Research Article

Anticancer Screening of Selected Apocynaceae, Simaroubaceae and Magnoliaceae of Indonesian Plants using Mechanism-Based Yeast Bioassay

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ABSTRACT

National Cancer Institute recommends some plant families as Families Of Special Interest (FOSI) that predicts abundance of anticancer agents. In present study, some FOSI of Indonesian plants were screened by mechanism-based yeast bioassay for their anticancer activity. The research result revealed that 6 of 23 species of Apocynaceae, Simaroubaceae and Magnoliaceae were potential as anticancer agents, having DNA damaging agent or a Topoisomerase inhibitor. The best extract as anticancer agents was bark extracts of *Funtumia elastic* (Preuss) Stapf, *Kibatalia arborea* (Blume) G. Don and *Michelia champaca* L. that showed bioactivity on *Saccharomyces cereviceae* strain 1140 (Topoisomerase I inhibitor) with IC_{12} values at 1590.78, 1657.67, 3424.54 μg/mL, respectively and bioactivity on *Saccharomyces cereviceae* strain 1353 (Topoisomerase II inhibitor) with IC_{12} values at 353.42, 931.39, 2124.42 μg/mL, respectively. Camptothecin and Nystatin were used as reference anticancer and antifungal agent.

Keywords: Indonesian plants, Apocynaceae, Simaroubaceae, Magnoliaceae, *Saccharomyces cereviceae*, Topoisomerase inhibitors, anticancer agents.

INTRODUCTION

Indonesian Health Department during July 2006-August 2008 was reported that malignant tumor prevalence recorded at 0.43% of 987,205 population and became the sixth death caused (5.7% of 4,552 death cases)[1]. Breast, cervic, lung and ovary cancers were 4 of 10 common cancer cases in 2010[2]. It was also reported that in the world, cancer was caused 7.6 million deaths (13% of 57 million death cases) in 2008, and lung, breast, colorectal, and prostate cancer were the major death of cancer cases[3].

Around 80% of population in Asia and Africa used natural product to cure their illnesses[4]. In Indonesia, 59.12% of populations were used *Jamu* (Indonesian herbal medicine)[5]. Natural product have a good prospect for chemotherapy of cancer and more than 50% of anticancer agent in clinical used were natural products or their derivate[6]. There were 6 of 26 drug approved/launched basically from plant during year 2000-2006 used to treat cancer diseases in U.S, U.K, Canada and Ireland[7]. Anticancer agent from plants that have been reported such as podophyllin from *Podophyllum rhizome,* vinca alkaloid from *Vinca rosea*, paclicatel/taxol from *Taxus brevifolia*, and camptothecin from *Camptotheca acuminata*[8].

Main target of many anticancer agents is DNA. Interaction of DNA and the agents can be detected in yeast that has been genetically engineered. As eukaryotic microorganism, genetic and biochemical resemblance of yeast are closest to mammalian cells[9]. In 1997 a collaboration between Developmental Therapeutics Program (DTP) of National Cancer Institute (NCI) and The Fred Hutchinson Cancer Research Center (FHCRC) were began drug discovery by used a panel of yeast strains (*Saccharomyces cerevisiae*) that screened almost 100,000 compounds collection of NCI-DTP with selective toxicity to mutant cells[10].

Regarding to plants used, during year 1960-1982, NCI has been screened antitumor activity of 35,000 species of plant extracts. From the reviews, there were reported special plant samples as Families Of Special Interest (FOSI) i.e Amaryllidaceae, Apocynaceae, Celastraceae (including Hippocrateaceae), Liliaceae, Magnoliaceae (and related families in Magnoliales), Rubiaceae, Rutaceae, Simaroubaceae and Thymelaeaceae[11]. The FOSI were families predicted abundance of antitumor active agents and some species of those families have been traditionally used in Indonesia as *Jamu*[12,13]. It emerged a hypothesis that Indonesian plants of those families might have anticancer activity.

According to these review, this research attempted to evaluate some extracts of Apocynaceae, Simaroubaceae and Magnoliaceae of Indonesian Plants using mechanism-based yeast bioassay for producing their anticancer activity[9-14].

MATERIALS AND METHODS

Plant material and extraction

Plant material consists of 17 species of Apocynaceae, 4 species of Simaroubaceae, and 2 species of Magnoliaceae. All species were collected and identified in The Bogor Botanic Gardens, Center For Plant Conservation, Indonesian Institute of Science. With the exception of *Plumeria alba* L. (Apocynaceae) and *Brucea*

javanica (L.) MERR (Simaroubaceae) were identified in Herbarium Bandungense, School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia.

Dried bark was used as part of all plant material to be tested except for *Brucea javanica*, fresh bark, leaf and fruit were used.

The barks were powdered using grinder and extracted using methanol maceration for 3x24 hours[9].

Filtrate was concentrated using vacuum rotavapor (BUCHI) and electrical dryer (PHILIPS).

Activity Screening Use Mechanism-Based Yeast Bioassay

The screening method of mechanism-based yeast bioassay was adapted from references[14-18] with modification.

Saccharomyces cerevisiae strains 1140, 1353, and 1138 were kindly donated from Professor Karlo H. Primavera (Department of Chemistry, University of The Philippines). Yeast cultures that grown in YPD broth at 30°C were suspended in sterile 0.9% saline solution (T=80% at 600nm).

20 ml of medium YPD agar was prepared with 1 ml of inoculum to produce 6mm layer in petri dish with 9cm diameter.

Seven wells with 6mm diameter were made on the plate using perforator. Each extracts were dissolved in a mixture of dimethyl sulfoxide-methanol (1:1) with variation concentrations. 50μL of each mixture were placed in each well.

All plates were then incubated at 30°C for 36-48 hours. Camptothecin and Nystatin (Sigma Aldrich) used as reference anticancer and antifungal agent.

The samples were considered active or contain DNA damaging agent if inhibition zone produced as the growth of yeast were inhibited.

Active extract should showed selective activity against one or more strains and has IC_{12} ranging from 8000μg/mL to 1000μg/mL.

IC_{12} value was referred to required concentration of sample (in μg/mL) that produced an inhibition zone of 12 mm around a well.

The value were determined by using linear regression from dose-response curves with log of dose as absis (Y) and zone size as ordinate (X). The IC_{12} value from samples were compared to value given by *rad52* deficient yeast cells (represented by *Saccharomyces cerevisiae* strain 1138). Activity against *Saccharomyces cerevisiae* strain 1353 means Topoisomerase II inhibition while activity against *Saccharomyces cerevisiae* strain 1140 means Topoisomerase I inhibition. These value should be equal to or minimal three times greater than in strain 1138[9,14,16].

RESULTS AND DISCUSSION

In this research, Indonesian plant extracts of Apocynaceae, Simaroubaceae and Magnoliaceae were screened using mechanism-based yeast bioassay to *Saccharomyces cereviceae* strain 1140, 1353 and 1138. Each extracts were tested at concentration of 8000, 4000, 2000, 1000, 500, 250 and 125μg/mL while Camptothecin and Nystatin as positive controls at concentration of 800, 400, 200, 100, 50, 25 and 12.5μg/mL were used. This bioassay was done in three replication. The IC_{12} value of each plant extracts against each yeast strains were showed in Table 1. This values has been calculated by regression analysis.

An important target of many clinically active anticancer agents is type I and type II Topoisomerases (DNA Topoisomerases). Covalent complexes of Topoisomerases-DNA that act in front of replication forks can cause irreversible DNA damage if there are present agent or DNA Topoisomerase-targeting drug. This agent inhibit DNA ligation reaction and enzyme activity, as well as Topoisomerase-mediated DNA damage leading into cell killing. Yeast has become a powerful tools for studying biological role of DNA Topoisomerases and mechanism of drug action targeting these enzyme[10].

There were 23 species for screening that representatively samples of Apocynaceae, Simaroubaceae and Magnoliaceae. The bioassay results was revealed that Bark extracts of *Quassia indica* (L) Nooteboom, *Plumeria alba* L., *Brusea javanica* (L.) MERR showed bioactivity on *Saccharomyces cereviceae* strain 1140 (Topoisomerase I inhibitor), while fresh leaves and fruit extracts of *Brusea javanica* (L.) MERR showed bioactivity on *Saccharomyces cereviceae* strain 1353 (Topoisomerase II inhibitor). Furthermore, bark extracts of *Funtumia elastic* (Preuss) Stapf, *Kibatalia arborea* (Blume) G. Don and *Michelia champaca* L. were active against all strains, means that these extracts contained DNA damaging agents with no specific mechanism, could be both as Topoisomerase I inhibitor or/and Topoisomerase II inhibitor[16-18].

Camptothecin as reference anticancer agent, also showed activity against all strains with IC_{12} value on 1140 strain is lower than 1353 strain, means more potent as Topoisomerase I inhibitor than Topoisomerase II inhibitor. It was so because Camptothecin acted as Topoisomerase I inhibitor that inhibited DNA synthesis by stabilizing complex of DNA-Topoisomerase I[6,10].

On the other hand, bark extracts of *Wrightia pubescens* Blume, *Picrasma javanica* Blume, *Picrodendron baccatum* Krug. & Urb. Ex Urb. and *Michelia alba* DC. were inactive as anticancer agents. These might be acted as antifungi compare to Nystatin as reference antifungal agent, that only showed bioactivity to strain 1140. Yet, it needed further testing using normal yeast or other fungi for verifying this possibilities.

Table 1: Screening Result by Mechanism-Based Yeast Bioassay

Family and Plants Species		Collection number	Form of Extract	% yield	IC_{12} (in µg/mL)		
					SC[1] 1140	SC 1353	SC 1138
APOCYNACEAE							
1	*Alstonia boonei* De Wild	IV.A. 151	Semi-solid; dark-brown	9.77	>8000	>8000	>8000
2	*Carissa carandas* L.	XXIV.A. XIII.20	Semi-solid; dark-red	12.39	>8000	>8000	>8000
3	*Cerbera manghas* L.	IV.A.159	Semi-solid; dark-brown	13.29	>8000	>8000	>8000
4	*Funtumia elastic* (Preuss) Stapf	XV.J.B. IX.15	Semi-solid; yellowish dark-brown	7.53	1590.78±1204.23	353.42±175.79	434.64±480.55
5	*Kibatalia arborea* (Blume) G. Don	XIX. M.43	Semi-solid; yellowish dark-brown	7.69	1657.67±912.02	931.39± 426.85	1667.79±415.76
6	*Kopsia arborea* Blume	IV.A.52	Semi-solid; dark-brown	6.72	>8000	>8000	>8000
7	*Kopsia fruticosa* (Ker) A. DC	IV.A.44	Semi-solid; dark-brown	6.71	>8000	>8000	>8000
8	*Ochrosia citrodora* Lauterb & K. Schum.	IV.A.200	Semi-solid; dark-brown	5.85	>8000	4429.47±981.93	>8000
9	*Plumeria alba* L.	12.131	Semi-solid; dark-brown	27.67	6641.38±3422.24	>8000	2089.92±1154.65
10	*Plumeria rubra* L.	II.O. III.45	Semi-solid; black	11.79	>8000	>8000	>8000
11	*Rauvolfia sumatrana* Jack	IV.A.167	Semi-solid; greenish dark- brown	5.75	>8000	>8000	>8000
12	*Stemmadenia gabatiana* (A. Rich.) Miers	IV.A. 151	Semi-solid; dark-brown	8.39	>8000	>8000	>8000
13	*Strophantus caudatus* (Burn.f.) Kurz	XVII.A. 131	Semi-solid; dark-brown	5.82	>8000	>8000	>8000
14	*Strophantus gratus* Baill	XX.D.19	Semi-solid; dark-green	10.52	>8000	>8000	>8000
15	*Tabernaemontana macrocarpa* Jack	IV.A.194	Semi-solid; yellowish dark-brown	5.57	339.72±78.75	>8000	2850±1536.41
16	*Thevetia peruviana* (Pers.) Merrs	XXIV.A.VIII.18	Semi-solid; black	15.76	>7600	>7600	>7600
17	*Wrightia pubescens* Blume*	XV.J. A.IV.5	Viscous liquid; dark-brown	6.65	262.51±201.51	>8000	3255.84±1803.44
SIMAROUBACEAE							
1	*Brusea javanica* (L.) MERR (bark)	12.129	Semi-solid; yellowish brown	3.63	7289.76±590.43	>8000	363.18±480.79
	Brusea javanica (L.) MERR (leaves)		Semi-solid; green	13.47	>8000	1457.21±1241.99	221.22±146.31
	Brusea javanica (L.) MERR (fruits)		Semi-solid; dark-brown	8.96	>8000	6555.73±356.33	482.20±430.18
2	*Picrasma javanica* Blume*	III.L.108	Semi-solid; dark-brown	6.75	221.26±201.43	>8000	1647.75±436.12
3	*Picrodendron baccatum* Krug. & Urb. Ex Urb.*	VI.B.102	Solid; brown	13.43	251.56±43.01	>8000	1108.91±792.32
4	*Quassia indica* (L) Nooteboom	VI.B.47	Semi-solid; yellowish dark-brown	3.98	1481.23±540.61	>8000	746.39±502.77
MAGNOLIACEAE							
1	*Michelia alba* DC.*	IV.F.38	Semi-solid; dark-brown	4.49	>4000	1678.1± 860.01	>4000
2	*Michelia champaca* L.	IV.F.138	Semi-solid; yellowish dark-brown	4.09	3424.54±2806.57	2124.42±450.40	542.6±102.45
REFERENCES							
1	Camptothecin				432.88±140.33	2828.99±494.43	95.58±53.04
2	Nystatin				283.41±151.68	>800	>800

Note: 1.SC:*Saccharomyces cereviceae;** IC_{12} value of strain 1140 or strain 1353 is less than IC_{12} value of strain 1138 (not qualified with method)

In line with hypothesis, this result research revealed that some Indonesian plants contained DNA damaging agents or active as anticancer agents. Bark extracts of, *Funtumia elastic* (Preuss) Stapf, *Kibatalia arborea* (Blume) G. Don and *Michelia champaca* L. were the most active extracts as anticancer agents. However, the lead compound as active ingredients should be further studied.

Some active anticancer agents were derived from Apocynaceae, Simaroubaceae and Magnoliaceae. Vinblastine (Velban® or Velbe®) and vincristine (Oncoven®), vinca alkaloid from Apocynaceae that has been used in treatment of different cancer type[6]. Bruceantin from Simaroubaceae is kind of old molecule with new uses. Phase I and phase II clinical trial was terminated in 1980es as it observed no objective responses to tumor regressions. Later studies of bruceantin suggested and supported bruseantin as agent for hematological malignancies treatment[6,19]. Magnolol and partenolide derived from Magnoliaceae were toxic to tumor cell lines. Magnolol caused mortality of prostate cancer cell lines (PC-3, DU-145, and LNCaP)[20]. Parthenolide was active as an antitumor and anti-angiogenic agents. Its derivate, dimethyl-amino pharthenolide, was used in phase I clinical trial on acute myelogenous leukemia, acute lymphoblastic leukemia and other blood-lymph tumors[21-22].

CONCLUSION

This research confirmed anticancer activity of selected Indonesian plants. Bark extracts of *Funtumia elastic* (Preuss) Stapf and *Kibatalia arborea* (Blume) G. Don from family Apocynaceae and *Michelia champaca* L. from family Magnoliaceae were the most active extracts as anticancer agents that contains DNA damaging agent or a Topoisomerase inhibitor.

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