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

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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 Helath 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 chlorampenicol (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, H_2SO_4, $(CH_3COO)_2Pb$, 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 to15daysold 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 10^6 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 250µg/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-triphenyltetrazolium 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-triphenyltetrazolium 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 *P. 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 Chantaranothai 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, 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, antinociceptive, hypoglycemic, gastroprotective, antimicrobial properties and cardioactivity (Suresh t 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 (Pelegrini et al. 2009), flavonoids (Ko et al. 2004) or others compound. The *V. pinnata* extracts inhibited *P. acnes* can be affected by iridoids, flavonoids, ecdysteroids, 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

Scientific name	Family	Local name	Traditional utilization	Plant parts
Lepisanthes amoena (Hassk.) Leenh., Blumea	Sapindaceae	Selekop	General: skin treatment Specific: reduce skin stained and anti acne	Young leaf
Vitex pinnata L.	Verbenaceae	Kelepapaaq	General: skin treatment Specific: anti acne	Young leaf
Crotalaria pallida Aiton	Fabaceae	Belaluq lasooq	Specific: anti acne	Leaf
Premna corymbosa (Burm.f.) Rotti.&Willd.	Lamiaceae	Singkil	General: skin and hair treatment Specific: anti acne (Leaf)	Leaf

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

Sample	Alkaloid	Flavonoid	Tannin	Steroid	Triterpene	Saponin
L. amoena	+	+	–	+	–	–
V. pinnata	+	+	+	–	+	–
C. pallida	+	+	+	+	–	–
P. corymbosa	+	+	+	+	+	–

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

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

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

Table 4. The Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against *P. acne*

Sampel	MIC (µg/mL)	MBC (µg/mL)
L. amoena	1250	>1250
C. pallida	156.25	156.25
V. pinnata	312.5	312.5
P. corymbosa	>1250	>1250
Chloramphenicol (positive control)	<78.125	<78.125

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.

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REFERENCES

Aires A, Mota VR, Saavedra MJ, Monteiro AA, Simões M, Rosa E, Bennett R. 2009. Initial *in vitro* Evaluations of the Antibacterial Activities of Glucosinolate Enzymatic Hydrolysis Products Against Plant Pathogenic Bacteria. J. Appl. Microbiol.106: 2096-2105.

Amasa W, Santiago D, Mekonen S, Ambelu A. 2012. Are Cosmetics Used in Developing Countries Safe? Use and Dermal Irritation of Body Care Products in Jimma Town, Southwestern Ethiopia.J Toxicol 2012: 1-8.

Ata A, Mbong N, Iverson CD, Samarasekera R. 2009. Minor chemical constituents of *Vitex pinnata*.Nat Prod Commun 4: 1-4.

Batubara I, Mitsunaga T, Ohashi H. 2009. Screening antiacne potency of Indonesian medicinal plants: antibacterial, lipase inhibition, and antioxidant activities. J Wood Sci 55: 230-235

Boonsuk B, Chantarathai P. 2016. A new record of *Lepisanthes* Blume for Thailand and lectotypification of two names in *Otophora* Blume (Sapindaceae). Thai For Bull Bot 44: 22-25

Bose LV, Varghese GK, Habtemariam S. 2013. Identification of acteoside as the active antioxidant principle of *Premna serratifolia* root wood tissues. Phytopharmacol 4: 228-236.

Cogni R, Trigo JR. 2016. Pyrrolizidine alkaloids negatively affect a generalist herbivore feeding on the chemically protected legume *Crotalaria pallida*. Neotropical Entomol 45: 252-257.

Dinant A, Boulos RA. 2016. Zolav (R): A New Antibiotic for the Treatment of Acne. Drug Des Dev Ther 10: 1235–1242.

Govindappa M, Bharath N, Shruthi HB, Sadananda TS, Sharanappa P. 2011. Antimicrobial, antioxidant and in vitro anti-inflammatory activity and phytochemical screening of *Crotalaria pallida* Aiton. Afr J Pharm Pharmacol 5 : 2359-2371.

Harbone JB. 1984. Phytochemical Methods. 2nd ed. Springer, Berlin.

Herman H. 2013. Antioxidant of several medicinal plants in East Kalimantan. J Trop Pharm Chem 2: 100-104. [Indonesian]

Kim SS, Kim JY, Lee NH, Hyun CG. 2008. Antibacterial and anti-inflammatory effects of Jeju Medicinal Plants against acne-inducing bacteria. J Gen Appl Microbiol.54: 101-106

Ko HH, Weng JR, Tsao LT, Yen MH, Wang JP, Lin CN. 2004. Anti-inflammatory flavonoids and pterocarpanoid from *Crotalaria pallida* and *C. assamica*. Boorg Med Chem Lett 14: 1011-1014.

Kokate CK. 2001. Pharmacognosy. 16th ed. Niali Prakasham, Mumbai, India

Kusuma IW, Arung ET, Rosamah E, Purwatiningsih S, Kuspradini H, Syafrizal, Astuti J, Kim YU, Shimizu K. 2010. Antidermatophyte and antimelanogenesis compound from *Eleutherine americana g*rown in Indonesia. J Nat Med 64: 223–226.

Kusuma IW, Murdiyanto, Arung ET, Syafrizal, Kim YU. 2014. Antimicrobial and antioxidant properties of medicinal plants used by the Bentian Tribe from Indonesia. Food Sci Hum Well 3: 191-196

Lunga PK, Qin XJ, Yang XW, Kuiate JR, Du ZZ, Gatsing D. 2014. Antimicrobial steroidal saponin and olenane-type triterpennoid saponins from *Paullinia pinnata.* BMC Compl Altern Med 14: 369-375.

Martins CH, Cunha BP, Solferini VN, Trigo JR. 2015. Feeding on host plants with different concentrations and structures of pyrrolizidine alkaloids impacts the chemical-defense effectiveness of a specialist herbivore. PLOS One 10: 1-27.

Meena AK, Singh U, Yadav AK, Singh B, Rao MM. 2010. Pharmacological and phytochemical evidences for the extracts from plants of the genus *Vitex* – A review. Intl J Pharmaceut Clin Res. 2: 1-9.

Otsuka H, Watanabe E, Yuasa K, Ogimi C, Takushi A, Takeda Y. 1993. A verbascoside iridoid glucoside conjugate from *Premna corymbosa* var. *abtusifolia.* Phytochemistry 32: 983-986.

Patil V, Bandivadekar A, Debjani D. 2012. Inhibition of *Propionibacterium acnes* lipase by extracts of Indian Medicinal Plants. Int J Cosmet Sci 34: 234-239.

Pelegrini PB, Farias LR, Saude AC, Costa FT, Bloch C Jr, Silva LP, Oliveira AS, Gomes CE, Sales MP, Franco OL. 2009. A novel antimicrobial peptide from *Crotalaria pallida* seeds with activity against human and phytopathogens. Curr Microbiol 59: 400-404.

Pramana MRA, Saleh C. 2013. Isolation and characterization steroid compound in n-hexane fraction of *Lepisanthes amoena* (Hassk.) Leenh.) leaf. J Kimia Mulawarman 10: 85-89.

Radhika S, Senthil KR, Sivalingam S, Arumugam P. 2013. Phytochemical investigation and evaluation of antihyperglycemic potential of *Premna corymbosa*. Intl J Pharm Pharmaceu Sci 5: 352-356.

Rattanasena P. 2012. Antioxidant and antibacterial activities of vegetables and fruits commonly consumed in Thailand. Pak J Biol Sci 15: 877-882.

Roosita K, Kusharto, Sekiyama CM, Fachrurozi M, Ohtsuka Y. 2008.Medicinal Plants Used by the Villagers of a Sundanese Community in West Java, Indonesia. J Ethnopharmacol 115: 72–81

Soedarsono RS, Harini SR. 2002. Jamu as traditional medicine in Java, Indonesia. South Pacific Study 23: 1-9.

Sparavigna A, Tenconi B, Ponti ID, Penna LL. 2015. An innovative approach to the topical treatment of acne. Clin Cosmet Invest Dermatol 8: 179-185.

Suresh G, Babu KS, Rao MSA, Rao VRS, Yadav PA, Nayak VL, Ramakrishna S. 2011. Premnalatifolin A, a novel dimeric diterpene from *Premna latifolia* Roxb. Tetra Lett 52: 5016-5019.

Wahyono S, Supriyati N, Mujahid R, Subositi D, Wahyu, Jokopriambodo et al. 2013. National Reports Exploration Ethnomedicine Local Knowledge and Community-Based Medicinal Plant in Indonesia 2012. Publishing Agency for Health Research and Development, Indonesian Ministry of Health, Jakarta. [Indonesian].

Wahyono S, Supriyati N, Mujahid R, Subositi D, Wahyu, Jokopriambodo et al. 2016. National Reports Exploration Ethnomedicine Local Knowledge and Community-Based Medicinal Plant in Indonesia2015. Publishing Agency for Health Research and Development, Indonesian Ministry of Health, Jakarta. [Indonesian].

Wang KH, Sipes BS, Schmitt DP. 2002. *Crotalaria* as a cover crop for nematode management: a review. Nematropica 32: 35-57.