

## Inhibition of Fibrosarcoma Growth by 5-Hydroxy-7-Ethoxy-Flavanons from *Kaempferia Pandurata* Roxb

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### ABSTRACT

This paper discusses the anticancer activity of 5-hydroxy-7-ethoxy-flavanones isolated from *Kaempferia pandurata* Roxb against mice fibrosarcoma resulted from benzopyrene induction. This study was initiated by isolating the 5-hydroxy-7-ethoxy-flavanones in the rhizome powder of *Kaempferia pandurata* Roxb followed by creating the fibrosarcoma in the nape of the mice through benzopyrene injection. Fibrosarcoma appeared approximately 1.5 months after the injection of benzopyrene. The mice with fibrosarcoma were then grouped into 3 treatment groups, namely: group I (negative control) those that were fed aquadest and CMC-Na only, group II those that were given 5-hydroxy-7-ethoxy-flavanones solution at a dose 80 of mg/kg body weight and group III those that were given the anticancer drug of at a dose of 13.33 mg/kg body weight. The treatment was carried out for about 1.5 months until the fibrosarcoma volume was about 100 mm<sup>3</sup>. The analysis was started with the surgery of the fibrosarcoma tissue in the infected mice and then weighing out the fibrosarcoma obtained. The identification results of the isolates with UV-Vis, IR, Hydrogen and Carbon NMR, MS and three-dimensional X-ray diffraction suggested that the isolates was the compound of 5-hydroxy-7-ethoxy-flavanones or 5-hydroxy-7-methoxy-flavanon. The fibrosarcoma tissue was then stored in a container containing 10% formalin. The result of histopathological analysis with Hematoxylin and Eosin (HE) on the fibrosarcoma tissue showed the existence of polichromatin. This suggested that there was a damage in the cells caused by fibrosarcoma infection. The weighing results of the cancer cells showed that the oral concentration of 5-hydroxy-7-ethoxy-flavanones of 80 mg/kg could inhibit the growth of fibrosarcoma with the mass of 68.62 % and with the cancer drug (control +) there was a resistance of 95.95 % compared to the negative control which was only given CMC-Na orally. The results of this study indicated that 5-hydroxy-7-ethoxy-flavanones is potential to developed as a cancer chemotherapy.

**Keywords:** 5-hydroxy-7-ethoxy-flavanones, *Kaempferia pandurata* Roxb, benzopyrene and fibrosarcoma.

### INTRODUCTION

As a traditional medicine, the rhizome of *Kaempferia pandurata* Roxb is used as a medicine for dry cough, ulcers, disorders of the colon, stomach swelling, inflammation of the uterus, diare, and cancer (Hembing, 1994).

The rhizome of *Kaempferia pandurata* Roxb extracted with diethyl ether produces relatively a large amount of 5-hydroxy-7-ethoxy-flavanones i.e. about 2,5% and alpinetin which is approximately 1%. 5-hydroxy-7-ethoxy-flavanones shows an antioxidants activity and relaxes smooth muscle.

The high 5-hydroxy-7-ethoxy-flavanones content in the *Kaempferia pandurata* Roxb rhizome causes the isolation of the compound as a pure substance can be carried out easily and quickly. The 5-hydroxy-7-ethoxy-flavanones structure that the polarity reduces as a result of the existence of intramolecular hydrogen bonding between the carbonyl group at C-4 with a hydroxyl group at C-5, could be extracted with the application of semipolar or nonpolar solvents such as ethylacetate and  $\text{CCl}_4$  (Parwata,1998).

5-hydroxy-7-ethoxy-flavanones shows a fairly high toxicity with  $\text{LC}_{50} = 63.5$  ppm (Parwata,2000). This compounds have an activity in inhibiting the growth of human breast cancer cells through the barrier activity of DNA topoisomerase I enzyme (Sukardiman *et.al.*,2006).

The death of the DNA of a cancer cells can affect the the process inside the cell, especially the process of cell replication and terminated with the death of cancer cells by apoptosis (Sukardiman, *et.al.*,2006). DNA damage cells is thought to activate tumor suppressor gene p53 besides inducing apoptosis (Whibley, 2009).

Activation and stabilization of p53 products by a flavonoid, is thought to inhibit the angiogenesis effect through the mechanism of down regulation by the decrease of VEGF expression, COX-2 and MMP-9 that involve in the metastasis process of cancer cells.. Based on this phenomenon the researchers suspect that 5-hydroxy-7-ethoxy-flavanones compound has the same activity as a therapy or chemopreventive to cancer on fibrosarkoma.

## MATERIALS AND METHODS

The materials used were as follows: rhizome *Kaempferia pandurata* Roxb powder obtained from Badung Market, Denpasar-Bali and male mice of 2 months old and weight of 20-25 grams. The chemicals used were: technical n-heksana, n-heksana (E Merck), methanol (E Merck), ethanol (E Merck), chloroform (E Merck), ethyl acetat (E Merck) aquadest, silica gel and Plate of Thin Layer Chromatography (TLC) Aluminium with silica Gel GF-254, E Merck), primary and

secondary antibodies p53 and VEGF, buffered formalin 10 %, PBS, DAB,  $\text{H}_2\text{O}_2$  3% and destilate water (DW).

The apparatus used were HPLC (High Performance Liquid Cromatography) and TLC (Thin Layer Cromatography)-Densitometer Shimadzu CS-930, UV-Vis Spectrophotometer Hitachi 557 and 365 NIR Shimadzu, IR Spectrophotometer Jasco 5300 FTIR, proton NMR spectrometers and Carbon NMR Hitachi FT-1500 and Mass Spectrometer HP 8890, light microscope, polilysin preparate and objects glass.

## Isolation and identification of 5-hydroxy-7-ethoxy-flavanones from the rhizome of *Kaempferia pandurata* Roxb

Approximately 2000 grams of the powder of *Kaempferia pandurata* Roxb rhizome, was macerated with n-hexane for 24 hours, the liquid maserat obtained was then evaporated by rotary evavorator. The n- hexane extract was allowed to arise crystalline amorphous. After that, the amorphous crystals was chromatographed with vacuum column with n- hexane as the developer. The results of this column chromatography were collected and then reevaporated with rotary evavorator until the crystals appeared. The crystals were filtered, then recrystallized with methanol 3 times. The crystals obtained in this study were shiny white. In the next step, the melting point of the crystals was determined by DTA and Melting Points Apparatus Fiesher & John and then its purity was tested by TLC (Thin Layer Chromatography) with the application of some eluents of various polarities, TLC-densitometer with multiple wavelengths as well as HPLC (High Performance Liquid Chromatography). When the test shows that 5-hydroxy-7-ethoxy-flavanones was pure, the work was continued with subsequent identification by UV- Vis spectroscopy with sliding reagents, IR,  $^{13}\text{C}$  and  $^1\text{H}$  NMR, MS and three-dimensional X-ray diffraction to find out the structure of 5-hydroxy-7-ethoxy-flavanones.

## Establishment of Fibrosarkoma on Mice by Benzopyrene Induction

Forty male mice were prepared to undergo a process of the research adaptation, then the mice had benzopyrene induction as much as 0.3 mg/0.2

mL in the oleum olivarium as a subcutaneous injection in the scapular area 5 times every two days. All mice were maintained in the same atmosphere and diet for two months/until the cancer formed in the neck. After the cancer volume reached  $\pm 100 \text{ mm}^3$ , the mice with cancer were randomly divided into 3 groups. Group I was the negative control which was only given CMC-Na. The second group was given 5-hydroxy-7-ethoxy-flavanones at a dose of 80 mg/kg; group III was given cyclophosphamide at a dose of 13.33 mg/kg. All of the test materials were administered orally and were given every day for 14 days. After that the mice were surgered for removing the fibrosarcoma tissue followed by weighing the tissue obtained.

## RESULTS AND DISCUSSION

### Isolation of 5-hydroxy-7-ethoxy-flavanones

Isolates obtained was in the form of needles white crystalline. Analysis of purity was

**Table 1: Analysis of purity isolates by Thin Layer Chromatography**

No.	Mobile phase	Result
1	BAW = 4:1:5	1 stain
2	$\text{CHCl}_3:\text{CH}_3\text{CH}_2\text{OH}=3:1$	1 stain
3	$\text{CHCl}_3:\text{CH}_3\text{CH}_2\text{OH}=15:1$	1 stain
4	$n\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3:$ $\text{CH}_3\text{COOCH}_2\text{CH}_3 = 1:1$	1 stain
5	$n\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3:$ $\text{CH}_3\text{COOCH}_2\text{CH}_3 = 3:1$	1 stain
6	$n\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3 :$ $\text{CH}_3\text{COOCH}_2\text{CH}_3 = 3 : 2$	1 stain
7	$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3 :$ $\text{CH}_3\text{COOCH}_2\text{CH}_3 = 7 : 3$	1 stain

Note : BAW : n-Butanol : Acetic acid : Water  
All the Mobile phase result one stain

**Table 2: Kualitatif Chemical Ingredients of isolates**

No	Reagent	Discoloration	Indicated
1.	<i>Willstater</i>	colorless to pink	Flavonoid
2.	NaOH 10%	colorless to yellow	Flavonoid
3.	$\text{FeCl}_3$	colorless to red	Flavonoid

Note : All the Reagent result flavonoid

performed by TLC using the mobile phase that different polarity. The results obtained showed one stain as shown in Table 1 below. Analysis of purity by TLC-densitometry at a wavelength [220 nm (I); 254 nm (II); 287 nm (III); 300 nm (IV); 320 nm (V) and 350 nm (VI)] resulted in one peak whereas by HPLC the purity of the compound obtained was of 94.83%. All this purity test results showed that the isolate was pure chromatographic.

Analysis of the melting point showed that the range of its melting point = 99-100°C (I); 99,5-101.5°C (II) and 99.5-100.5° C (III), whereas with DTA melting point observed was of 97°C, and the substance stability was up to a temperature of 150°C. These results indicated that the isolates were pure based on its physical properties.

Analysis of chemical constituents showed that isolates indicated positive flavonoids of flavanones, as shown by the following Table 2 : Identification with UV-Vis spectrophotometer showed the I max = 287 nm (band II) and shoulder = 325 nm (band I).

**Table 3: Identification isolates by IR spectroscopy**

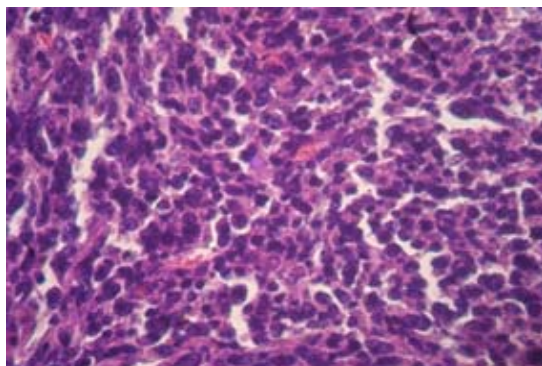
No.	Wavenumber ( $\text{cm}^{-1}$ )	Indicated
1	3437.46	-OH at C-5
2	3061.31	-C-H stretch of aromatics
3	1645.43	-C=O ketones at C-4
4	1622.26 and 1579.64	-C=C- of aromatic
5	3000-2800	-C-H of methyl
6	1450-1350	-C-H of methylene
7	1250 and 1290	-C-O

**Table 4: Identification Isolates by Proton NMR spectroscopy**

No.	Chemical shift (d), ppm	Indicated
1	3.75 (s)	$\text{ArOCH}_3$ (8H)
2	6.019 (s)	H-6 and H-8 (2H)
3	11.948 (s)	-OH (1H)
4	2.817 to 3.029 (m)	H-3 cis or trans (2H)
5	5,284	H-2 (1H)

Subsequent analysis when isolates in methanol was coupled with  $AlCl_3$ , the absorption band II has a bathochromic shift of 23 nm. Identification by IR spectroscopy showed some peaks as shown by the data in the following Table 3 : Identification Isolates by Proton NMR spectroscopy resulted some peaks as shown by the data in the following Table 4 :

Identification Isolates by Carbon NMR spectroscopy resulted some peaks as shown by the data in the following Table 5 : Analysis of isolates by mass spectroscopy resulted in molecular ion with  $m/e=270$  and the fragmentation as shown by the data in the following Table 6 :



**Fig. 1: Histopathology of Fibrosarcoma tissue**

**Table 5: Identification Isolates by Karbon NMR spectroscopy**

No.	Chemical shift ( $\delta$ ), ppm	Indicated
1	55.583	O-CH <sub>3</sub> (1C)
2	79.121	C-2 (1C)
3	43.326	C-3 (1C)
4	195.440	C-4 (1C)
5	163.944	C-5 (1C)
6	95.037	C-6 (1C)
7	167.755	C-7 (1C)
8	94.153	C-8 (1C)
9	162, 572	C-9 (1C)
10	103.025	C-10 (1C)
11	138.241	C1 '(1C)
12	138.241	C2'and C6 '(2C)
13	128.667	C3'-C5 '(3C)

### Surgery of fibrosarcoma

After one month of benzopyrene injection, fibrosarcoma started to grow and approximately after 2 months the volume of fibrosarcoma was 100 mm<sup>3</sup>. Prior to the treatment, one of the infected mice was sacrificed to prove the existence of fibrosarcoma. Justification of this was carried out by observing the histopathological tissue of the fibrosarcoma with HE colouring. The weight of the surgical result obtained was 3.4756 grams and the histopatology observation with 400x magnification showed a lot of chromatin (polychromatin) proving the damage of the cells due to fibrosarcoma as shown by the following After one month of benzopyrene injection, fibrosarcoma started to grow and approximately after 2 months the volume of fibrosarcoma was 100 mm<sup>3</sup>. Prior to the treatment, one of the infected mice was sacrificed to prove the existence of fibrosarcoma. Justification of this was carried out by observing the histopathological tissue of the fibrosarcoma with HE colouring. The weight of the surgical result obtained was 3.4756 grams and the histopatology observation with 400x magnification showed a lot of chromatin (polychromatin), as shown by the following figure 6.

After 14 days of treatment, the mice with fibrosarcoma were sacrificed to take the cancer tissue for subsequent examination i.e. the weight

**Tabel 6: Identification Isolates by Massa spectroscopy**

No.	m/e	Indicated
1	270	M <sup>+</sup>
2	252	M <sup>+</sup> -H <sub>2</sub> O
3	242	M <sup>+</sup> -CO
4	227	M <sup>+</sup> -CO-CH <sub>3</sub>
5	213	M <sup>+</sup> -CO-C <sub>2</sub> H <sub>5</sub>
6	193	release of ring B/phenyl
7	166	M <sup>+</sup> -C <sub>2</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>5</sub>
8	152	M <sup>+</sup> -C <sub>2</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>5</sub> -CH <sub>3</sub>
9	123	M <sup>+</sup> -C <sub>2</sub> H <sub>5</sub> -C <sub>6</sub> H <sub>5</sub> -CO-CH <sub>3</sub>
10	77	C <sub>6</sub> H <sub>5</sub>

Note : M<sup>+</sup> indicated Massa Molekul Relatif of 5-hydroxy-7-ethoxy-flavanones

of the cancer tissue, as shown by the data in the following table :

### DISCUSSION

Isolates obtained was in the form of needles white crystalline. Purity analysis with chromatographic by TLC , TLC - densitometry and HPLC showed one peak with a stain and a different polarity eluent. This shows that all this purity test results showed that the isolate was pure chromatographic. Analysis of one of the physical properties of the isolates are melting with Melting Points Apparatus and DTA showed that isolates said to be purely due to their melting point range < 2°. Based on these results further isolates identified spectroscopically.

Phytochemical screening reagents Wilstatter color, FeCl<sub>3</sub> and NaOH 10% indicated that the isolates do indeed flavonoids flavanones.

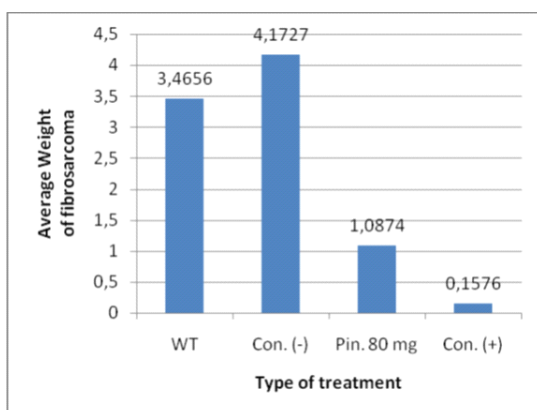


Fig. 2: The Effect of 5-hydroxy-7-ethoxy-flavanones on Fibrosarcoma Weight

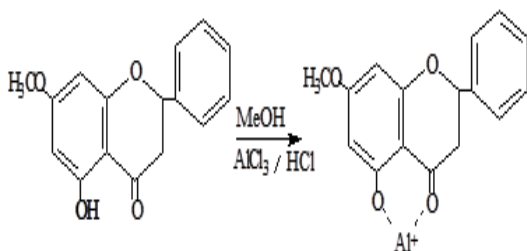


Fig. 4: Structure of 5-hydroxy-7-ethoxy-flavanones

Identification with UV-Vis spectrophotometer showed the I max = 287 nm (band II) and shoulder = 325 nm (band I). This was in agreement to the literature in which flavonoids flavanones show two absorption bands i.e. between 275-295 nm (band II) and a shoulder at 300-330 nm that caused by the cinnamoyl and benzoyl groups of flavanones as shown in the structure below (Silverstein *et al.*, 1981; Parwata, 1998):

Bathochromic shift indicates that flavonoids flavanones contains an -OH group at C-5 as shown in the structure below (Silverstein *et al.*, 1981): Identification by IR spectroscopy proved that isolates containing -OH groups, -CH, -C=O and -C-O. The results of the Proton and Carbon NMR spectra interpretation showed that 5-hydroxy-7-ethoxy-flavanones has 14 H atoms and 16 C, as shown by the following structure :

Analysis with mass spectroscopy resulted in molecular ion with m/e =270 that indicated the

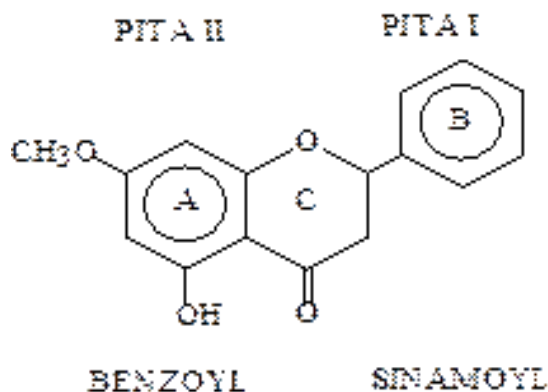


Fig. 3: Structure of flavanone

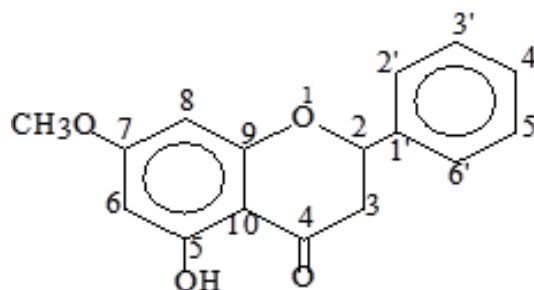
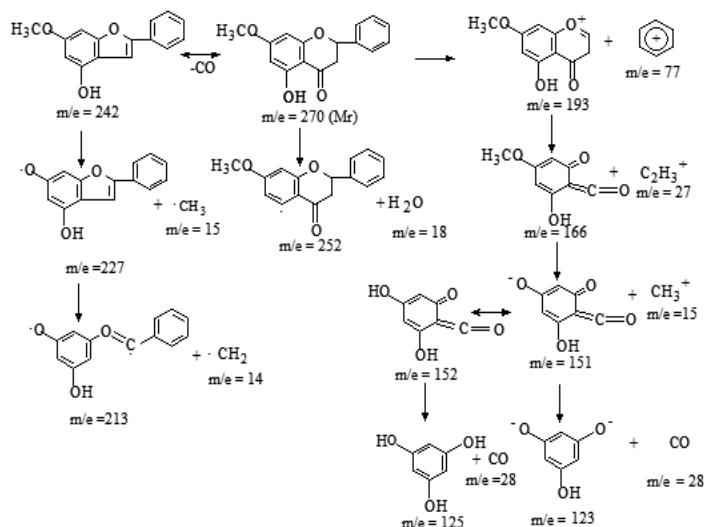
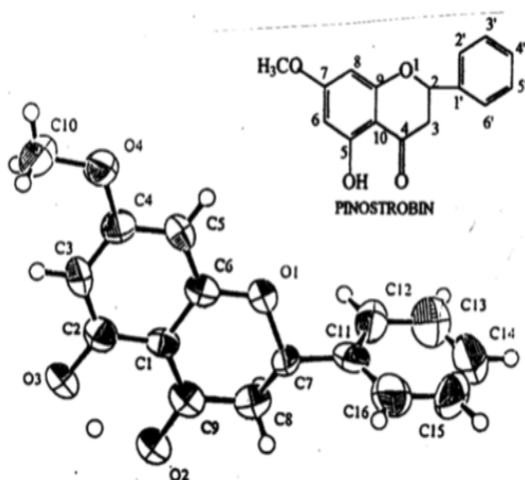


Fig. 5: Numbering Structure of 5-hydroxy-7-ethoxy-flavanones



**Fig. 6: Pragmentasi of 5-hydroxy-7-ethoxy-flavanones**



**Fig. 7: 5-hydroxy-7-ethoxy-flavanones structure with three-dimensional X-ray diffraction**

relative molecular mass of 5-hydroxy-7-ethoxy-flavanones with some fragmentation. This is illustrated by the following fragmentation schemes (Silverstein *et al.*, 1981)

The result of the three-dimensional X-ray diffraction analysis showed that isolates as 5-hydroxy-7-ethoxy-flavanones have a structure as follows: The weight of the surgical result obtained was 3.4756 grams and the histopatology

observation with 400x magnification showed a lot of chromatin (polychromatin), proving the damage of the cells due to fibrosarcoma.

After 14 days of treatment, the mice with fibrosarcoma were sacrificed to take the cancer tissue for subsequent examination i.e. the weight of the cancer tissue. The result obtained was that oral 5-hydroxy-7-ethoxy-flavanones concentration as much as of 80 mg/kg body weight could inhibit the growth of fibrosarcoma with a weight of 68.62% grams while with the cancer drug (control +) there was a barrier of 95.95% compared to the negative control which was only given CMC-Na orally. This suggested that 5-hydroxy-7-ethoxy-flavanones is potentially be developed as a cancer chemotherapeutic agent.

## CONCLUSION

Based on the results of the research, it can be concluded as follows:

1. Isolates produced are correct is 5-hydroxy-7-ethoxy-flavanones
2. Results of histopathological analysis fibrosarcoma cells with HE staining showed there are many chromatin on fibrosarcoma as evidence of the destruction of normal cells due to the occurrence of fibrosarcoma
3. 5-hydroxy-7-ethoxy-flavanones has a

significant effect in inhibiting the growth of fibrosarcoma.

4. 5-hydroxy-7-ethoxy-flavanones oral concentration of 80 mg/kg body weight could reduce as much as 68.62 % fibrosarcoma weight while with a cancer drug (control +) there was a decrease of 95.95 % , this suggested that 5-hydroxy-7-ethoxy-flavanones is potentially be developed as a cancer chemotherapy agent.

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#### REFERENCES

1. Adi Parwata, 1998, Isolasi, Identifikasi Senyawa 5-hydroxy-7-ethoxy-flavanones pada Rimpang Temu Kunci (*Kaempferia pandurata* Roxb) dan Standarisasi Ekstrak Etanol berdasarkan Kadar 5-hydroxy-7-ethoxy-flavanonesnya dengan KLT Densitometri. Thesis, PPS Unair, Surabaya.
2. Adi Parwata, 2000, Uji Toksisitas Senyawa 5-hydroxy-7-ethoxy-flavanones pada Rimpang Temu Kunci (*Kaempferia pandurata* Roxb), DIK/DIKS, Lemlit Unud, Bali.
3. Anonim, 2005, Fibrosarkoma, <http://medika-online.blogspot.com/2005/11/Fibrosarkoma.html> , diakses, 28 September 2011
4. Bail, L., Aubourg L., Habrioux G., 2000. Effect of 5-hydroxy-7-ethoxy-flavanones on estrogen metabolism and estrogen reseptor transactivation, *Cancer Lett*, Aug, 1;156910;37-44.
5. Banerjee S., Carlos ,B.R and Bharat B.A.(2002) Suppression of 7,12-Dimethylbenz(a) anthracene-induced Mammary Carcinogenesis in Rats by Resveratrol , *Carcinogenesis*, 62, 4945-4954.
6. Chrestella J., 2009. Gambaran Immunoekspresi Matrix Metallo- proteinase 9 (MMP-9) pada Lesi-Lesi Prakanker dan Karsinoma serviks invasif Thesis, Medan Universitas Sumatra Utara.
7. Cumming, J., Smyth, J.F., 1993. DNA Topoisomerase I and II as target of Rational Design of New Anticancer Drugs, *Ann Oncology*, Aug, 3(7), 533-534.
8. Harborne J.B. and Mabry, T.J., 1992, *Flavonoids, Advances and Research*, Chapman and Hall, London.
9. Hembing W., 1994, *Tanaman Berkhasiat Obat di Indonesia* , Penerbit Pustaka Kartini, Jilid III.
10. Jianguo, M., Karin, Reed, A., and James, M., Gallo, 2002, Cells Designed to Deliver Anticancer Drug by Apoptosis, *Cancer Research*, 62, 1382-7.
11. Manahan, D., and Wienberg, R.A., 2002. The Hallmarks of Cancer, *Cell*, 100 : 57-70.
12. Markam K.R., 1988, *Cara Identifikasi Flavonoid*, terjemahan Penerbit ITB Bandung, 1-59
13. Nagata ,S. 1997. Apoptosis by Death Factor, *Cell*. 88 : 355 – 365. Pai, S., Kaustubh, D. and Debabrata, M., 2001. Central Role of p53 Regulation of Vascular Permeability Factor/ Vascular Endothelial Growth Factor (VPF/ VEGF) Expression in Mammary Carinoma, *Cancer Research*, 61, 6952-6957.
14. Pratiwi D., dkk., 2009, Ekstrak Etanol Jeruk Nipis (*Citrus aurantiiifolia* (C) Swingle) meningkatkan ekspresi p53 pada sel payudara tikus galur spague dawley terinduksi 7,12-dimetilbenzene-[A] Antrasena, *Cancer Chemoprevention Research Center, Fakultas Farmasi, UGM, Yogyakarta*.
15. Ravi, R., Bijoyesh, M., Zaver M.B., Carrie. H.S., Dmitri, A., 2000. Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1a, *Gene and Development*, Vol 14.
16. Samiasih S., 2010. Perbedaan Ekspresi

- VEGF Sel Adenokarsinoma Kolorektal Tikus Sprague Dawley Dengan dan Tanpa Pemberian Ekstrak Phyllanthus niruri, Thesis, Semarang, UNDIP.
16. Silverstein, Bassler and Morrill, 1981, Spectrometric Identification of Organic Compounds, John Willey and Sons, New York.
  17. Sukardimana, Hadi Poerwono., Sofia Mubarika., Sismindari . 2000. Penapisan Senyawa Antikanker dari Tanaman Obat Indonesia dengan Molekul Target Enzim DNA Topoisomerase, Laporan Penelitian Domestic Collaborative Research Grant (DCRG).
  18. Sukardimanb, Darwanto A, Tanjung M., Parwarta Oka Adi ., 2000. Cytotoxic Mechanism of Flavonoid from Temu Kunci (*Kaempferia pandurata*) in Cells Culture of Human Mammary Carcinoma, Clinical Hemorheology and Microcirculation, **23**,185-190.
  19. Sukardiman, Noor Cholies Z, Sismindari ,2006. Induksi Apoptosis dan Peningkatan Ekspresi p53, Bax serta Aktivasi Enzim Caspase Sel Kanker Payudara Manusia oleh 5-hydroxy-7-ethoxy-flavanones dari *Kaempferia pandurata* Roxb, Laporan Penelitian Hibah Bersaing Tahun I, Lembaga Penelitian Universitas Airlangga.
  20. Wittmann S, Bali P, Donapaty S, Nimmanapalli R, Guo F, Yamaguchi H, Huang M, Jove R, Wang HG & Bhalla K. (2003). Flavopiridol down-regulates antiapoptotic proteins and sensitizes human breast cancer cells to epothilone B-induced apoptosis. *Cancer Res* **63**: 93-99.
  21. Whibley C., 2009, p53 polymorphisms: cancer implications, *Nature Revlew Cancer*, Vol. **9**, Februari 2009, p. 95-107, <http://www.google.co.id /p53+cancer+search>, Diakses, 6 Desember 2011.