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6,7-dimethoxydihydrocoumarin Compound from Ethyl Acetate Extracts from Steambarks *Dysoxylum Alliaceum* and Cytotoxic Activity Against P-388

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Abstract

Dysoxylum is a genus that has a variety of secondary metabolites. Research on various species of this genus is always growing and producing compounds that have interesting structures and activities, until now many compounds of the terpenoid group, chroman alkaloids, limonoids, sesquiterpenes, flavonoids, steroids, protolimonoids and sulfur have been reported. which is very interesting. One of the *Dysoxylum* species that has a variety of secondary metabolites is *D. alliaceum*. The purpose of this study was to obtain secondary metabolites of *D. alliaceum* bark. The bark of *D. alliaceum* was macerated successively with n-hexane, ethyl acetate and methanol. The ethyl acetate extract was separated and purified by various chromatographic techniques and was characterized using spectroscopic methods including, ultraviolet, infrared, NMR and mass spectroscopy and guided by thin layer chromatographic analysis to obtain the compound 6,7-dimethoxydyhidrocoumarin and the proposed biogenesis. The chemical structure of these compounds has been determined based on the interpretation of spectroscopic data and compared with spectral data from previous studies. Cytotoxic activity test against P-388 MTT leukemia cells obtained an IC_{50} of 39.210 g/mL and was declared inactive.

Keywords: *Dysoxylum* alliaceum, phenol, Bark, spectroscopy, P-388

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1 Introduction

Dysoxylum is a genus of Meliaceae which consists of ± 80 species found in Asia, Australia, and eleven endemic species in China [1]. Research on various species of this genus is always developing and producing compounds that have interesting structures and activities, until now many compounds of the terpenoid group, chroman alkaloids, limonoids, sesquiterpenes, flavonoids, steroids, protolimonoids and sulfur compounds have been reported and have activity and very interesting [2]. Sesquiterpenoid Prostanterol from the bark of *Dysoxylum excelsum* has been isolated [3]. Six triterpenes and triterpene glycosides were isolated from the methanolic extract of the leaves of *D. cumingianum* [4]. Phenolic derivatives 5-hydroxy-7-methoxy-2-methyl-4H-chromene-4-one and squalene were isolated from *D. macrocarpum* [5]. Isolating a new steroid type grandol from the leaves of *D. grande* [6]. 2,3,2″,3″-tetrahydroamentoflavone compounds were isolated from *D. cauliflorum* [7]. Kumingianol triterpenoid isolated from *D. cumingianum* can inhibit P-gp in MCF-7 cancer cells so that it can be used as an agent for chemotherapy [8]. According to [9] rohitukin in *Dysoxylum* is a precursor to flavopiridol, a potential anticancer compound [9]. Flavopiridol in *D. binectariferum* is an inhibitor of MMP-2 and MMP-9 in MCF-7 breast cancer cells[10]. The bark of *D. binectariferum* produces rohitukin which is highly effective against ovarian and breast cancer [11]. Steroid Compound from *D. alliaceum* is an active Compound for breast cancer MCF-7 [12]. Various secondary metabolites have been found and seeing their many activities, researchers are interested in obtaining secondary metabolites from the bark of *D. alliaceum* and its activity against P-388.

2 Experimental Section

2.1 Isolation of Plant Material

This research is a deductive experimental research, starting from a literature review from various journal sources or scientific articles, then conducting laboratory experiments. This process consists of three stages, extraction of bark *D.alliaceum*, separation and purification of isolates from the target fraction and characterization of pure isolates. The bark of *D. alliaceum* (2.5 kg) was ground and then extracted by maceration using methanol as a solvent. The resulting methanol extract was evaporated using a rotary evaporator under vacuum at a temperature of ± 40°C to obtain a concentrated methanol extract. This extract was then partitioned with n-hexane, ethyl acetate and methanol as solvents. The obtained n-hexane, ethyl acetate and methanol extracts were concentrated using a rotary evaporator to obtain concentrated extracts of n-hexane (30 g), ethyl acetate (14 g) and methanol (20 g). The ethyl acetate fraction (4 g) was separated by vacuum liquid chromatography silica gel G60 stationary phase, using n-hexane, ethyl acetate and gradient methanol with an increase in polarity of 10% resulting in six fractions (1-6). All fractions were analyzed by TLC using the eluent n-hexane:EtOAc (4:6). This fraction was chosen for further separation in the hope that there are compounds that are antagonistic so that the isolate activity is better than the extract activity. Fraction 3 (4 g) was separated by column chromatography of silica gel G60 (70-230 mesh) with a gradient of 2.5% n-hexane:EtOAc eluent to produce five fractions (3A-3E). The 3D fraction (400 mg) was separated by column chromatography of silica gel G60 (70-230 mesh) gradient system 1% eluent n-hexane:acetone yielded six fractions (3DA-3DF). The 3DD fraction (115 mg) was separated by isocratic system using n-hexane eluent:EtOAc (4:6) yielded four fractions (3DD1-3DD4). Each fraction was analyzed using TLC with the eluent of n-hexane:EtOAc (3:7) so that compound was obtained in the fraction (3DD3) as much as 10 mg.

2.2 Cytotoxic assay using P-388 murine leukemia cells

The P-388 cells were seeded into 96-well plates at an initial cell density of approximately 3×10^4 cells cm^{-3}. After 24 h of incubation for cell attachment and growth, varying concentrations of samples were added. The compounds added were first dissolved in DMSO at the required concentration. Subsequent six desirable

concentrations were prepared using PBS (phosphoric buffer solution, pH = 7.30–7.65). Control wells received only DMSO. The assay was terminated after a 48 h incubation period by adding MTT reagent [3-(4,5- dimethylthiazol- 2-yl)-2,5- diphenyl tetrazolium bromide; also named as thiazol blue] and the incubation was continued for another 4h, in which the MTT-stop solution containing SDS (sodium dodecyl sulphate) was added and another 24h incubation was conducted. Optical density was read by using a micro plate reader at 550 nm. IC_{50} values were taken from the plotted graph of percentage live cells compared to control (%), receiving only PBS and DMSO, versus the tested concentration of compounds (mg/mL). The IC_{50} value is the concentration required for 50% growth inhibition. Each assay and analysis was run in triplicate and averaged.

3 Results and Discussion

The compound obtained is a colorless crystal. The molecular formula of the compound was determined as $C_{11}H_{10}O_4$ (Mr = 206.170 g/mol) based on the ES-MS- data (m/z = 205.99 $([MH]^+)$ together with the ^1H and ^{13}C-NMR data (Table 1). It has seven degrees of unsaturation. This compound fluoresces at UV 365 nm which indicates that this compound has a conjugated double bond. The infrared spectrum of this compound shows absorption bands originating from aliphatic CH (2924 cm^{-1}), C=C range (1603 cm^{-1}), medium C=O, CO range (1090 cm^{-1}), and aromatic substitution (824 cm^{-1}). Analysis of the ^1H-NMR data showed a proton doublet signal at *δH* 7.59 (1H,*d*,9Hz) which was matched with a proton doublet *δH* 6.27 (1H,*d*,9Hz) at the ortho position. At *δH* 6.93 ppm and *δH* 6.88 indicated the presence of protons from the aromatic ring. Protons at *δH* 3.81 and *δH* 3.82 ppm with singlet multiplicity indicate protons originating from oxygenated CH_3. The ^{13}C-NMR spectrum showed 11 carbon signals consisting of two oxygenated sp^3 carbons (OCH_3) at *δC* 56.2 and *δC* 56.4 ppm, nine sp^2 carbons *δC* 113.5, 107.5 166.3, 165.3, 89 .9, 143.5, 180.3, 103.3, and 162.8 ppm. Signal *δC* 180.3 ppm indicates the presence of conjugated carbonyl. The presence of eight sp^2 *δC* carbons 113.5, 107.5 166.3, 165.3, 89.9, 143.5, 103.3, and 162.8 ppm indicates the presence of an aromatic ring in which one of the rings is bonded to an oxygen atom so that give a greater chemical shift. So that the chemical structure of this compound can be identified as shown in Figure 1.

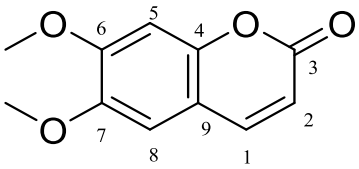

Figure 1. Chemical structure of 6,7-dimethoxydihydrocoumarin.

To strengthen the identification of the structure of this compound, a comparison was made with the compound 6,7-dimethoxydihydrocoumarin reported by [13]. The following is the NMR data for this compound and the comparison compound 6,7-dimethoxydihydrocoumarin is listed in Table 1.

Table 1. NMR Data (600 MHz for 1H and 150 MHz for 13C, in acetone-d6) this compound

C position	Compound* ^1H-*NMR* $\delta_{H\ (ppm)}$ (Int; mult; *J*= Hz)	^{13}C-*NMR* $\delta_{C\ (ppm)}$	comparison compound** ^1H-*NMR* $\delta_{H\ (ppm)}$ (Int; mult; *J*= Hz)	^{13}C-*NMR* $\delta_{C\ (ppm)}$
1	7,59 (1H;d;9)	162,8	7,69 (1H;d;9,6)	162,8
2	6,27 (1H;d;9)	103,3	6,15(1H;d;9,6)	103,3
3		180,3		180,32
4		143,5		143,5
5	6,93 (1H,s)	89,9	6.92 (1H,br s,)	90,5
6		165,3		165,3
7		166,3		166,3
8	6, 80(1H,s)	107,5	6.03 (1H,br s,)	107,5
9		113,5		113,6
OMe	3,81 (3H, s)	56,2	3,80 (3H, s)	56,5
OMe	3,82 (3H, s)	56,4	3,85(3H, s)	56,6

4 Conclusions

Phenolic compounds from the bark D.alliaceum is 6,7-dimethoxy-2H-chromene-2-on. Cytotoxic activity test against P-388 MTT leukemia cells obtained an IC50 of 39.210 g/mL and was declared inactive.

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6 Conflicts of Interest

The authors declare no conflict of interests. None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

7 Author Contributions

The names of the authors listed in this journal contributed to this research.

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