Cytotoxicity and antioxidant activity of combination noni (*Morinda citrifolia*) and periwinkle (*Catharanthus roseus*) extracts as anticancer candidate

D W Agustina and M Rifa’i

Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University

E-mail: rifa.123@ub.ac.id

Abstract. The research of herbal compounds and conventional anti-cancer compounds has been widely explored. Noni fruits are well known as an herbal medicine for cancer. In the meantime, periwinkle develops two strong alkaloids, vinblasts and vincristins. In this study, we evaluate the cytotoxicity and antioxidant activity of a combination of Noni fruits (*Morinda citrifolia*) and Periwinkle Madagaskar leaves (*Catharanthus roseus*), which in this study called Moriseus extract as an anticancer drug candidate. Noni fruit ethanol extracts and periwinkle extract. DPPH tests were used to assess the antioxidant of the extracts. The cytotoxicity of the MCF-7 breast cancer extracts was performed using the MTT procedure. In a nutshell, cells were seeded in 96 well plates at various extract concentrations. Cells were incubated for 24 hours and tetrazolium salt solution measured their viability. After 2h medium aspiration, DMSO was applied and the absorbance with an ELISA plate reader was estimated at 540 nm. Cytotoxicity was considered when a decrease in cell survival of more than 50 percent was observed. Combination Noni (*M. citrifolia*) and Periwinkle (*C. roseus*) extract significantly decreased the viability of MCF7 cells in a concentration-dependent manner and have IC value 53.84 ppm with the scavenging activity of combination extract are 69.91%. Combination *M. citrifolia* and *C. roseus* extract have strong cytotoxicity and antioxidant activity which could be high potential as a candidate of anticancer drug.

1. Introduction
Cancer still is the most mortality cause in the world. Based on GCO (Global Cancer Observatory) 1.8 million new cases up came around the world [1]. Prevalence cancer case in Indonesia is up to 400,000 new cases per year with Lung and Breast cancer as the most cancer type that occurs both sexes and ages [2]. Recent cancer treatments have limited such negative side effect and resistance of cancer cell to anticancer agent lead to seeking alternative treatment for cancer therapy [3]. An anticancer agent from herbal substant is strongly recommended. In the present study, some plant components have been successfully used in the treatment of cancer. Experimental studies show that phytochemical substances with antioxidative and anti-inflammatory properties can inhibit cancer formation and development [4]. Indonesia is well known for the biodiversity of plant, that’s made many studies has been done to explore potential Indonesia herbal as an anticancer candidate.

Noni (*M. citrifolia*) and Periwinkle Madagaskar (*C. roseus*) are herbal that widespread and easily found in every region in Indonesia [5]. Both noni and periwinkle are well known as a traditional herb to treat various diseases such as hypertension, diabetes, and cancer [6,7]. *M. citrifolia* has more than 160 identified chemical compounds; the main components are terpene compounds, anthraquinones,
morindone, morindin, asperuloside, acubin, caproic acid, caprylic acid, dammcanthal, scopeletin, polysaccharide, and alkaloids [6]. C. roseus is an abundant useful, diabetes, blood pressure, asthma, constipation, cancer, and menstrual problems medicined plant of Apocynaceae. Vinblastine and vincristine are two strong natural anticancer drugs, which fall under the Terpenoid Indole Alkaloid category [7]. Based on the pharmaceutical benefit of both plants, this study was conducted to evaluate the anticancer potency combination of both plants. A combination of those herbal is expected to have good synergy which boasts anticancer activity and be a good anticancer agent candidate.

2. Material and method

2.1. Extract preparation
Simplicia of M. citrifolia fruits and C. roseus leaves were obtained from Materia Medica, Batu. Crude extracts of the M. citrifolia fruit and C. roseus leave were carried out using ethanol extraction. Dried samples were homogenized and maceration at room temperature for 24 hours in 70% ethanol. This procedure was followed by vacuum filtration, and crude extracts of M. citrifolia fruit and C. roseus were then obtained using a rotary evaporator. Crude extract of both plants then combined with ratio 1:1 makes Moriseus extract (Combination M. citrifolia fruits and C. roseus leaves extract).

2.2. Cell culture
MCF-7 cell line was obtained from Laboratorium Sentral Ilmu Hayati (LSIH) Brawijaya University. Cell line in the Roswell Park Memorial Institute (RPMI) was preserved in 1640 medium, complemented by 10% FBS and 1% penicillin/streptomycin. The cell was maintained in an incubator at 37°C with 5% CO2.

2.3. DPPH assay
The extract was prepared at 100 mg/L concentration. DPPH concentration was 50 mg/L. Methanol was used as a black sample and to dissolve DPPH and M. citrifolia L. extract (paste form). Then, 3 ml of DPPH solution was mixed with 2 ml of M. citrifolia L. extract sample solution and shaken well (quickly). This is because the DPPH and antioxidant reaction begin instantaneously. Then, this solution is quickly moved into the cuvette using a pipette. After that, the cuvette was put into the spectrometer and the absorbance was measured at 515 nm. The DPPH radical scavenging in term of percentage is calculated [8].

2.4. Determination total phenolic content
Total phenol content (TPC) was measured by the Folin-Ciocalteu method [9]. Firstly 20 μL of sample extracts were mixed with 100 μL of Folin-Ciocalteu reagent in a 96-well microplate then incubated for 5 min. after that 75 μL of sodium carbonate solution (75 g/L) was added then incubate for 2 h in a dark place at room temperature. The absorbance was measured at 740 nm with a microplate reader. Galic acid (100–1000 μM) was used as normal for calibration, and water was used as blank for the construction of a linear regression line. The total phenolic content was measured as mg GAE/g of dry extract.

2.5. Determination total flavonoids content
Total flavonoid contents (TFC) were measured according to Sasipriya & Siddhuraju method with modification [10]. Briefly, 50 μL Moriseus extracts were added with 70 μL of distilled water and 15 μL of 5% sodium nitrite solution into a 96-well microplate. At room temperature, the solutions were well combined and incubated for 5 minutes. The mixture was then fitted with 15 μL of 10 percent aluminum chloride solution. 100 μL of 1 M of sodium hydroxide solution was added after 6 minutes of incubation. Then the absorbance was measured at 510 nm with a microplate reader. The total flavonoid contents were estimated from quercetin (200–1000 μM) standard curve, and the results were expressed as mg quercetin equivalent (mg QE)/g of dry extract.

2.6. Cytotoxicity Assay
Briefly, 100 μl of cell suspension (2 × 10⁴ cell/ml) was seeded in 96-well microplates except for the last row which contained only 100 μl of Roswell Park Memorial Institute medium (RPMI) 1640 which was considered as the blank. After 24 h of incubation in the previously mentioned conditions, the medium was replaced using 100μl medium treatment with various doses (0, 10, 20, 40, 80, 160 pm). All plates were incubated for 24 h. After that medium was replaced with 100 μl MTT reagent with a concentration 0.5 μg/mL and incubated for 4h. Formazan crystal that have been formed dissolve by adding 100 μl DMSO for each well plate then incubated for 30 minutes. An ELISA platform reader then calculated the absorbance at 540 nm.

3. Result and discussion

3.1. Antioxidant activity, total phenol, and flavonoids of moreseus extract

Measured in terms of DPPH free radical function, the antioxidant activity has been focused on the constant concentration calculation of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in a methanol solution, after adding the mixture of sample extract [9]. As shown in Table 1 scavenging activity of moriseus extract in highest concentration dose 100 μ g/mL is 69.92% is lower than scavenging activity of ascorbic acid as control which showed scavenging activity up to 92.63%. Although have lower scavenging activity, with IC₅₀ as 43.11 μ g/mL, moriseus extract still categorized as the strong antioxidant agent which bellow concentration 50 μg/mL could inhibit 50% free radical [11]

| Sample          | Concentration (μg/mL) | Absorbance (517 nm) | % Scavenging |
|-----------------|-----------------------|---------------------|--------------|
| Moriseus Extract| 0                     | 0.678               | 0            |
|                 | 20                    | 0.275               | 59.44        |
|                 | 40                    | 0.265               | 60.92        |
|                 | 60                    | 0.239               | 64.75        |
|                 | 80                    | 0.222               | 67.25        |
|                 | 100                   | 0.204               | 69.92        |
| Ascorbic Acid   | 0                     | 0.678               | 0            |
|                 | 20                    | 0.129               | 80.97        |
|                 | 40                    | 0.098               | 85.55        |
|                 | 60                    | 0.082               | 87.91        |
|                 | 80                    | 0.066               | 90.27        |
|                 | 100                   | 0.05                | 92.63        |

Table 2. Antioxidant, total phenol and flavonoids

| Sample          | IC₅₀ (μg/mL) | Gallic Acid (mg GAE)/g | Quercetin (mg QE)/g |
|-----------------|--------------|------------------------|---------------------|
| Moriseus Extract| 43.11        | 590.76±0.32            | 554.28±3.06         |

An antioxidant is needed in cancer therapy. Antioxidant plays an important role to maintain homeostatic of the immune system [12]. Cancer is linked to the inability of the immune system to respond to tumor antigens leading to uncontrollable of proliferation of cancer cells [13]. The antioxidant helps to repair damaged cells and prevent angiogenesis of cancer cells [14]. M. citrifolia fruits contain high flavonoid, vitamin C, gallic acid, ascorbic acid, beta carotene that makes M. citrifolia known as antioxidant sources [6]. C. roseus contain high phenolic acid such as gallic acid which is used to neutralize free radicals and inhibit lipid oxidation, it could inhibit the formation of toxins such as malondialdehyde (MDA) [7]. Those study relevant with the finding in this study showed moriseus extract which contains M. citrifolia and C. roseus have the abundance of total phenolic (gallic acid) and total flavonoid (quercetin).

3.2. Cytotoxic activity of moriseus extract
The cytotoxic effect of Moriseus extract against MCF-7 human breast cancer cell line was determined by Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. This assay is based on the reaction between mitochondrial dehydrogenase enzymes in viable cells and the tetrazolium rings of a soluble reagent which results in the production of violet formazan crystals. Crystals are solubilized in DMSO [15]. The number of surviving cells is proportional to the amount of formazan and is represented by the color of the solution. The color is quantified using a multi-well scanning spectrophotometer (ELISA reader). As shown in Table 2, the increasing concentration extract resulting from the decreasing of viability of MCF-7 cells. Cytotoxic activity of moriseus extract showed IC$_{50}$ as 53.82 µg/mL. Cytotoxic activity for herb extract divide into 3 categories, High (IC50 ≤50 µg/mL) and moderate (IC50 50 µg/mL - 100 µg/mL) and low ≥100 µg/mL [16].

| Concentration Extract | Viability Cell (%) |
|-----------------------|--------------------|
| 0 ppm                 | 100 ± 0.0          |
| 10 ppm                | 85.8 ± 2.54        |
| 20 ppm                | 71.2 ± 1.64        |
| 40 ppm                | 59.6 ± 5.72        |
| 80 ppm                | 38.2 ± 2.91        |
| 160 ppm               | 27.3 ± 1.13        |

A previous study showed that anthraquinonoid and quercetin compounds in *M. citrifolia* act as antitumors in Lewis lung peritoneal carcinomatosis cell line enhancing the immune system through the production of tumor necrosis factor-alpha (TNF), interleukin-1beta, interleukin-10, interleukin-12, interferon-gamma, and nitric oxide [17]. Meanwhile, *C. roseus* produce secondary metabolite such as vinblastine and vincristine which are known as potent antitumor activity [21]. Vinblastine has anticancer activity with specific targets Tubulin Beta Chain (TUBB2A) which plays a role in the mitotic cell, including in the induction of apoptosis of cancer cells [18].

4. Conclusion
In conclusion, the combination of *M. citrifolia* and *C. roseus* or in this study called moriseus extract showed strong antioxidant and cytotoxic activity which could be a good potential as an anticancer candidate. The result of this study could be preliminary information to evaluate the anticancer potency of moriseus extract for further study in discovering drug candidates.

References
[1] Global Cancer Observatory 2020 Cancer Today GBO, Access on on 27 July 2020

https://gco.iarc.fr/

[2] National Cancer Institute 2020 Cancer statistic NCI, Access on 27 July 2020

https://www.cancer.gov/about-cancer/understanding/statistics

[3] Irfan A R, Wee Y K, Woon K P, Jeonghui L 2017 The sources of chemical contaminants in food and their health implications Front Pharmacol 2017 1-7

[4] Elyjoba A A, Odeleye O M, Ogunyemi C M 2005 Traditional medicine development for medical and dental primary health care delivery system in Africa African Journal of Traditional, Complementary and Alternative Medicines 2 46-61.

[5] Karthikeyan B, Joe M M, Jaleel C A, Deiveekasundaram M 2010 Effect of root inoculation with plant growth promoting rhizobacteria (PGPR) on plant growth, alkaloid content and nutrient control of Catharanthus roseus (L) G. Don. Nat. Croat. 19:205–212.

[6] Sharma K, Pachauri S D, Khandelwal K, Ahmad H, Arya A, Biala P, Agrawal S, Pandey A, Srivasta A, Sivastav A, Sexena J K, Dwivedi A K 2016 Anticancer effects of extracts from the fruit of morinda citrifolia (noni) in breast cancer cell lines. Drug Res 66 141-147.

[7] Gajalakshmi S, Vijayalakshmi S, Devi R V 2013 Pharmacological activities of Catharanthus roseus: A perspective review International Journal of Pharmaceutical Science 4 431-439.
[8] Marghitas L A, Stanciu O G, Dezmiorean D S 2009 In vitro antioxidant capacity of honeybee-collected pollen of selected floral origin harvested from Romania Food Chemistry 115 878–883

[9] Royer M, Diouf P N, Stevanovic T 2011 Polyphenol contents and radical scavenging capacities of red maple (Acer rubrum L.) extracts Food and Chemical Toxicology 49 2180–2188.

[10] Sasipriya G, Siddhuraju P 2012 Effect of different processing methods on antioxidant activity of underutilized legumes, Entada scandens seed kernel and Canavalia gladiata seeds Food and Chemical Toxicology 50 2864–2872

[11] Royer M, Diouf P N, Stevanovic T 2011 Polyphenol contents and radical scavenging capacities of red maple (Acer rubrum L.) extracts Food and Chemical Toxicology 49 2180–2188

[12] Shahin M N, Ramazan A K N, Parviz A A 2016 Potent Antioxidant Properties of rolB-transformed Catharanthus roseus (L.) G. Don, Iranian J Pharm 15(2):537-550

[13] Nerin C, Alfaro P, Aznar M, and Dome-o, C. (2013). The challenge of identifying non-intentionally added substances from food packaging materials: a review. Anal. Chim. Acta 775, 14–24.

[14] Alamode T T 2013 An overview of the anti-cancer properties of some plants used in traditional medicine in Nigeria Journal of Biochemistry and Bioinformatics 3 7–14

[15] Morgan D M L 1998 Tetrazolium (MTT) assay for cellular viability and activity Methods Mol Biol. 79 179-84.

[16] Berridge M V, Herst P M, Tan A S 2005 Tetrazolium dyes as tools in cell biology: New insights into their cellular reduction Biotechnol Annu Rev. 11 127-52.

[17] Brown A C 2012 Anticancer Activity of Morinda citrifolia (Noni) Fruit: A Review Phytotherapy Research 26 1427-40

[18] Tang, K X, Xing S H, Guo X B 2011 Induction and flow cytometry identification of tetraploids from seed-derived explants through colchicine treatments in Catharanthus roseus (L.) G.Don Journal of Biomedicine and Biotechnology, 793198.