibitory**

Table 1 presented the selected plants that use by the Dayak tribe (indigenous people of Borneo/ Kalimantan island) for cosmetic or skin treatment in their daily life and all of them is used for anti-acne. Therefore, anti *P. acnes* bacteria assay was performed to validate its application use as seen in Table 3.

Extracts of *C. pallida*, *V. pinnata*, and *P. corymbosa* had a zone inhibition effect on the growth of *P. acnes* with range were about 11-17 mm, and *L. amoena* was about 10 mm at the highest concentration while Chloramphenicol as positive control was 29.44 mm (Table 3). Extracts of *C. pallida* has not been reported to inhibit the growth of *P. acnes*. The *V. pinnata* extract against *P. acnes* has not been reported, but Patil et al. (2012) reported that extracts of *V. negundo* inhibit the activity of the enzyme lipase in *P. acnes* by 50% at a concentration of 19 μg/mL. The ethanol extract of *Premna emblica* reported by Rattanasena (2012) to inhibit *P. acnes* without mentioning the value of inhibition and concentration. Extract of *P. corymbosa* also not yet been reported by other researchers. Over all, the inhibition of *P. acnes* growth in order was *V. pinnata>*C. pallida>*P. corymbosa>*L. amoena*, respectively. Based on Aires et al. (2009), all extracts were classified in the classification of moderate because the inhibitory zone was 0 < inhibition zone samples < antibiotic inhibition zone. In addition, MIC and MBC of all extract was done and none of them was reported previously (Table 4), and the MIC and MBC order were *C. pallida>*V. pinnata>*L. amoena>*P. corymbosa* respectively. Related to MIC and MBC several medicinal plants of India (Patil et al. 2012), Korea (Kim et al. 2008b), and Indonesia (Batubara et al. 2009) were reported to have MIC and MBC potential against *P. acnes*. The inhibition of leaf extract from *C. pallida* may cause by some compounds contained in this plant such as pyrrolizidine alkaloids (Martins et al. 2015), peptides (Pelegreni et al. 2009), flavonoids (Ko et al. 2004) or others compound. The *V. pinnata* extracts inhibited *P. acnes* can be affected by iridoids, flavonoids, ecldysteroids, pinnata sterones or others compound (Ata et al. 2009; Meena et al. 2010).
Table 1. The traditional uses of selected medicinal plants from Kalimantan island

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Family</th>
<th>Local name</th>
<th>Traditional utilization</th>
<th>Plant parts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepisanthes amoena (Hassk.)</td>
<td>Sapindaceae</td>
<td>Selekop</td>
<td>General: skin treatment</td>
<td>Young leaf</td>
</tr>
<tr>
<td>Leenh., Blumea</td>
<td></td>
<td></td>
<td>Specific: reduce skin stained and anti acne</td>
<td></td>
</tr>
<tr>
<td>Vitex pinnata L.</td>
<td>Verbenaceae</td>
<td>Kelepapaq</td>
<td>General: skin treatment</td>
<td>Young leaf</td>
</tr>
<tr>
<td>Crotalaria pallida Aiton</td>
<td>Fabaceae</td>
<td>Belaluq lasoq</td>
<td>Specific: anti acne</td>
<td>Leaf</td>
</tr>
<tr>
<td>Premna corymbosa (Burm.f.)</td>
<td>Lamiaceae</td>
<td>Singkil</td>
<td>General: skin and hair treatment</td>
<td>Leaf</td>
</tr>
<tr>
<td>Rotti.&amp;Wild.</td>
<td></td>
<td></td>
<td>Specific: anti acne (Leaf)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Phytochemical analysis of plant extracts having anti-acne properties

<table>
<thead>
<tr>
<th>Sample</th>
<th>Alkaloid</th>
<th>Flavonoid</th>
<th>Tannin</th>
<th>Steroid</th>
<th>Triterpene</th>
<th>Saponin</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. amoena</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>V. pinnata</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>C. pallida</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>P. corymbosa</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
</tbody>
</table>

Note: (+) presence, (−) absence. All experiments was repeated twice.

Table 3. The effect of plants extracts on growth inhibition of Propionibacterium acnes

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (μg/well)</th>
<th>Inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. amoena</td>
<td>1000</td>
<td>9.33</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>8.56</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>8.89</td>
</tr>
<tr>
<td>V. pinnata</td>
<td>1000</td>
<td>16.44</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>15.89</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>13.44</td>
</tr>
<tr>
<td>C. pallida</td>
<td>1000</td>
<td>13.78</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>11.33</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>11.11</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>10.67</td>
</tr>
<tr>
<td>P. corymbosa</td>
<td>1000</td>
<td>11.00</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>9.44</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>0.00</td>
</tr>
<tr>
<td>Chloramphenicol (positive control)</td>
<td>500</td>
<td>29.44</td>
</tr>
</tbody>
</table>

In conclusion, the interest is huge to know whether medicinal plants used for skin treatment especially for anti acne and it might be useful in modern formulations. In the present study, the evaluation of L. amoena, C. pallida, V. pinnata, and P. corymbosa extracts against P. acnes bacteria was in line with the application of these plants by local inhabitants life in East and North Kalimantan. However, the active compounds still unknown and further analysis is needed.

ACKNOWLEDGEMENTS

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