INTRODUCTION

During the second half of the 20th century, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the classical antibiotics led researchers to investigate the antimicrobial activities of medicinal plants. Antimicrobials of plant origin have the enormous therapeutic potential [1], they are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials.

Red bulb plant or “bawang dayak” (Eleutherine Americana Merr.) have been widely used as traditional medicine. Empirically the local community of Central Kalimantan, bulb of the plants has been used against cancer, anti-diabetic, anti-fungal, and anti-inflammatory. Studies demonstrated that bulbs of Eleutherine (E. bulbosa and E. Americana) contain naphtoquinones (elecancine, eleutherine, eleutherol, and eleutheronine) [2-6]. Tawas ut (Ampelocissus rubiginosa) tubers empirically were used by Palangka Raya people in Central Kalimantan for treating malaria. Research from Arnida et al. [7] Tawas ut (A. rubiginosa) tubers in vitro, antiplasmodial activity was active.

Medicinal plants are known to contain several compounds with antimicrobial properties, and the uses of these types of compounds are being increasingly reported from different parts of the world [8]. Combination of antimicrobial agents has expressed significant interactions and two or more compounds interact to produce mutual enhancement, amplification of each other’s effects when combined. These combinations could enhance the efficacy of the other antimicrobial agents and acted as an alternative to treating infections caused by multidrug-resistant microorganisms having no effective therapy [9,10]. Some of the bioactive compounds could hinder the life processes of disease-causing bacteria, either by itself or in combination with other therapeutic agents [11]. Therefore, an attempt has been made to study the preliminary phytochemical screenings and antibacterial activity of bawang dayak (Eleutherine Sp.), tawas ut (Ampelocissus Sp.), and a combination of both.

METHODS

The materials procured for this in vitro test compounds were bawang dayak (Eleutherine bulbosa) and Tawas ut (A. rubiginosa). The test bacteria were Propionibacterium acnes, and Mueller-Hinton agar (MHA) plate was used.

Preparation of plant extracts

The healthy and fresh bulb of the plant bawang dayak (Eleutherine bulbosa) and root of Tawas ut (A. rubiginosa) were bought from a traditional market in Palangka Raya, Central Kalimantan. The plant materials were dried under the sun for 5–7 days. The dried plant materials were crushed by grinder without adding any solvent into it. The powder of the plant materials was extracted with 70% ethanol using a Soxhlet extractor and once the process was finished, all extracts were concentrated in a rotary evaporator.

Phytochemicals screening

The prepared extract was subjected to phytochemical screening to detect the presence/absence of secondary metabolites [12].

Evaluation of antimicrobial activity by a zone of inhibition by well diffusion method

The bacterial isolates were subcultured into a nutrient broth. The 24-h-old bacterial culture was standardized using McFarland standard (10^6 cfu/mL of 0.5 McFarland standard).
MHA was used for bacteria bioassay. MHA was prepared by dissolving 38 g in 1000 ml of distilled water and brought to boil to completely dissolve. Sterilization was achieved by autoclaving at 121°C for 15 min [13].

MHA plates were prepared, and bacterial strains were inoculated by cotton swab and then antibiotic and extract with various concentration applied in it. The plates were incubated at 37°C for 24 h, and the zone of inhibition was measured [14] and recorded later on.

RESULTS AND DISCUSSION

Preliminary phytochemical screenings

In general, secondary metabolites compound is widely distributed in plants and contribute significantly toward biological activities or pharmacological effects including antibacterial and antioxidant. In this present study ethanolic extract Bawang Dayak (Eleutherine Sp) and ethanolic extract Tawas Ut (Ampelocissus Sp) could be potential antibacterial against Propionibacterium acnes. Furthermore, this study needs more research with variant concentration so that may be possible to be used as natural anti-acne formulations.

Tannins have amazing stringent properties. They are known to hasten the healing of wounds and inflamed mucous membranes [15], it is good for anti-acne agent. Furthermore, flavonoids as a potent antioxidant which prevent oxidative cell damage and terpenoids are also known to possess antimicrobial and antifungal properties. The preliminary phytochemical screening of ethanolic extracts of bawang Dayak (Eleutherine Sp) dan Tawas Ut (Ampelocissus Sp) mainly revealed the presence of flavonoid, alkaloid, saponin, tanin, steroid and triterpenoid (Table 1).

Antibacterial activity

In few last decades, there has been especial interest in the use of abundant naturally occurring antimicrobials and antioxidants such as plants, fruits for medicinal applications. In the present study was conducted antibacterial evaluations of ethanolic extract Bawang Dayak (Eleutherine Sp.), ethanolic extract Tawas Ut (Ampelocissus Sp.), and a combination of both. The antimicrobial activities can be classified into three levels [18]: Weak activity (inhibition zone <12 mm), moderate activity (inhibition zone between 12 and 20 mm), and strong activity (inhibition zone >20 mm). The results of antimicrobial activity revealed that significant antibacterial activity showed against Propionibacterium acnes in comparison with positive control or standards clindamycin (Table 2) Fig 1.

The highest anti-acne effect was found for ethanolic extract TU (Ampelocissus Sp.) with 16.3 mm zone of inhibition which means moderate activity Fig 2, while BD (Eleutherine Sp.) has a low zone

### Table 1: Secondary metabolites of an ethanolic extract of Bawang Dayak (Eleutherine Sp.) and Tawas Ut (Ampelocissus Sp.)

| Secondary metabolites | Ethanolic extract of bawang dayak (Eleutherine Sp.) | Ethanolic extract of tawas Ut (Ampelocissus Sp.) | References |
|-----------------------|---------------------------------------------------|-------------------------------------------------|------------|
| Flavonoid             | -                                                 | +                                               | The presence of flavonoids was indicative if pink or magenta-red color developed within 3 min [16]. |
| Alkaloid              | +                                                 | -                                               | The samples were then observed for the presence of turbidity or precipitation [16]. |
| Saponin               | +                                                 | +                                               | The presence of saponin was positive if froth ≥1.2 cm [16]. |
| Tannins               | +                                                 | +                                               | Positive tests are confirmed by the addition of the FeCl₃ solution to the extract and should result in a characteristic blue, blue-black, green or blue-green color and precipitate (phenolic compounds) [16]. |
| Steroid              | +                                                 | +                                               | Formation of red color ring confirmed the presence of steroid [17]. |
| Triterpenoid          | -                                                 | +                                               | If reddish violate color appeared, the existence of triterpenoids was confirmed [17]. |
of inhibition with the same concentration (50 mg/ml) Fig 3 but it is can be potential strong activity if the concentration was increased so ethanol extract TU (*Ampelocissus* Sp.) and this requires further research. Difference zone of inhibition is possible due to the content of triterpenoid and flavonoid in Tawas Ut (*Ampelocissus* Sp.) so zone of inhibition larger than Bawang Dayak (*Eleutherine* Sp.).

One study stated that plants containing terpenoid showed a significant inhibitory activity of bacteria. Terpenoid compound treated microbes resulted in the leakage of reducing sugars and proteins through the membrane. It also induced the activity of respiratory chain dehydrogenase. Therefore, it was justified that terpenoid compound was able to destroy the permeability of the bacterial membrane [19]. Flavonoid significantly contributed to the antibacterial properties [20].

Test of combination ethanolic extract Bawang dayak (*Eleutherine* Sp.) and ethanolic extract Tawas Ut was also done by comparison. The

### Table 2: Antibacterial against Propionibacterium acnes effect of positive control, ethanolic extract BD (*Eleutherine* Sp.), ethanolic extract TU (*Ampelocissus* Sp.) and a combination of both by well diffusion method

| Name of sample                  | Concentration (mg/ml) | Zone of inhibition (mm) | X±SD  |
|---------------------------------|-----------------------|-------------------------|-------|
|                                 |                       | I       | II      | III     |         |
| Clindamycin (positive control)  | 25                    | 30.9    | 29.5    | 30.6    | 30.3±0.74 |
|                                 | 50                    | 33.5    | 36.5    | 30.8    | 33.6±2.85 |
| BD (*Eleutherine* Sp.)          | 25                    | 3.5     | 2.2     | 4       | 3.2±0.93  |
|                                 | 50                    | 6.1     | 6.7     | 10.6    | 7.8±2.44  |
| TU (*Ampelocissus* Sp.)         | 25                    | 7.5     | 10.6    | 11.9    | 10.0±2.26 |
|                                 | 50                    | 18.8    | 12.7    | 17.3    | 16.3±3.18 |
| Combination BD+TU               | 1:1 (25:25)           | 5.5     | 4.7     | 9.9     | 6.7±2.80  |
|                                 | 1:2 (25:50)           | 4.5     | 4.3     | 2.9     | 3.9±0.87  |
|                                 | 2:1 (50:25)           | 3.3     | 3.7     | 3.9     | 3.63±0.31 |

![Fig. 1: Zone of Inhibition of Clindamycin (Positive Control): A. concentration is 50 mg/ml, B. concentration is 25 mg/ml](image1)

![Fig. 2: Zone of Inhibition of ethanolic extract Tawas Ut (*Ampelocissus* Sp): A. concentration is 50 mg/ml, B. concentration is 25 mg/ml](image2)

![Fig. 3: Zone of Inhibition of ethanolic extract Bawang Dayak (*Eleutherine* Sp): A. concentration is 50 mg/ml, B. concentration is 25 mg/ml](image3)

![Fig. 4: Zone of Inhibition of a combination ethanolic extract of both (Bawang Dayak & Tawas Ut) with comparison of concentration : A. 1:1, B. 1:2, C. 2:1](image4)
highest zone of inhibition is ratio 1:1 with the same concentration (25 mg/ml) in weak activity category (6.7 mm) (Fig 4) but still has potential as antibacterial against Propionibacterium acnes and may be better inhibitory if given a concentration >25 mg/ml which will later be the basis of further research.

CONCLUSION

Ethanolic extract Bawang Dayak (Eleutherine Sp.), ethanolic extract Tawas Ut (Ampelocissus Sp.) and a combination of both can be potential antibacterial effects against Propionibacterium acnes. Ethanolic extract Tawas Ut (Ampelocissus Sp.) are containing flavonoid, saponin, tannins, and steroid, and triterpenoid have a larger zone of inhibition than the ethanolic extract of Bawang Dayak (Eleutherine Sp.) that are containing alkaloid, saponin, tannins, and steroid. The greatest ratio combination of both is 1:1 (25 mg/ml). Furthermore, this present study needs more research by raising the concentration or with variant concentration so that may be possible to be used as natural anti-acne formulations.

ACKNOWLEDGMENT

The author would like to express her great appreciation to the Program Bantuan Seminar Luar Negeri Ditjen Penguatan dan Pengembangan, the author would like to express her great appreciation to the Program Bantuan Seminar Luar Negeri Ditjen Penguatan dan Pengembangan, Kemenristekdikti of Indonesia to facilitate to the 6th International Conference on Biological and Medicinal Sciences (ICBMS) 2018 in Seoul, South Korea.

REFERENCES

1. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564-82.
2. Hara H, Maruyama N, Yamashita S, Hayashi Y, Lee KH, Bastow KF, et al. Elecanacin, a novel new naphthoquinone from the bulb of Eleutherine americana. Chem Pharm Bull 1997;45:1714-6.
3. Alves TM, Kooos H, Zani CL. Eleutheronine, a novel fungitoxic naphthoquinone from Eleutherine bulbosa (Iridaceae). Mem Inst Oswaldo Cruz 2003;98:709-12.
4. Jinzhong X, Feng Q, Wenjuan D, Gexia Q, Naili W, Xinsheng Y. New bioactive constituents from Eleutherine americana. Chem J China 2006;26:320-3.
5. Nielsen LB, Wege D. The enantoioselective synthesis of elecanacin through an intramolecular naphthoquinone-vinyl ether photochemical cyclodaddition. Org Biomol Chem 2006;4:868-76.
6. Han AR, Min HY, Nam JW, Lee NY, Wiyawatan A, Suprapto W, et al. Identification of a new naphthalene and its derivatives from the bulb of Eleutherine americana with inhibitory activity on lipopolysaccharide-induced nitric oxide production. Chem Pharm Bull (Tokyo) 2008;56:1314-6.
7. Arnida A, Wahyono W, Mustofa M, Asmahusudarti R. In vitro antiplasmodial activity of ethanol extracts of borneo medicinal plants (Hydrolea spinosa, Ampelocissus rubiginosa, Urraria crinita, Angelopeteris excuta). Int J Pharm Pharm Sci 2015;7:72-5.
8. El-Shoumy WA, Nanis GA, Maha AE, Afwat MH. Antibacterial response of combination between antibiotics and some plant extracts against multidrug resistant bacteria. Adv Biol Res 2016;10:51-7.
9. Kamatou GP, Viljoen AM, van Vuuren SF, van Zyl RL. In vitro evidence of antimicrobial synergy between Salvia chameleseagana and Leonotis leonurus. South Afr J Bot 2006;72:634-6.
10. Ariyegoro O, Adewusi A, Oyedemi S, Akinpelu D, Okoh A. Interactions of antibiotics and methanolic crude extracts of Afzelia africana (Smith.) against drug resistance bacterial isolates. Int J Mol Sci 2011;12:4477-503.
11. Sivananthan M. Antibacterial activity of 50 medicinal plants used in folk medicine. Int J Biosci 2013;3:104-21.
12. Darshpreet K, Prasad SB. Anti-acne activity of acetone extract of Plumbago indica Root. Asian J Pharm Clin Res 2016;9:285-7.
13. Mhatre J, Smita N, Shradhita K. Formulation and evaluation of antibacterial activity of a herbal ointment prepared from crude extracts of Aegle marmelos, (BAEL). Int J Pharm Pharm Sci 2014;6 Suppl 2:575-9.
14. Bhalodia NR, Shukla VJ. Antibacterial and antifungal activities of leaf extracts of Cassia fistula L.: An ethnomedicinal plant. J Adv Pharm Technol Res 2011;2:104-9.
15. Yadav M, Chatterji S, Gupta SK, Watal G. Preliminary phytochemical screening of six medicinal plants used in traditional medicine. Int J Pharm Pharm Sci 2014;6:539-42.
16. Mejia F, Kamalinejad M, Ghaderi N, Vahidpour HR. Phytochemical screening of some species of Iranian plants. Iran J Pharm Res 2003;2:77-82.
17. Ghosal M, Mandal P. Phytochemical screening and antioxidant activities of two selected ‘bihi’ fruits used as vegetables in darjeening Himalaya. Int J Pharm Pharm Sci 2012;4:567-74.
18. Shahbaz Y. Antibacterial and antioxidant properties of methanolic of apple (Malus pumila), grape (Vitis vinifera), pomegranate (Punica granatum L.) and common fig (Ficus carica L.) Fruits. Pharm Sci 2017;23:308-15.
19. Bama SS, Kingsley JS, Kamaranarayan SS, Bama P. Antibacterial activity of different phytochemical extracts from the leaves of T. Procumbens Linn.: Identification and mode of action of the terpenoid compound as antibacterial. Int J Pharm Pharm Sci 2012;4 Suppl 1:557-64.
20. Alghazeer R, Elmansori A, Sidati M, Gammoudi F, Azwai S, Naas H, et al. In vitro antibacterial activity of flavonoid extracts of two selected Libyan algae against multi drug resistant bacteria isolated from food products. J Biol Med 2017;5:26-8.