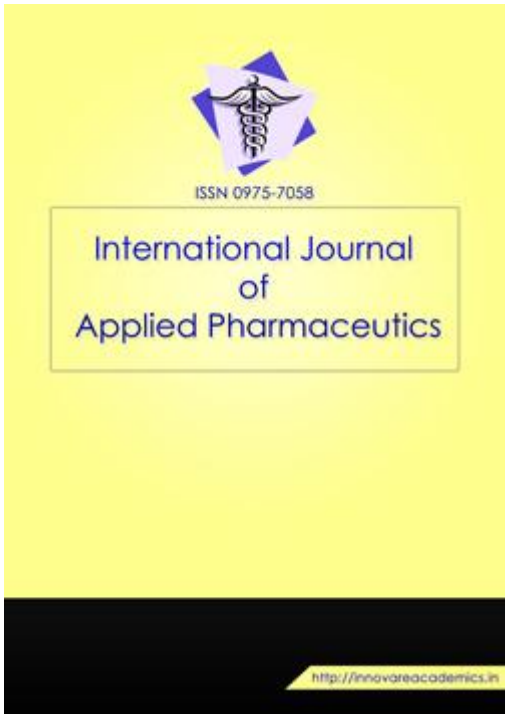

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Research Article

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 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 (10^6 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.)

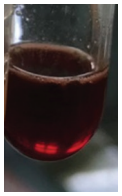
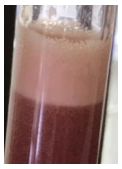
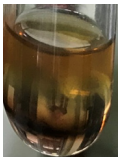
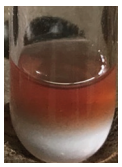
Secondary metabolites	Ethanolic extract of bawang dayak (*Eleutherine* Sp.)		Ethanolic extract of tawas Ut (*Ampelocissus* Sp.)		References
Flavonoid	–		+		The presence of flavonoids was indicative if pink or magenta-red color developed within 3 min [16].
Alkaloid	+		–		The samples were then observed 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 $FeCl_3$ 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 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	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

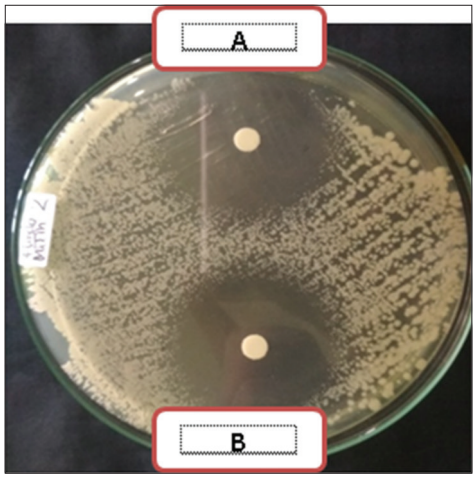


Fig. 1: Zone of Inhibition of Clindamycin (Positive Control): A. concentration is 50 mg/ml, B. concentration is 25 mg/ml

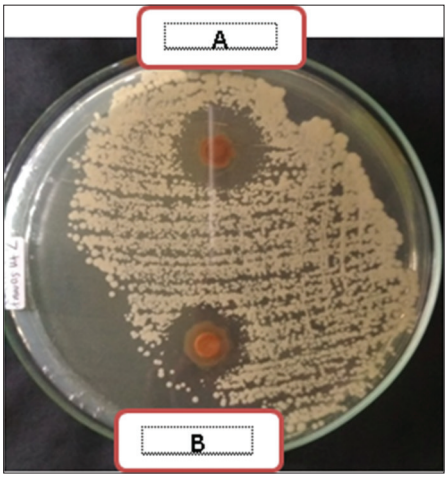


Fig. 2: Zone of Inhibition of ethanolic extract Tawas Ut (*Ampelocissus Sp*): A. concentration is 50 mg/ml, B. concentration is 25 mg/ml

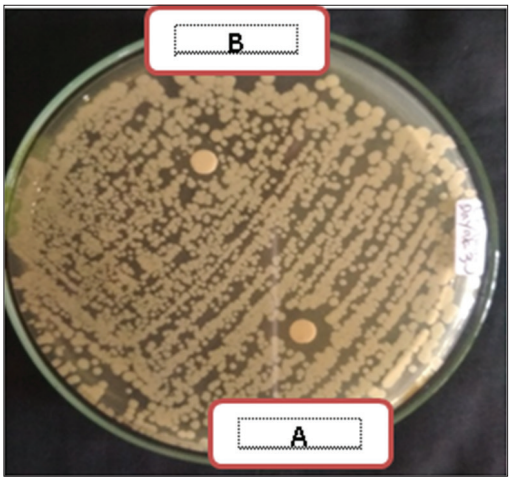


Fig. 3: Zone of Inhibition of ethanolic extract Bawang Dayak (*Eleutherine Sp*): A. concentration is 50 mg/ml, B. concentration is 25 mg/ml

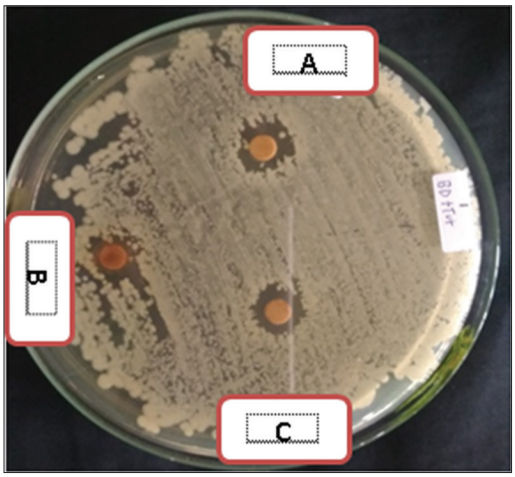


Fig. 4: Zone of Inhibition of a combination ethanolic extract of both (Bawang Dayak & Tawas Ut) with comparison of concentration : A. 1:1, B. 1:2, C. 2:1

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

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 (*Ampelocissu*s 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.

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