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Original Research Article

Characterization of volatile compounds of *Albertisia papuana* Becc root extracts and cytotoxic activity in breast cancer cell line T47D

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Abstract

Purpose: *To evaluate the cytotoxic activity of chloroform and water root extracts of Albertisia papuana Becc. on T47D cell line and identify the volatile compounds of the extracts.*
Methods: *The plant roots were extracted with chloroform and water using maceration and boiling methods, respectively. The cytotoxicity of the extracts on T47D were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Doxorubicin was used as reference drug in the cytotoxicity test while Probit analysis was used to calculate the Median Growth Inhibitory Concentration IC_{50} of the extracts. The volatile compounds in the chloroform and water root extracts were analyzed using Gas Chromatography-Mass Spectrophotometry GC-MS.*
Results: *The IC_{50} of the chloroform and water extracts were 28.0 ± 6.0 and 88.0 ± 5.5 µg/mL, respectively whereas that of doxorubicin was 8.5 ± 0.1 µg/mL. GC-MS results showed that there were 46 compounds in the chloroform extract, out of which the five major components are ethyl linoleate (49.68 %), bicyclo (3.3.1) non-2-ene (29.29 %), ethyl palmitate (5.06 %), palmitic acid (3.67 %) and ethyl heptadecanoate (1.57 %).The water extract consisted of three compounds, butanoic acid (15.58 %); methyl cycloheptane (3.45 %), and methyl 2-O-methylpentofuranoside (80.96 %).*
Conclusion: *The chloroform root extract of A. papuana Becc. had a fairly potent anticancer activity against breast cancer cells and may be further developed as an anticancer agent. Its major components were fatty acids and fatty acid esters.*

Keywords: *Albertisia papuana Becc., Cytotoxicity, Breast cancer, T47D cell lines, Methyl 2-O-methylpentofuranoside*

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INTRODUCTION

Cancer is considered to be the most killer disease in the world and has become a serious problem for the society, both in the developed countries and developing countries. The ineffectiveness of cancer treatment and side effects from the use of current cancer drugs have encouraged the search of alternative cancer drugs from natural products. Many studies have been carried out to obtain new alternative drugs for cancer treatment. Plants which usually used as traditional medicines have been exploited as a source of active compounds with anticancer activity [1,2].

Albertisia papuana Becc., from the family of Menispermaceae, is recognized as a traditional

medicinal plant in Sumatra and Kalimantan, Indonesia [3]. Dayak, a tribe in Kalimantan, usually use the root of *A. papuana* Becc. for cancer treatment by boiling it. This plant demonstrated cytotoxic activity on HeLa cell [4]. Some plants from the genus of *Albertisia* also showed many pharmacological activities. *Albertisia delagoensis* showed anti-plasmodium and cytotoxic activities on breast cancer, blood cancer, and kidney. It contained alkaloid as bioactive compound [5]. Alkaloid from *A. vilosa* had antibacterial, antifungal, anti-plasmodium, and cytotoxic activities [6]. Alcoholic extract of *A. laurifolia* had antitumor activity [7].

Based on these reports, *A. papuana* Becc. could be a potential source in the discovery of bioactive compound for anticancer. Most of the active compounds were alkaloids. In this study, we evaluated the cytotoxic activity of the chloroform and water extracts of *A. papuana* Becc. root on T47D cell lines using MTT assay and identified the volatile compounds which might be the active compounds for anticancer activity. The volatile chemical contents were identified using GC-MS analysis.

EXPERIMENTAL

Materials

Albertisia papuana Becc was collected from Dayak, East Kalimantan, Indonesia in April 2014. The plant was identified by Dr. Joeni Setijo Rahajoe, a taxonomist of Herbarium Bogoriense, Biology Research Center, Bogor, Indonesia. A voucher specimen (No. 001/2014/FB-UKSW/KHT) was deposited in the herbarium of Laboratory of Primary Biology, Faculty of Biology, Universitas Kristen Satya Wacana, Salatiga, Indonesia. T47D cell line was obtained from Parasitology Laboratory, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Preparation of root extract of *A. papuana* Becc.

The roots of about 50 cm in height of *A. papuana* plants were cleaned using tap water and cut into small pieces. The sample was air-dried for at least a week then dried in an oven at 40 °C for 5 h. The dried root was ground using a blender (Philip HR1538). The chloroform root extract (CE) was macerated using chloroform (1:5 w/v) and then soaked for 24 h. This procedure was repeated four times until the resultant supernatant became clear. The water root extract (WE) was prepared by the boiling method. The powdered sample was boiled with water (1:5 w/v) for five minutes. Each extract was filtered and then dried in a rotary evaporator (Rotavapor R-114 Buchi) under vacuum (Eyela A-1000S) at 40 °C. The crude extracts were stored at 4 °C before use.

Cytotoxicity assay

The *in vitro* cytotoxicity of chloroform and water extracts and doxorubicin on T47D were determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT) assay with a slight modification. We used SDS instead of DMSO to stop the formation of formazan crystal. After that, the plate was incubated overnight without rotation on shaker. Briefly, an aliquot of 100 µl cell suspension (±1 x 10^4 T47D cells) was loaded into each well of 96-well plate and incubated for 24 h at 37°C in a 5 % CO_2 incubator (Heraeus).The various concentrations of treatments (extracts 0-500 µg/mL and doxorubicin 0-100 µg/mL) were added to each well and then incubated for 24 h at the same condition. Each concentration tested was in triplicates. At the end of treatment, the medium was removed and 10 µLMTT solution 5 mg/mL (Sigma) was added. The plate was incubated in the dark for 3 to 4 h. The reaction was stopped by the addition of 100 µL 10 % SDS solution in 0.01 N HCl (Sigma) and then incubated overnight at room temperature. The absorbance of each well was measured using ELISA reader (SLT 240 ATC) at 595 nm. IC_{50} values (mean ± SD) were calculated using Probit analysis (SPSS 16.0 for Windows).

Identification of chemical compounds

The chemical compounds of extracts were analyzed by GC-MS(Agilent GC 6890N 5975B MSD).The capillary column was Agilent 19091S-433 model, HP-5MS 5 % Phenyl Methyl Siloxane. The oven temperature was programmed as follows: initial temperature at 100 °C, initial time for 1.00 min, final temperature at 300 °C for 10.0 min. The conditions of front inlet mode splitless were as follows: initial temperature at 300 °C, pressure was 10.45 psi for CE and 9.32 psi for WE, purge flow was 50.0 ml/min, purge time for 0.0 min, total flow 53.8 ml/min, saver flow for 20.0 ml/min, saver time was 2.0 min, and carrier gas was Helium. The sample was dissolved in pure ethanol and injected using a split technique. Identification of components in sample used Wiley7Nist05.L database.

RESULTS

The cytotoxic activity of both extracts on T47D cell lines are shown in Table 1. The cytotoxicity of CE (IC_{50}, 28.0 µg/mL) was three-fold higher than that of WE (IC_{50}, 88.0 µg/mL). Both extracts were less cytotoxic than doxorubicin hydrochloride (IC_{50}, 8.5 µg/mL).

Based on the GC-MS chromatogram, there were forty-six compounds in the chloroform root extract of *A. papuana* Becc. (Table 2). The five major components of this extract were ethyl linoleate (49.68 %), bicyclo (3.3.1) non-2-ene (29.29 %), ethyl palmitate (5.06 %), palmitic acid (3.67 %), ethyl heptadecanoate (1.57 %). The other components had a relative concentration less than 1 %. The GC-MS chromatograms of water root extract of *A. papuana* Becc. consisted of three compounds (Table 3). These were methyl 2-O-methylpentofuranoside (80.98 %), butanoic acid (15.58 %), and methyl cycloheptane (3.45 %).

DISCUSSION

Natural products are considered as potential sources for drugs in several human diseases including cancer [12]. Many anticancer agents are plant-based compounds. *Albertisia papuana* is one of the endogenous plants in Dayak, East Kalimantan. Dayak people usually use *A. papuana* Becc for cancer treatment by boiling the root of the plant using water. In this study, the difference in solvents caused the difference of yield rendemen and cytotoxic activity of the extracts. The cytotoxic activity of the water extract was three time lower than the chloroform extract. Some alkaloids have been reported as heat labile compounds, so it is very possible that the alkaloids in the water extract were decomposed during the water extraction process [9].

In vitro cytotoxicity screening is often used to select the potential medicinal properties of a matter. This method was used to determine the inhibition of the growth of cells caused by an anticancer agent. According to The National Cancer Institute (NCI) USA, the IC_{50} values of 30 µg/mL is the upper limit of the crude extract which is qualified for further purification [12], so the chloroform root extract was promising for further purification because its IC_{50} value was 28.0 mg/mL. On other hand, to extract the root of the plant by boiling with water was not recommended. It showed that the extraction method is one of the crucial steps to obtain the active compound which have medicinal properties.

The medicinal properties of plants are caused by the presence of active compounds of therapeutic value. The active compounds can be extracted from plants by an extraction method with certain solvents. The traditional medicines usually use water as a solvent, but modern medicines use various organic solvents in order to exploit the various compounds in herbal medicines [10]. The use of chloroform would extract non polar compounds, whereas water would extract polar compounds. The majority of compounds from the chloroform extract were organic acids, a long chain of saturated and an unsaturated hydrocarbon, or triterpene and sesquiterpene [11-14].

Based on the GC-MS chromatogram, it seems that the anticancer activity of chloroform root extract of *A. papuana* Becc. might be because of the action of the major compounds of this extract such asethyl linoleate, bicyclo (3.3.1) non-2-ene, ethyl palmitate, palmitic acid, and ethyl heptadecanoate. It has been reported that some of these compounds were also found in chloroform leaf extract of *Finlaysonia obovata* [12] and *Acacia nilotica* L [11]. Ethyl linoleate is a derivative of linoleic acid (LA). Various studies have reported that has biological beneficial effects, including anticancer activity. LA inhibits the initiation, promotion, and progression phases in mammary tumors [14].In addition, LA showed anticancer activity [16], especially by its antiproliferative activity [17] and by inducing apoptosis in breast cancer cells [18].LA was demonstrated to not only inhibit the growth of hepatoma cell but also induce apoptosis in colorectal cell line [19,20]. A study of human leukemic cells MOLT-4 showed that palmitic acid inhibited DNA topoisomerase I and induced apoptosis [20], however in human lung adenocarcinoma cell line A549,

Table 1: Cytotoxicity of*A. papuana* Becc. chloroform and water root extracts on T47D cell line

Plant (part)	Solvent	Code	IC_{50} (µg/mL)
Root	Chloroform	CE	28.0 ± 6.0
Root	Water	WE	88.0 ± 5.5
-	-	Doxorubicin (DX)	8.5 ± 0.1

Table 2: Compounds in chloroform root extract of *A. papuana* Becc analyzed using GC-MS

No.	Retention time	Compound	Formula	Mol wt	Area (%)
1	5.949	7-Methyl-2-decene	$C_{11}H_{22}$	154	0.17
2	6.053	2-Acetylcyclopentanone	$C_7H_{10}O_2$	154	0.15
3	6.164	4-Methyl-2-decene	$C_{11}H_{22}$	128	0.26
4	8.069	2,3,5,8-Tetramethyldecane	$C_{14}H_{30}$	198	0.07
5	8.179	2,6-Di-tert-butyl-4-methylene-2,5-cyclo hexadiene-1-one	$C_{15}H_{22}O$	218	0.06
6	8.589	2,4-Bis(1,1-Dimethylethyl)-phenol	$C_{14}H_{22}O$	206	0.42
7	8.706	1-Cyclopentylethanone	$C_7H_{12}O$	112	0.11
8	9.784	1-(Dodecyloxy)ethanol	$C_{14}H_{30}O_2$	230	0.10
9	8.920	1,2,4-Trimethylcyclohexane	C_9H_{18}	126	0.13
10	9.044	1,1'-Oxybis decane	$C_{20}H_{42}O$	298	0.11
11	10.578	n-Heptadecane	$C_{17}H_{36}$	240	0.10
12	10.643	2,6,11,15-Tetramethyl hexadecane	$C_{20}H_{42}$	282	0.10
13	11.039	1,3,5-Trimethylcyclohexane	C_9H_{18}	126	0.07
14	11.215	Tetratetracontane	$C_{44}H_{90}$	619	0.25
15	11.345	1,2,4-Trimethylcyclohexane	C_9H_{18}	126	0.36
16	11.429	1-Bromohexadecane	$C_{16}H_{33}Br$	305	0.18
17	11.657	n-Octadecane	$C_{18}H_{38}$	254	0.36
18	12.684	Nonadecane	$C_{19}H_{40}$	268	0.25
19	13.250	Hexadecenoic acid	$C_{16}H_{30}O$	254	0.44
20	13.497	Palmitic acid	$C_{16}H_{32}O_2$	256	3.67
21	13.653	Ethyl palmitate	$C_{18}H_{36}O_2$	284	5.06
22	13.822	Diethylmethylborane	$C_5H_{13}B$	84	0.13
23	14.394	Heptadecanoic acid	$C_{17}H_{34}O_2$	270	0.35
24	14.576	Ethyl heptadecanoate	$C_{19}H_{38}O_2$	298	0.58
25	15.323	Ethyl linoleate	$C_{20}H_{36}O_2$	308	49.68
26	15.570	Bicyclo (3.3.1) non-2-ene	C_9H_{14}	122	29.29
27	15.830	(Cyclohex-2-enyl)acetic acid	$C_8H_{12}0_2$	140	0.36
28	15.882	Methyl-6-octadecynoate	$C_{19}H_{36}O_2$	296	0.43
29	15.999	(Cyclohex-2-enyl) acetic acid	$C_8H_{12}0_2$	140	0.42
30	16.136	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280	0.25
31	16.357	Ethyl nonadecanoate	$C_{21}H_{42}O_2$	326	0.55
32	16.981	2-Methylene-cyclododecanone	$C_{13}H_{22}O$	194	0.80
33	17.033	Tetradec-13-en-11-yn-1-ol	$C_{14}H_{24}O$	208	0.52
34	17.189	Ethyl heptadecanoate	$C_{19}H_{38}O_2$	298	1.57
35	17.351	9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	278	0.39
36	17.644	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280	0.18
37	17.988	1-Methyltridecyl methoxyacetate	$C_{17}H_{34}O_3$	286	0.21
38	18.463	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	0.13
39	18.749	1-Chlorooctadecane	$C_{18}H_{37}Cl$	288	0.22
40	19.484	Eicosane	$C_{20}H_{42}$	282	0.16
41	20.199	Ethyl palmitate	$C_{18}H_{36}O_2$	284	0.29
42	20.888	Ethyl heptadecanoate	$C_{19}H_{38}O_2$	298	0.22
43	21.577	7h-dibenzo-c,g-carbazole	$C_{20}H_{13}N$	281	0.20
44	22.006	N-(3-methoxyphenyl)-2,2-dimethylpropanamide	$C_{12}H_{17}NO_2$	207	0.21
45	24.398	Beta-stigmasterol	$C_{29}H_{48}O$	412	0.18
46	25.094	Gamma-sitosterol	$C_{29}H_{50}O$	414	0.19

Table 3: Compounds in water root extract of *A. papuana* Becc. analyzed using GC-MS

No.	Retention time	Compound	Formula	Mol wt	Content (%)
1	14.524	Butanoic acid	$C_4H_8O_2$	88	15.58
2	14.758	Methylcycloheptane	C_8H1_6	112	3.45
3	16.513	Methyl 2-O-methylpentofuranoside	$C_7H_{114}O_5$	178	80.96

palmitic acid only inhibited DNA topoisomerase I without inducing apoptosis [21].

Palmitic acid, an active compound from *Marthasterias glacialis* L. showed apoptotic activity in neuroblastoma cell line by a ceramide-independent mechanism [22].In this study, a minor compound of water root extract of *A. papuana* Becc., butanoic acid was reported to have anticancer activity. Previous studies

demonstrate that butyric acid induced apoptosis in some cancers cells [23]. According to this study, the volatile compounds in both of the extracts were had anticancer activity. The chloroform extract contains forty six compounds, some of which have been known to have cytotoxic activity. While, water extract contains three compounds and only one compound has cytotoxic activity. Therefore, the water extract was less toxic than the chloroform extract.

CONCLUSION

The chloroform root extract of *A. papuana* Becc. has a fairly potent anticancer activity with some promise. Further purification and isolation of the bioactive anticancer compounds may yield a more cytotoxic agent. The major components are fatty acids and fatty acid esters. The water root extract of *A. papuana* which contains butanoic acid also has some anticancer activity.

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CONFLICT OF INTEREST

No conflict of interest associated with this work.

CONTRIBUTION OF AUTHORS

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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