ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Toxicity Of Selected Apocynaceae, Magnoliaceae And Simaroubaceae Of Indonesian Plants Using Brine Shrimp Lethality Bioassay.

Ade Zuhrotun[1,2*], Asep Gana Suganda[2], Komar Ruslan Wirasutisna[2], and Marlia Singgih Wibowo[2]

[1]Faculty of Pharmacy Universitas Padjadjaran, Jl Raya Bandung-Sumedang KM 21,5 Jatinangor 45363 West Java, Indonesia.
[2]School of Pharmacy Institut Teknologi Bandung, Jl Ganeca No 10 Bandung 40132 West Java Indonesia

ABSTRACT

World Cancer Report 2014 showed that cancer prevalence has increased from 12.7 million in 2008 to 14.1 million in 2012, and was predicted to 25 million over the next two decades. Whereas in Indonesia, cancer prevalence was 14 ‰ of 1,027,763 samples surveyed in 2013.These high prevalence has emerge many researches to discover new anticancer or cytotoxic agents from natural products. Brine shrimp lethality bioassay is simple, cheap and fast test for toxic compounds. This bioassay result have correlation with cytotoxic activity to human cancer cell lines of any agents. In this research, methanol extracts of selected Indonesian plants consist of 17 species of Apocynaceae, 3 species of Simaroubaceae, and 2 species of Magnoliaceae were screened using this method for their toxicity to *Artemia salina* L. The results revealed that the most active extract were *Michelia alba* DC., *Quassia indica* (L) Nooteboom and *Strophantus caudatus* (Burn.f.) Kurz. with LC_{50} values were 37, 118 and 118 μg/ml respectively. All tested plants of these families contained flavonoids, kuinons, polyphenols, saponins, triterpenoids and few plants contained tannins and alkaloids. With the exeption of simaroubaceae was not detected alkaloids and magnoliaceae was not detected kuinons or tannins.
Keywords: toxicity, *Artemia salina*, *Michelia alba, Quassia indica, Strophantus caudatus*

Corresponding author

ISSN: 0975-8585

INTRODUCTION

World Cancer Report 2014 showed that cancer prevalence has increased from 12.7 million in 2008 to 14.1 million in 2012, and was predicted to 25 million over the next two decades. There were 32.6 million people living with cancer and recorded 8.2 million cancer deaths (58.3% of cancer cases). Breast, prostate, lung, colorectum and cervix utery is the five common cancer in 2012 [1]. Whereas in Indonesia, cancer prevalence was 14 ‰ of 1,027,763 samples surveyed in May-Juni 2013. This incidence in women is higher than in men [2]. These high prevalences encourage many researcher to discover new anticancer or cytotoxic agents from natural product.

During year 1960-1982, the National Cancer Institute of USA has been screen 35.000 plants species for antitumor activity. There where reported that Apocynaceae, Simaroubaceae and Magnoliaceae were included in list of plant families categorized as Famlily Of Special Interest (FOSI), i.e plants families that predicted contain anticancer agents [3]. In this research, selected Indonesian Plants of this families were screened using brine shrimp lethality bioassay (BSLB) for their toxycity to *Artemia salina* L. In addition, qualitative phytochemical screening was done to know secondary metabolites that contains in plants.

Brine shrimp lethalitty bioassay (BSLB) is simple, cheap and fast test for toxic compounds from natural product [4]. It was applied in preliminary bioassay of natural antitumor agents, cytotoxic compounds [5], detection of poisonous environmental pollutants (e.g pesticides, fungal toxins, cyanobacterial toxins, heavy metals) [6], mycotoxin, stream pollutants, anesthetics, dinoflagellate toxins, morphine-like compounds, toxicity of oil dispersants, cocarcinogenicity of phorbol esters, and toxicants in marine environments [7].

MATERIALS AND METHODS

Plant materials:

Selected Indonesian plants in this research were consisted of 17 species of Apocynaceae, 3 species of Simaroubaceae, and 2 species of Magnoliaceae. Samples were collected and identified in The Bogor Botanic Gardens, Center For Plant Conservation, Indonesian Institute of Science, except *Plumeria alba* L. (Apocynaceae) was identified in Herbarium Bandungense, School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia.

Here are the plant lists and their collection number: *Alstonia boonei* De Wild (IV.A. 151), *Carissa carandas* L. (XXIV.A. XIII.20), *Cerbera manghas* L. (IV.A.159), *Funtumia elastic* (Preuss) Stapf (XV.J.B. IX.15), *Kibatalia arborea* (Blume) G. Don (XIX. M.43), *Kopsia arborea* Blume (IV.A.52), *Kopsia fruticosa* (Ker) A. DC (IV.A.44), *Michelia alba* DC. (IV.F.38), *Michelia champaca* L. (IV.F.138), *Ochrosia citrodora* Lauterb & K. Schum. (IV.A.200), *Picrasma javanica* Blume (III.L.108), *Picrodendron baccatum* Krug. & Urb. Ex Urb. (VI.B.102), *Plumeria alba* L.(12.131), *Plumeria rubra* L. (II.O. III.45), *Quassia indica* (L) Nooteboom (VI.B.47), *Rauvolfia sumatrana* Jack (IV.A.167), *Stemmadenia galeottiana* (A. Rich.) Miers. (IV.A. 151), *Strophantus caudatus* (Burn.f.) Kurz (XVII.A. 131), *Strophantus gratus* Baill (XX.D.19), *Tabernaemontana macrocarpa* Jack (IV.A.194), *Thevetia peruviana* (Pers.) Merrs (XXIV.A.VIII.18), *Wrightia pubescens* Blume (XV.J. A.IV.5)

Experimental animals:

Artemia salina L. cysts (brine shrimp eggs from local store)

Chemical materials:

sea salt (from fish store), methanol (CV. Vadillah) , aquadest, ammonia (Merck), chloroform (Bratachem), petroleum ether (Merck), hydrochloric acid (Merck), kalium hydroxide, ferric chloride, Mayer reagent, Dragendorf reagent, gelatin-salt reagent, Lieberrnann-Burchard reagent, magnesium metal

Instruments:

small plastic chamber with dividing dam, cover, lamp, pump, 96-wells microplate, and microscope.

Methods:

Phytochemical screening

Qualitative phytochemical screening were conducted by standard methods [8,9] with a little modification procedures as followed:

1. Alkaloid
   2 g crude drug was extracted by ammoniacal chloroform (5 ml ammonia 25 % and 20 ml of chloroform. Chloroform extract filtered by pipeting into a small test tube and then extracted with an equal volume of 1N hydrochloric acid. The separated acid extract was treated with Mayer's and Dragendorf"s reagents. Turbidity or precipitation after the addition of these reagents was confirmed as positive or the presence of alkaloids
2. Flavonoid
   10 g powdered crude drug was added 100 ml of hot water, boiled for 5 minutes then filtered. Filtrate was then called solution A. Magnesium powder, 1 ml of concentrated HCl, and 2 ml amylalcohol were added in solution A, then shake well. The occurrence of a red or orange colour that extracted to amylalcohol was indicative of the flavonoids.
3. Kuinon
   A few drops of 0.5 N KOH was added to 5 ml solution A. Positive reaction or the presence of kuinon is evidenced by the formation of yellow or red color in filtrate layer.
4. Saponin
   10 ml solution A was vigorously shaken for 10 second. The appearance 1 cm of honeycomb froth for 10 min which persists after addition of two drops of 2 N HCl were indicated for the presence of saponins.
5. Tannin and Polyphenol
   5 ml of solution A was added reagent 1% ferric chloride. Blue colour indicated as positive for polyphenol. 2 ml of solution A was added a few drop of gelatin-salt reagent. The precipitation or gummy consistency like mucilage indicated that sample contain tannin.
6. Triterpenes dan steroid
   1 g of crude drug was extracted by petroleum ether 5 ml at room temperature for 2 hours. The extract filtered then evaporated to a dry mass. A few drop of Lieberrnann-Burchard reagent was added to recidue. A bluish green or blue colour that formed indicated for the presence of steroids and red, pink, or purple colors for terpenes.

Extraction

The barks of all tested plants were powdered using grinder and extracted using methanol maceration for 3x24 hours [9]. Filtrate was concentrated using vacuum rotavapor (BUCHI) and electrical dryer (PHILIPS).

Brine Shrimp Lethality Bioassay (BSLB)

Bioassay was done using standard method [4-7] with modification. Artificial seawater was prepared by diluting sea salt 40g into 1L of aquadest, suplemented with 6mg/L dried yeast. This seawater then filtered and put in hatching chamber oxygenated with aquarium pump. Dry brine shrimp eggs (100 mg/3L) were added to one side of the hatching chamber then covered. Allow in room temperature for two days for the shrimp to hatch and mature as nauplii. Nauplii were collected with a pasteur pipette after attracting to one side of the chamber with a light source.

All extracts were dissolved in artificial seawater and then transferred into the wells of the 96-well microplates, except for water insoluble compound were adding 1% DMSO as cosolvent. Serial dilutions were made up to 1 mg/ml in 100 µL artificial sea water. Afterward, 100 µL of nauplii suspension containing 4-17 organism was added to each well. Artificial seawater with DMSO were taken as controls wells. Then the microplates were incubated under light source in room temperature for 24 h without covers.

Plates were then examined under microscope (magnification10x4) and number of dead (non-motile) nauplii in each well were counted. A100 µL methanol were then added to each well and after 15 minutes the total number of shrimp in each well were counted. LC_{50} value were then calculated by probit analysis. In cases

where data were insufficient for this technique, the dose-response data were transformed into a straight line by means of a logit transformation; the LD50 was derived from the best fit line obtained by linear regression analysis.

RESULTS AND DISCUSSION

Phytochemical screening results:

Phytochemical screening results as can be seen in Table 1. showed that all tested plants of these families contained flavonoids, kuinons, polyphenols, saponins, triterpenoids and few plants contained tannins and alkaloids. With the exeption of simaroubaceae was not detected alkaloids and magnoliaceae was not detected kuinons or tannins.

Table 1. Phytochemical Screening Results

	Family and Plants Species	F	K	T	P	S	A	Tr	St
APOCYNACEAE									
1	*Alstonia boonei* De Wild.	+	+	-	+	+	+	+	-
2	*Carissa carandas* L.	+	+	-	+	+	-	+	-
3	*Cerbera manghas* L.	+	+	+	+	+	-	+	-
4	*Funtumia elastic* (Preuss) Stapf.	+	+	+	+	+	+	+	-
5	*Kibatalia arborea* (Blume) G. Don.	+	+	+	+	+	+	+	-
6	*Kopsia arborea* Blume.	+	+	-	+	+	+	+	-
7	*Kopsia fruticosa* (Ker) A. DC.	+	+	-	+	+	+	+	+
8	*Ochrosia citrodora* Lauterb & K. Schum.	+	+	-	+	+	+	+	-
9	*Plumeria alba* L.	+	+	-	+	+	-	+	-
10	*Plumeria rubra* L.	+	+	-	+	+	-	+	-
11	*Rauvolfia sumatrana* Jack.	+	+	-	+	+	+	+	-
12	*Stemmadenia galeottiana* (A. Rich.) Miers.	+	+	-	+	+	+	+	-
13	*Strophantus caudatus* (Burn.f.) Kurz.	+	+	-	+	+	+	+	-
14	*Strophantus gratus* Baill.	+	+	-	+	+	-	+	-
15	*Tabernaemontana macrocarpa* Jack.	+	+	-	+	+	+	+	-
16	*Thevetia peruviana* (Pers.) Merrs.	+	-	+	-	+	+	+	-
17	*Wrightia pubescens* Blume.	+	+	-	+	+	-	+	-
SIMAROUBACEAE									
1	*Picrasma javanica* Blume	+	+	+	+	+	-	+	-
2	*Picrodendron baccatum* Krug. & Urb. Ex. Urb.	+	+	+	+	+	-	+	-
3	*Quassia indica* (L) Nooteboom	+	+	-	+	+	-	+	-
MAGNOLIACEAE									
1	*Michelia alba* DC.	+	-	-	-	+	+	+	-
2	*Michelia champaca* L.	+	-	-	+	+	+	+	-

Note:
+ = Detected, - = Not detected, F = Flavonoid, K = Kuinon, T = Tannin, P = Polyphenol, S = Saponin, A = Alkaloid, Tr = Triterpenoid, St = Steroid

These phytochemical screening results were in line with literatures. Plants species of Apocynaceae and Magnoliaceae generally were contained alkaloid [10]. Vinchristine and vinblastine were alkaloid isolated from *Vinca rosea* L., Apocynaceae [11]. Whereas liriodenine was an alkaloid from *Michelia champaca* L., Magnoliaceae

ISSN: 0975-8585

[12]. Plants species of Simaroubaceae generally were contained simaroubalid, a form of triterpene lactone such as quasin [10].

Brine Shrimp Lethality Bioassay Results:

Table 2. Brine Shrimp Lethality Bioassay Results

	Family and Plants Species	LC_{50} (μg/mL)
APOCYNACEAE		
1	*Alstonia boonei* De Wild.	>1000
2	*Carissa carandas* L.	>1000
3	*Cerbera manghas* L.	>1000
4	*Funtumia elastic* (Preuss) Stapf.	>1000
5	*Kibatalia arborea* (Blume) G. Don.	>1000
6	*Kopsia arborea* Blume.	>1000
7	*Kopsia fruticosa* (Ker) A. DC.	>1000
8	*Ochrosia citrodora* Lauterb & K. Schum.	>1000
9	*Plumeria alba* L.	>1000
10	*Plumeria rubra* L.	>1000
11	*Rauvolfia sumatrana* Jack.	>1000
12	*Stemmadenia galeottiana* (A. Rich.) Miers.	>1000
13	*Strophantus caudatus* (Burn.f.) Kurz.	118
14	*Strophantus gratus* Baill.	569
15	*Tabernaemontana macrocarpa* Jack.	588
16	*Thevetia peruviana* (Pers.) Merrs.	>1000
17	*Wrightia pubescens* Blume.	>1000
SIMAROUBACEAE		
1	*Picrasma javanica* Blume	362
2	*Picrodendron baccatum* Krug. & Urb. Ex. Urb.	>1000
3	*Quassia indica* (L) Nooteboom	118
MAGNOLIACEAE		
1	*Michelia alba* DC.	37
2	*Michelia champaca* L.	546
REFERENCES		
1	*Camptothecin*	2
2	*Nystatin*	297

The results of BSLB as can be seen in Table 2. showed that some species of Apocynaceae, Simaroubaceae and Magnoliaceae were active or have toxic activity to *Artemia salina* L (LC_{50} < 1000 μg/ml). These active plants species were *Strophantus caudatus* (Burn.f.) Kurz., *Strophantus gratus* Baill., and *Tabernaemontana macrocarpa* Jack. from Apocynaceae, *Picrasma javanica* Blume and *Quassia indica* (L) Nooteboom from Simaroubaceae and *Michelia alba* DC. dan *Michelia champaca* L. from Magnoliaceae. The most active extract were *Michelia alba* DC., *Quassia indica* (L) Nooteboom and *Strophantus caudatus* (Burn.f.) Kurz.

The BSLB may not specific for antitumor agents, but there is correlation since 14 of 24 species that active to brine shrimp also have cytotoxic activity to 9PS cell lines [7]. Furthermore, cytotoxicity of cell lines (ED_{50} values) are usually one-tenth to BSLB (LC50 values). Over 300 novel antitumor and pesticidal natural products that had been isolated were screened by BSLB [4]. Quasinoid brusein A, B, D and brusatol were toxic to *A. salina*. These compounds were also have cytotoxic and antiplasmodial activity [5]. Related to this study, it has been reported that some species of Apocynaceae, Simaroubaceae and Magnoliaceae were potential as anticancer agents or a Topoisomerase inhibitor [13]. This results in line with NCI review that reported these plants families as Families Of Special Interest (FOSI).

CONCLUSIONS

It can be concluded that some species of Apocynaceae, Simaroubaceae and Magnoliaceae were toxic to *Artemia salina* L (LC_{50} < 1000 μg/ml). The most active extract were *Michelia alba* DC. (Magnoliaceae), *Quassia indica* (L) Nooteboom (Simaroubaceae) and *Strophantus caudatus* (Burn.f.) Kurz. (Apocynaceae). All tested plants of these families contained flavonoids, kuinons, polyphenols, saponins, triterpenoids and few plants contained tannins and alkaloids. With the exeption of simaroubaceae was not detected alkaloids and magnoliaceae was not detected kuinons or tannins.

ACKNOWLEDGEMENTS

The authors was thank to Theresia Ginting for her support in this experiment.

REFERENCES

[1] Stewart, B.W. dan Wild, C.P. World Cancer Report 2014, International Agency for Research on Cancer, WHO Press, 1993

[2] Badan Penelitian dan Pengembangan Kesehatan. Riset Kesehatan Dasar: Riskesdas 2013 (Basic Health Research Year 2013). Balitbangkes, 2013.

[3] http://www.worldbotanical.com/images/ARS%20NCI%20Active%20Plants/Review%20of%20plants%20collectod%20for%20antitumor%20screening-Spjut-2010.pdf [Accessed on: January 2013].

[4] McLaughlin JL, Rogers LL, Anderson JE. Ther Innov Regul Sci 1998; 32: 513-524. DOI: 10.1177/009286159803200223

[5] Solis PN, Wright CW, Anderson MM, Gupta MP, Phillipson JD. Planta Med 1993; 59: 250-252. DOI: 10.1055/s-2006-959661

[6] Zhang Y, Mu J, Han J and Gu X. Toxicology Mechanisms and Methods 2012; 22(1): 23–30. DOI: 10.3109/15376516.2011.583297

[7] Meyer, BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE McLaughlin, JL. Planta Med 1982; 45: 31-34. DOI: 10.1055/s-2007-971236

[8] Farnsworth NR. J Pharm Sci 1966; 55(3): 243-268. DOI: http://dx.doi.org/10.1002/jps.2600550302

[9] Kemenkes RI (2008): Farmakope Herbal Indonesia, Edisi I, Departemen Kesehatan RI, Jakarta, 64-67, 115-118.

[10] Cronquist, A. *An Integrated System of Classification of Flowering Plants*, Columbia University Press, New York, 1981

[11] Cragg GM, Kingston DGI, Newman DJ. Anticancer Agents From Natural Products, Taylor And Francis Group: CRC Press, 2005

[12] Monteiro MCM, Leptokarydis IH, Silva GH, da Silva VC, Bolzani VS, Young MCM, Lopes MN. Ecl Quim 2007;32(3):13-8. DOI: http://dx.doi.org/10.1590/S0100-46702007000300002

[13] Zuhrotun, A., Suganda, A.G., Wirasutisna, K.R., Wibowo, M.S. Int. J. Pharm. Sci. Rev. Res.2015; 35 (2): 90-94