Piceatanol: Anti-Cancer Compound From Gewang Seed Extract

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ABSTRACT

Piceatanol (Compound 1), brownish white solids compound, is a stilbene compound that has been isolated from methanol seed extract of Corypha utan Lamk. Isolation and purification conducted by chromatographic methods. Structure elucidation deduced on the basis of spectroscopic data (UV spectrometer, FTIR, NMR and HRMS). MTT assay method of cytotoxicity activity showed that Compound 1 has a very strong cytotoxic activity against Murine leukemia P-388 cell lines with IC50 value 1.56 ppm.

INTRODUCTION

Research and development of drugs is a very important part of health development, requires the development of new compounds as ingredients of medicines. Natural products are important sources of new structures, especially for the discovery of compounds that are efficacious drugs. Presently, natural products drug development focused on the search and analysis of the new compounds that might be useful as a medicine. The selection of suitable plants is an important and decisive step, can be done several ways, among others, the use of traditional, chemical constituents, toxicity, random selection of a combination of several criteria (Gudrun et al., 2010). Corypha utan Lamk. is a type of palm plant that grows wild in the savanna of East Nusa Tenggara (NTT), used as fish poison by the people of Timor Island. Murine leukemia P-388 is one of the tumor cells type that serve as a cytotoxic test protocol by NCI (National Cancer Institute) America. Test results using these cells are often used as the basis of the tests in order to obtain further compounds or candidate cancer models (Hoetetman and Hamburger, 1991). A pure compound categorized as anticancer active compound if it has IC50 value <2 ppm (very active), IC50 2-4 ppm (active) and IC50 > 4 ppm (inactive). The purpose of this study is to isolate anticancer active compound from the seed of Corypha utan Lamk.

MATERIALS AND METHODS

Extraction and Isolation

3.9 kg of Corypha utan Lamk fresh fruit collected from Buat So’e area, District of Timor Tengah Selatan, Nusa Tenggara Timur Province, Indonesia. Separate the seed from the flesh, crushed, and then macerated with 3 liters of methanol for 3 days. Liquid methanol extract filtered and concentrated using vacuum rotary evaporator at ± 40 °C temperature. 20 g methanol extract fractionated using vacuum liquid chromatography (silica gel GF254) with n-hex-EtOAc 1:1; 4:6; 3:7; 2:8 and EtOAc as mobile to obtain five fractions (A1-A5). Fraction A5 further chromatographed over silica gel column, eluted successively with n-hex-EtOAc (2:8), to give 50 mg of brownish white solid compound (Compound 1).

Anticancer Activity Test Against murine leukemia P-388

The principle of the measurement of the cytotoxic properties of murine leukemia cancer cells P-388 are as follows: the activity of the compounds and Antonin E (positive control) is expressed by the IC50 which is sample concentration or comparison is needed to inhibit 50% tumor cells Murine leukemia P-388 cell
line through MTT reagent staining, which was observed with a micro plate reader at 540 nm. Approximately 3 x 104 cell cm-3 of P-388 Murine leukemia cells were plated in 96-well culture dishes, and incubated for 24 h. Various concentrations of the samples were added. Six desirable sample concentrations were prepared using PBS (phosphoric buffer solution, pH = 7.30-7.65), except control. After 48 h incubation, the test was stop by adding MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide]. Incubation continue for next 4 h before the addition of MTT stop solution containing sodium dodecyl sulphate (SDS), the incubation continue for next 24 h. Optical density measured using microplate reader at 540 nm. IC50 value calculated using extrapolation of 50% absorption lines in the positive control sample on the uptake curve against sample concentration.

RESULTS AND DISCUSSION

Compound 1, a brownish white solid with a melting point of 226°C. UV (MeOH, λmax)(log ε) nm: 221 (tape conjugates) and 327 (tape benzene). These data indicated that in this compound under electronic transition π → π* which characterizes a chromophore of an aromatic substitute with auxochrome and under bathochromic shift with the addition of NaOH reagent (λmax)(log ε) nm: 309 and 347, showed that compound 1 has free OH group (Figure 1). IR spectrum showed conjugation absorption bands (vmax cm⁻¹) for hydroxyl groups (3348), =C=–C= of aromatic ring (1650) supported by α-CH alkenes and aromatics (652, 800, and 960) (Figure 2). 1H-NMR spectrum (Figure 3) indicate the presence of three ABX system proton aromatic signals of A ring at δH 6.82 (1H, d, C-6'); δH 7.01 (1H, d, C-2'), and δH 6.77 (1H, s, C-5'), three proton aromatic signal of B ring at δH 6.5 (2H, d), and δH 6.25 (1H, t), and two proton signals at δH 6.74 (1H, d), and δH 6.89 (1H, d) belong to a system of a typical trans vinyllic stilbenoid group.

C-NMR spectrum show six aromatic carbon at δC 102.5; 105.8 (2C); 113.7; 116.3; 120.3; 126.7; 129.5; and two olefinic carbon at δC 146.02 (2 C), 2 carbon chemistry shift value at δC ≈ 159.08 (2C) and two aromatic carbon quartenary at δC 130.9 and 141.17 (Figure 4). Mass spectroscopy analysis showed that compound 1 has a molecular weight (m/z) 245 and molecular formula C14H12O4. Further identification of compound 1 was determined by HMQC and HMBC (Figure 5), showed that protons at δH 7.01 correlated with δC 120.3 (C-6') and δC 145.9 (C-4'). The opposite correlation also showed between δH 6.82 with δC 113.7 signal (C-2'). Proton at δH 6.82 showed correlation with the two aryl carbon δC 145.9 (C-4') and δC 146.0 (C-3'), δH 6.77 also has correlation with δC 145.9 (C-4'), δC 146.0 (C-3'), and quaternary aromatic carbon δC 130.9 (C-1'). Proton signals δH 6.74 (C-7) had a trans coupling with δH 6.89 (C-8). HMBC spectrum also showed other correlation between δH 7.01 and δH 6.77 with δC signal (C-1'), and δH 6.89 with δC (C-7) and carbon quartenary (C-1) second aromatic ring in unit A. HMQC and HMBC correlation of compound 1 shown in Table 1. The relationship between proton-carbon neighbor within 2 ties and 3 ties of the HMBC spectrum of compound 1 is shown in Figure 5. Based on the 1D- and 2D-NMR data, supported with mass spectroscopic data and compared with a reference (Brinker and Seigler, 1991) can be concluded that compound 1 is Piceatannol. Cytotoxicity activity against Murine leukemia P-388 cell lines of compound 1 has been done. Compound 1 showed strong activity with IC50 1.56 ppm compared to Artonin E (IC50 0.3 ppm) as positive control.

CONCLUSION

Anticancer active compound contained in Corypha utan Lamk. seeds successfully isolated and identified as piceantannol, which also has very strong cytotoxic activity against Murine leukemia P-388 cells with IC50 values 1.56 ppm.
Table 1: 1D- and 2D-NMR Data of Compound 1 and Reference (Brinker and Seigler, 1991).

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<td>2.6</td>
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<tr>
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Fig. 2: IR spectrum of compound 1.

Fig. 3: ¹H-NMR spectrum of compound 1.
REFERENCES


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