BIOASSAY-GUIDED FRACTINATION OF ANTIMITOTIC COMPOUND FROM ONGKEA CORTEX (*MEZZETTIA PARVIFLORA BECC*) TOWARDS SEA URCHIN EGGS

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ABSTRACT

Ongkea cortex, the wood bark of *Mezzettia Parviflora* Becc, is a traditional medicine originated from Southeastern Sulawesi (Indonesia). It has been empirically known to have antitumor property. In this study, we examined the antiproliferative activity and obtained the antimitotic compound of ongkea cortex. Antimitotic activity was ultimately determined by the inhibition of cleavage-stage of newly fertilized sea urchin (*Lytechinus variegatus*) eggs. A bioassay-guided fractination was performed in order to find the bioactive substance of ongkea cortex. The IC_{50} values of methanolic extract, ethyl acetate-soluble part of metanolic extract and ethil acetat insoluble part of metanolic extract were 1221.68 μg/mL, 2.69 μg/mL, and 15.15 μg/mL, respectively. Ethyl acetate-soluble part of metanolic extract was further investigated. It was partitionated using vacuum liquid column chromatoghraphy with different solvent system by increasing their polarities. There were three different fractions obtained. Fraction III exerted the highest inhibition activity with IC_{50} value of 1.33 μg/mL. It was separated subsequently to result four groups of compounds. III-C group presented the most potent inhibition activity with IC_{50} value of 0.7147 μg/mL. It was then subjected to preparative TLC and yieldedsix groups of subfractions. III-C-3 subfraction was indicated as the most potent compound with IC_{50} value of 0.3378 μg/mL. It was ten times weaker compared with antimitotic activity of Vincristine with IC_{50} of 0.0351 μg/mL. As a conclusian, ongkea cortex might have antimitotic property with the highest rate inhibition activity exhibited by III-C-3 compound.

Keywords: ongkea cortex, *Mezzettia Parviflora* Becc, sea urchin eggs, antimitotic compound, antiproliverative activity

INTRODUCTION

Ongkea cortex (*Mezzettia Parviflora* Becc.), belongs to annonaceae family, is a traditional substance that has been empirically used in Southeast Sulawesi (Indonesia) to treat some diseases such as hipercholesterolemia, asthma, diabetes mellitus and tumor [1].

The familiy of annonacea has a broad range of pharmacological activities such as antimicrobial, antifungal, antitumor, and insecticidal properties. It has some various chemical compounds such as *benzylisoquinoline*, acetogenin, c-benzil-flavonoid and diterpenoid alkaloids [2]. Based on the concept of chemotaxonomy, chemical compounds of plants from the same familial groups are mostly similar.

Previous studies have reported that ongkea cortex has antiatherosclerotic, antiplatelet and free radical scavenging activities [3, 4, 5]. Yet, there is no research that has been performed to investigate its antimitotic property. Therefore, the present work is

the first study trying to evaluate the possibility of ongkea cortex to be developed as an anticancer agent.

To analyse theantiproliferative activity of ongkea cortex, we used an inhibition of sea urchin (*Lytechinus variegates*) eggs development assay. It is a proper bioassay model to study biological activities such as antimitotic, teratogenic and antineoplastic activities [6].

In this study, the antitumor activity of ongkea cortex was evaluated to provide scientific based information as a traditional medicine. A bioassay-guided fractination was performed in order to find the bioactive compound of ongkea cortex.

METODE PENELITIAN

Samples Collection

Ongkea cortex was obtained from Buton regency, Southeast Sulawesi. Samples were cleaned, shattered, dried and powdered.

Samples Extraction

Dried powdered portions of 1.6 Kg of ongkea cortex were extracted by maceration method with 12 Litres of methanol for 3x3x24 h. The results were collected and rotoevaporated to yield viscous methanol extract.

Partition of methanol extract

Methanol extract was partitionated with ethil acetate using liquid-solid partition method. It produced ethyl acetate-soluble part of metanolic extract and ethil acetat insoluble part of metanolic extract. The chemical compound profiles of extracts were monitored by thin layer chromatography. Antimitotic activities of extracts were investigated by an inhibition of sea urchin (*Lytechinus variegates*) eggs development assay.

Fractination of active extract

The most antimitotically active extract was fractionated using vacuum liquid column chromatoghraphy with diffrent solvent with increasing polarity; hexane, hexane:ethyl acetate (15:1; 10:1, 5:1), methanol. Fractions with similar chemical profiles based on thin layer chromatography were grouped and tested against sea urchin (*Lytechinus variegates*) eggs.

Determination of Antiproliferative Activity

Antimitotic effect was determined by the ability of samples to prevent the cleavage-stage of newly fertilized sea urchin (*Lytechinus variegatus*) eggs. The procedure was based on the study demostrated by Jiminez [7]. Gamete elimination was induced by injecting 1 mL KCl 10 % in the perivisceral cavity. Fertilization was undertaken by mixing 1 mL of a sperm suspension and 4 mL of eggs in the chamber filled with 50 mL of filtered seawater. Methanolic extract, ethyl acetate-soluble part of metanolic extract and ethil acetat insoluble part of metanolic extract plus filtered sea water were added to 100 µl of fertilized eggs in the eppendorf to make concentrations 1, 10 and 100 ppm in a final volume of 1 mL. The mixtures were incubated at 15-20 °C with intermitten shaking. The observation of dividing cells was carried out after 2 h incubation. Filtered seawater and DMSO were used as negative controls. Vincristine (0.01 ppm, 0.1 ppm, and 1 ppm) was used as a positive controls. Experiment was carried out in triplicate.The same examination was applied to test the activities of fractions but with different series concentration i.e. 0.2, 1.5, 25 and 125 ppm. The number of divided and non divided cells were counted, analyzed, and displayed with IC_{50}(*Inhibitory Concentration 50%*).

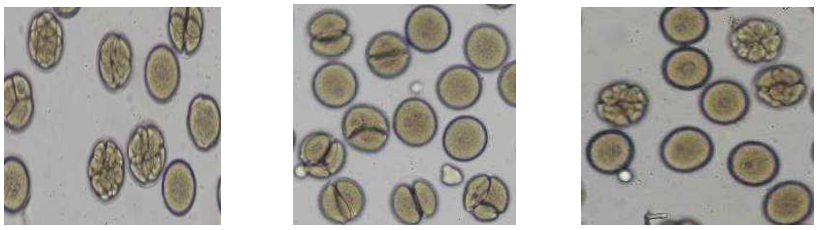
RESULTS AND DISCUSSSION

The antiproliferative assay using sea urchin eggs has been well known to have a good sensitivity against toxic substance. It can help to assess the ability of natural compounds to be developed as an anticancer. The inhibition of growth cycle of sea urchin eggs can be associated with several steps such as DNA and RNA synthesis, protein synthesis, and mitotic spindle assembly [8].

A first screening showed that the IC_{50} values of methanolic extract, ethyl acetate-soluble part of metanolic extract and ethil acetat insoluble part of metanolic extract were 1221.68 μg/mL, 2.69 μg/mL, and 15.15 μg/mL, respectively (Table 1). Ethyl acetate-soluble part of metanolic extract possesed the highest inhibition rate. Figure 1 showed its effect on the initial development of sea urchin embryos. In order to find the active principles possibly responsible for antimitotic activity of ongkea cortex, ethyl acetate-soluble part of metanolic extract was further investigated by bioassay-guided fractination.

Table 1. Antiproliferative activity on sea urchin egg development of methanolic extract, ethyl acetate-soluble part of metanolic extract and ethil acetat insoluble part of metanolic extract of ongkea cortex

Samples	IC_{50} (μg/mL)
Methanolic Extract	1221.68
Ethyl acetate-soluble part of metanolic extract	2.69
Ethil acetat insoluble part of metanolic extract	15.15



(a) (b) (c)

Figure 1. The effect of ethyl acetate-soluble part of metanolic extract of ongkea cortex on the initial development of sea urchin (*Lytechinusvariegatus*) embryos. (a) 1 µg/ml (44.34 % inhibition); (b) 10 µg/ml (54.4 % inhibition); (c) 100 µg/ml(76.28 % inhibition).

Table 2. Antiproliferative activity on sea urchin egg development of Fraction 1,II and III.

Samples	IC_{50} (µg/mL)
Fraction I	7.39
Fraction II	7.48
Fraction III	1.33

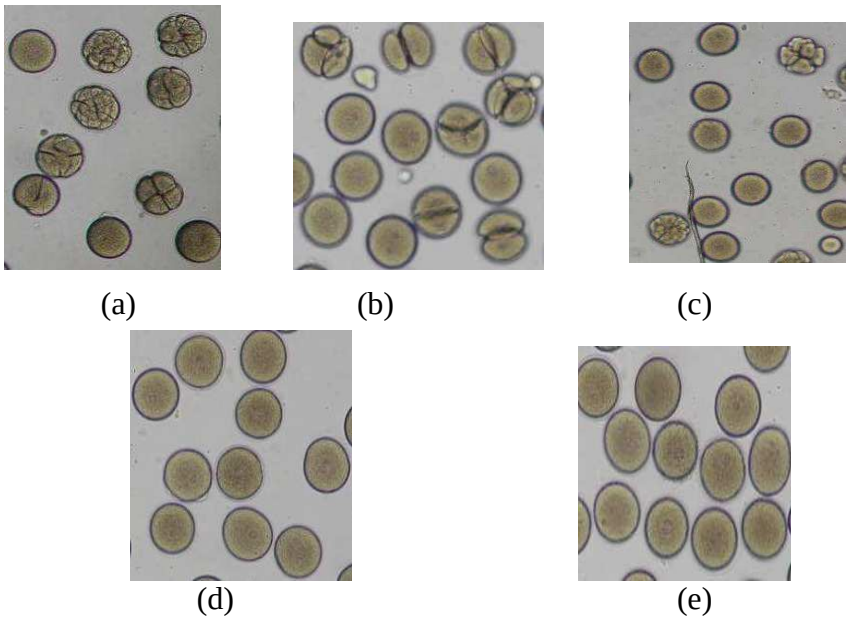


Figure 2. The inhibition effect of fraction III against sea urchin development. (a) 0.2 µg/mL (3.37 % inhibition); (b) 5 µg/mL (55.95 % inhibition); (c) 25 µg/mL (84.5 % inhibition); (d) 25 µg/mL (100 % inhibition); and (e) 125 µg/mL (100 % inhibition)

Table 3. Antiproliferative activity on sea urchin egg development of Fraction III-A,III-B, III-C and III-D.

Samples	IC_{50} (µg/mL)
Fraction III-A	1.2971
Fraction III-B	1.9480
Fraction III-C	0.7147
Fraction III-D	3.2569

It was partitionated using vacuum liquid column chromatoghraphy with different solvent system by increasing their polarities yielded three different group of bioactive fractions. Fraction III exerted the highest inhibition activity with IC_{50} value of 1.33 μg/mL while Fraction I and II had IC_{50} of 7.39 μg/ml and 7.48 μg/ml, respectively (Table 2). The inhibition effect of fraction III on sea urchin eggs was demonstrated by figure 2.

Fraction III was separated subsequently to result four groups of compounds that were then exposed to sea urchin eggs. Fraction III-A, III-B, III-C and III-D exerted high toxicity activities with IC_{50} 1.2971 μg/mL; 1.9480 μg/mL; 0.7147 μg/mL; and 3.2569 μg/mL, consecutively (Table 3). The most bioactive substance was seemingly in Fraction III-C. Furthermore, it was subjected to preparative TLC and yielded six groups of subfractions. Their IC_{50} were 3.4411 μg/mL; 3.7153 μg/mL; 0.3378 μg/mL; 59.827 μg/mL; 85.2903 μg/mL; 40,794.3483 μg/mL for III-C-1, III-C-2, III-C-3, III-C-4, III-C-5, and III-C-6, respectively (Table 4). Among them, subfraction III-C-3 exhibited the most potent compounds. Although, it was ten times weaker compared with antimitotic activity of Vincristine with IC_{50} of 0.0351 μg/mL.Negative controls, filtered water and DMSO, had no inhibition effect.

Table 4. Antiproliferative activity on sea urchin egg development of subfraction III-C-1,III-C-2, III-C-3, III-C-4, III-C-5 and III-C-6.

Samples	IC_{50} (µg/mL)
Subfraction III-C-1	3.4411
Subfraction III-C-2	3.7153
Subfraction III-C-3	0.3378
Subfraction III-C-4	59.827
Subfraction III-C-5	85.2903
Subfraction III-C-6	40,794.3483
Vincristine	0.0351
Filtered seawater	0
DMSO	0

Jacobs reported that if there are compounds presenting 100% inhibition in sea urchin eggs assay at a concentration 16 μg/mL or less, they should be very potential to be investigated as anticancer agents in in vivo experiment [9]. Based on this information, it can be stated that subfraction III-C-3 has a strong antiproliferative activity. Therefore, the potency of subfraction III-C-3 of ongkea cortex as a natural antimitotic agent and its mechanisms should be further elaborated.

CONCLUSION

Finally, it can be concluded that ongkea cortex might have potent antitumoral property with the highest rate inhibition activity presented by III-C-3 compound. We suggest to evaluate the cytotoxicity effect of III-C-3 compound using human cell lines and in vivo experiment with animal model.

REFERENCES

1. Fernandes-Silva CC, Freitas JC, Salatino A, Salatino MLF. 2013. Cytotoxic Activity of Six Samples of Brazilian Propolis on Sea Urchin (*Lytechinusvariegatus*) Eggs. *Evidence-Based Complementary and Alternative Medicine*. Article ID 619361.
2. Hakim, E.H., Achmad, S.A., Makmur, L., Mujahidin, D., dan Syah, Y.M. 2001. Profil Kimia Annonaceae. *Bull Soc. Nat. Prod. Chem. (Indonesia).* VolI : 1. Januari-Juni 2001.
3. Jacobs RS, White S, Wilson L. 1981. Selective compounds derived from marine organisms: effects on cell division in fertilized sea urchin eggs. *Fed Proc.* **40** (1). 26-29.
4. Jimenez PC, Fortier SC, Lotufo TMC, Pessoa C, Moraes MEA, Moraes MO, Costa-Lotufo LV. 2003. Biological activity in extracts of ascidians (Tunicata, Ascidiacea) from the Northeastern Brazilian coast. *J. Exp. Mar. Biol. Ecol*. 287, 93D101.
5. Militao GCG, Pinheiro SM, Dantas INF, Pessoa C, De Moraes MO, Costa-Lotufo LV, Lima MAS, Silveira ER. 2007. Bioassay-guided fractionation of pterocarpans from roots of *Harpalycebrasiliana*Benth. *Bioorganic & Medicinal Chemistry*. 15: 6687–6691.
6. Mufidah, Manggau MA, Tayeb R, Alam G. 2007. Free radical scavenging activity of ongkea (*Mezzetia parviflova* becc.) Woodbark. *POKJANAS TOI*. Airlangga University.
7. Mufidah. 2011. Aktivitas antiaterosklerosis ekstrak terstandar klikaongkea (*mezzetia parviflora* becc.) pada tikus wistar yang diberi asupan kolesterol: kajian efek anti-oksidan dan anti-kolesterol terhadap penghambatan mcp-1 dan disfungsi

endotel. *Disertasi*. Program Studi Ilmu Kedokteran. Universitas Hasanuddin.

8. Mufidah, Manggau MA, Alam G, Bahar MA, Kasim S, Rusdi M. 2012. Efek Antiagregasi Platelet Fraksi Klik a Ongkea (*Mezzettia Parviflora* Becc.). *Majalah Farmasi dan Farmakologi*, **16**. (1). 51 – 54.
9. Mufidah M, Wahyudin E, Lawrence GS, Subehan, Manggau MA, Alam G. 2012. Phytochemical analysis and antioxidant activity of *Mezzetia parviflora* Becc. Woodbark extract. *Phcog J*, **4**, (34).