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Phytochemical Screening and Antibacterial Activity of *Dendrobium* from Papua Against *Eschericia coli* and *Staphylococcus aureus*

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ABSTRACT

Orchid found as one of medicinal plant in some areas, especially *Dendrobium*. Some species were investigated its bioactive compound, and antibacterial activity, but the information about antibacterial activity of Papuan *Dendrobium* species is still limited. The aims of this research were to determine the phytochemical constituents and the antibacterial activity of ethanolic extract and fraction of *Dendrobium* species from Papua against *Escherichia coli* and *Staphylococcus aureus*. There were three species of *Dendrobium* namely: *D. spectabile*, *D. violaceoflavens*, and *D. antennatum* used in this study. Dried simplisia was macerated using ethanol, then tested for the phytochemical content. Total ethanolic extract was fractionated with three different solvents, ethanol, ethyl acetate and hexane. Disc diffusion assay was used to examine the antibacterial activities of the total extract and the fractions. Total extract were positive for flavonoid, alkaloid and tanin, except *D. spectabile* stem extract only alkaloid and tannin. No saponin was found in the plants. The ethyl acetate fraction of leaves of *D. spectabile* showed the highest antibacterial activity against *S. aureus* with diameter of inhibition zone 20.54 $\pm$ 1.47 mm (strong category). Other plant extract and fraction tested had moderate antibacterial activities against both *E.coli* and *S aureus* with diameter zone 6-9 mm. The present work indicates that the ethyl acetate fraction of *D. spectabile* leaves is potential to be developed in antibacterial drug design research.

Key words: antibacterial activity; Papua; *Dendrobium*; *E. coli*; *S. aureus*.

INTRODUCTION

Genus *Dendrobium* (Orchidaceae) with more than 1100 species has been used as a traditional medicine over centuries all over the world such as Asia, Europe and Australia (Rosa, 2010; Xue *et al*., 2013). They contain some pharmaceutical properties likes phenolic, alkaloids, tannins, lignins, terpenoids and other phytochemical contents (Hoque *et al*., 2015). The pharmacological profile of species of Dendrobium already reported, namely *D. formosum*, *D. signatum*, *D. nobile*, *D. nutantiflorum*, *D. panduratum*,, *D. crumenatum* were demonstrated as anticancer, antioxidant and antimicrobial as well (Devi *et al*., 2009; Johnson & Janakiraman, 2013; Prasad & Koch, 2014; Rashmi *et al*., 2015; Chimsook, 2016). Study on isolation and structural characterization of bioactive compounds of *Dendrobium* was also already conducted such as *D. macraei* (Esha *et al*., 2016), *D. ovatum* (Ganapaty *et al*., 2013), and *D. moniliforme* (Lin *et al*., 2001).

Phytochemicals play a role for study the activity of bioactive compound in most species of Orchidaceae including *Dendrobium*. In China where *D. crysanthum* is widely found, their

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powder of the dry leaves used as antipyretic agent (Li *et al*., 2001). Bi *et al.* (2003) reported that in Japan, people used leaves paste of *D. fimbriatum* to promote body fluid. It is applied to the fractured of bone. Study of cytotoxic activity of some *Dendrobium* extracts were also done recently namely *D. brymerianum*, *D. ellipsophyllum*, *D. lasianthera* to test their cytotoxic activity (Klongkumnuankar *et al*., 2015; Tanagorumeatar *et al*., 2014).

Certain phytochemicals that easily found in orchidaceae are phenolics, alkaloids, flavonoids,terpenoids,and tannins. *Dendrobium* were widely explored for their bioactivity as anticancers (Prasad *et al.*, 2014; Laurentius *et al*., 2016). One compound of many that can be extracted from stem of most *Dendrobium* is *Denbinobin* a natural chemical constituent *phenanthroquinone* (Singh & Duggal, 2009). It is inducing apoptosis in cancer cells. Papua has almost 500 species of *Dendrobium* from total around 1200 species in the world. Four species of genus *Dendrobium* used in this study are all epiphytic, beautiful spray orchid, and commonly found in the retail trade.

In Papua, *Dendrobium* are distributed from the low land to the high land, from coast to mountain. It becomes incredible sources of highly valued bioactive compounds, and therefore methodology are being developed to get the need. The aim of the present study was to determine the phytochemical content and antibacterial activity on the extract of the three species of *Dendrobium* from Papua.

MATERIALS AND METHODS

Material

Fresh plant materials of three *Dendrobium* namely *D. spectabile* (Blume) Miq., *D. violaceoflavens* J.J.Sm., and *D. antennatum* Lindl. were collected from Brian Orchid, Jayapura, Papua (Figure 1). Plant identification was done at Plant Systematics Laboratory, Biology Department, Faculty of Math and Sciences, Cenderawasih University, Jayapura.

Preparation of Plant Extract

The part of plant used in this research were stem and leaves, except *Dendrobium antennatum* only stem. The collected materials were washed with tap water and then rinsed with distilled water two times. The stem and leaves were cut into small pieces and dried in oven at 50 ^{o}C for 48 hours. The dried materials were powdered using laboratory blender (Warring Commercial). Ethanol, ethyl acetat, n-hexane was purchased from Merck. All solvents and chemicals used were from local sources.

Figure 1. Morphology of *D. spectabile* (Blume) Miq (left), *D. violaceoflaven*s J.J.Sm. (center) and *D. antennatum* Lindl (right).

The powdered were extracted using ethanol by maceration method. Each maceration process was done for 72 hours, while stirred every 24 hours. Macerat filtered with Whatman filter paper number 1. The maceration process was continue until the macerat was colorless. To remove the solvent, the extract was evaporated using rotary evaporator. This extract was called as ethanolic extract. The ethanolic extract then fractionated using separating funnel into three fraction, they are ethanol, n-hexane and ethyl acetate fractions.

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 2 M. Water was added to the solution, then filtered. The filtrate was poured into two test tubes. 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 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 1 mL of 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.

Table 1. Phytochemistry of crude ethanol extract of the *Dendrobium*.

Part of plant	Flavonoid	Alkoloid	Tanin	Saponin
D. spectabile (L)	+	+	+	-
D. spectabile (S)	-	+	+	-
D. violaceoflavens (L)	+	+	+	-
D. violaceoflavens (S)	+	+	+	-
D. antennatum (S)	+	+	+	-

Notes: + = present, - = absent, L = leaf , S = stem.

Table 2. Antibacterial activity of different extracts of selected *Dendrobium* orchids.

No	Name of orchids	Inhibition zone (mm)							
		S.aureus				*E.coli*			
		T	NH	EA	ET	T	NH	EA	ET
1.	*D. spectabile* (L)	7,38	7,14	20,54	6,83	6,13	6,69	6,23	7,46
2.	*D. spectabile* (S)	6,94	6,15	6,16	6,69	6,40	7,02	7,45	7,35
3.	*D. violaceoflaven* (L)	7,15	7,47	8,17	7,72	6,10	8,25	7,64	7,95
4.	*D. violaceoflaven* (S)	7,39	6,10	7,11	8,25	6,48	7,60	8,07	7,37
5.	*D. antennatum* (S)	7,32	6,75	8,24	8,94	6,25	7,41	9,21	6,77
6.	Ciprofloxacin	25,94	25,94	25,94	25,94	42,33	42,33	42,33	42,33

Notes: L= leaves, S= stem, T= total crude extract, NH = N-hexane, EA = ethyl acetate, ET= ethanol.

Test for Saponin

0,1 g extract was mixed with 1 mL of 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.

Collection of Strain

Human pathogenic bacteria *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (strain collected from Microbiology Lab of Dr. Daniel Lantang, Biology Department, Faculty of Mathematics and Sciences, Cenderawasih University, Jayapura, Papua. Antimicrobial susceptibility disks blank and ciprofloxacin 5 µg bought from Thermo Scientific Oxoid, Fisher Scientific.

Antimicrobial Activity Assay

Gram positive bacteria, *Streptococcus aureus* and gram negative *Escherichia coli* were used. Approximately 20 ml of NA were poured into each sterilized petridishes and left to solidify at room temperature for about one hour prior to inoculation. Bacterial suspension containing approximately two inoculating loop (ose) which is equivalent to standard Mc-Farland 0.5 x 10^8 CFU/mL was spread on the agar over the entire surface of the medium using cotton swab.

Sterile susceptibility disc blank impregnated with 50 µl extract ethanol and three fractions of ethanol, ethyl acetate, n-hexane (concentration 1 mg/L) were placed on the agar separately. The plates were incubated at room temperature for 24 hours. The zone of inhibition were measured in cm using digital caliper. The experiment was carried out in triplicate. The positive control used in this study was ciprofloxacin 5 µg.

RESULTS AND DISCUSSION

The extraction method used in this work was cold extraction which was suitable for isolation of various compounds, especially for crude extract from plant materials. The recovery of extract from two different plant organs isolated using ethanol, n-hexane, ethyl acetate varied considerably. It showed that some bioactive compounds were present in the part of the three *Dendrobium* tested in this study viz alkaloid, flavonoid, tannin except in the stem of *D. spectabile*, flavonoid were not detected (Table 1).

Dendrobium species with many structures of alkaloid, flavonoid and other nitrogen compounds have demonstrated many pharmacological activities (Devi *et al*., 2009; Johnson & Janakiraman, 2013; Prasad & Koch, 2014; Rashmi *et al*., 2015; Chimsook, 2016; Esha *et al*., 2016). In this study, alkaloids and tannins were present in the stem and leaves of the three plants. It is also reported that alkaloids are one of the major phytochemicals found within orchids, including *Dendrobium* which indicates an significantly importance in the pharmaceutical industry (Singh & Duggal, 2009). The chemical constituent which found in all the plant tested were tanins. Tanins are another important class of bioactive molecular that has a potential antibacterial, antiviral, and anti parasitic (Kolodziej *et al*., 2005). Beside alkaloids and tannins, other compound possessing several bioactives are polyphenolic compounds including flavonoids. In this study, flavonoid constituent was found in all leaves of three *Dendrobium* species, whereas in stem of *D. spectabile*, flavonoid was absent. The result agrees with the findings of Williams (1978) who had conducted a major research of leaf flavonoid in orchids. It found that the most common constituents were flavon, c-glycoside and flavonols. New phenolic compounds namely quercetin and pentamethoxybibenzyl also found in *D. capillipes* and *D. secundum* separately (Phechrmeekha *et al*., 2012). Most bioactives of phenolics are due to their antioxidant nature. In this work, *saponin*, were however absent in all part of the plant used in this work. This was different with research done by Yang *et al*. (2018) which found furostanol saponin from *D. chrysanthum*, and Sandrasagaran *et al*. (2014) on *D. crumenatum* for bioactive compound saponin.

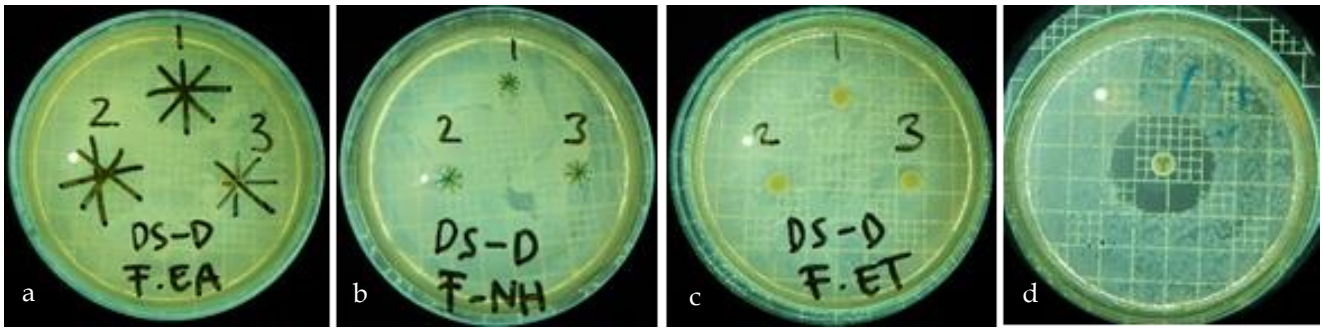

Figure 2. The inhibition zones of three different fractions of *D. spectabile* leaves against gram positive bacterium *S. aureus*. a. EA fraction (20.54 mm), b. NH fraction (7.14 mm), c. ET fraction (6.83 mm), and d. Ciprofloxacin (25.94 mm).

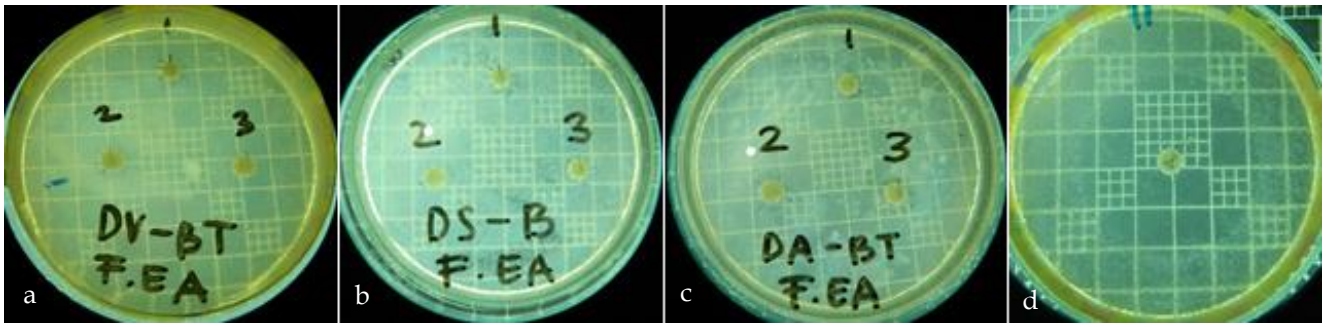

Figure 3. Inhibition zone against gram negative bacteria *E. coli* in ethanol fractions of stem. a. *D. violaceoflavens* (DV) 7.37 mm, b. *D spectabile* (DS) 7.35 mm, c. *D. antennatum* (DA) 6.77 mm., and d. positive control ciprofloxacin is 42.33 mm.

In the present study, ethanol, ethyl acetate and n-hexane extract from stem and leaf of *D. spectabile*, *D. violaceoflavens* and *D. antennatum* were tested against two human pathogenic bacteria namely *S. aureus* and *E. coli* to determine the antimicrobial potentiality of the orchids (Figure 2; 3). The results showed that gram positive bacteria (*S. aureus*) and gram negative bacteria (*E. coli*) were inhibited varied by all extracts of orchids respectively (Table 2). The ethyl acetate extract of leaves of *D. spectabile* showed the high antibacteria activity, 20.54 mm against *S.aureus*, it is comparable to reference antibiotic (25.94 mm). It followed by less activities of stem *D. antennatum* and *D. violaceoflavens* ethanol fraction which are 8.94 mm and 8.25 mm separately. Moderate activities also found in ethanol fraction of *D. antennatum* stem (9.21 mm) and n-hexane fraction of *D. violaceoflavens* leaves (8.25 mm) against *E. coli*. Moderate results also found on extract of *D. crepidatum* against *E. coli* 11 mm and 9.67 mm for *S aureus* (Paudel *et al*., 2018). Ciprofloxacin was used as a positive control. Other publication about orchids for antimicrobial activity, mostly used chloramphenicol, ampicillin, which has inhibition zone around 20-22 mm (Devi *et al.,* 2009; Paul *et al*.. 2013; Paudel *et al*., 2018).

Study on *D. nobile* by Devi *et al*. (2009) also found that the stem extract showed inhibition zone of 6 mm against *E. coli* whereas in the present study it was about 6.4 mm for *D. spectabile*, 6.48 mm for *D. violaceoflavens*, and 6.25 mm for *D. antennatum. E. coli* appeared to be less sensitive to the extracts. Similar antimicrobial activities had been found which were reported by Sandrasagaran *et al*. (2014) who worked on *D. crumenatum.*

The antimicrobial activity of leaf and stem of all extracts used in this study against *E. coli* and *S.*

aureus was generally low, which exhibited in different degrees. Although studies of phytochemistry and antimicrobial activity on *Dendrobium* are still limited, it seemed equitable to believe that the three species used in this study have shown sufficient result to carry out with more work.

CONCLUSSION

In the present study, the biological activities of ethanol, ethyl acetate and n-hexane extracts of three orchids namely *D. spectabile, D. violaceoflavens,* and *D. antennatum* are the first reported. Among those three species, *D. spectabile* has great potential as antibacterial agent against *S. aureus*. The others have moderate antibactaerial activity. All plants tested in this study have moderate antibacterial activity against *E. coli*. However, the results of antbacterial activity of the *Dendrobium* tested here primarily forms further study on phytochemical and pharmacology.

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