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Phytochemical Screening and Antibacterial Activity of Leaves Extract Balangla (*Litsea cubeba* (Lour) Pers.) from Malinau, East Borneo

Hetty Manurung[1,a], Rudy Agung Nugroho[2,b] and Elvi Marina[3,c]

[1,2,3] Department of Biology, Faculty of Mathematics and Natural Sciences Mulawarman University, Samarinda. Indonesia

ahetty_manroe@ymail.com, brudyagung.nugroho@fmipa.unmul.ac.id, celvimarina92@gmail.com

Abstract. Balangla (Litsea cubeba) plant is used as traditional medicine by Dayak Kenyah tribe in East Kalimantan. It contains active compounds that are efficient to treat many human diseases and believed to have an antibacterial activity. The purposes of this study were to determine the phytochemical compounds of the balangla leaves and to investigate the antibacterial activity of ethanol extract of leaves *L. cubeba*. Respectively, various levels of ethanol extract of *L. cubeba* leaves viz: 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% were used to examine its antibacterial activity against bacteria gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) by using pitting diffusion method. The results indicated that alkaloids, flavonoids, phenols and steroids have been found as phytochemical compounds in the ethanol extracts of *L. cubeba* leaves. Meanwhile, the antibacterial activities of ethanol extracts of *L. cubeba* leaves against the test organisms had been determined and significantly inhibited the growth of S. aureus and *E. coli*, forming a wide inhibition zone (15.91±0.950 mm) for S. aureus and (16.23±0.416 mm) for *E. coli*. Further, antibacterial activity of (*L. cubeba*) in-vitro had been justified on its utility in traditional medicines for the treatment of infections of bacterial origin.

Keywords: Balangla (*Litsea cubeba*), Lauracea, Antibacterial, Phytochemical Screening.

Introduction

Litsea cubeba (Lours.) Pers., locally known as Balangla belongs to the Lauraceae plant family. *L. cubeba* is a 3- to 10 m evergreen tree or shrub widely distributed in South-eastern Asia, Southern China, Japan, and Taiwan [1, 2]. In Indonesia. *L. cubeba* distributed in Sumatera, Java, and Borneo. This plant is one of the common medicinal plants in Borneo. *L. cubeba* can be used as a flavouring or herbal medicine. As a flavouring, it gives a unique flavour resembling that of a mixture of pepper, ginger, and citrus. It is popular as a flavour enhancer in foods, cosmetics, and cigarettes [3, 4]. Balangla by the people of East Kalimantan especially Dayak Kenyah tribe used as traditional medicine to cure for coughs, colds, migraine headaches, skin diseases, pain reliever, diarrhoea, fever, aromatherapy, and even benefit as seasoning (spices) and believed to have an antibacterial activity. Recent study has been reported that an aqueous EtOH extract of the barks of *L. cubeba* has yielded five novel isoquinoline alkaloids, namely (+)-*N*-(methoxylcarbonyl)-*N*-nordicentrin, (+)-*N*-(methoxycarbonyl)-*N*-norpredicentrine, (+)-*N*-(methoxyl-carbonyl)-*N*-norbul-bodione, and (+)-*N*-(methoxycarbonyl)-*N*-norisocorydione, and (+)-8-methoxyl-isolaurenine *N*-oxide, and one known compound, (+)-*N*-(methoxycarbonyl)-*N*-norglaucine [5]. Another study [6] showed that the essential oil in the leaves of *L. cubeba* containing sineol, sitronellol, oinen alpha, beta-pinene, and limonene sitronellal. Previous studies revealed that essential oil and bioactive compound were shown potential as an anticancer, antioxidant, and antibacterial. Antibacterial compounds usually found in plant parts such as leaves, twigs, bark, and other parts. Antibacterial is useful to eliminate bacteria potentially harmful bacteria to health and pathogens such as *Staphylococcus aureus* and *Escherichia coli*. *E. coli* generate bladder infections and diarrhoea [7] while S. aureus can cause purulent infection. *S. aureus* led to various types of diseases such as infections of the polikel hair, sweat glands, ulcers, skin infections, and wound infections [8]. The purposes of this study were to determine the phytochemical compounds that found in the balangla leaves and to investigate the antibacterial activity of ethanol extract of leaves *Litsea cubeba*.

Material and Methods

Sample Collection

The leaves of *L. cubeba* were collected locally from Gunung Seribu (Can batu) Mahak Baru, district Sungai Boh Malinau, East Borneo, in December 2014. The plants collected were identified botanically in Plant Anatomy and Taxonomy Laboratory, Biology Department Mulawarman University and deposited in the herbarium for future reference.

Preparation of plant material

The fresh leaves were washed with tap water and then thoroughly cleaned with distilled water and shade dried for a week. Then the dried leaves (1500 g) were grinded to a fine powder by using blender. The powder was taken and macerated with 95% EtOH. They were kept at room temperature for 5 days. Thereafter the mixtures were filtered by using Whatmann filter paper no .1. The supernatant were pooled together, concentrated in rotary evaporator at 40°C. The dried extract (57.283 g) was used directly for the determination of a presence of phytochemicals and antibacterial activity.

Qualitative Analysis of Phytochemicals

Phytochemical examinations were carried out for leaves extract *L. cubeba* as per the standard methods [9].

Test for Alkaloids

Dragendorff's test: A-2 mL of extract was added with 5 mL of chloroform-ammonia 0.005 M, then homogenized and filtered. The filtrate was added with a few drops of 2M sulfuric acid and shaken to form two layers of acids and bases. The layer acid (found on the top layer), added with a few drops of reagent Dragendorff's. Formation of deposits of red-brown colour indicated the presence of alkaloids.

Test for Flavonoids

A-2 mL crude extract was added with 5 mL of water, boiled for 5 minutes and filtered. Two mL filtrate was added with 0.05 mg of Mg powder and 1 mL chloride acid, then shaken until homogeneous. A yellow or red colouration indicates the presence of flavonoids.

Test for phenols

Lead acetate test: To 2 mL of the extract, few mL of 1 % lead acetate solution was added. The formation of bluish black precipitate indicated the presence of tannins and phenolic compounds.

Test for saponins

Frothing test: A-2 mL of filtrate was diluted with 5 mL of distilled hot water and the mixture was shaken vigorously and observed for persistent foam which lasted for at least 10 mins which indicated the presence of saponins.

Test for Terpenoids

Liebermann - Burchard's test: Extract was treated with a few drops of acetic anhydride, boiled and cooled. Concentrated sulfuric acid was added from the sides of the test tube which showed a brown ring at the junction of two layers, and the formation of deep red colour indicated the presence of terpenoids.

Test for Antimicrobial Activity

Source of Bacterial

Two microbial isolates were chosen for antimicrobial investigation: Gram positive bacteria (*S. aureus*) and Gram negative bacteria (*E. coli*). Both microbial were purchased from Laboratory of Microbiology, Faculty of Sciences Mulawarman University, Samarinda, Indonesia.

Testing for Antibacterial Activity

The test organisms were sub cultured by streaking them on nutrient agar, followed by incubation for 20 hr at 37 °C. These were subcultured prior to each experiment and were used for antibacterial. The pitting-diffusion method of two species of bacteria *Staphylococcus aureus* and *Escherichia coli* was performed in in-vitro antibacterial activity. Medium that was used in the antimicrobial activity was MHA (Mueller Hinton Agar), an inoculum containing 107 bacteria/mL was incorporated. After the

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suspension of bacteria seep into the media, then created pit in the media using the sterile iron cork borer (6 mm diameter). Using sterilized dropping pipettes, different concentrations (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% /well) of leaves extract *L. cubeba* was carefully added into the wells The plates were then incubated at 37 °C for 24–48 hr. Chloramphenicol was used as positive control for antibacterial activities while DMSO was used as the negative control. The diameter of the inhibition zone was measured for antibacterial activities testing. Experiments were performed in triplicate, and the results were presented as the mean values of the diameters of the inhibitory zones from three runs. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone [10, 11]. The diameters of the inhibitory zones value were used as criteria to judge the antimicrobial activity (strong active: the diameters of the inhibitory zones ≥ 20 mm, active: the diameters of the inhibitory zones 10-20 mm, moderately active: the diameters of the inhibitory zones 5-10 mm, not active: the diameters of the inhibitory zones are invisible or ≤ 5 mm) [12].

Results and Discussion

Phytochemical Analysis

The results of the phytochemical analysis were carried out in extracts of *Litsea cubeba*. The experiment showed the presence of secondary metabolites such as alkaloids, flavonoids, phenols and steroids. The results of the phytochemical analysis of *L. cubeba* are shown in Table 1. The presence of alkaloids in leaves extract is likely to be responsible for antimicrobial activity effects observed. Alkaloid is one of the phytochemical compounds in *Litsea cubeba* that act as an antibacterial activity [13, 14].

Table 1. Qualitative phytochemical analysis of leaves extract *L. cubeba*

No	Parameters	Leaves extract
1	Alkaloid	+
2	Flavonoid	+
3	Phenol	+
4	Saponin	-
5	Steroid	+

(+indicates presence, - indicates absence)

Antimicrobial Activity

Antimicrobial activity is used to test whether the leaf extract has capability to control the growth of the bacterial. The zone of inhibition had been obtained for both bacterials *Staphylococcus aureus* and *Escherichia coli*. The result obtained, the zone of inhibition was recorded at ten concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% in Table 2.

Table 2. Antimicrobial activity of leaves extract of Litsea cubeba

Sample extract Conc. (%)	Zone inhibition (mm)	
	Staphylococcus aureus	Escherichia coli
10%	9.58±0.36	9.63±0.32
20%	10.71±0.62	10.65±0.75
30%	11.36±0.41	11.07±0.40
40%	12.40±0.40	12.41±0.18
50%	12,71±0.70	13.62±0.11
60%	13.06±0.29	13.16±0.68
70%	13.63±0.27	14.00±0.37
80%	14.03±0.81	14.56±0.66
90%	13.80±0.69	15.08±0.32
100%	15.91±0.95	15.23±0.41
Chloramphenicol (25%)	18.33±0.10	18.63±0.18

In general Table 2 showed that increasing concentrations of leaf extracts enhanced inhibitory zone on both bacteria. The extracts displayed relative antimicrobial activities against bacterial tested with the diameter of inhibition zones ranging between 9.58±0.36 to 15.91±0.95 mm (S. aureus) and 9.63±0.32 to 18.63±0.18 mm (*E. coli*). The highest antibacterial activity in both bacteria was found at 100 % concentration and the lowest at 10 % concentration of leaves extract. The results obtained was indicated of the presence of broad spectrum antimicrobial compounds or metabolic toxins in *L. cubeba* leaves extract that could be exploited in treating infections associated with the aforementioned bacterial. Previous studies on antimicrobial activities of medicinal plants indicated that inhibition zones of 10 mm or greater were taken to represent good activity of such plants [15, 16].

Conclusion

Balangla leaf extract can be used as a source of secondary metabolites such as alkaloids, Flavonoids, Phenol, and steroids. The results suggest that the antibacterial activity of leaves extract *L. cubeba* may contribute to prevent some of the diseases caused by S. aureus and *E. coli*.

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