INTRODUCTION

Chemical and pharmacological study of traditional medicinal plants from Kendari, Indonesia has been conducted. The plants were Dipterocarpaceae [1-3], Jatropha [4-6], Annonaceae [7], Pongamia [8], Imperata [9], Polygonum [10,11], and Dillenia [12]. In continuing our study of both aspects, Dryobalanops genera (Dipterocarpaceae) is still interesting. Dryobalanops is a minor genus of Dipterocarpaceae and comprises 7 species. Conventionally, this plant used for stomachache and antioxidant [13]. The benefits showed that the chemical content and pharmacological aspects of Dryobalanops plants are very important.

Phytochemical studies of Dryobalanops, like other plants in Dipterocarpaceae such as Dicerandra [14] and Hoplea [15], produced stilbene oligomers. Some Dryobalanops plants which have studied on chemical content are D. aromatica, resulted ε-viniferin, α-viniferin, laevopinol, amelopinol E, malaysianol A, flexuosol A, vaticanol B, vaticanol C, diptoidindonesin A, and bergenic [16,17]. Dryobalanops beccari gawe malaysianol D, malaysian A, ε-viniferin, diptoidindonesin A, flexuosol A, vaticanol B, vaticanol C, bergenic, 4-O-galloylbergenic, scopoletin, 4-O-methylgalbanopholin, methyl gallocate, and gallic acid [18]. Cis- diptoidindonesin B and trans-diptoidindonesin B were isolated from Dryobalanops lanceolata [19]. Moreover, D. lanceolata from Malaysia yielded malaysianol B, hopeaphenol, stenophyllol, nepalesinol B, vaticanol B, vaticanol C, upunaphenol D and flexuosol A [20]. Pharmacological study showed that ε-viniferin is the most active compound against Hel-60 cell lines [16] and vaticanol C is active toward A549 cell lines [18]. Upunaphenol D and flexuosol A displayed interesting potency toward bacteria Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus xylosus, Shigella flexneri, Salmonella typhimurium, and Escherichia coli [17]. One of the Dipterocarpaceae plants which grow in Pohara Forest, Kendari is D. lanceolata or baoti (Tolatinese name).

D. lanceolata is one of seven species of Dryobalanops plants, and chemical and pharmacological aspects of D. lanceolata from Indonesia have been reported by Wibowo et al. [21]. This article reports isolation and structure determination of five stilbene oligomers from acetone extract of D. lanceolata stem barks from Kendari, Indonesia and their biological properties as antibacterial and antioxidant. Three of five compounds from Kendari's Dryobalanops including malaysianol, ε-viniferin, and α-viniferin are the first time reported from the plant. In addition, biological activities toward bacteria E. coli ATCC 35219, S. aureus ATCC 25923, and the cytotoxic property against human breast cancer cells T-47D have not been published yet.

METHODS

General procedures

Melting point (MP) of the isolated compounds was determined by “micro MP apparatus; Fisher John.” The optical rotation established by polarimeter Perkin-Elmer 341 in MeOH. Ultraviolet (UV) and Infrared (IR) spectra were measured with Cary varian 100 concentration and Perkin-Elmer Spectrum One FT-IR spectrophotometer, respectively. spectra of H and D nuclear magnetic resonance (NMR) were determined by spectrophotometer JEOL LTD ECP 400, operated at 400 MHz (H) and 100.53 MHz (D), used acetone-d5 as solvent and TMS as internal standard. Separation and purification used thin-layer chromatography, vacuum liquid chromatography (VLC), and radial chromatography (Rf).

RESULTS

Five stilbene oligomers have been isolated and identified from acetone extract of D. lanceolata stem barks namely balanocarpol (1), ε-viniferin (2), α-viniferin (3), vaticanol B (4), and hopeaphenol (5). The inhibition zone value (in mm) of ε-viniferin, balanocarpol, α-viniferin, hopeaphenol, and vaticanol B is 34.13±0.15, 98.17±0.41, 84.79±0.24, 52.04±0.26, and 119.30±0.54, respectively.

Conclusions: ε-viniferin is the most active compound toward E. coli and human breast cancer cells T-47D. Biological activity against bacteria S. aureus indicated that balanocarpol is the most potential compound.

Keywords: Dipterocarpaceae, Dryobalanops lanceolata, Stilbenoids, Antibacterial, Cytotoxic.
Sample
Stem barks of *D. lanceolata* got from Pohara Forest, Kendari, Sulawesi Tenggara. The plant was identified by staffs of Herbarium Bogoriense, Bogor, Indonesia.

Extraction and isolation
Powder of stem barks of *D. lanceolata* (2.0 Kg) was extracted by acetone of 3×5 L for 3-4×2 hrs. The acetone extract was concentrated by rotary evaporator to get brown dark gum (116 g). All of acetone extract was fractionated by VLC using column with Φ 10 cm, adsorbent: Si-gel (150 g) and mixture of ethyl acetate: N-hexane (30%: 100-100%: 0%, MeOH 100%) as eluent, produced 5 main fractions that are F1-F5 (3.0, 8.5, 11.0, 17.0, and 19.0 g). Separation of F2 (8.5 g) used VLC with column of Φ 10 cm, gave 5 fractions F21-F25 (0.4, 1.2, 1.3, 1.1, and 2.7 g). Purification of F22 using RC with eluent 10% MeOH-CHCl3 yielded compound 1 (40 mg).

Further separation of F24 employing the same procedure as F22 produced compound 2 (28 mg). Partition of F3 using VLC gave four fractions that are F31 (0.4 g), F32 (1.3 g), F33 (3.1 g), and F34 (3.1 g). Purification of F33 using VLC and RC yielded compound 3 (74 mg) and 4 (34 mg), respectively. Compound 5 (50 mg) came from separation and purification of F4 fraction which used the same method as purification of F33 fraction.

Biological activities evaluation

The antibacterial test was conducted by the agar dilution method using the general procedure outlined by Thakurta et al. [21]. The general procedure was adapted by Sahidin et al. (2007) [22]. The cultural concentration of bacteria was *E. coli*: 2×10^9 cfu/mL and *S. aureus*: 3×10^9 cfu/mL. The cytotoxic property toward human breast cancer cell line T-47D was evaluated using MTT assays methods about 1×10^4 cells/well [22].

**RESULTS**

Data of spectroscopy and physical properties of isolated compounds

**Compound 1**, a yellow powder, MP 180-183°C [α]_D^20 = -12° (C 0.1 MeOH).

Spectra of UV (MeOH) λ_max (log ε) 205 (5.03), 220 (4.96), 284 nm (4.38).

Spectra of IR (KBr) ν_max (cm^-1) 3448 (OH), 1614, 1516 (C=C aromatic), and 834 (C=C aromatic). Spectra of 1H NMR (MeCO-CD_3, 400 MHz) and 13C NMR (MeCO-CD_3, 100 MHz) (Table 1).

**Compound 2**, a yellow powder, MP 172-176°C [α]_D^20 = -44° (C 0.1 MeOH).

Spectra of UV (MeOH) λ_max (log ε) 205 (5.05), 230 (4.87), 324 nm (4.57).

Spectra of IR (KBr) ν_max (cm^-1) 3393 (OH), 1601, 1513, 1443 (C=C aromatic), and 832 (C=C aromatic).

**Compound 3** and mixture of ethyl acetate: N-hexane (30%: 100-100%: 0%, MeOH 100%) yielded compound 1 (40 mg). Further separation of F24 employing the same procedure as F22 produced compound 2 (28 mg). Partition of F3 using VLC gave four fractions that are F31 (0.4 g), F32 (1.3 g), F33 (3.1 g), and F34 (3.1 g). Purification of F33 using VLC and RC yielded compound 3 (74 mg) and 4 (34 mg), respectively. Compound 5 (50 mg) came from separation and purification of F4 fraction which used the same method as purification of F33 fraction.

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160.6 (C-1a), 102.7 (C-12a), 158.2 (C-13a), 107.4 (C-14a), 138.2 (C-1b), 130.1 (C-2/6b), 114.9 (C-3/5b), 155.1 (C-4b), 44.1 (C-7b), 53.4 (C-8b), 142.2 (C-9b), 117.7 (C-10b), 159.1 (C-11b), 95.3 (C-12b), 156.9 (C-13b), and 110.2 (C-14b).

**Biological activities data**
The biological activities data of isolated compounds against bacteria and T-47D cancer cell lines are displayed in Table 1.

**DISCUSSION**
Stilbene monomer (resveratrol) comprises 14 carbon atoms and structure pattern C₆-C₆-C₆. The research isolated and identified five stilbene oligomers from acetone extract of *D. lanceolata* stem barks. A total of 2 compounds have 28 carbon atoms or stilbene dimers, a compound has 42 carbon atoms or stilbene trimer, and two compounds have 56 carbon atoms or stilbene tetramer. The compound structures are displayed in the Fig. 1.

All isolated compounds are known compounds so the structures are determined by comparing the spectroscopic data of isolated compounds with similar data from references. For example, isolate 1, the spectrum data of ¹H NMR and ¹³C NMR has a high similarity parameter with balanocarpol (1*) [23]. It can be concluded that compound 1 is balanocarpol as displayed in Fig. 1.

In the same way as structure determination of balanocarpol, the compounds 2, 3, 4, and 5 are α-viniferin [24], α-viniferin [25], vaticanol B [26], and hopeaphenol [27], respectively. Refers to a previous study [20], three compounds were isolated from *D. lanceolata* from Kendari, namely, balanocarpol, α-viniferin, and α-viniferin, not reported from Malaysia’s *D. lanceolata*. These data complemented the diversity of *D. lanceolata*’s stilbene oligomers. Two compounds, vaticanol B and hopeaphenol, have been reported previously that thought to be characteristic of *D. lanceolata*.

According to biological activity data in Table 1, biological activity against bacteria indicated that α-viniferin is the most active compound toward *E. coli*, which followed by balanocarpol, α-viniferin, hopeaphenol, and vaticanol B. While the activity against *S. aureus*, balanocarpol is the most active compound followed by α-viniferin, hopeaphenol, α-viniferin, and vaticanol B. α-Viniferin and balanocarpol are stilbene dimers, consist of two unit stilbenes, means having a smaller molecular size than the others. It is estimated that activity of stilbene derivative against bacteria is influenced by the size of the molecule which can affect molecular penetration [26].

In general, stilbene derivatives have cytotoxic potency toward cancer cell lines not only from Dipterocarpaceae but also from Gnetaceae, for example, gnetin C and gnemonoside A, two stilbene dimers from *Gnetum gnemon* [29] or other phenolic compounds such as curcumin [30]. The cytotoxic properties against human breast cancer cell lines T-47D showed that all isolated compounds are less active than standard (doxorubicin). For isolated compounds, α-viniferin is the most active compound, followed by hopeaphenol, α-viniferin, balanocarpol, and vaticanol B. α-Viniferin has intact stilbene unit and all carbon atoms have orbital hybrid sp². Consequently, α-viniferin becomes richer phi electrons with the equitable electrons distribution from the ring B1 to B2 through electron delocalization. It will produce a more stable radical that can inhibit cancer cell growth [31]. Biological activity of hopeaphenol and α-viniferin against human breast cancer cell lines T-47D thought to be caused by the density of the compound [32]. Both of these compounds have a symmetrical plane in their structure. This causes the density of molecules into larger.α-viniferin, balanocarpol has a small volume identical with stilbene dimer, but its molecular weight as a tetramer. Hopeaphenol as a tetramer has a molecular weight greater than two times the dimer, because there is a plane of symmetry in the structure hopeaphenol. Hopeaphenol is a plane of symmetry in the structure hopeaphenol, this compound has a small volume identical with stilbene dimer but its molecular weight as a tetramer. Hopeaphenol has a molecular weight greater than two times the dimer because there is a plane of symmetry in the structure hopeaphenol. Hopeaphenol volume increases more and more active compound against cancer cell lines. The sequence of cytotoxic properties of stilbene oligomers toward T-47D cell lines was identical to the sequence of the cytotoxic properties of the compounds against murine leukemia P-388 cells that are hopeaphenol (5.7±0.3 µM), α-viniferin (25.8±0.7 µM), balanocarpol (33.6±8.3 µM), and vaticanol B (56.8±2.3 µM) [1].

**CONCLUSION**
Five stilbene oligomers have been isolated and identified from the stem barks of *D. lanceolata*, namely, balanocarpol (1), α-viniferin (2) (stilbene dimers), α-viniferin (3) (stilbene trimer), hopeaphenol (4) (stilbene tetramer), and vaticanol B (5) (stilbene tetramer).
### Table 2: Spectra of $^1$H NMR of balanocarpol (1)

| Number of component | $\delta_c$ (mult., J in Hz) | $\delta_h$ | 1 | 1* |
|---------------------|-----------------------------|-----------|----|----|
| 1a                  |                            |           |    |    |
| 2 (6)a              | 7.48 (2H, d, 8.4)           | 7.50 (2H, d, 8.3) | 133.2 | 133.5 |
| 3 (5)a              | 6.94 (2H, d, 8.4)           | 6.95 (2H, d, 8.3) | 113.9 | 114.2 |
| 4a                  |                            |           |    |    |
| 7a                  | 5.69 (1H, d, 9.5)           | 5.69 (1H, d, 9.3) | 72.9  | 73.2  |
| 8a                  | 5.15 (1H, d, 9.5)           | 5.16 (1H, d, 9.3) | 50.0  | 50.3  |
| 9a                  |                            |           |    |    |
| 10a                 |                            |           |    |    |
| 11a                 |                            |           |    |    |
| 12a                 | 6.09 (1H, d, 2.2)           | 5.96 (1H, d, 2.3) | 104.2 | 104.4 |
| 13a                 |                            |           |    |    |
| 14a                 | 5.95 (1H, d, 2.2)           | 6.09 (1H, br s) | 94.8  | 95.1  |
| 1b                  |                            |           |    |    |
| 2 (6)b              | 6.73 (2H, d, 8.4)           | 6.75 (2H, d, 8.3) | 130.3 | 130.5 |
| 3 (5)b              | 6.41 (2H, d, 8.4)           | 6.42 (2H, d, 8.3) | 116.2 | 116.4 |
| 4b                  |                            |           |    |    |
| 7b                  | 4.89 (1H, br s)             | 4.90 (1H, br s) | 52.1  | 52.3  |
| 8b                  | 5.38 (1H, br s)             | 5.40 (1H, br s) | 93.3  | 93.5  |
| 9b                  |                            |           |    |    |
| 10b                 |                            |           |    |    |
| 11b                 |                            |           |    |    |
| 12b                 | 6.18 (1H, d, 2.2)           | 6.20 (1H, br s) | 101.8 | 102.0 |
| 13b                 |                            |           |    |    |
| 14b                 | 6.24 (1H, d, 2.2)           | 6.26 (1H, d, 2.0) | 106.5 | 106.8 |
| 0H                  | 4.41 (br d, 4.4)            | 4.36 (d, 4.4, 4b) | 7.74  | 7.85 |
|                     | 8.65; 8.09; 8.06; 7.91; 7.81 (br s) | 7.79 (br s, C-13a) | 8.04 (br s, C-13a) | 8.56 (br s, C-4b) |

Measured in acetone-d$_6$ ($^1$H, 400 MHz; $^{13}$C NMR 100 MHz) *[23], NMR: Nuclear magnetic resonance.

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REFERENCES

1. Sahidin, Hakim EH, Juliawaty LD, Syah YM, Din LB, Ghisalberti EL, et al. Cytotoxic properties of oligosteroids from the tree bark of Hopea dryobalanoides. Z Naturforsch 2005;60(9-10):723-7.
2. Juliawaty LD, Sahidin, Hakim EH, Achmad SA, Syah YM, Latip J, et al. A 2-arylbensofuran derivative from Hopea mengarana. Nat Prod Commun 2009;4(7):947-50.
3. Muhammad N, Din LB, Sahidin I, Hashim SF, Ibrahim N, Zakaria Z, et al. Acuminatol and other antioxidative resveratrol oligomers from Uvaria rufa (Annonaceae). MJAS 2013;17(1):50-8.
4. Al Muqarrabun LM, Ahmat N, Ruzaina SA, Ismail NH, Sahidin I. Medicinal uses. Phytochemistry and pharmacology of Pangamia pinnata (L.) Pierre: A review. J Ethnopharmacol 2013;150(2):395-402.
5. Ruslin M, Asmawi MZ, Rianse U, Sahidin I, Dhianawaty D, Soemantri AA, et al. Anti-hypertensive activity of Alang-alang (Imperata cylindrica) (L.) Beauv. root methanolic extract on male Wistar rat. Int J Res Pharm Sci 2013;4(4):337-42.
6. Sahidin I, Nohong N, Sani A, Manggau MA, Sukohar A, Widodo H, et al. Radical scavenging activity of triterpene steroids from stem of Polygonum pulchrum Bl. Int J Pharm Pharm Sci 2014;6(8):350-4.
7. Sahidin I, Suwandi A, Nohong N, Manggau MA. Profile of anticancer and radical scavenging activities of steroids from stems of Polygonum pulchrum. IJPSR 2015;6(5):2178-84.
8. Jalil J, Sabandar CW, Ahmat N, Jamal JA, Jantan I, Aladdin NA, et al. Inhibitory effect of triterpenoids from Dillenia serrata (Dilleniaceae) on prostaglandin E2 production and quantitative HPLC analysis of its koeltpic acid and betulinic acid contents. Molecules 2015;20(2):3206-20.
9. Ashton PS. Flora Malesiana Spermatophyta I. The Hague: Martinus Nijhoff; 1983. p. 391-436.
10. Aslam MS, Ahmad MS, Mamat AS. A phytochemical, ethnomedicinal and pharmacological review of genus Dipterocarpus. Int J Pharm Pharm Sci 2015;7(4):27-38.
11. Shettar AK, Vedhamurthy AB. Studies on in vitro antioxidant activities of Hopea longa and Fites leucosylon. Int J Pharm Pharm Sci 2015;9(2):263-7.
12. Wibowo A, Ahmat N, Hamzah AH, Ahmad R, Jafar FM. Resveratrol oligomers from the stem bark of Dryobalanops aromatica. Fitoterapia 2011;82:676-81.
13. Wibowo A, Ahmat N, Hamzah AH, Ismail NH, Ahmad R, Hamzah MA, Lukman. Cytotoxic potency of diterpenes from Jatropha plants. Int J Pharm Pharm Sci 2013;5(3):3-6.
14. Sabandar CW, Ahmat N, Jafar FM, Sahidin I. Medicinal property, phytochemistry and pharmacology of several Jatropha species (Euphorbiaceae): A review. Phytochemistry 2013;85:7-29.
15. Rosandy AR, Din LB, Yaacob WA, Yusoff NI, Sahidin I, Latip J, et al. Isolation and characterization of compounds from the stem bark of Uvaria rufa (Annonaceae). MJAS 2013;17(1):50-8.
16. Saatchd M, Amini NS, Hakim EH, Amini N, Kitajima M, Takayama J, et al. Two oligostilbenes, cis- and trans-diptoindonesin B from Dryobalanops oblongifolia. Phytochemistry 2003;63:913-7.
20. Wibowo A, Ahmat N, Hanzah AS, Low AL, Mohamad SA, Khong HY, *et al.* Malaysianol B, an oligostilbenoid derivative from *Dryobalanops lanceolata.* Fitoterapia 2012;83:1569-75.

21. Thakurta P, Bhownik P, Mukherjee S, Hajra TK, Patra A, Bag PK. Antibacterial, antisecretory and antihemorrhagic activity of *Acacia rachita indica* used to treat cholera and diarrhea in India. J Ethnopharmacol 2007;111:607.

22. Soundararajan R, Prabha P, Rai U, Dixit A. Antileukemic potential of *Momordica charantia* seed extracts on human myeloid leukemic HL-60 cells, evidence-based. Complement Altern Med 2012;10:1-10.

23. Tanaka T, Ito T, Ido Y, Nakaya K, Iinuma M, Chelladurai V. Hopeafuran and a C-glucosyl resveratrol isolated from stem wood of *Hopea utilis.* Chem Pharm Bull 2001;49(6):785-7.

24. Oshima Y, Ueno Y. A new hydroxystilbene tetramer named isohopeaphenol from *Vitis vinifera.* Heterocycles 1997;45(9):1809-13.

25. Tanaka T, Ito T, Ido Y, Son TK, Nakaya K, Iinuma M, *et al.* Stilbenoids in stem bark of *Hopea parviflora.* Phytochemistry 2000;53:1009-14.

26. Ebejer JP, Charlton MH, Finn PW. Are the physicochemical properties of antibacterial compounds really different from other drugs? J Cheminform 2016;8:30.

27. Mun’im A, Munadhil MA, Puspitasari N, Azminah, Yanuar A, Angiotensin converting enzyme inhibitory activity of Melinjo (*G. gnemon*) seed extracts and molecular docking of its stilbene constituents. Asian J Pharm Clin Res 2017;10(3):243-8.

28. Steffi PF, Srinavasan M. Curcumin, a potent anticarcinogenic polyphenol-a review. Asian J Pharm Clin Res 2014;7(Suppl 2):1-8.

29. Khaledi H, Alhadi AA, Yehye WA, Ali HM, Abdulla MA, Hassandarvish P. Antioxidant, cytotoxic activities, and structure-activity relationship of gallic acid-based indole derivatives. Arch Pharm Chem Life Sci 2011;344:703-9.

30. Chu C, Xu P, Zhao H, Chen Q, Chen D, Hu H, *et al.* Effect of surface ligand density on cytotoxicity and pharmacokinetic profile of docetaxel loaded liposome. Asian J Pharm Sci 2016;11:665-71.