

Anti Cancer Activity of Active Substances from Mangosteen pericarp (*Garcinia mangostana* Linn) against T47 D Cell Lines

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ABSTRACT

Background: *Garcinia mangostana* Linn (Guttiferae) or commonly know as mangosteen has been reported scientifically as anticancer. In developing a pericarp of *G.mangostana* as the anticancer phytopharmaceutical product, bioassay-guided fractionation approach was chosen to get active substances to be used as raw material breast-cancer drugs. **Objectives:**The aim of this study is to get the anticancer substances from the pericarp of *G.mangostana* that are active against breast cancer cells T47D. **Method:** Separation of active substances was carried out by column chromatography techniques. Based on the principle of bioassay-guided fractionation, a cytotoxicity assay of ethanolic extract and fractions was performed against T47D human breast cancer cell lines. **Results:**Cytotoxicity assay of the extract, fraction 2, subfraction 2.2, subfraction 2.2.4, and subfraction 2.2.4.4 provided IC₅₀ values 8.96 ug/mL; 1.80 ug/mL; 1.76 ug/mL; 1.12 ug/mL; and 6.93 ug/mL respectively

Keywords: *Garcinia mangostana* Linn, bioassay-guided fractionation, active substance against breast cancer

INTRODUCTION

Cancer is a disease which characterized by a change of control mechanism that regulates the proliferation and differentiation of cells. Mortality caused by cancer is approximately about 13% from all (WHO, 2012). Breast cancer is the type of cancer that is characterized by high level of mortality. Cancer treatments are aimed to treatment, prolong and improve patient quality of life.

Garcinia mangostana Linn or commonly known as mangosteen is a plant from Guttiferae family and have been used traditionally for the treatment of skin infections and wound, amoebic dysentery and inflammation (Chaverri et al. 2008; Chin & Kinghorn 2008). These plants contain xanthone-type compounds, flavonoids, triterpenoids, benzophenones, biphenyl compounds, pyrrole, benzofuran, tannins, and saponins (Chaverri et al. 2008; Hutapea 1994). Anticancer research of *G.mangostana* pericarp ethanol extract using cultured human breast cancer cells (SKBR3) have been given the IC₅₀ value of 15.45 ± 0.5 ug/ml (Moongkarndi 2004). The Breast-cancer activity of γ -mangosteen and α -mangosteen

compounds using cultured human breast cancer cells (MCF-7) showed IC₅₀ values of 0.85 and 10.5 µg/ml, respectively (Ahmat et al. 2010, Chitra et al. 2010).

Based on the previous studies, the pericarp of *G.mangostana* has active compounds as anticancer and is prospective as a phytopharmaceutical product. In developed of *G.mangostana* pericarp into phytopharmaceutical product, then the study to get the active substances as raw material for breast cancer. The processes include extraction and fractionation. Based on the principle of bioassay-guided fractionation, a cytotoxicity assay of ethanolic extract and fractions against T47D human breast cancer cell lines were performed.

METHOD

Materials and cell

Ethanol, n-hexane, ethyl acetate, chloroform, methanol, dichloromethane, aqua bidest, silica gel 60 Merck 0,063-0,200 mm, silica gel 60 G Merck. Breast cancer cell lines T47D collected from Parasitology Laboratory, Medicine Faculty UGM, medium RPMI, fetal bovine serum 10%, penicillin-streptomycin, 1%, fungison 0,5 %, Phosphate Buffer Saline 20 %; Dimethyl sulfoxide; 3-(4,5-Dimethyliazol-2-il)-2,5-diphenyltetrazolium bromide; Sodium Dodecyl Sulphate 10 %.

Plant material

Garcinia mangostana pericarp was obtained from Blitar.

Fractionation

5.5 Kg mangosteen's pericarp powder macerated using 14.5 L ethanol 96% for 48 hours. Then filtered and the filtrate was separated. The filtrate was collected and evaporated with a vacuum rotary evaporator to obtain a viscous extract which was dried in an oven at 40° C. Separation used vacuum chromatography column method with stationary phase silica gel and the mobile phase gradient solvent hexane: ethyl acetate, chloroform: methanol, methanol, and ethanol in order to obtain 12 fractions. The results of cytotoxicity assay used T47D cells, proved that the fraction 2 was active. The next separation was performed on fraction 2 using open column chromatography with stationary phase silica, mobile phase gradient solvent hexane: ethyl acetate, ethyl acetate: chloroform, chloroform: methanol, and methanol to obtain 7 subfractions. The results of cytotoxicity assay used T47D cells, proved that the fraction 2 was active. Then, subfraction 2.2, separated by vacuum column chromatography used stationary phase silica, mobile phase gradient solvent hexane: dichloromethane, dichloromethane: ethyl acetate and methanol to obtain 8 subfractions. The results of cytotoxicity assay used T47D cells, proved that the fraction 2 was active. Further separation was performed on the SF2.2.4 with open column chromatography used stationary phase silica, mobile phase hexane: dichloromethane, dichloromethane: methanol and methanol to obtain 7 subfractions. The results of cytotoxicity assay used T47D cells, indicated that subfraktion 2.2.4.4 and 2.2.4.5 are active.

Sample preparation

The sample for cytotoxicity assay was dissolved in DMSO.

Cell Viability

Cells were distributed into 96 well of the plate and then were incubated in 5% CO₂ conditions at 37°C for overnight. Test solution with a certain concentration incorporated into cancer cell cultures 5x10³ cells/ml each well and were incubated under conditions of 5% CO₂ at 37 ° C for 24 hours. The fluid was pipetted and was discarded for each well and then were rinsed

with PBS solution then shaken and thrown away. 100 ml of culture media were added to each well. Added 10 μ l solution of MTT into PBS at concentration 5 mg/ μ l. Incubated under conditions of 5% CO₂ at 37°C for 4-6 hours, then added *stopper* reagent (10% SDS). Incubated overnight at room temperature and stored in the dark place. The spare was read by ELISA *reader* at 550–600 nm wavelengths.

Statistical Analysis

The percentage of live cells was calculated to obtain the IC₅₀ value. The data were analyzed with probit analysis.

RESULT AND DISCUSSION

This study is one part of the process to develop mangosteen pericarp (*Garcinia mangostana* Linn.) into phytopharmaceutical products to treat breast cancer. Introduction and development of several new and highly specific in vitro bioassay techniques, chromatographic methods, and spectroscopic techniques have made it much easier to screen, isolate and identify the *lead compound* quickly and precisely. The drug discovery from natural materials could use *bioassay-guided isolation* approach (Sarker et al. 2006). This research aims to obtain anticancer ingredients that originally are from *Garcinia mangostana* Linn pericarp which is active against human breast cancer cells T47D.

The first stage was extraction process. *G. mangostana* pericarp was chopped and dried in the oven at 50 °C, then grounded into powder. 5.5 Kg dried powder were macerated by ethanol. The macerate then vaporized using a rotary evaporator at 60° C to obtain a yellowish brown viscous extract.

TLC profiles at 254 nm UV light observations showed a dark brown color, the observation of 366 nm UV light showed brown, yellow, blue and purple stain. The plate then sprayed with 10% sulfuric acid and gave a yellow to brown colored stain. Flavonoids observed with UV will give a-characteristic colors such as yellow, orange, brown, blue and purple fluorescent (Harborne 1987). Meanwhile, phenolic compounds of carboxylic acid group observed with UV will also give a-characteristic colors such as yellow, brown, blue or turquoise fluorescent (Debenedetti 2009). The TLC densitometry analysis of the plate was conducted at 254 nm wavelength and showed 2-3 peaks in 250-500 nm area. This result indicated that the compound contained the conjugated aromatic group (Harborne 1987). The result of in vitro assay of *G. mangostana* ethanolic extract against T47D breast cancer cells showed the IC₅₀:8.96 μ g/mL (Figure 1). According to the *National Cancer Institute* (NCI), the extracts that had IC₅₀ values less than 20 μ g/mL against cancer cell cultures could be considered effective as anticancer (Ampasavate et al. 2010). So based on the provision of the NCI, the ethanolic extract of *G mangostana* pericarp is effective as against breast cancer.

Bioassay guided isolation principle, as much as 7.5 grams of ethanol extracts were fractionated by vacuum column chromatography using silica gel G60 stationary phase with a mobile phase gradient of the solvent mixture of hexane and ethyl acetate, chloroform: methanol, methanol and finally 96% ethanol. The solvents had been used each gradient was 100 ml and the fractions that collected and was combined based on similar TLC profiles, so were obtained 12 fractions with respective weights of 357.2 mg; 2639.7 mg, 1093.4 mg, 477.2 mg; 298.5 mg, 214.1 mg, 181.6 mg; 1005.5 mg, 393.8 mg, 25.9 mg, 10 mg and 43 mg.

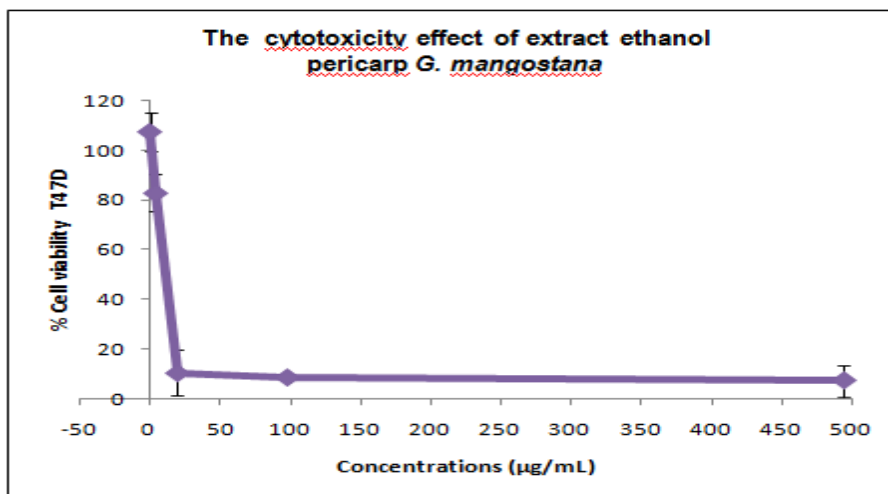


Figure 1 Cell viability T47D cell lines

Anticancer activity results of fractions 1-12 as in vitro assay against T47D breast cancer cells, showed IC_{50} : 6.02; 1.80; 3.43; 6.09; 7.44; 10.44; 21.19 ; 411.52; 542.55; 5746.05; 1005.62; 360.81 µg/mL (Figure 2). Based on provisions of the NCI, the fractions 1-6 were declared effective against breast cancer. Based on those data, further separation of the most active fraction of the fraction 2 with IC_{50} values of 1.80 µg/mL will be done.

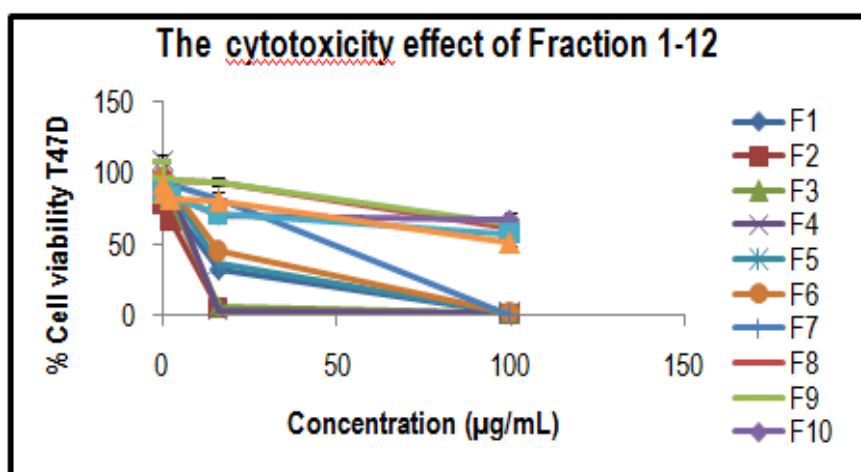


Figure 2 Cell viability T47D cell lines

Separation of the fraction 2 as much as 2 grams used an open column chromatography with the stationary phase silica gel 60 and the mobile phase gradient solvent mixture hexane: ethyl acetate, ethyl acetate: chloroform, chloroform: methanol and the last used methanol. Eluates were collected every 10 ml, fractions that collected were combined based on TLC profiles, thus obtained 7 subfractions with respective weights of 237.6 mg, 736.5 mg, 278.6 mg, 567.3 mg, 45.3 mg; 9.4 mg, 148.1 mg. Anticancer activity in vitro assay result of subfraction 1-7 against T47D breast cancer cells, showed IC_{50} : 7.59; 1.76; 3.58; 4.46; 8.07; 23.64; 31.75 µg/mL (Figure 3). Based on provisions of the NCI, the subfraction 2.1-2.5 were defined as effective against breast cancer. Based on those data further separation of the most active subfraction the subfraction 2.2 with IC_{50} values of 1.76 µg/mL will be done.

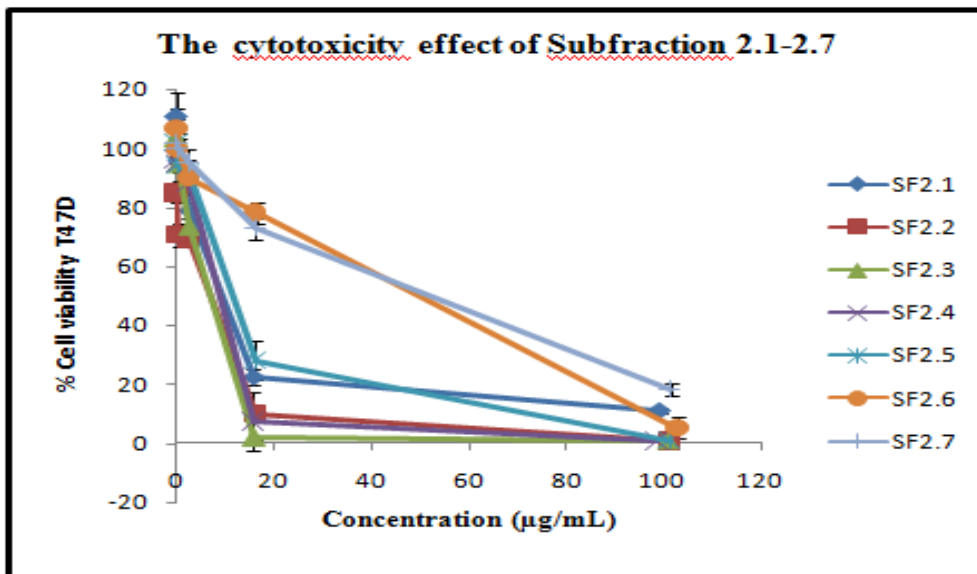


Figure 3 Cell viability T47D cell lines

Separation process of 1 gram subfraction 2.2 by vacuum column chromatography with the stationary phase silica gel G 60 and the mobile phase is a mixture of hexane solvent gradient and dichloromethane, then mixed dichloromethane and ethyl acetate and the last used methanol. The solvent was used in each gradient was 10 ml and fractions were collected and were combined based on similar TLC profiles, so obtained 8 subfractions with respective weights of 10.7 mg, 29.5 mg, 8.0 mg, 283.0 mg, 454.4 mg, 30.0 mg, 21.0 mg, 4.6 mg. Anticancer activity in vitro assay results of subfraction 2.2.1-2.2.8 against T47D breast cancer cells showed IC₅₀ values of 5.33; 3.14; 5.79; 1.12; 2.54; 4.72 ; 3.13; 10.86 µg/mL respectively (Figure 4). Based on provisions of the NCI, the fractions 2.2.1-2.2.8 were declared as effective against breast cancer. Based on those data further separation of the most active subfraction the subfraction 2.2.4 with IC₅₀ values of 1.12 µg/mL was done.

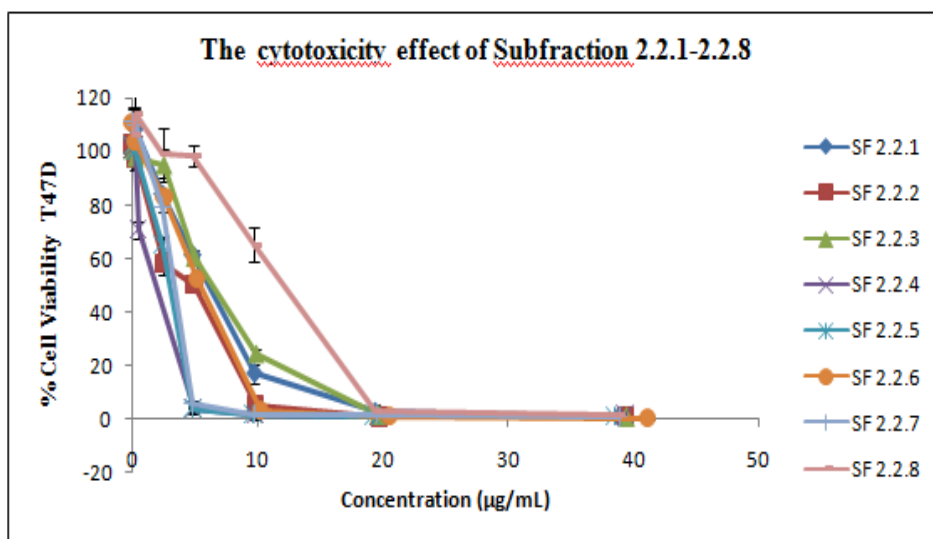


Figure 4 Cell viability T47D cell lines

Against subfraction 2.2.4 as much as 100 mg, had been separated by using an open column chromatography with stationary phase silica gel 60 and the mobile phase gradient solvent hexane: dichloromethane, dichloromethane: methanol and 100% methanol. Eluates were collected every 1 ml, fractions that collected were combined based on TLC profiles, so

obtained 7 subfractions with respective weights of 15.4 mg, 3.5 mg, 13.2 mg, 9.4 mg, 24 mg; 16.2 mg, 11.3 mg. Anticancer activity in vitro assay results of subfraction 2.2.4.1-2.2.4.7 against T47D breast cancer cells showed IC₅₀ values amounted to 694.95; 324.62; 190.30; 6.93; 7.97; 98.87; 198.97 µg/mL (Figure 5). Based on provisions of the NCI, the subfractions 2.2.4.4 and 2.2.4.5 were declared as effective against breast cancer. Subfraction 2.2.4.4 and 2.2.4.5 had anticancer activity not too different, as shown by the IC₅₀ values at 6.93 and 7.97 µg/mL. The results of TLC subfractions 2.2.4.4 and 2.2.4.5 indicated that the profiles have no significant differences showed by dominant stains on Rf value 0.31 and 0.34 of each.

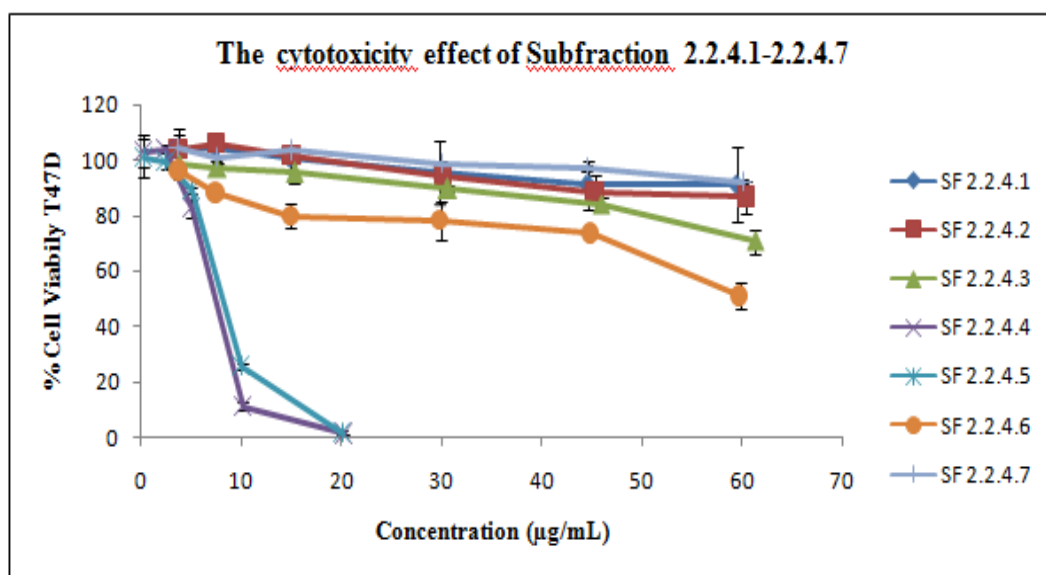


Figure 5 Cell viability T47D cell lines

The results of anti-breast-cancer activity assay showed that the ethanolic extract of *G. mangostana* pericarp and some fractions were effective against T47D breast cancer cell lines. The IC₅₀ values are 8.96 µg/mL for ethanolic extract; 1.80 µg/mL for F2; 1.76 µg/mL for SF2.2; 1.12 µg/mL for SF2.2.4 and 6.93 µg/mL for SF2.2.4.4. Bioactivity-guided fractionation result of the ethanol extract until had been obtained SF2.2.4 showed the decrease in IC₅₀ values. It's showed that from this separation process was obtained the fraction which had compounds that were responsible for the breast-cancer activity. However, there was an odd phenomenon when the further separation was done to SF 2.2.4 producing SF2.2.4.4 as subfraction product. The IC₅₀ value of SF 2.2.4.4 was higher than its mother fraction SF 2.2.4. This phenomenon also occurred in the research that had been done by Widyawaruyanti (2007) and Tumewu (2009) about the antimalarial activity of Cempedak bark (*Artocarpus champeden* SPRENG). From those studies was known that Cempedak bark ethanol extract and fraction 4 which from the separation of the ethanol extract, could inhibit in vitro of Plasmodium falciparum growth at IC₅₀ values at 1.90 µg/mL and 6.69 µg/mL, respectively.

In general, the activity of the active component would provide good results because it has inert compound (Yadav & Dixit 2008). This inert compound does not affect the mechanism of pathology directly, but these compounds may affect the bioavailability and the excretion of the active compounds, which leads to the increasing of the active compounds stability and decreasing of the side effects.

CONCLUSION

Anticancer substances derived from *Garcinia mangostana* Linn pericarp which are active against human breast cancer cells T47D are ethanol extract, fraction 2, subfraction 2.2, subfraction 2.2.4 and subfraction 2.2.4.4.

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