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Forest betel (*P. acre* Blume) a Wild Plant from East Kalimantan, Indonesia Potentially as Resources of Drug Compounds for Cancer

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Abstract

Cytotoxic and antioxidant activities from a compound has potency as a cancer drug, cytotoxic activities can cause death cancer cells and antioxidants as regeneration function of damaged cells due to the death of these cells. Forest betel (*P. acre*) plants a wild plant from East Kalimantan, Indonesia could potentially as sources of compounds for cancer drugs, water extract from leaves shown cytotoxic activity with LC_{50} values <30 ppm against *A. salina*, and antioxidant activities against DPPH radical compounds with IC_{50} values <100 ppm. Toxicity assay in experimental animals shown water extract of *P. acre* also proved not caused liver necrosis based on changes levels of SGPT and SGOT in experimental animals. Cytotoxic and antioxidant activities these probably derived from the class of flavonoids and saponins compounds as a dominant secondary metabolites.

Keyword : Antioxidant, Anticancer, cytotoxic, *P. acre.*

INTRODUCTION

The general principle treatment cancers are cancer cell killer as a abnormal cells and regenerates these cells into normal cells but not easily accomplished because the molecule is generally killer cells can not regenerate cells but caused cell death. Killer cells using a chemical compound called with chemotherapy or chemical therapy.

Cells damaged regeneration by chemical treatment only expect enzymatic metabolism in cells through administration stimulant for these enzymatic process. However, chemical therapy is still relatively low level of success, so that cancers was impressed not have rational medicine for healing.

Use of chemical molecules for the treatment of cancer started utilize natural compounds biodiversity from plant and animal.Natural compound made by enzymatic process expected to be eliminated in the metabolism because of chemical environment in every chemical bond metabolites of natural materials can be eliminated by a non-specific enzyme as a receptor in treatment system. Common problems utilization of natural molecules for therapeutic purposes is used a mixture of compounds in form of extracts which are difficult to ascertain the mechanism of action that required. In addition, the separation of chemical molecules in an extract to be accepted by the receptors may not occur because the digestive system does not support that the mechanism of action that is not expected to occur. Furthermore, use of of pure molecules from natural compounds also may be difficult because limitations natural molecule separation technology to get adequate amounts.

Scientific estimates for cancer therapy is the utilization of natural molecules that have cytotoxic and antioxidant activity.

Cytotoxic activity of killer cells while acting as an antioxidant as cell regeneration. This activity can be performed by a single molecule or several molecules in a natural material extract. Molecules that act as cytotoxic and antioxidants preferably can be revealed from an extract even though the utilization in the form of extracts. Due to natural substance that has two activities namely cytotoxic and antioxidant potential as a source of molecules for cancer drugs.

Molecules that have the potential as a cancer drug is a molecule that selectively against cancer cells. Aqueous extracts from leaves of *P. acre*, has proven potential as a cancer drug by *in vitro* assay. Results of research on plants leaves water extract potentially cytotoxic because LC_{50} values <30 ppm against larvae of A. salina and potential antioxidant with IC_{50} values < 100 ppm. Two of these activities with very strong power is considered a prospect for utilization as a source of cancer drug compounds.

Class of compounds which are thought to contribute as an antioxidant and cytotoxic are flavonoids and saponins which are main secondary metabolites (Rine, 2011).

Figure 1. Forest betel (P. acre Blume) plant

MATERIAL AND METHODS

Preparation of plant Material

Leaves of Forest betel plant (*P. acre*) were collected from Samarinda, East Borneo, Indonesia. They were identified by the staff of the Biosystematics Laboratory LIPI-Cibinong in 2014. Assay performed to fresh leaves, while testing for dry leaves, dried with oven at temperatures 40-60 °C until it reaches water content 3-5%.

Water extraction

Extraction method by steeping, the sample poured boiling using boiling water. Termination extraction process of steeping is done after no discoloration, and the residue that is added hot water to anticipate there are chemical components that have not been extracted.

Determination water extract concentration as stock solutions

Determination concentrations of stock solution extraction results performed with a gravimetric technique. Furthermore, measurements concentrations performed by dilution from stock solution.

Cytotoxic assay against *A.salina*

Cytotoxic test performed *in vitro* use bioindicator *Artemia salina* larvae. *A. salina* mortality observations carried out for 24 hours with a frequency of observation every 6 hours. Extract concentrations for testing were found in determining concentrations shown in the following table 1.

Table 1. Cytotoxic assay against *A. salina* concentration

No	Sample	Concentration (ppm)				
1	Dry leaves steeping	10	20	30	40	50
2	Dry leaves stew	10	20	30	40	50
3	Fresh leaves steeping	5	15	25	35	45
4	Fresh leaves stew	5	15	25	35	45

Antioxidant assay

Antioxidants assay also performed *in vitro* against DPPH free radical compounds. Concentration were determined by determining concentrations series are shown in Table 2 below. Assay process similar to that commonly done in assays of antioxidants *in vitro*.

Table 2. Antioxidant assay Concentration

No	Sampel	Concentration (ppm)				
1	Dry leaves steeping	20	30	40	50	60
2	Dry leaves stew	20	30	40	50	60
3	Fresh leaves steeping	10	20	30	40	50
4	Fresh leaves stew	10	20	30	40	50

Stability assay of cytotoxic and antioxidant activities

Stability assay of cytotoxic and antioxidant activities performed *in vitro* with the same process as cytotoxic and antioxidant assay. Extracts were tested for the water extract from dried leaves that have been made safe for 4 months starting May to August 2014. Stability test experimental design shown in table 3.

Storage of samples carried out on the container and left in a storage closet during the experiment period. Storage time is done every month ranged 27-30 days and subsequently samples were extracted and tested for cytotoxic and antioxidant assay.

Table 3. Stability treatment design of cytotoxic and antioxidant activities with stew and steeping technique of forest betel (*P. acre*) leaves.

Storage time (Month)	Date and repetition assay	Information
1 – 27 May 2014	28– 31 May 2014 I II III	4 times assay with 12 repetition
1 – 27 June 2014	28 – 30 June 2014 I II III	
1 – 27 July 2014	28 – 31 July 2014 I II III	
1 – 27 August 2014	28 – 31 august 2014	

Extract sample Toxicity assay

Toxicity assay performed *in vivo* animal assay using rats. Observations on liver cell necrosis with parameter levels increase of SGOT and SGPT from animal blood. Tests conducted for 5 weeks with oral administration of samples continuously once a day every day. SGOT and SGPT measurement is conducted every 7 days, testing was conducted for 35 days or 5 weeks.

Total extracts were tested as much as four samples consisted from two extracts from the dried leaves and two extracts from fresh leaves. Extraction conducted every when the fresh leaves will be tested while the extract from the dried leaves are also performed so as to avoid fungal invasion of the extract. The number of test animals used were 48 animals including animal control. The dose used was randomly assigned or attempted is 50 mg; 100 mg; and 200 mg. The doses representing a category of low-dose, medium and high doses.

RESULT AND DISCUSSION

Cytotoxic potency

Indicators of determination cytotoxic potential was if the LC_{50} values <30 ppm, then extract are set for potential cytotoxic. Results of testing the cytotoxic potential by *in vitro* by using bio-indicators *A. salina* shown in Table 4.

Based on predetermined criteria then the entire water extract of leaves *(P. acre)* showed good cytotoxic potential that with LC_{50} value of the average < 30 ppm.

Table 4. Cytotoxic potential of forest betel leaf aqueous extract (*P. acre*) *in vitro* with bioindikator larvae of *A. salina*

Sample	LC_{50} Value (ppm)	Information
Dry leaves steeping	24,54	Potentially cytotoxic
Dry leaves stew	24,88	Potentially cytotoxic
Fresh leaves steeping	28,66	Potentially cytotoxic
Fresh leaves stew	28,83	Potentially cytotoxic

Antioxidant potency
Indicators of determination antioxidant potential was if the IC_{50} value less from 200 ppm expressed antioxidant potential. The test results of antioxidant activity water leaves extract (*P. acre*) are shown in Table 5. Based on the criteria for determining the antioxidant potential then entire water extract of leaves (*P. acre*) declared potential as a source of antioxidants because test results showed an average IC_{50} value <200 ppm and even <100 ppm.

Table 5. Antioxidant potency extract water and ethanol extracts of betel leaf forest (*P. acre*) *in vitro* against DPPH radical compound

Sampel	IC_{50} Value (ppm)	Information
Dry leaves steeping	31,54	Antioxidant potentially
Dry leaves stew	28,98	Antioxidant potentially
Fresh leaves steeping	43,55	Antioxidant potentially
Fresh leaves stew	44,04	Antioxidant potentially

Potency of Cytotoxic and Antioxidant Extract stability
Criteria for determining the stability of cytotoxic and antioxidant extracts based on LC_{50} and IC_{50} values change before and after storage.
If there is no significant change in then potential cytotoxic and antioxidant extracts declared stable during storage has been done. Results of stability test cytotoxic and antioxidant extracts shown in Table 6 and 7.
Based on criteria for determining stability of cytotoxic and antioxidant extracts, the water extract of forest betel (*P. acre*) stable during storage sixteen weeks or four months.

Table 6. The results of stability assay *in vitro* cytotoxic activity of the water extract of the dried leaves of betel forest (*P. acre*)

Sample Extraction method	LC_{50} value on storage time (week) 0	4	8	12	16
Steeping	32,66	46,88	45,65	47,62	48,08
stew	32,83	48,54	48,44	48,04	48,12

Table 7. Results of the stability assay antioxidant activity *in vitro* against water extract of dried leaves of betel forest (*P. acre*)

Sample Extraction method	LC_{50} value on storage time (week) 0	4	8	12	16
steeping	43,55	47,56	48,44	48,86	47,88
stew	44,04	45,43	45,36	45,22	47,76

Forest betel Leaves water Extract toxicity (*P. acre*)
In vivo toxicity assay conducted forest betel leaves extract with liver necrosis assay using animal model white mice with parameter increased levels of SGOT and SGPT animals blood test. Criteria for determining toxic levels of SGOT and SGPT based on the up to 20 times or more than normal levels of SGOT and SGPT. Results shown on Table 8-11.
Results from toxosity assay against liver cells, water extracts of forest betel (*P. acre*) does not cause necrosis to liver cells.

Table 8. Results of *in vivo* toxicity assay water extract of fresh leaves Forest betel (*P. acre*) with stew extraction technique

Dose	replication	SGOT level (IU) Treatment Period (week)				
		I	II	III	IV	V
0 mg	1	56,4	55,0	60,3	50,8	48,6
	2	54,6	58,8	59,4	56,8	47,8
	3	55,6	58,0	54,8	55,8	49,2
50 mg	1	102,4	130,4	100,3	70,4	67,8
	2	107,2	134,5	112,8	68,8	72,3
	3	101,8	132,4	121,3	73,4	72,8
100 mg	1	143,4	163,4	148,4	90,6	68,8
	2	144,5	154,5	134,5	88,9	67,4
	3	154,8	174,8	144,8	92,4	67,8
200 mg	1	221,5	254,5	210,8	80,4	75,8
	2	230,8	263,6	198,8	78,9	73,9
	3	242,4	268,1	234,8	86,8	79,8
Dose	**replication**	**SGPT level (IU) Treatment Period (week)**				
		I	**II**	**III**	**IV**	**V**
0 mg	1	28,8	24,2	27,5	23,7	20,3
	2	23,8	23,4	26,6	23,2	23,6
	3	26,9	22,5	23,7	22,2	22,2
50 mg	1	45,8	44,8	32,4	29,4	28,8
	2	47,1	46,8	30,9	27,8	27,1
	3	50,2	51,3	31,7	28,1	27,6
100 mg	1	88,2	102,1	121,8	81,2	74,4
	2	87,1	111,9	120,4	79,3	72,0
	3	80,8	120,8	123,7	82,8	70,2
200 mg	1	123,7	134,4	123,6	90,4	76,8
	2	130,8	112,4	128,9	89,8	78,9
	3	141,2	123,5	124,5	92,6	80,9

Table 9. Results of *in vivo* toxicity assay water extract of fresh leaves Forest betel (*P. acre*) with steeping extraction technique

Dose	replication	SGOT level (IU) Treatment Period (week)				
		I	II	III	IV	V
0 mg	1	52,4	55,6	63,2	49,8	45,8
	2	51,1	58,1	56,4	50,6	45,4
	3	52,8	53,0	52,6	53,8	44,6
50 mg	1	108,6	140,1	109,3	71,4	64,8
	2	102,6	144,3	122,8	64,1	70,7
	3	101,4	142,8	121,5	71,6	71,9
100 mg	1	145,4	168,4	138,4	91,4	58,1
	2	148,5	154,5	138,5	87,8	69,4
	3	153,8	174,6	143,8	95,7	67,4
200 mg	1	241,5	264,7	210,8	80,4	76,3
	2	240,8	26,6	198,8	78,9	74,1
	3	242,4	267,1	234,8	86,8	78,8
Dose	**replication**	**SGPT level (IU) Treatment Period (week)**				
		I	**II**	**III**	**IV**	**V**
0 mg	1	24,5	25,2	26,3	22,7	20,6
	2	23,4	23,5	26,6	21,4	21,3
	3	24,9	24,1	23,1	22,2	23,4
50 mg	1	42,3	42,8	31,5	23,4	26,8
	2	43,3	44,5	30,6	24,8	26,1
	3	51,2	51,3	33,5	22,1	25,1
100 mg	1	82,2	105,5	121,8	83,2	74,3
	2	86,4	114,1	122,4	79,4	72,4
	3	81,5	124,2	121,7	81,8	70,4
200 mg	1	124,5	131,1	121,6	91,4	76,4
	2	131,4	114,5	122,9	83,8	75,6
	3	144,3	122,4	122,5	94,6	84,1

Table 10. Results of *in vivo* toxicity assay water extract of dried leaves Forest betel (*P. acre*) with stew extraction technique

Dose	replication	SGOT level (IU) Treatment Period (week)				
		I	II	III	IV	V
0 mg	1	50,1	52,6	65,2	46,2	43,1
	2	53,1	58,3	55,3	51,6	45,6
	3	54,2	53,4	55,5	52,3	43,7
50 mg	1	107,1	143,1	104,3	70,4	63,1
	2	101,3	140,5	122,4	64,5	70,5
	3	102,1	141,8	123,5	75,0	73,1
100 mg	1	145,4	165,2	134,4	90,4	54,1
	2	144,5	150,3	135,1	86,5	64,5
	3	153,5	170,3	145,5	94,5	64,6
200 mg	1	244,5	254,5	211,8	78,4	73,6
	2	245,8	265,5	196,2	68,4	71,5
	3	245,4	263,1	237,1	80,5	74,1
Dose	**replication**	**SGPT level (IU) Treatment Period (week)**				
		I	II	III	IV	V
0 mg	1	20,3	24,2	23,3	21,3	22,3
	2	21,4	23,6	25,7	25,7	24,1
	3	22,4	21,6	22,6	21,7	22,3
50 mg	1	44,3	40,8	29,5	22,4	25,8
	2	40,4	45,1	30,1	21,8	25,1
	3	54,0	50,6	32,5	23,6	25,1
100 mg	1	85,2	102,2	121,6	80,2	71,3
	2	83,4	112,4	120,4	78,4	74,4
	3	81,5	120,3	121,3	82,8	70,5
200 mg	1	122,5	132,1	123,6	91,1	70,4
	2	130,5	115,3	122,3	84,8	71,6
	3	134,6	121,4	122,5	94,5	78,1

Table 11. Results of *in vivo* toxicity assay water extract of dried leaves Forest betel (*P. acre*) with steeping extraction technique

Dose	replication	Kadar SGOT level (IU) Treatment Period (week)				
		I	II	III	IV	V
0 mg	1	49,3	52,6	64,1	46,6	42,1
	2	52,4	57,3	55,3	50,6	44,6
	3	51,1	53,4	54,6	52,1	43,2
50 mg	1	107,6	143,5	103,3	70,1	61,1
	2	103,3	142,5	122,2	63,5	70,3
	3	102,5	141,1	123,3	75,4	73,3
100 mg	1	143,4	165,5	131,4	90,6	51,1
	2	144,1	152,3	132,1	81,4	64,1
	3	154,5	170,1	141,5	90,5	62,6
200 mg	1	243,5	253,5	201,8	78,4	71,6
	2	245,1	255,5	196,1	66,4	73,5
	3	242,4	263,1	227,1	80,2	72,1
Dose	**replication**	**SGPT level (IU) Treatment Period (week)**				
		I	II	III	IV	V
0 mg	1	20,1	23,2	23,4	21,5	22,4
	2	21,2	23,2	24,7	24,7	24,4
	3	21,4	22,6	23,4	21,2	23,3
50 mg	1	44,1	40,3	28,5	21,4	24,8
	2	42,1	44,1	31,1	20,8	24,1
	3	50,1	46,6	31,5	23,3	25,5
100 mg	1	86,2	106,2	123,6	80,4	72,3
	2	83,6	112,4	123,4	78,1	74,3
	3	81,6	121,1	122,3	83,8	70,5
200 mg	1	121,5	131,1	123,3	90,1	71,4
	2	124,5	112,3	122,4	84,2	71,2
	3	130,6	122,4	124,5	94,1	78,3

CONCLUSION

Forest betel leaves aqueous extract (*P. acre* Blume) potential as a source of cancer drug compounds with primary activity of cytotoxic and antioxidant, with LC_{50} values <30 ppm against *artemia salina* and antioxidant assay with IC_{50} values <100 ppm, and does not cause necrosis of liver cells in long-term use. Utilization of Forest betel leaves aqueous extract (*P. acre*) can be used in dosage forms dried simplisia tea or fresh leaves are immediately boiled or brewed with hot water, because it has proven stable in a wide range of heating and storage. Secondary metabolites are suspected as the source of cytotoxic activity and antioxidants are flavonoids and saponins which is the primary secondary metabolites in the plant leaves

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