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Phytochemical Screening, Antibacterial and Cytotoxic Activity of *Dendobium lasianthera* from Papua

VERENA AGUSTINI[1,*], EVA S. SIMAREMARE[2], ELSYE GUNAWAN[2], JANE AWOM[2], SUSAN WOPI[2]

[1]*Biology Study Program, Department of Biology, Faculty of Mathematics and Natural Sciences, Cenderawasih University, Jayapura*
[2]*Pharmacy Study Program, Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Cenderawasih University, Jayapura*

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ABSTRACT

The aims of the study are to evaluate bioactive compounds, antibacterial and cytotoxic potential of *D. lasianthera*. This orchid grows well all over New Guinea Island as an ornamental plant because of their beautiful flowers. Orchids also known rich of its phytochemical compounds which already used as a traditional medicine in many countries around the world. However, research in pharmacological fields is still limited. In this study, leaves and stem of *D. lasianthera* were powdered and extracted with ethanol followed by fractionated using n-hexane, ethyl acetate, and ethanol solvent. Extract as well as fraction were tested for phytochemical screening and determined antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* using Disc Diffusion Method. Brine Shrimp Lethality Test (BSLT) was used to observe cytotoxic potential of leaves and stem extract and fraction at 10, 50, 100, 250, 500, 750, and 1000 ppm. The results showed that the leaves and stem extract contained tannin and alkaloids, separately. The ethanol extract of *D. lasianthera* showed 7.35 mm (leaves) and 7.43 mm (stem) inhibition zone against *S. aureus* Furthermore, the maximum inhibition zone of ethanol fraction of leaves and n-hexane fraction of stem were 8.42 mm and 8.10 mm separately. The LC_{50} of stem extract and fractions in these study were 699.3 ppm (ethanol extract), 602.1 ppm (ethyl acetate), 329.6 ppm (n-hexane fraction) and 676 ppm (ethanol fraction), whereas for leaves, only ethyl acetate fraction has toxict activity with an average LC_{50} 833.2 ppm.

Key words: *D. lasianthera*; phytochemical screening; antibacterial; cytotoxicity; Papua.

INTRODUCTION

Orchidaceae is known as an ornamental plant because of their beautiful flowers. Since years ago Chinese and Indian used orchids as traditional healing of sickness. Research in ethnomedicine has been proved the pharmacology activities (Jorim *et al*., 2012; Bulpitt *et al*., 2007; Jalal *et al*., 2010; Behera *et al*., 2013; Kong *et al*., 2003; Panda *et al*., 2013).

Orchids are known to produce some active compounds like alkaloid, flavonoids, glycosides, carbohydrates and other phytochemical compounds. The study of the potential compounds in orchids as anti-inflammatory, antitumor, antimicrobial, antitoxin, antioxidant and anticancer have been carried out by many countries. Therefore, orchids are uncontrolled hunting by collectors for business either for ornamental plants (Agustini *et al*., 2013; 2015), while other used it for for traditional medicine (Rajendran *et al*., 1997; Bulpitt *et al*., 2007; Ganapaty *et al*., 2013, Khan *et. al*. 2019). More over in the last decade research in biocative potential of

* *Corrsponding author:*
Department of Biology, Faculty of Mathematics and Natural Sciences, Cenderawasih University
Jl. Kamp Wolker Waena, Jayapura, Papua.
E-mail: verena.agustini@gmail.com,

some genus of orchidaceae are also developed by some researcher like Shanmugavalli *et al. (*2009) studied on antimicrobial activity of *Vanilla planiflora*. Paul *et al.* (2013) worked on three species of orchids namely *Areides odorata*, *Acampe papilosa* and *Acampe ochracea* against ampicillin resistant srain of *E. coli*. Besra *et al*. (2011) was evaluated of antioxidant of tuber *Geodorum laxiflorum*.

More detail Dendrobium, the third largest genus of the Orchidaceae family, has been reported containing compounds that have the potential to be developed as antimicrobials namely *D. nutantiflorum* (Rashmi *et al.,* 2015), *D. crumenatum* (Sandrasagaran *et al.,* 2014), *D. amoenum, D. crepidatum, D. moniliforme, D. longicomu* (Paudel *et al*., 2018), *D. nobile* as an antitumor (Devi *et al.,* 2009), antioxidant (Prasad, *et al.,* 2016), antitoxin (Shrestha *et al.,* 2015). *Dendrobium* also contains many chemical and bioactive compounds that have the potential as drugs.

Distribution of *D. lasianthera* in Papua are Sorong, Fak-Fak, Nabire, Jayapura, and Merauke; whereas in Papua New Guinea is around tributaries of the Sepik River, and also the Ramu Valley. The flower is quite large, the inflorescentia can reach more than 3 meters. With 10 to 30 flowers. (Smart, 2008). *D. lasianthera* have weighed bunches of flowers (many flower buds), so sometimes the plant were fallen by strong winds (Cahyo & Dinarti, 2015). Not on for its beautiful flowers, in Indonesia *D. lasianthera* has been studied by Nugroho, et.al. in 2016 has anticancer activity, three vegetative organs root, stem and leaf of *D. lasianthera* are toxic. Because of the large area of distribution of the species it has opportunity to develop more research of *D. lasianthera* in pharmacology such as antibacterial and toxicity activities.

MATERIALS AND METHODS

Plant Material

D. Lasianthera were collected from Nimbokrang, Jayapura and determined in Biology Department, Cenderawasih University, Papua. The collected leaves and stem were sun dried and then put it in oven at 50 °C. The materials were blended, sieved with 100 µm pore and stored in a sealed container.

Phytochemical Screening

Phytochemical screening of the total extract revealed the presence of alkaloid, flavonoid, saponin and tannins.

Test for Alkaloid

0,1 g extract was diluted into1 ml HCl 2M. Water was added to the solution, then filtered. The filtrate was poured into two test tube. A few drops of Dragendorf's reagent was added to the extract solution in the first tube. The positive result indicated by the forming of reddish brown precipitate. The extract in the second tube was used to test the the extract with Mayer test. A few drops of Mayer's reagent was added to the extract solution. Creamy white precipitate was indicated

Table 1. The water contain of stem and leaf of *D. lasianthera.*

Sample	Fresh weight (g)	Dry weight (g)	water contain (%)
Stem	1000	200	80
Leaf	400	40	90

Table 2. Results of ethanolic extract *D. lasianthera*.

Sample	Weight		Color	Forming
	(g)	(%)		
Stem	3,9563	1,98	Blackish green	Paste, thick
Leaves	5,6196	14,05	Blackish green	Paste, thick

the presence of the alkaloid in the extracts.

Test for Flavonoid

Shinoda test was used to determine the presence of flavonoid. 3 ml methanol was poured into 0,1 g extract in the test tube. A little magnesium powder was added to the solution. Four to five drops of concentrated HCl was dropped into the mixture. Yellowish, yellow-orange occasionally orange color appears after few minutes indicated the presence of flavonoid.

Test for Tannins

0,1 g extract was diluted with a little water in a test tube, the heated for 5 minutes, then filtered. Filtrate was added with a few drops of $FeCl_2$ 1 %. Positive tannin could be seen by the forming of the blue-green color.

Test for Saponin

0,1 g extract was mixed with a little water, warmed for 5 minutes, then filtered. Filtrate was shaked for 10 seconds. The forming of foam that was not vanished with the addition of HCl 1% indicated saponin was positive.

Preparation of Plant Extracts and Fraction

A total of 200 g of simplicia of stem was weighed and soaked in 1300 ml 96% ethanol then stirred occasionally and 40 g simplicia of leaves soaked in 200 ml ethanol. Maceration process were repeated 3 times for 3 days. Concentrated extract was obtained by filter paper, evaporated at 40 ^oC then stored. Extract was fractionated in 3 different solven with n-hexane, ethyl acetate, and ethanol separately. Three fraction of n-hexane, ethyl acetate, and ethanol fraction were prepared.

Antibacterial activity

28 g of Nutrient Agar (NA) mixed in 1000 ml of distilled water. The mixture was heated and then sterilized using an autoclave at 121 ^oC for 15 minutes. 15-20 ml of NA was poured into a petri dish, and allowed to dry. After solidifacation of media, inoculum of strain *Escherichia coli* (ATCC 25923) and *Staphylococcus aureus* (ATCC 25922) (collection of Microbiology Laboratory Cendera-wasih University, Indonesia) were swabbed uniformly and keep for about 5 minutes to dry, then incubated in an incubator 37 ^oC for 24 hours. Bacterial colonies from the subculture medium were taken by one end of the loop, suspended in 5 ml of 0.9 % Natrium chloride brine in the test tube until their turbidity were same with McFarland's standard (108 CFU/ml). McFarland turbidity standard was made of 0.5 mL of 1% Barium chloride and 9.5 mL of 1% H_2SO_4 (Hastari, 2012).

The antibacterial activity of the extract and fraction of leaves and stem *D. lasianthera* was evaluated by using disc diffusion method against *E. coli* and *S. aureus.* The concentration of ethanol extract used in this study was 1000 ppm. 10 µL test solution is then dropped onto sterile disc paper and allowed the solvent to evaporate, then placed on the surface agar medium. The media was then incubated at 37 for 24 hours then examinated. The antibacterial activity expressed of inhibition zone diameter in mm, and analyzed statistically using a Completely Randomized Design (CRD) method and further tested using BNJ test.

Brine Shrimp Lethality Test (BSLT)

The cytotoxic activity of extracts and fractions of leaves and stem *D. Lasianthera* was tested using BSLT method. One hundred mg of the extract was added to the 100 mL flask and dissolved with ethanol to get 1000 ppm stock solution. The various sample concentrations (10, 50, 100, 250, 500, 750, and 1000 ppm) were used in the study. Larvae of *Artemia salina* Leach were used as a test organisms. Three grams of shrimp eggs were placed and hatched using 2 L sea water from Amai beach Sentani with pH 7 for 48 hours under the light and room temperature. A vial containing 50 µl DMSO in 10 mL of sea water and 10 living larvae were used as the negative control.

Taken 5 ml of each test sample (10, 50, 100, 250, 500, 750, and 1000 µg/ml) in vials, let the solvent evaporated, after that added 1% 50µl DMSO solution, and sea water to the limit. A total of 10 larvae were inserted into each vial. After 24 hours, observed the number of death shrimp larvae. The LC_{50} was calculated using probit

method by transforming the percent of deaths in the probit table.

RESULTS AND DISCUSSION

The fresh weight of 1000 g of stems and 400 g leaves of *D. lasianthera* were dried to keep the simplicia stable. The dry weight of stem simplicia was 200 g and 40 g for the leaf. The percent of water content is useful in order to determine the resistance of the material during storage and to avoid the growth of microorganisms (Table 1).

Extract yield

Extraction method used is maceration

Table 3. Results of fractionation of *D. lasianthera*.

	Solvent	Weight (g)	Recovery (%)
Stem	Ethanol	0.6381	42.00
	Etyl acetate	0.3932	25.89
	n-hexane	0.3033	19.97
Leaves	Ethanol	0.5615	21.89
	Etyl acetate	0.8234	32.10
	n-hexane	0.9107	35.51

Table 4. Test results of phytochemical screening stem and leaves of *D. lasianthera*

Group of compounds	Stem extract	Leaves extract
Alkaloids	+	+
Flavonoids	-	-
Quinones	-	-
Saponins	-	-
Tannin	+	+
Triterpenoids	-	-

Note : (+) Detected, (-) Not detected

Table 5. Test extracts and fractions of leaf and stem of *D. lasianthera* on *S. aureus*.

Sample		Zone of Inhibition (mm) (mean)	Notation
Ethanol extract	Leaves	7.35	bc
	Stem	7.43	bc
Fraction (Leaf)	Ethanol	8.42	b
	Eyil Acetate	7.84	bc
	N-hexane	7.98	b
Fraction (stem)	Ethanol	6.77	bc
	Eyil Acetate	7.17	bc
	N-hexane	8.10	b
K (+)	Sifrofloxasin	25.70	a
K (-)	Aquabidest	0	c
	$BNJ_{0,05}$ = 0,57	$BNJ_{0,01}$ = 0.82	

extraction using 96% ethanol. During the maceration process, the solvent diffuses into the sample and dissolves compounds that have the same polarity as the solvent. The advantage of maceration is it not require high temperature, so all temperature sensitive compounds are not decomposed. The Table 2 showed colours and formed 200 g fine powder of the stem *D. lasianthera*, blackish green, paste and thick as much as 3.9563 g with extract levels of 1.98 %. While 40 grams of leaves *D. lasianthera*, was blackish green color, paste and viscous form as much as 5.6196 g with extract content of 14.05 %.

The extract was fractionated by Liquid-Liquid Extraction (ECC) method using three different solvents ethyl acetate, n-hexane, and ethanol separately. Ethyl acetate will dissolve semi polar compounds, n-hexane for non-polar compounds, the residual results, ethanol solvents containing polar compounds. The purpose of fractionation is to separate compounds based on their polarity. In principle, polar compounds are extracted with polar solvents while non-polar compounds are extracted with non-polar solvents (Harbone, 1987). Ethanol as polar, ethyl acetate as semi-polar, and n-hexane as non-polar solvents. The results of the fractionation of *D. lasianthera* stem and leaf extract were shown in Table 3.

The highest recovery in ethanol fraction was 42.00 %, this is because the thick extract of the *D. lasianthera* stem containing more polar compounds. Methanol solvents were able to extract components derived from alkaloids, phenolics, steroids, triterpenoids, tannins, and saponins. (It is known that solvents of methanol and ethanol are alcohol groups and have polar properties. Whereas the *D. lasianthera* leaves fraction was the most abundant in n-hexane solvent is 35.51%. This informed that *D. lasianthera* orchid leaf contains more non-polar compounds. N-hexane is a non-polar solvent that capable of dissolving water-insoluble compounds such as lipids, chlorophyll, essential oils, carotenoids and waxes. Recovery number of water extract of leaves and stem *D. lasianthera* reported in Laurentius *et al.* (2016) paper were 25.6 % and 11.9 % separately. Moreover much lower result also reported in other solvent namely chloroform 4.8 % (stem), 4.2 % (leaf) and methanol 3.35 % (stem), 6.65 % (leaves).

Results of Preliminary Phytochemical Screening

Phytochemical screening has been carried out to determine the group of secondary metabolites in *D. lasianthera*. This purpose of the preliminary study was to see if this orchid has the potential as a raw material for medicine. Phytochemical screening results of extracts and fractions of the stem and leaves of *D. lasianthera* were shown in Table 4.

Based on the results of phytochemical screening, it can be seen that the ethanol extracts of the stem and leaf of *D. lasianthera* contained tannin and alkaloid compounds. And no class of saponins, flavonoids, triterpenoids and quinones were detected. Study using chloroform and methanol extract of organs of *D. lasianthera* showed that the bioactive compound were terpenoid and phenolic (Laurentius *et al.,* 2016). Research in phytochemical *D. crumenatum* found saponin, terpenoid and alkaloid compounds (Sandrasagaran *et al*., 2014). Effectiveness of the bioactive constituents might related to the solvents used for their extraction and fraction. Alkaloids and tannins are known to function as antioxidants, so it is very good for the prevention of cancer and alkaloids itself is also good for analgesic, anti-inflammatory, anti-fungal, anti-cancer, and antioxidant activities.

Antibacterial Activity Test

Antibacterial activity test was done using disc diffusion method. Disk diffusion is a testing method to see antibacterial activity by seeing whether there is a clear area (inhibitory zone) around the disc paper on bacterial growth in solid media. The presence of antibacterial activity is indicated by the formation of inhibitory zones. In this experiment gram-positive bacteria were represented by *Staphylococcus aureus* and gram-negative bacteria represented by *Escherichia coli*. Stem and leaf extracts, as well as the fraction of *D. lasianthera* were used in this experiment.

According to Davis & Stout, 1971 the criteria for the strength of antibacterial inhibition as

Table 6. Percent of mortality of *A. salina* in stem ethanol extract and fraction of *D. lasianthera*.

Concentrat (ppm)	Log Concentrat	Ethanol extract		Hexene fraction		Ethyl acetate fraction		Ethanol fraction	
		% Mortality	Probit value	% Mortality	Probit value	% Mortality	Probit value	% Mortality	Probit value
10	1.0	6.67	3.45	6.67	3.45	13.33	3.87	10.00	3.72
50	1.7	16.67	4.01	10.00	3.72	20.00	4.16	20.00	4.16
100	2.0	20.00	4.16	20.00	4.16	23.33	4.26	23.33	4.26
250	2.4	23.33	4.26	36.67	4.64	30.00	4.48	26.67	4.36
500	2.7	43.33	4.82	60.00	5.25	40.00	4.75	43.33	4.82
750	2.9	56.67	5.15	66.67	5.41	53.33	5.05	53.33	5.08
1000	3.0	63.33	5.33	83.33	5.95	73.33	5.41	66.67	5.41

Table 7. Percent death average of *A. salina* Leach to leaves ethanol extract and fraction of *D. lasianthera*.

Concentrat (ppm)	Log Concentrat	Ethanol extract		Hexane fraction		Ethyl acetate fraction		Ethanol fraction	
		% Mortality	Probit value	% Mortality	Probit value	% Mortality	Probit value	% Mortality	Probit value
10	1.0	6.67	3.45	6.67	3.45	3.33	3.12	0.00	0
50	1.7	10.00	3.72	16.67	4.01	20.00	4.16	3.33	3.12
100	2.0	16.67	4.01	20.00	4.16	23.33	4.26	6.67	3.45
250	2.4	23.33	4.26	30.00	4.48	30.00	4.48	10.00	3.72
500	2.7	26.67	4.36	36.67	4.64	36.67	4.64	13.33	3.87
750	2.9	30.00	4.48	40.00	4.75	43.33	4.82	16.67	4.01
1000	3.0	33.33	4.56	46.67	4.9	60.00	5.25	20.00	4.16

Table 8. LC_{50} of leaves ethanol extract and fraction of *D. lasianthera* on *A. salina*.

Part of Plant	Extract/Fraction	Linear regression equation	R2	LC50 (ppm)
Stem	Extract	Y=0.092x + 2.434	0.903	699.3
	Fraction n-hexane	Y=1.241x + 1.875	0.924	329.6
	Fraction Ethyl acetate	Y=0.749x + 2.918	0.830	602.1
	Fraction ethanol	Y= 0.771x + 2.818	0.898	676.0
Leaves	Extract	Y=0.569x + 2.844	0.986	6153.1
	Fraction n-hexane	Y=0.699x + 2.775	0.995	1524.4
	Fraction Ethyl acetate	Y=0.897x + 2.380	0.993	833.2
	Fraction ethanol	Y=0.770x + 1.822	0.992	13396.7

follows, 5 mm inhibition zone diameter or less are categorized as weak, 5-10 mm inhibition zones are categorized as moderate, 10-20 mm inhibition zones are categorized as strong, and inhibition zones are 20 mm or more categorized as very strong. The results of the antibacterial activity test of extracts and fractions of *D. lasianthera* stems and leaves is in table 5. It showed the ethanol fraction of the leaves exhibited strong activity against *S. aureus*, with inhibition zone 8.42 mm. and no inhibitory activity against *E. coli* (there is no clear zone so the results are zero). One way ANOVA was conducted to assess the significance of different treatment against gram positive bacteria *S. aureus*.

In the table 5, a variety analysis of the antibacterial activity of *S. aureus* against extracts and fractions of *D. lasianthera* leaves and stems, F value = 192.10564 > F table 0.05 = 2.39, followed by BNJ test. Based on the results of the analysis of the variance above, it can be seen that the bacterial F value > F table, then proceed with the BNJ Test. Based on the results of the testing data, extracts and fractions of orchid stems and leaves *D. lasianthera* provided inhibitory zones for gram-positive bacteria (*S. aureus*) and negative results for gram-negative bacteria (*E. coli*). The antimicrobial activity of leaves and stem against *S. aureus* was generally in moderate category, the highest was the leaf ethanol fraction 8.42 mm. Similar results of methanolic extract of *D. crumenatum* against same bactaeria has been reported by Sandrasagaran *et al.* (2014). It has low antimicrobial activity (1-3 mm). In contrast, study on stem extract of *D. nobile* against the *E. coli* and *S. aureus* found better results 0.6 cm and 1.03 cm (ethanol), 0.26 cm and 1.0 cm (chloroform), and 0.46 cm and 1.2 cm (aqueous) (Devi *et al*., 2009). It is proved that solvent has an important role to make extract and fraction more effective.

Brine Shrimp Lethality Test Results

It is common that in the study of drug development process, an extract of crude plant firstly screened for their cytotoxic activity, after that they were assayed using cancer cell lines. In this study BSLT method was used to test toxic activity of *D. lasianthera*. The experiment used the seawater that taken from Amay beach (Depapre District) with pH= 7.0, and not polluted. Total seven graded concentration and three replications were used in the experiment. They were used for stem and leaves samples with 3 different fractions, namely ethanol, ethyl acetate and n-hexane fractions.

One extract and three fractions obtained were tested for toxicity using the *Brine Shrimp Lethality Test* (BSLT) method with a concentration of 10, 50, 100, 250, 500, 750, and 1000 ppm. This was determined the LC_{50} value of the samples to obtain the most toxict one. The results showed that the n-hexane fraction of stem *D. lasianthera* had toxic effects on *A. salina* larvae with LC_{50} = 329.6 ppm, compared with ethanol extracts of stem LC_{50} = 699.3 ppm, ethanol fraction of stem had LC_{50} values = 676.0 ppm, ethyl acetate fraction, LC_{50} = 602.1 ppm (Table 6; Table 7). These results indicate that the n-hexane fraction has a greater toxic effect than extracts and other fractions. A compound is toxic and has the potential as an anti-cancer candidate in BSLT testing if it has an LC_{50} value of 24 hours is less than 1000 µg/ml (Meyer *et al*., 1982).

The results (Table 8) showed that the sample of ethyl acetate fraction which had a toxic effect on *A. salina* larvae with LC_{50} = 833.2 ppm because it had LC_{50} values below 1000 ppm, compared with ethanol extract of leaves with LC_{50} = 6153.1 ppm, ethanol fraction of leaves had LC_{50} = 13396.7 ppm, n-hexane fraction with LC_{50} = 1524.4 ppm. These results indicate that only the ethyl acetate fraction has a toxic effect among others.

Other study using Chloroform as a solvent to extract stem of *D. lasianthera* demonstrated cytotoxic against T47D breast cancer cells (Laurentius *et al*., 2016). Research in 2009 by Devi *et al*. found that flower extract of *D. nobile* has an antitumor activity. More research in *Dendrobium* genus also indicated that the plant has great potential as antitoxic as well as antimicrobial agents. The present of alkaloids and flavanoids caused the lethality of the larvae of *A. Salina*. Alkaloid (vincristine and vinblastin) compounds inhibited the growth of cancer cells.

CONSLUSSION

Phytochemical screening of *D. lasianthera* indicated the presence of two bioactive compounds alkaloids and tannins. This results ensures further detailed investigations such as the isolation and purification of active chemical compounds. The study showed moderate antibacterial activity against *S. aureus* which means that the antibacterial effect can be further investigated. Study on toxicity found that the extract and fraction of *D. lasianthera* stem had great potential as a cytotoxic compound, wherein n-

hexane fraction of stem with average LC_{50} 329.9 ppm followed by ethyl acetate fraction LC_{50} 602.1 ppm, ethanol fraction LC_{50} 676 ppm, and ethanol exract with LC_{50} 699.3 ppm. Results on leaves extract and fraction showed non cytotoxic effect with LC_{50} greater than 1000 ppm, unless ethyl acetate fraction with average LC_{50} 833.2 ppm.

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