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Annona muricata leaves have strongest cytotoxic activity against breast cancer cells

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ABSTRACT

BACKGROUND

Plant-derived herbal compounds have a long history of clinical use, better patient tolerance and acceptance. They are freely available natural compounds that can be safely used to prevent various ailments. Plants have been the basis of traditional medicine throughout the world for thousands of years and are providing mankind with new remedies. The objective of this study was to determine the cytotoxicity of soursop (*Anona muricata* Linn*)* leaves and pearl grass (*Hedyotis corymbosa* (L.) Lam.) on the hormone-dependent human breast carcinoma Michigan Cancer Foundation-7 (MCF-7) cell line.

METHODS

This study used two types of solvents (water and ethanol) in the extraction process and two incubation times (24 hours and 48 hours) in the MTT assays to analyze the cytotoxic effects of both plants.

RESULTS

Preliminary results showed that the ethanolic extract of soursop leaves (SE) displayed cytotoxic effects against MCF-7 on 24- and 48-hour incubation times with IC_{50} values of 88.788 μg/ml and 14.678 μg/ml, respectively. Ethanolic pearl grass extract (PE) showed similar results, with IC_{50} values of 65.011 μg/ml on 24-hour incubation time and 52.329 μg/ml on 48-hour incubation time against MCF-7 cell line. However, the water extract of both plants displayed lower cytotoxic effect against MCF-7 cell line.

CONCLUSION

The ethanolic extract of both plants displayed cytotoxic effect against MCF-7. Soursop (*Anona muricata Linn)* leaves have the strongest cytotoxic activity against MCF-7 breast cancer cell line.

Keywords: Microculture tetrazolium salt assay, *Hedyotis corymbosa, Annona muricata,* MCF-7

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Daun sirsak mempunyai aktifitas sitotoksik paling kuat terhadap kultur sel kanker payudara

ABSTRAK

LATAR BELAKANG

Senyawa-senyawa yang diturunkan dari tumbuhan memiliki sejarah yang panjang dalam penggunaan klinis, toleransi pasien yang lebih baik dan juga penerimaannya. Senyawa-senyawa tersebut adalah senyawa alami yang tersedia secara bebas yang dapat digunakan secara aman untuk mencegah berbagai penyakit. Tanaman menjadi dasar sistem pengobatan tradisional di seluruh dunia selama ribuan tahun dan memberikan obat baru kepada manusia. Penelitian ini dilakukan untuk menentukan efek sitotoksisitas daun sirsak (Annona muricata Linn) dan rumput mutiara (Hedyotis corymbosa (L.) Lam.) terhadap kultur sel karsinoma payudara manusia tergantung hormon Michigan Cancer Foundation-*7 (MCF-7).*

METODE

Dalam studi ini, kami telah menggunakan dua jenis pelarut (air dan etanol) dalam proses ekstraksi dan dua jenis waktu inkubasi (24 jam dan 48 jam) dalam tes *MTT untuk menganalisis efek sitotoksik dari kedua tanaman .*

HASIL

Hasil awal menunjukkan bahwa ekstrak etanol daun sirsak (SE) yang ditampilkan efek sitotoksik terhadap MCF - 7 pada waktu 24 jam dan 48 jam inkubasi dengan masing-masing nilai IC_{50} adalah 88,788 µg/ml dan 14,678 µg/ml. Di sisi lain, ekstrak etanol rumput mutiara (PE) juga menunjukkan hasil yang serupa dengan nilai IC_{50} 65,011 µg/ml pada 24 jam waktu inkubasi dan 52,329 µg/ml pada waktu 48 jam inkubasi terhadap MCF-7. Namun, ekstrak air dari kedua tanaman menampilkan efek sitotoksik yang lebih rendah terhadap MCF-7.

KESIMPULAN

Daun sirsak (Annona muricata Linn) mempunyai aktifitas sitotoksik paling kuat terhadap kultur sel kanker payudara MCF – 7.

***Kata kunci**: Uji microculture tetrazolium (MTT), Hedyotis corymbosa, Anona muricata, MCF-7*

INTRODUCTION

Breast cancer incidence in Asia is escalating more rapidly than in the West. Breast cancer is one of the most prevalent cancers in Indonesian females.[1] In the other hand, the plant derived herbal compounds have a long history of clinical use, better patient tolerance and acceptance. They are freely available natural compounds that can be safely used to prevent various ailments. Plants have been the basis of traditional medicine throughout the world for thousands of years and are providing mankind with new remedies.[2] In the case of human cancers, thus far, nine plant-derived compounds have been approved for clinical use as anticancer drugs in the United States. In the cancer drug discovery program, a paradigm based on ethnobotanical and ethnopharmacological data would be more economical and beneficial for identifying potential anticancer molecules than mass screening of plant species.[3] Indonesians have known a lot of traditional medicinal plants, among which are soursop and pearl grass.

Pearl grass (*Hedyotis corymbosa* (L.) Lam.) (synonym: *Oldenlandia corymbosa* Linn.)

of the Rubiaceae family, is a weedy annual herb. The plant is known to clear heat and toxins, activate blood circulation, promote diuresis and relieve stranguria. It is also active against appendicitis, hepatitis, pneumonia, cholesystitis, urinary infection, cellulites and snake bite. Chinese folk medicine prescribes the plant for treatment of skin sores, ulcers, sore throat, bronchitis, gynecologic infections and pelvic inflammatory diseases.[4] The anticarcinogenic properties of methanolic extracts of pearl grass have been published in our previous study.[5] In the present study we have continued our study using different solvents (water and ethanol) to achieve better results.

Annona muricata (common name: soursop) is a lowland tropical fruit-bearing tree in the Annonaceae family. Other common names are graviola and guanábana (sometimes shortened to guanába). Related species include cherimoya (*A. cherimola*) and sugar-apple (*A. squamosa*), and paw paw (*Asimina triloba*). The soursop is native to tropical Central and South America and the Caribbean, but is now widely cultivated in tropical areas worldwide, including southern Florida and Southeast Asia, from sea level to altitudes of around 1150 meters. Soursop has numerous traditional medicinal uses in South America and the Caribbean, and has become a popular nutritional medicinal supplement. Fruit, seeds, bark, leaves, and roots have all been used to treat intestinal parasites, coughs (including asthma and bronchitis), liver ailments, inflammation, diabetes, and hypertension. The seeds are insecticidal and a preparation from the leaves has been used to kill headlice and bedbugs.[6] Numerous studies on plants from the Annonaceae family have been carried out. *A. montana* has cytotoxic effect on the human liver cancer cell line HepG2.[7] The seeds of *A.crassiflora* have high antioxidant activity.[8]

The purpose of this study was to determine the cytotoxic effects of pearl grass and soursop leaf extracts using different solvents by the microculture tetrazolium salt (MTT) assay on the hormone-dependent human breast carcinoma Michigan Cancer Foundation-7 (MCF-7) cell line.

METHODS

This study has been done using an experimental research design and conducted in Integrated Research Laboratory, YARSI University Jakarta from January to October 2013.

Plant materials and extractions

For preparing the ethanolic extract, dried whole pearl grass (1 kg) and soursop leaves (1 kg) were extracted with 70% ethanol at room temperature. The extracts were then filtered through a Whatman No. 1 filter paper. The collected filtrates were evaporated to dryness under vacuum at 40°C using a rotary evaporator (N-1000V-W, Eyela, Japan). The extraction methods were according to Ali et al. [9] with slight modifications. After evaporation, the yield of dried ethanolic extracts (SE and PE) was about 10% of the original plant samples.

For preparing the water extract, dried whole pearl grass (1 kg) and soursop leaves (1 kg) were extracted with water at room temperature. The extracts were then filtered through a Whatman No. 1 filter paper. The filtrates were freeze-dried and the dried water extracts (SW and PW) were collected.

Cell cultures

The MCF-7 cell line was obtained from the American Type Culture Collection (ATCC, USA). The cells were grown in Dulbecco's Modified Eagle medium (Gibco, USA) supplemented with 10% of fetal calf serum, 100 IU/ml penicillin and 100 μg/ml of streptomycin (Gibco, USA) using 25 cm^2 flasks (Nunc, Denmark), in a CO_2 incubator (Sanyo, Japan) at 37°C.

MTT assay

The viability of cells was determined with trypan blue. Exponentially growing cells were

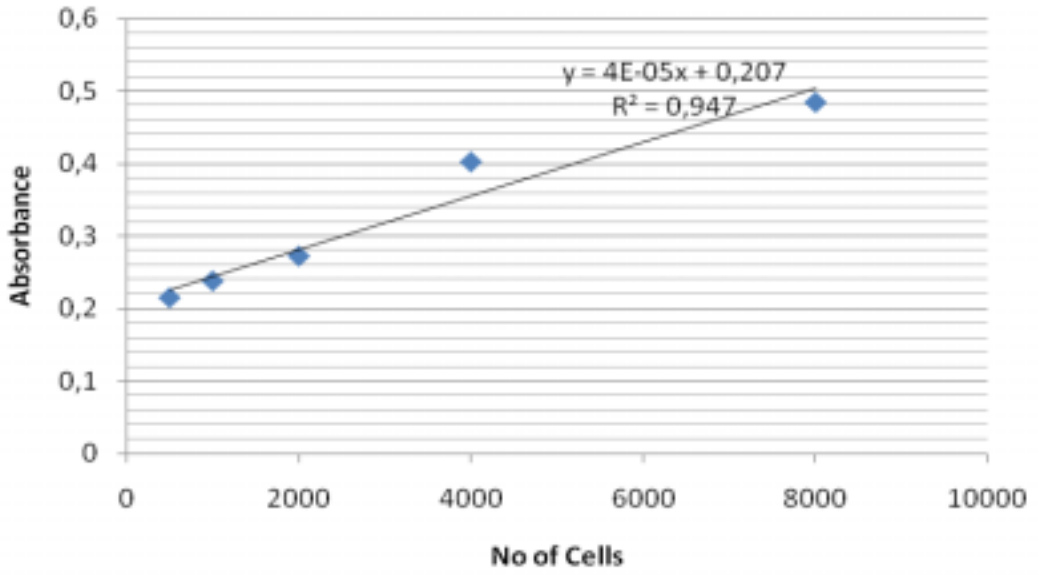

Figure 1. Standard curve of MCF-7

harvested, counted by hemocytometer and diluted with medium, yielding a concentration of 1 x 10^5 cells/ml. From this cell suspension, 100 µl was pipetted into 96-well microtiter plates (Nunc, Denmark) and incubated for 24 h in a 5% CO_2 incubator (Sanyo, Japan) at 37°C. The diluted range of test extracts were 0.468, 0.937, 1.875, 3.750, 7.5, 15 and 30 µg ml-1. After adding the extract samples, new medium was added to make up to a final volume of 200 µl per well. The plate was then incubated in the 5% CO_2 incubator at 37°C for 24 and 48 h. Then 20 µl of MTT reagent (Roche, USA) was added into each well. The plate was incubated again for 4 h in the Sanyo CO_2 incubator at 37°C. After incubation, 200 µl solubilization solution (Roche, USA) was added into each well. The cell was then left overnight at 37°C in the 5% CO_2 incubator. Finally, the absorbance was read with the ELISA reader (LX-800).

RESULTS

As mentioned above, the ethanolic extracts and the water extracts of the studied plants were analyzed for their cytotoxic effects on the MCF-7 cell line by the MTT assay using different incubation times. The MCF-7 standard curve used for calculation of IC_{50} values is shown in Figure 1. The IC_{50} values against the MCF-7 cell line of the ethanolic extract of soursop leaves (SE), the water extract of soursop leaves (SW), the ethanolic extract of pearl grass (PE) and the water extract of pearl grass (PW) for different incubation time (24 hours and 48 hours) are shown on Table 1. Doxorubicin has been used

Table 1. IC_{50} values of extracts on 24 hours and 48 hours incubation time

Treatment	IC_{50} (24 hours incubation) (µg/ml)	IC_{50} (48 hours incubation) (µg/ml)
Ethanolic extract of soursop (SE)	88.79^a	14.68^a
Water extract of soursop (SW)	682.88^b	538.22^b
Ethanolic extract of pearl grass (PE)	65.01^c	52.33^c
Water extract of pearl grass (PW)	354.33^d	475.71^d

There were significant differences between groups a, b, c, and d at 24 and 48 hours. P-value was calculated using a one-way Anova test with a value of less than 0.05 indicating significance

as a positive standard, with IC_{50} values of 0.147 μM and 0.000325 μM against MCF-7 on 24- and 48-hour incubation times, respectively. There were significant differences between groups of IC_{50} values ($p<0.05$). The strongest cytotoxic activity was shown by soursop leaves.

DISCUSSION

This study has shown that the ethanolic extract of soursop leaves (SE) has potential cytotoxic effects on MCF-7 cells and that the effect increased with incubation time. The highest cytotoxic effect of this extract was displayed by the lowest IC_{50} value that has been achieved. Rachmani et al.[10] and Fidianingsih et al.[11] have reported that *Annona muricata* was cytotoxic against T47D cells. Paul et al.[2] found that HeLa cells treated with 75 μg of a crude leaf extract of *Annona muricata* showed 80% cell inhibition. They also detected in the crude leaf extract of *Annona muricata* several bioactive compounds, such as anonaine, friedelin, isolaureline, annonamine, anomurine, kaempferol, asimilobine, quercetin, and xylopine. However, the water extract of soursop leaves (SW) did not display the potential cytotoxic activities.

On the other hand, pearl grass has also displayed similar results. The ethanolic extract of pearl grass (PE) has potential cytotoxic effect on MCF-7 cell lines. However, the cytotoxic effect of its ethanolic extract was lower compared to the methanolic extract from our previous study.[5] Lee et al.[12] reported that pearl grass extract had a significantly inhibited cell growth and induced apoptosis in COLO 205 (colon cancer), Hep3B (hepatocellular carcinoma) and H460 (lung cancer) cell lines. Andriyani et al.[13] have reported that pearl grass extract displayed cytotoxicity against T47D cells. The ethanolic extract of pearl grass leaves has shown significant anticancer activity on k562 human leukemia cell lines.[14] Based on our previous study, the anticarcinogenic properties of pearl grass could be due to its high antioxidant activities. According to Sasikumar et al.,[15] pearl grass extract exhibited high antiradical activity against ABTS, nitric oxide and hydroxyl radicals, with EC_{50} values of 150, 130, and 170 ìg/ml, respectively. The pearl grass extract also enhanced the hepatoprotective in albino rats.[16] The alcoholic extract of pearl grass has also shown significant antiulcer activity against aspirin in rats.[17] Pearl grass extract were reported to have a number of common bioactive constituents, including ferulic acid, flavonols, flavones, vanillic acid, syringic acid, and caffeic acid.[18] Another study has also reported that the methanolic extract of pearl grass contains many bioactive chemical constituents including alkaloids, glycosides, terpenoids, steroids, flavonoids and tannins.[19] Two groups of isolated compounds from whole plants are the genoposides and iridoid glycosides.[20] The iridoid glycosides have shown a variety of pharmacological activities, such as antioxidant, analgesic, antibacterial, antimalarial, anticancer and hepatoprotective effects.[21]

The mechanisms of the cytotoxic effects of both plants are being studied. The different IC_{50} values from different solvent extraction methods may have been due to the bioactive compounds of both plants. The anticarcinogenic properties of a combination of both plants are being studied to explore better treatments for breast carcinoma with inflammation.

CONCLUSIONS

The strongest cytotoxic properties against the MCF-7 cell line were displayed by soursop leaves with ethanol as a solvent in the extraction process and 48-hour incubation time. The different IC_{50} values from different solvent extraction methods may have been due to the bioactive compounds of both plants.

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