

Anticancer Effect Of Pinostrobin From (*Kaempferia Pandurata* Roxb) In Benzo(A)Pyrene – Induced Fibrosarcoma In Mice

Sukardiman^{a*}, Dinar Charisma^b, Hani Plumeriastuti^c, Lusiana Arifianti^a

^{a,b} Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University Surabaya Indonesia

^c Faculty of Medicinal of Veterinary, Airlangga University Surabaya Indonesia

*Corresponding author: Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University Surabaya Indonesia

Email : sukardiman@unair.ac.id

Abstract.

Pinostrobin, a flavonoid purified from the Indonesian herb (Kaempferia pandurata Roxb) . was tested for antitumor properties in several model system. In vitro, pinostrobin inhibited proliferation of primary human breast cells, human breast cells line MC7, and mieloma cells . The aim of this study was to investigated the inhibitory effect of pinostrobin isolated from Kaempferia pandurata Roxb on the growth of solid tumor of benzop(a)pyrene – induced fibrosarcoma in mice in vivo. The inhibition of solid tumor was measured by weight of tumor and histological observation. These result indicated that pinostrobin showed inhibitory effects on the growth of solid tumor of benzop(a)pyrene – induced fibrosarcoma in mice.

Key Word : Anticancer, *Kaempferia pandurata* Roxb . pinostrobin . fibrosarcoma .

INTRODUCTION

Kaempferia pandurata (Zingiberaceae) is a plant from Indonesia, known as “temu kunci” in folk medicine, and used as an antitusive, cholic, diuretic, inflammation and disentry (Heyne, 1987). In search for naturally occurring antitumor activity, flavonoid compound as pinostrobin from *Kaempferia pandurata* rhizome has been done antitumor activities testing *in vitro* on cell culture of primary human breast cancer (Ermawati, 1996) human breast cancer cell line (MC7) (Bail *et al.*, 2000) mieloma cell line (Sukardiman, 2003) The critical biochemical target has been identified . mechanism for the cytotoxicity in cell culture of human breast cancer by inhibition in the activity of DNA Topoisomerase I (Sukardiman *et al.*, 2000) and pinostrobin inhibition aromatase receptor not inhibition estrogen receptor. DNA topoisomerase alter the topological state of DNA, thereby carrying out function essential for several cellular processes including DNA replication, transcription, recombination, DNA repair, and chromosome segregation at mitosis . There are two types of DNA topoisomerase : type I enzymes changes the DNA linking number by transiently breaking one strand of duplex DNA, while tipe II enzymes transiently breaks both strand (Knut and James, 1992; Cumming and Smyth, 1993; Zahir, 1996)

The *in vitro* activity is not general correlated with *in vivo* activity, because the *in vivo* activity many other factor are influence as metabolism effect from bioactive compound if used oral administration (Constantinu, 1995). In the present study, we investigated the inhibitory effect of pinostrobin isolated from *Kaempferia pandurata* Roxb on the growth of solid tumor of benzo(a)pyrene – induced fibrosarcoma in mice *in vivo*.

2. Material and Methods

2.1. Plan material. *Kaempferia pandurata* rhizoma used in this study were purchased from Ponorogo, East Java. Voucher specimen are deposite at Laboratory of Pharmacognosy . Faculty of Pharmacy, Airlangga University Surabaya.

2.2. General experimental prosedur. Melting point were measured with DTA Analysis. FTIR spectra were recorded on a Jasco FTIR 5300. NMR spectra were recorded on a 138 Tessia Spectrofotometer running at 450 MHz for ¹³C and ¹H, with DMSO as solvents. Chemical shift were measured with tetramethylsilane (TMS) as internal standar. MS spectra measured with a Hawlett – Packard.

2.3. Extraction and isolation of pinostrobin. The pulverized rhizome of *Kaempferia pandurata* (300 g) were extracted at room temperature with n-hexane and the extracts concentrated to dryness under reduced pressure, yielding residues of 47 g, respectively. The residue was subjected to flash column chromatography on silica gel with EtOAc – n Hexane. The compound was recrystallized by hot MeOH. The molecular weight was confirmed by MS. The structure was established by aggreement of ¹H-NMR and ¹³C –NMR and data with those of literatur.

2.4. Induced of fibrosarcoma in mice by benzopirena and analysis effect of pinostrobin on inhibit ion growth on solid tumor. Fibrosarcoma were induced in 8 week - old male mice by s.c injection of benzo(a)pyrene from Sigma 0.3 % (b/v) every two days until ten injection. All animal were housed in light and temperature room and fed standart laboratory diet and tap water *ad libitum*.. The solid tumor of fibrosarcoma allowed to grow for 3 month . a size of ~ 100mm³ and were devided into three groups of three animal each, i.e . the control animal were treated with 0.9% NaCl solution. Thereafter . pinostrobin 0.2 mg/bw / day and 0.4mg/bw/day was injected i.p into the mice on daily basis . At the end 2 and 3 week . the mice were solid tumor, and the tumor were dissected, photographed weighed and histological observation. Histological examination was carried out in all specimen by HE exclusion . benzo(a)pyrene – induced fibrosarcoma were classified into five grades . i.e., a scoring 0 for 0% the growth of solid tumor, a scoring 1 for 25% the growth of solid tumor, a

scoring 2 for 50% the growth of solid tumor. a scoring 3 for 75% the growth of solid tumor. a scoring 4 for 100% the growth of solid tumor (Sakamoto, 1993).

The data of weighed of solid tumor were expressed as :
Inhibition % = [1 – (mean weight of tumor test / mean weights of tumor control)] x 100% (Yang, 2003).

2.5. Statistical analysis. The statistical significance of difference between group was evaluated by one way anova for data of weighed of solid tumor. and by Kruskal-Wallis rank test for data of histological of solid tumor and $P < 0.05$ was considered significant (Yang, 2003).

3. Result and Discussion

3.1. Identification of pinostrobin from *Kaempferia pandurata* rhizoma.

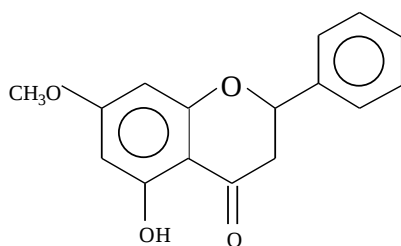


Fig.1 Structural formula of pinostrobin

Pinostrobin crystallized as colorless needles from MeOH. mp 101.8°C. The EIMS showed the molecular ion $[M]^+$ at m/z 270, corresponding the molecular formula of $C_{18}H_{14}O_4$. IR and NMR data suggested a fully aromatic flavanone ring system. The 1H -NMR spectrum displayed signal for one methoxyl group at δ 3.76 ppm, five aromatic proton constituen by one singlet at 7.38 ppm, one hydroxyl groups at δ 12 ppm. Assignment of the 16 carbon signals of ^{13}C -NMR spectrum.

3.2. Inhibition of growth of solid tumor of benzo(a)pyrene- induced fibrosarcoma by pinostrobin.

3.2.1. The percent change of tumor weight . The solid tumor of fibrosarcoma allowed to growth for 3

month. a size of $\sim 100mm^3$. fig. 2 mice with benzo(a)pyrene- induced fibrosarcoma . On this basis . we chose a dose 0.2 mg/bw/ day . i.p . the injection were given daily because pinostrobin has short half life. Figure 3 solid tumor of all dose. after were dissected.



Figure 2. Benzo(a)pyrene- induced fibrosarcoma in mice

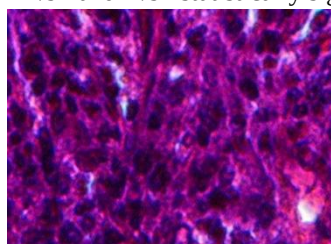


Figure 3. Effect of pinostrobin on growth solid tumor of Benzo(a)pyrene- induced fibrosarcoma in mice

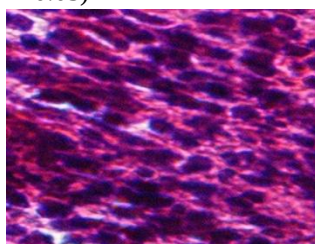
Table 1. Inhibition percent of tumor weight by pinostrobin

Eksperimental groups	Sampel (n)	Meanof tumor weight	inhibition % of growth
(1) Control	3	2.3164 ± 1.6126	0 ^a
(2) Dose 0.2mg/bw/day	3	0.7971 ± 0.4912	62.6 ^b
(3) Dose 0.4mg/bw/day	3	0.5373 ± 0.4893	76.8 ^c

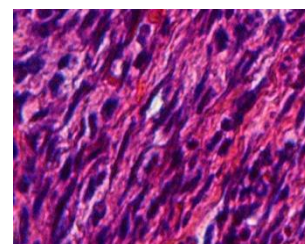
^a vs ^b and ^a vs ^c statistically significant ($P < 0.05$)



a. Control



b. Pinostrobin
dose 0.2mg/bw/day



c. Pinostrobin dose
0.4mg/bw/day

Figure 4. Histological examination in experimental group

Table 2. Inhibition percent of growth of solid tumor by pinostrobin with histological examination. ($Z_{\text{calculated}}$ with average of rank)

Experimental groups	$R_{i1} - R_{i2}$	$Z_{\text{calculated}}$	p
Control – Dose 0.2mg/bw/day	3.66667	1.33888	0.0918
Control – Dose 0.4mg/bw/day	5.33333	1.94746	0.0256*
Dose 0.2mg/bw/day - Dose 0.4mg/bw/day	1.66667	0.60858	0.2709

* : significant with Table Z (Siegel, 1990)

Pinostrobin treatment increase inhibition % growth of solid tumor of benzo(a)pyrene- induced fibrosarcoma in mice. approximately 60 – 80 % of that of control mice ($P < 0.05$) (table1). Benzo(a)pyrene treatment for 3 months induced fibrosarcoma in mice.

3.2.2. Histology observation. Histological examination was carried out in all specimen by HE exclusion . benzo(a)pyrene – induced fibrosarcoma were classified into five grades . i.e.. a scoring 0 for 0% the growth of solid tumor. a scoring 1 for 25% the growth of solid tumor. a scoring 2 for 50% the growth of solid tumor. a scoring 3 for 75% the growth of solid tumor. a scoring 4 for 100% the growth of solid tumor. This result show in figure 4.

Pinostrobin treatment increase inhibition % growth of solid tumor of benzo(a)pyrene- induced fibrosarcoma in mice by histological data . approximately 48 – 57 % of that of control mice. and by Kruskal-Wallis rank test was statistically significant between control and dose 0.4 mg/bw/day ($P < 0.05$) (table2).

4.Conclusion. Pinostrobin was isolated from 'temu kunci' (*Kaempferia pandurata* Roxb) shown antitumor effect by inhibits the growth of solid tumor of benzo(a)pyrene – induced fibrosarcoma in mice in vivo.

Acknowledgement. Prof.Dr.Rer.Nat.Gunawan Indrayanto.Apt and Dr.R.Ebel for helpfully to analysis spectroscopy H- CNMR.

REFERENCES

- Bail, L. Aubourg L, Habrioux G., 2000. Effect of pinostrobin on estrogen metabolism and estrogen reseptor transactivation. *Cancer Lett.* Aug. Vol.10, p:37-44.
- Constantinu A., 1995. Flavonoids as DNA Topoisomerase Antagonists and Poison : Structure-Activity Relationship. *J. Natural Product.* Vol : 58 (2): 217-22
- Cumming, J. and Smyth, J.F. 1993. DNA Topoisomerase I and II as target of Rational Design

of New Anticancer Drugs. *Ann Oncology.* Vol 3(7): 533-534.

Ermawati M., 1996. Cytotoxic Activity Test of Flavonoid from *Kaempferia pandurata* againts Cell Culture of Human Mammary Carcinoma. *Tesis.*

Heyne, K.1987. *The Plant Used.* Jakarta : Ruygrok & Co, p. 2273.

Knut, R.M. and James, J.C. 1992. Overexpression of Human Topoisomerase I in Baby Hamster Kidney Cells : Hypersensitivity of Clonal Isolates to Camptothecin. *Cancer Research.* Vol 52 (2): 525-53

Sakamoto S., 1993. Effect of Kampo (Japanase Herbal) Medicine “ Sho-Saiko-To” on DNA-Synthesing Enzym Activity in 1,2-Dimethylhydrazine-Induced Colonic Carcinoma. *Planta Medica .* Vol 59, pp 152-153.

Sukardiman, 2003. Anticancer Activity of Pinostrobin from Temu Kunci from *Kaempferia pandurata* againts Cell Culture of Mieloma. *Media Kedokteran Hewan.*

Sukardiman, Darwanto A., Darmadi O.M., 2000. Cytotoxic mechanism of flavonoid from Temu Kunci (*Kaempferia pandurata*) in cell culture of human mammary carcinoma . *Clinical Hemorheologyand Microcirculation.* Vol 23, pp 185-190.

Woerdenbag. H.J.. 1987. Investigation of The Antitumor Action of Euopatoripicrin Againts The Lewis Lung Tumour. A Fundamental Study On The Sytostatic Action of Sesquiterpene Lactone from *Eupatorium cannabinum* L.. *Planta Medica.* 53. p. 49.

Yang S., 2003. Triptolide Inhibits the Growth and Metastasis of Solid Tumors. *Molecular Cancer Therapeutics.* Vol 2, p. 65-67

Zahir, A. 1996. DNA Topoisomerase I Inhibitors : Cytotoxic Flavones from *Lethedon tannaensis.* *J.Natural Product.* Vol 59, p.701-703.