Journal of Applied Pharmaceutical Science Vol. 5 (01), pp. 110-113, January, 2015
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2015.50119
ISSN 2231-3354

Short Communication

Piceatanol: Anti-Cancer Compound From Gewang Seed Extract

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ARTICLE INFO

Article history:
Received on: 22/09/2014
Revised on: 11/11/2014
Accepted on: 17/01/2015
Available online: 30/01/2015

Key words:
Corypha utan Lamk, *Murine leukemia* P-388, Cytotoxic, Chromatography, Piceatannol.

ABSTRACT

Piceatannol (**Compound 1)**, brownish white solids compound, is a stilbene compound has been isolated from methanol seeds 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 IC_{50} 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 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 IC_{50} value <2 ppm (very active), IC_{50} 2-4 ppm (active) and IC_{50} > 4 ppm (inactive). The purpose of this study is to isolate anticancer active compound from the seed of *Corypha utan* Lamk.

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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 GF_{254}) 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 IC_{50} 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. IC_{50} 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 (ν_{maks} cm^{-1}) for hydroxyl groups (3348), -C=C- of aromatic ring (1650) supported by =CH alkenes and aromatics (652, 800, and 960) (Figure 2). ^{1}H-NMR spectrum (Figure 3) indicate the presence of three ABX system proton aromatic signals of A ring at δ_H 6.82 (1H, dd, C-6'); δ_H 7.01 (1H, d, C-2'), and δ_H 6.77 (1H, s, C-5'), three proton aromatic signal of B ring at δ_H 6.5 (2H, d), and δ_H 6.25 (1H, t), and two proton signals at δ_H 6.74 (1H, d), and δ_H 6.89 (1H, d) belong to a system of a typical trans vinyilic stilbenoid group. ^{13}C-NMR spectrum show six aromatic carbon at δ_C 102.5; 105.8 (2C); 113.7; 116.3; 120.3; 126.7; 129.5; and two olefinic carbon at δ_C 146.02 (2 C), 2 carbon chemistry shift value at δ_C = 159.08 (2C) and two aromatic carbon quarterner at δ_C 130.9 and 141.17 (Figure 4). Mass spectroscopy analysis showed that **compound 1** has a molecular weight (m/z) 245 and molecular formula $C_{14}H_{13}O_4$. Further identification of **compound 1** was determined by HMQC and HMBC (Figure 5), showed that protons at δ_H 7.01 correlated with δ_C 120.3 (C-6 ') and δ_C 145.9 (C-4'). The opposite correlation also showed between δ_H 6.82 with δ_C 113.7 signal (C-2'). Proton at δ_H 6.82 showed correlation with the two aryl carbon δ_C 145.9 (C-4 ') and δ_C 146.0 (C-3'), δ_H 6.77 also has correlation with δ_C 145.9 (C -4'), δ_C 146.0 (C-3'), and quaternary aromatic carbon δ_C 130.9 (C-1'). Proton signals δ_H 6.74 (C-7) had a trans coupling with δ_H 6.89 (C-8). HMBC spectrum also showed other correlation between δ_H 7.01 and δ_H 6.77 with δ_C signal (C-1'), and δ_H 6.89 with δ_C (C-7) and carbon quaternary (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 IC_{50} 1.56 ppm compared to Artonin E (IC_{50} 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 IC_{50} values 1.56 ppm.

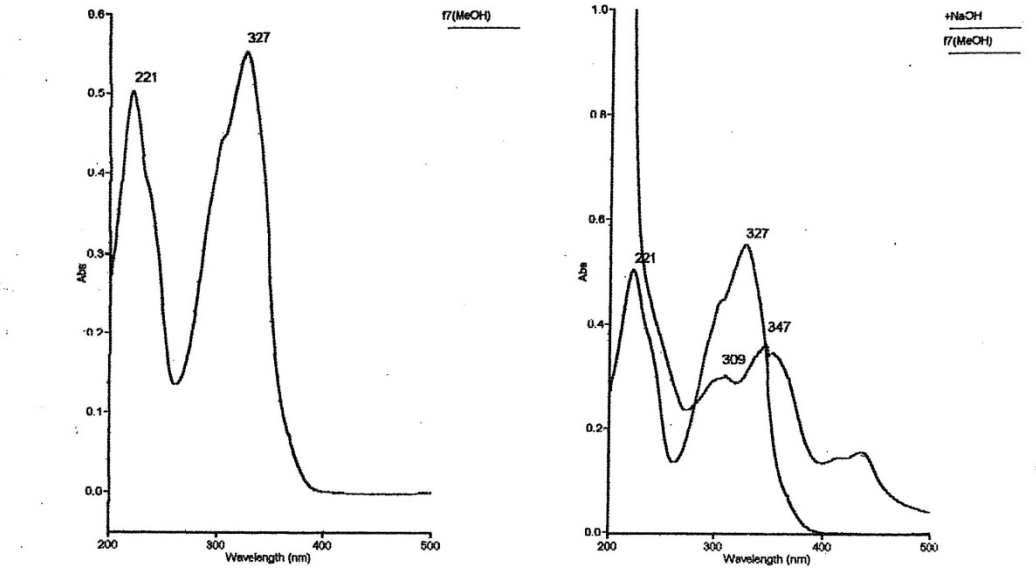

Fig. 1: UV spectrum of compound 1.

Table. 1: 1D- and 2D-NMR Data of **Compound 1** and Reference (Brinker and Seigler, 1991).

No	δH (int, mult, J = Hz) 500 MHZ (ppm)		HMQC	HMBC		
	1* (Brinker and Seigler, 1991)	1				
1			141.17			
2.6	6,43 (2H,d)	6,50 (2H,d)	105,84 (2C)	102.5	126.76	
3.5			159,08 (2C)			
4	6,15 (1H,t)	6,25 (1H,t)	102.5	105.84	159.08	
7	6,73 (1H,d)	6,74 (1H,d)	126.76	105.84	130.91	
8	6,89 (1H,d)	6,89 (1H,d)	129.59	126.76	141.17	
1'			130.91			
2'	6,73 (1H,d)	6,77 (1H,s)	116.38	130.91	145.98	146.02
3'			146.02			
4'			145.98			
5'	6,83 (1H,dd)	6,82 (1H,dd)	120.3	113.75	145.98	146.02
6'	6,97 (1H,d)	7,01 (1H,d)	113.75	113.75	120.3	145.98

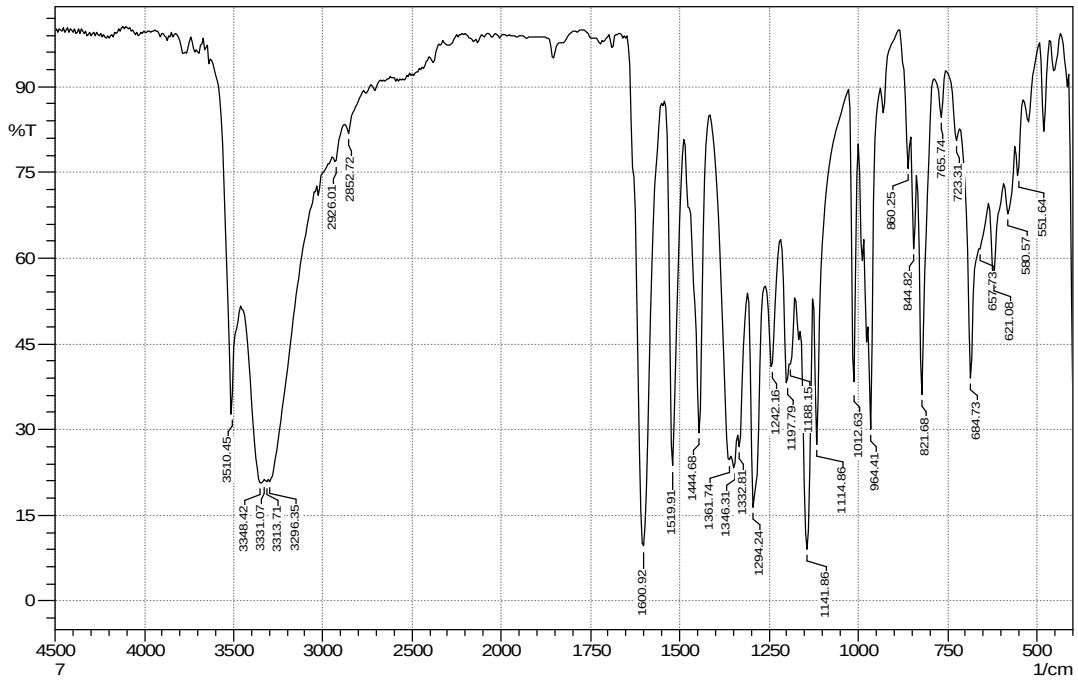

Fig. 2: IR spectrum of compound 1.

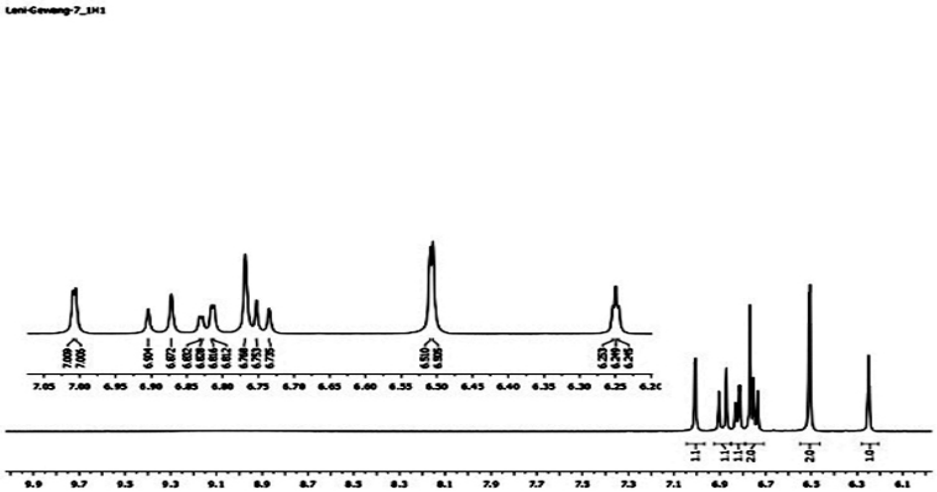

Fig. 3: ^{1}H-NMR spectrum of **compound 1.**

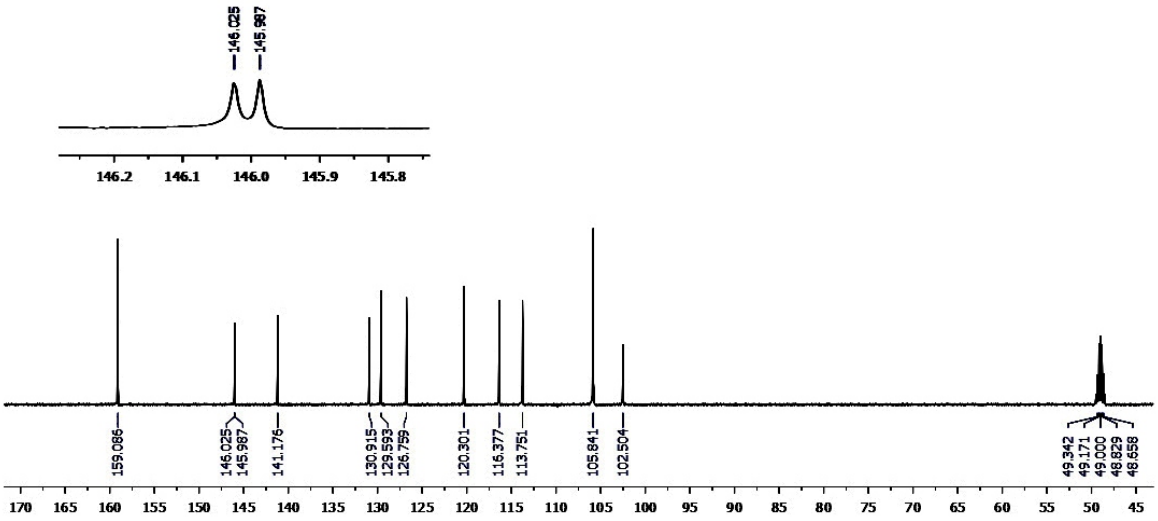

Fig. 4: 13C-NMR spectrum of compound 1.

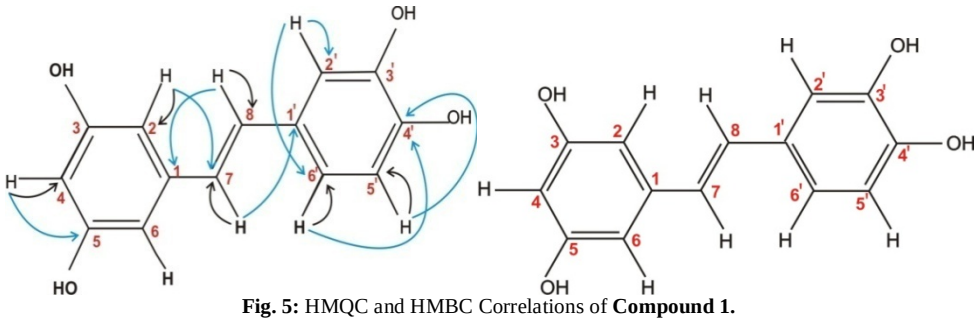

Fig. 5: HMQC and HMBC Correlations of **Compound 1.**

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How to cite this article:

Leny Heliawati, Agus Kardinan, Tri Mayanti, Roekmi-ati Tjokronegoro. Piceatanol: Anti-Cancer Compound From Gewang Seed Extract. J App Pharm Sci, 2015; 5 (01): 110-113.