BUKTI KORESPONDENSI

Lampiran	:	Peer review proses korespondensi submit Publikasi Jurnal Ilmiah Internasional Bereputasi
Nama Jurnal	:	International Journal of Applied Pharmaceutics Vol. 11 Special Issue 3 2019 Hal: 7-10
Index	:	Terindeks pada database internasional bereputasi dan berfaktor dampak (SCOPUS Q3)
Judul Jurnal	:	Antibacterial Activity of Bawang Dayak (<i>Eleutherine</i> sp.) and Tawas Ut (<i>Ampelocissus</i> sp.) from Central Kalimantan against <i>Propionibacterium</i> acnes

No	ltem	Tanggal	Halaman
1	Register/Submission pada konferensi	15 Mei 2018	1
	internasional ICBMS 2018 (International		
	Conference on Biological and Medical		
	Sciences) di Seoul, Korea Selatan 22-24		
	Agustus 2018		
2	Artikel accepted pada jurnal AJPCR (Asian	04 Juni 2018	2
	Journal of Pharmaceutical and Clinical		
	Research) (M0010)		
3	Notification dan Review artikel	05 Juni 2018	2
4	Review process (AJPCR)	16 Februari 2019	7
5	Artikel accepted pada jurnal AJPCR namun	13 Maret 2019	15
	jurnal tersebut masuk dalam kategori		
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8	Ethical approval	03 Februari 2018	28

1. Register/Submission pada konferensi internasional ICBMS 2018 (International Conference on Biological and Medical Sciences) di Seoul, Korea Selatan 22-24 Agustus 2018

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First of all, thank you for your concern. 2018 6th International Conference on Biological and Medical Sciences (ICBMS 2018) review procedure has been finished. We are delighted to inform you that your manuscript has been accepted for presentation at 2018 6th International Conference on Biological and Medical Sciences (ICBMS 2018) in Seoul, South Korea during August 22-24, 2018. Your paper was tripling blind-reviewed and based on the evaluation. The reviewers' comments are enclosed.

The conference received papers from about 10 different countries and regions during the submission period. And there are about 77 papers accepted by our reviewers who are the international experts from all over the world. The selected papers could be published in our conference journal with high quality. According to the recommendations from reviewers and technical program committees, we are glad to inform you that your paper identified above has been selected for publication and oral presentation. You are invited to present your paper and studies during our ICBMS conference that would be held on August 22-24, 2018, Seoul, South Korea.

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Paper of ICBMS 2018 will be published in Asian J Pharm Clin Res (AJPCR, Print ISSN- 0974-2441, Online ISSN- 2455-3891), and be indexed by SCOPUS, Google Scholar, Elsevier, EBSCO, EMBASE, SCI mago(SJR), CNKI, CAS, CASSI (American Chemical Society), Directory of Open Access Journal (DOAJ), Index Copencieus, ICAAP, Scientific commons, PSOAR, Open-J-Gate, Indian Citation Index (ICI), Index Medicus for WHO South-East Asia (IMSEAR), OAI, LOCKKS, OCLC (World Digital Collection Gateway), UIUC. Impact- 0.40 (SCImago, SJR 2016).

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Again, congratulations. We are looking forward to seeing you in Seoul, South Korea.

Yours sincerely,

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Vol 12, Special Issue 2, 2019



Online - 2455-3891 Print - 0974-2441 <u>Research Article</u>

ANTIBACTERIAL ACTIVITY OF BAWANG DAYAK (ELEUTHERINE SP.) AND TAWAS UT (AMPELOCISSUS SP.) FROM CENTRAL KALIMANTAN AGAINST PROPIONIBACTERIUM ACNES

SYAHRIDA DIAN ARDHANY*

Department of Pharmacy Faculty of Health Science, Muhammadiyah University of Palangkaraya, Central Kalimantan, Indonesia. Email: chass501@gmail.com

Received: ???, Revised and Accepted: ???

ABSTRACT

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Objective: The aim of the present study was to investigate phytochemical screenings and the *in vitro* effect antibacterial of BD (*Eleutherine* Sp.) and TU (*Ampelocissus* Sp.) against Propionibacterium acnes.

Methods: The antibacterial activity was investigated against Propionibacterium acnes by well diffusion method.

Results: Preliminary phytochemical screenings of BD ethanolic extract were found positive alkaloid, saponin, tannins, and steroid while TU positive flavonoid, saponin, tannins, steroid, and triterpenoid. Antibacterial activity against Propionibacterium acnes of ethanolic extract BD with concentration 25 mg/ml and 50 mg/ml showed the zone of inhibition 3.23 mm and 7.8 mm with category weak activity while ethanolic extract TU with same concentration showed zone of inhibition 10 mm (weak activity) and 16.3 mm (moderate activity) which mean ethanolic extract TU have better antibacterial activity. A combination ethanolic extract of both with variant ratio showed a zone of inhibition 6.7 mm (1:1), 3.9 mm (1:2), and 3.63 mm (2:1).

Conclusion: In this present study showed the highest potential antibacterial activity against propionibacterium acnes is an ethanolic extract of TU and The best ratio combination is 1:1. Furthermore, this study needs more research with variant concentration so that may be possible to be used as natural anti-acne formulations.

Keywords: Ampelocissus, Antibacterial, Eleutherine, Propionibacterium acnes.

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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, antidiabetic, antifungal, and anti-inflammation. Studies demonstrated that bulbs of *Eleutherine (E. bulbosa and E. Americana*) contain naphtoquinones (elecanacine, eleutherine, eleutherol, and eleutherinone) [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 *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 had 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 phytochemicals 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 bulbosci*) and Tawas ut (*A. rubiginosci*). 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° cfu/mL of 0.5 McFarland standard).

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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

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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 showed secondary metabolites that ethanolic extract of Bawang Dayak (Eleutherine Sp.) positive alkaloid, saponin, tannins and steroid while ethanolic extract of Tawas Ut (Ampelocissus Sp.) positive flavonoid, saponin, tannins, steroid and triterpenoid.

Tanning 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.) and Tawas Ut (Ampelocissus Sp.) mainly revealed the presence of flavonoid and alkaloid et al. (Table 1).

Antibacterial activity

In few past decades, there has been special interest in the use of abundant naturally occurring antimicrobials and antioxidants such as plants and fruits, et al. 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).

The highest anti-acne effect was found for ethanolic extract TU (Ampelocissus Sp.) with 16.3 mm zone of inhibition which means moderate activity, while BD (Eleutherine Sp.) has a low zone of inhibition with the same concentration (50 mg/ml) but it is can

Secondary metabolites	Ethanolic extract of bawang dayak (<i>Eleutherine</i> Sp.)	Ethanolic extract of tawas Ut (<i>Ampelocissus</i> 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 of precipitation [16].
Saponin	Ť		The presence of saponin was positive if froth ≥1.2 cm [16].
Tannins	·		Positive tests are confirmed by the addition of the FeCl ₃ 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 triterpenoid was confirmed [17].

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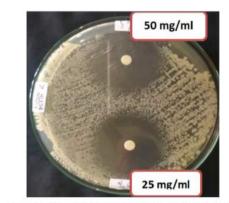
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Table 2: Antibacterial against Propionibacterium acnes effect of positive control, ethanolic extract BD (Eleutherine Sp.), ethanolic extract TU (Ampelocissus Sp.) and a combination of both by well diffusion method

Name of sample	Concentration (mg/ml)	Zone of inhibition (mm)			X±SD
		I	п	ш	
Clindamycin (positive control)	25	30.9	29.5	30.6	30.3±0.7
	50	33.5	36.5	30.8	33.6±2.8
BD (Eleutherine Sp.)	25	3.5	2.2	4	3.2±0.93
5	50	6.1	6.7	10.6	7.8±2.44
TU (Ampelocissus Sp.)	25	7.5	10.6	11.9	10.0±2.2
	50	18.8	12.7	17.3	16.3±3.1
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±0.3



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Fig. 1: Zone of inhibition of clindamycin (positive control)



Fig. 2: Zone of inhibition of ethanolic extract tawas Ut (Ampelocissus Sp.)

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

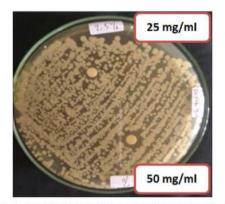


Fig. 3: Zone of Inhibition of ethanolic extract Bawang Dayak (Eleutherine Sp.)

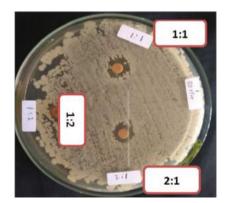


Fig. 4: Zone of Inhibition of a combination ethanolic extract of both

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 highest zone of inhibition is ratio 1:1 with the same concentration (25 mg/ml) in weak activity category (6.7 mm) 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.

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CONCLUSION

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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, 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, Kemenristekdikti of Indonesia to facilitate to the 6^{th} International Conference on Biological and Medicinal Sciences (ICBMS) 2018 in Seoul, South Korea.

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plants (Hydrolea spinosa, Ampelocissus rubiginosa, Uraria crinite, Angiopteris evecta). Int J Pharm Pharm Sci 2015;7:72-5.

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Name & mail address of corresponding author: Syahrida Dian Ardhany, chass501@gmail.com Highlighted Corrections

S.No.	Asked query no.	Details
1	AQ1	Received: 05 June 2018, Revised and Accepted: 16 February 2019
2	AQ2	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.
3	AQ3	 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).
		2. 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
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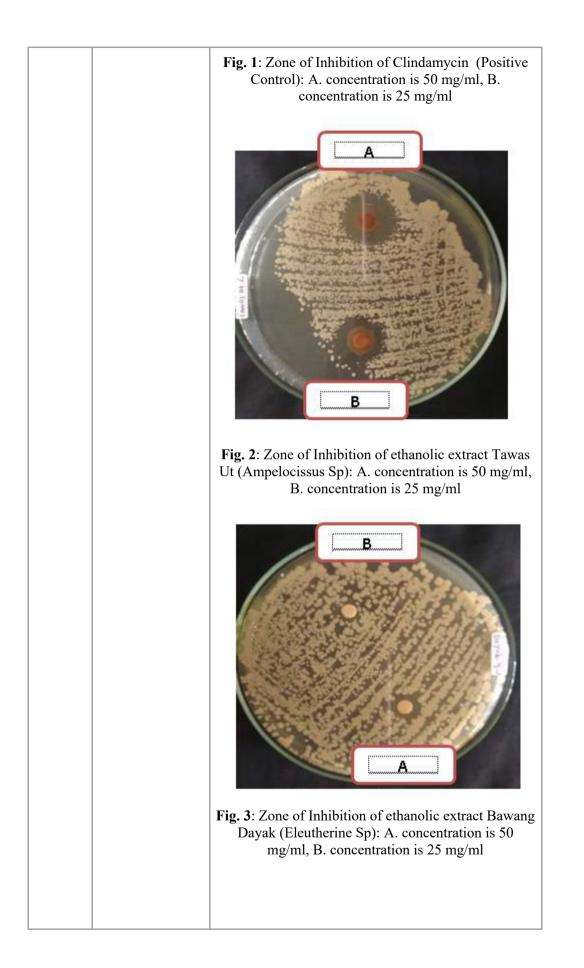
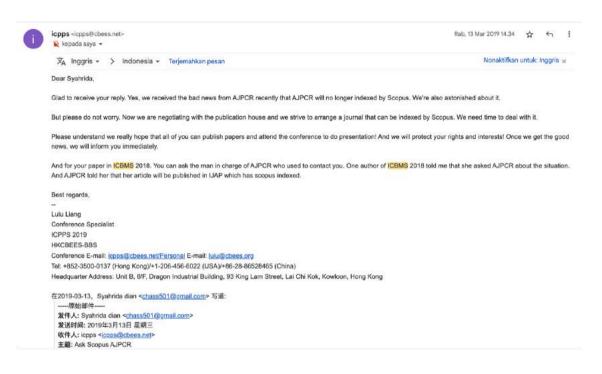


Fig. 4 : Zone of Inhibition of a combination ethanolic extract of both (Bawang Dayak & Tawas Ut) with comparison of concentration : A. 1:1, B. 1:2, C. 2:1

Other corrections

Page No.	Column(left or right)/Section /Paragraph/line no or talbe or figure	Incorrect text or matter	Corrected text

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Research Article

ANTIBACTERIAL ACTIVITY OF BAWANG DAYAK (*ELEUTHERINE* SP.) AND TAWAS UT (*AMPELOCISSUS* SP.) FROM CENTRAL KALIMANTAN AGAINST PROPIONIBACTERIUM ACNES

SYAHRIDA DIAN ARDHANY*

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Received: 05 June 2018, Revised and Accepted: 16 February 2019

ABSTRACT

Objective: The aim of the present study was to investigate phytochemical screenings and the in vitro effect antibacterial of BD (Eleutherine Sp.) and TU (Ampelocissus Sp.) against Propionibacterium acnes.

Methods: The antibacterial activity was investigated against Propionibacterium acnes by well diffusion method.

Results: Preliminary phytochemical screenings of BD ethanolic extract were found positive alkaloid, saponin, tannins, and steroid while TU positive flavonoid, saponin, tannins, steroid, and triterpenoid. Antibacterial activity against Propionibacterium acnes of ethanolic extract BD with concentration 25 mg/ml and 50 mg/ml showed the zone of inhibition 3.23 mm and 7.8 mm with category weak activity while ethanolic extract TU with same concentration showed zone of inhibition 10 mm (weak activity) and 16.3 mm (moderate activity) which mean ethanolic extract TU have better antibacterial activity. A combination ethanolic extract of both with variant ratio showed a zone of inhibition 6.7 mm (1:1), 3.9 mm (1:2), and 3.63 mm (2:1).

Conclusion: In this present study showed the highest potential antibacterial activity against propionibacterium acnes is an ethanolic extract of TU and The best ratio combination is 1:1. Furthermore, this study needs more research with variant concentration so that may be possible to be used as natural anti-acne formulations.

Keywords: Ampelocissus, Antibacterial, Eleutherine, Propionibacterium acnes.

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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, antidiabetic, antifungal, and anti-inflammation. Studies demonstrated that bulbs of *Eleutherine (E. bulbosa and E. Americana*) contain naphtoquinones (elecanacine, eleutherine, eleutherol, and eleutherinone) [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 *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 had 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 phytochemicals 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° cfu/mL of 0.5 McFarland standard).

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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 (<i>Eleutherine</i> Sp.)	Ethanolic extract of tawas Ut (<i>Ampelocissus</i> 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 \ge 1.2 cm [16].
Tannins	·	•	Positive tests are confirmed by the addition of the FeCl _s 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].

 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	п	ш	
Clindamycin (positive control)	25	30.9	29.5	30.6	30.3±0.74
, a ,	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

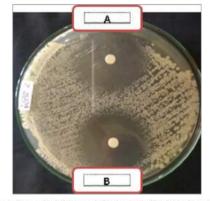


Fig. 1: Zone of Inhibition of Clindamycin (Positive Control): A. concentration is 50 mg/ml, B. concentration is 25 mg/ml



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

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



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

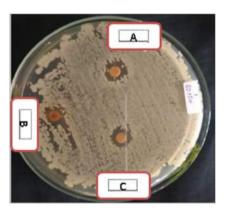


Fig. 4: Zone of Inhibition of a combination ethanolic extract of both (Bawang Dayak & Tawas Ut) with comparison of concentration : A. 1:1, B. 1:2, C. 2:1

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

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, 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 concentrations.

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7. Article Published (IJAP)



ANTIBACTERIAL ACTIVITY OF BAWANG DAYAK (ELEUTHERINE Sp) AND TAWAS UT (AMPELOCISSUS Sp) FROM CENTRAL KALIMANTAN AGAINST PROPIONIBACTERIUM ACNES

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ABSTRACT

Objective: The aim of the present study was to investigate phytochemical screenings and the in vitro effect antibacterial of BD (Eleutherine Sp) and TU (Ampelocissus Sp) against Propionibacterium acnes

Methods: The antibacterial activity was investigated against Propionibacterium acnes by well diffusion method

Results: Preliminary phytochemical screenings of BD ethanolic extract were found positive alkaloid, saponin, tannins and steroid while TU positive flavonoid, saponin, tannins, steroid and triterpenoid. Antibacterial activity against P.acnes of ethanolic extract BD with concentration 25 mg/ml and 50 mg/ml showed the zone of inhibition 3.23 mm and 7.8 mm with category weak activity while ethanolic extract TU with same concentration showed zone of inhibition 10 mm (weak activity) and 16.3 mm (moderate activity) which mean ethanolic extract TU have better antibacterial activity. A combination ethanolic extract of both with variant ratio showed zone of inhibition 6.7 mm (1:1), 3.9 mm (1:2) and 3.63 mm (2:1).

Conclusion: in this present study showed the highest potential antibacterial activity against P.acnes is an ethanolic extract of TU and The best ratio combination is 1:1. Furthermore this study needs more research with variant concentration so that may be possible to be used as natural anti acne formulations.

Keywords: Ampelocissus, Antibacterial, Eleutherine, Propionibacterium acnes

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.

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MATERIAL AND METHODS

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

Preparation of Plant extracts

The healthy and fresh bulb of the plant bawang dayak (Eleutherine bulbosa) and a root of Tawas ut (Ampelocissus rubiginosa) was 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 by 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 Zone of Inhibition by Well Diffusion Method

The bacterial isolates were subcultured into a nutrient broth. The 24-hour-old bacterial culture was standardized using McFarland standard (10^6 cfu/mL of 0.5 McFarland standard).

Mueller Hinton Agar (MHA) was used for bacteria bioassay. MHA was prepared by dissolving 38g in 1000ml of distilled water and brought to boil to completely dissolve. Sterilization was achieved by autoclaving at 121° C for 15 minutes [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 hours and the zone of inhibition was measured [14] and recorded later on.

RESULTS AND DISCUSSION

Preliminary phytochemical screenings

In general secondary metabolites compound are widely distributed in plants and contribute significantly toward biological activities or pharmacological effects including antibacterial, antioxidant, etc. In this present study showed secondary metabolites that ethanolic extract of Bawang Dayak (Eleutherine Sp) positive alkaloid, saponin, tannins and steroid while ethanolic extract of Tawas Ut (Ampelocissus Sp) positive flavonoid, saponin, tannins, steroid and triterpenoid.

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 dan 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).

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].

Table 1. Secondary Metabolites of an Ethanolic extract of Bawang Dayak (Eleutherine Sp) & Tawas Ut (Ampelocissus Sp).

Saponin	+	+	The presence of saponin was positive if froth ≥ 1.2 cm [16].
Tannins	+	+	Positive tests are confirmed by the addition of the FeCl ₃ solution to the extract and should result in a characteristic blue, blue black, green or bluegreen color and precipitate (phenolic compounds) [16].
Steroid	+	+	Formation of red colour ring confirmed the presence of steroid [17].
Triterpenoid	-	+	If reddish violate colour appeared, the existence of triterpenoids was confirmed [17].

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 lower than 12 mm), moderate activity (inhibition zone between 12 and 20 mm) and strong activity (inhibition zone higher than 20 mm). The results of antimicrobial activity revealed that significant antibacterial activity was showed against Propionibacterium acnes in comparision with positive control or standards clindamycin (Table 2).

The highest anti acne effect was found for ethanolic extract TU (Ampelocissus Sp) with 16.3 mm zone of inhibition which means moderate activity, while BD (Eleutherine Sp) has a low zone of inhibition with the same concentration (50 mg/ml) but it is can be potential strong activity if the concentration was increased so are ethanol extract TU (Ampelocissus Sp) and this requires further research. Difference zone of inhibition is possible because of 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 significanly 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 highest zone of inhibition is ratio 1:1 with the same concentration (25 mg/ml) in weak activity category (6.7 mm) but still has potential as antibacterial against propionibacterium acnes and may be better inhibitory if given a concentration greater than 25 mg/ml which will later be the basis of further research.

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	Zone of Inhibition (mm)			$\overline{\mathbf{X}} \pm \mathbf{S}\mathbf{D}$
	(mg/ml)	Ι	II	III	$\Lambda \pm SD$
Clindamycin (Positive	25	30.9	29.5	30.6	30.3 ± 0.74
Control)	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

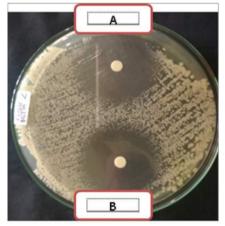


Fig. 1: Zone of Inhibition of Clindamycin (Positive Control): A. concentration is 50 mg/ml, B. concentration is 25 mg/ml

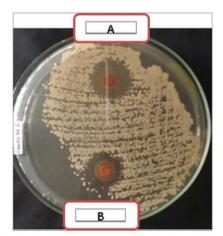


Fig. 2: Zone of Inhibition of ethanolic extract Tawas Ut (*Ampelocissus Sp*): A. concentration is 50 mg/ml, B. concentration is 25 mg/ml

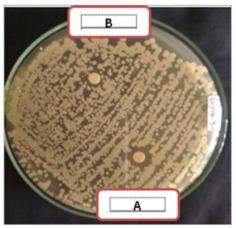


Fig. 3: Zone of Inhibition of ethanolic extract Bawang Dayak (*Eleutherine Sp*): A. concentration is 50 mg/ml, B. concentration is 25 mg/ml

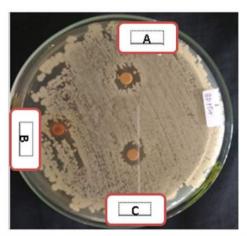


Fig. 4: Zone of Inhibition of a combination ethanolic extract of both (Bawang Dayak & Tawas Ut) with comparison of concentration : A. 1:1, B. 1:2, C. 2:1

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, 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 rasio 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.

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	Banjarmasin, 03 Februari 2018
No. SK	: 002.1/KE-LPPM/STIKES-SM/II/2018
Lampiran	:-
Perihal	: Rekomendasi Penelitian
	Sehubungan dengan telah dilaksanakannya sidang Etik Penelitian
kepada	a:
Nama	a : Syahrida Dian Ardhany
NIK	: 14.0601.033
Judul	: Uji aktivitas antibakteri Bawang Dayak (Eleutherine Sp.) dan Tawas
	ut (Ampelocissus Sp.) asal Kalimantan Tengah terhadap
	Propionibacterium acnes
Telah	DISETUJUI untuk dilanjutkan penelitiannya.
	Demikian surat persetujuan ini diterbitkan untuk dipergunakan dengan
penuh	tanggung jawab.
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