Ethanol extract of mangosteen (*Garcinia mangostana* Linn) peel effect in inhibiting the growth of human tongue cancer cells Supri's clone 1, invitro

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ABSTRACT

The incidence of tongue cancer in Indonesia reached 1.01% of all cancers and 42% of oral cavity cancer. Tongue cancer therapies including chemotherapy, radiotherapy, surgery, and all three combined therapy. Search for anti-cancer drugs currently switched on herbal plants, one of which is the mangosteen. Has the properties of mangosteen peel extract inhibited the growth of cancer cells. The purpose of the study, obtain IC_{50} of ethanol extract of mangosteen peel in inhibiting the growth of human tongue cancer cells SP-C1. Research carried out on 96 preparations of human tongue cancer SP-C1 were incubated with ethanol extract of mangosteen peel, preparations were classified in two groups of incubation time (24 hours and 48 hours) and each group will be given preferential treatment over 6 randomly different concentrations: 0 (control), 62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL and 1000 µg/mL. Model experiments were 2 x 6 factorial experiment with eight replication for each cell. Test results with ANAVA, incubation (24 and 48 hour) SP-tongue cancer cells with various concentrations of C1 ethanol extract of mangosteen peel gives a highly significant, indicating differences cancer cell growth inhibition. Incubation time factor showed the long incubation effect on cancer cell growth inhibition. Furthermore, by Newman Keuls test, showed 500µg/mL concentrations of 24-hour incubation had the best effect. Conclusion of the study of ethanol extract of mangosteen peel could achieve with IC_{50} values of cell growth resistance 50.3% at a concentration of 500 µg/mL and an incubation time of 24 hours.

Key words: Ethanol extract, *Garciana mangostana* Linn, Supri's Clone 1 (SP-C1)

ABSTRAK

Insidensi kanker lidah di Indonesia mencapai 1,01% dari seluruh jenis kanker dan 42% dari kanker rongga mulut. Terapi kanker lidah meliputi kemoterapi, radioterapi, pembedahan, dan terapi gabungan ketiganya. Pencarian obat anti kanker saat ini beralih pada tanaman herbal, salah satunya adalah manggis. Ekstrak kulit manggis mempunyai sifat menghambat pertumbuhan sel kanker. Tujuan penelitian ini adalah untuk mendapatkan IC_{50} dari ekstrak etanol kulit manggis dalam menghambat pertumbuhan sel kanker lidah manusia SP-C1. Penelitian dilakukan terhadap 96 sediaan kanker lidah manusia SP-C1

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yang diinkubasi dengan ekstrak etanol kulit manggis, sediaan dikelompokkan dalam 2 kelompok waktu inkubasi (24 jam dan 48 jam) dan tiap kelompok akan mendapatkan perlakuan secara acak atas 6 konsentrasi yang berbeda; 0 (kontrol); 62,5 µg/mL; 125 µg/mL; 250 µg/mL; 500 µg/mL; dan 1000 µg/mL. Model eksperimen berbentuk eksperimen faktorial 2x6 dengan 8 replikasi untuk tiap sel. Hasil pengujian dengan ANAVA, inkubasi (24 dan 48 jam) sel kanker lidah SP-C1 dengan berbagai konsentrasi ekstrak etanol kulit manggis memberikan hasil yang sangat bermakna, yang menunjukkan adanya perbedaan hambatan pertumbuhan sel kanker. Faktor lama inkubasi berpengaruh terhadap hambatan pertumbuhan sel kanker. Selanjutnya, dengan uji Newman Keuls, konsentrasi 500 µg/mL dengan inkubasi 24 jam mempunyai efek yang paling baik. Simpulan penelitian ini adalah ekstrak etanol kulit manggis dapat mencapai IC_{50} dengan nilai hambatan pertumbuhan sel sebesar 50,3% pada konsentrasi 500 µg/mL dan waktu inkubasi 24 jam.

Kata kunci: *Ekstrak etanol, Garciana Mangostana Linn, Supri's Clone 1 (SP-C1)*

INTRODUCTION

High incidency rate of tongue cancer accompanied by high mortality rate, mostly that is evolving in developing countries like Indonesia, is a problem that has to be solved. Different method of therapy, including surgery, radiation, chemotherapy, and/or the combination of those, have been applied to treat human tongue cancer. However, the incidence of tongue cancer has not yet decreased.[1,2] In these past four decades, the exploration of herbal anti-cancer drugs have been invigorated with hope that herbal drugs have better effectiveness with lower side effects.[3] Ethanol extract of mangosteen peels is one of the herbal material that is studied and it is believed to be efficacious as anti-inflammatory, anti-oxidant, anti-proliferation as well as apoptosis induction.[4]

The research of anti-cancer activity done by Elya[5] and Matsumoto et al.[6] was 6 Xanton (α, ß, γ mangostin, mangostinone, E garcinone dan 2-isoprenyl-1,7-dihidroksi-3-metoksi Xanton) that was extracted from mangosteen peel, has a potency of inhibiting the proliferation of cancer cells as well as increasing the apoptosis process of cancer cells.

Research results showed that breast cancer cells proliferation was inhibited by the mangosteen peel extract, and the inhibition depends on the concentration. The mangosteen peel extract concentration of 6.25-25 µg/ml was effective to inhibit the cell proliferation. Therefore, mangosteen peel has been proved as a strong cell proliferation inhibitor towards breast cancer activity IC_{50} as much as 9.25 ± 0.64 µg/ml.[6] The most important thing about the therapeutic effect finding from the extracted mangosteen peel with ethanol as the solvent was the finding of herbal material as proliferation inhibitor of cancer cells, including human tongue cancer *Supri's Clone 1* (SP-C1).[6,7]

Materials used in this research include; Fetal bovain serum/FBS (Gibco, Australia), Fungizone liquid (Hyclone, Japan), Penicillin-streptomycin, erathanol extract of mangosteen peels (Tasikmalaya, Indonesia), Trypsin-EDTA (Gibco, Australia), MTT solution (Sigma Aldrich, USA), Isopropanol, Alcohol 70%, PBS, and RPMI 1640.

Instruments used for this research are; Bio Rad microplate reader (Bio Rad, USA); Microplate 96 well (Iwaki, Japan); Adjustable volume digital pippetner (Eppendorf, Germany); Connical tube 15 ml dan 50 ml (Iwaki, Japan); Shaker (Lab Line, Japan); Waterbath (Eyela, Japan); Eppendorf mini tube (Iwaki, Japan); Incubator 37^{0}C; CO_2 5% (Sanyo, Japan); Refrigerator 4^{0}C, -20^{0}C, and -30^{0}C (Sanyo, Japan); electronic digital scale (Melter, Switzerland); Filter (Corming, Germany); Syringe 10 ml (Terumo, Filipina); Vortex (Maxi mix II, USA); Clean bench (Sanyo, Japan); methylated spirit lamp; Flash (Iwaki, Japan); Petri dish (Iwaki, Japan); Microscope (Nikon, Japan); Suction (Assamica, Japan); and Haemocitometer (Assistant, Germany).

This research was conducted in *Laboratorium Riset Terpadu* (Research Centre Laboratory) Faculty of Dentistry Universitas Gadjahmada from April 2010 to October 2010, using the *Supri's Clone 1* (SP-C1) tongue cancer cells.

METHODS

The assessment of *Supri's Clone 1* tongue cancer cells inhibition was done using *microplate 96 well* invitro. The research was conducted by counting the average proliferation of living SP-C1 tongue cancer cells after being incubated using various concentration of ethanol extract of mangosteen peels, with two groups of incubation time length, 24 and 48 hours. The results interpretation was completed using 450 nm-wave length-Bio Rad *microplate reader*.

Ethanol extract of mangosteen peels was divided into 6 (six) test groups according to its concentration, as follow: First group was the control with 0 (zero) concentration of ethanol extract of mangosteen peels; Second group used 62.50 µg/ml concentrate of ethanol extract of mangosteen peels; Third group used the 125 µg/ml concentrate; Fourth group used the 250 µg/m concentrate; Fifth group used the 500 µg/ml concentrate; and the sixth group used the 1000 µg/ml concentrate.

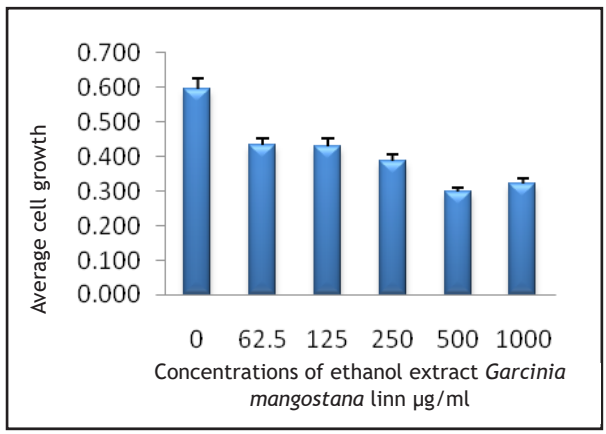

Figure 1. Average tongue cancer cell growth at incubation time 24 hours.

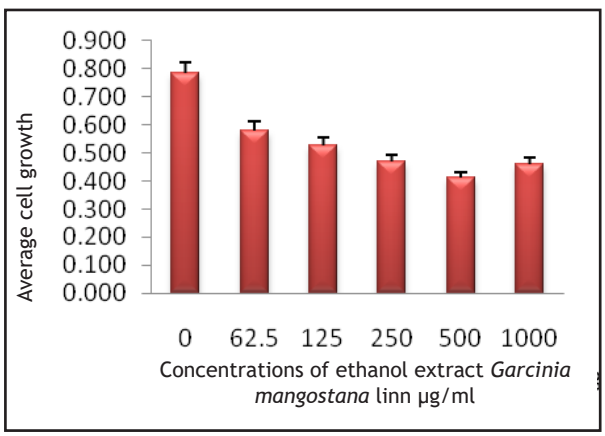

Figure 2. Average tongue cancer cell growth at incubation time 48 hours.

The average of proliferation of SP-C1 tongue cancer cells was obtained using BioRad *microplate reader.* The cell viability percentage showed the interval number using the *microplate 96 well* filled with the *Supri's Clone 1* (SP-C1) cancer cells cultures that was added various concentration of ethanol extract of mangosteen peels. The results were then examined with statistical analysis with ANOVA. If ANOVA were significant, Newman Keuls test was then performed.

RESULTS

Obtained from this study, the ethanol extract of mangosteen peel could inhibit tongue cancer cell growth of SP-C1 from the lowest concentration, 62.5 ug/mL. In the Figure 1 showed that 0 (zero) concentrations was a control and showed an average growth of 100% of cells. Average reduction of the tongue cancer cell growth occurred from the concentration of the ethanol extract of mangosteen peel 62.5 µg/mL (mean, 72.3% cell growth). At a concentration of 125 µg/mL (median 72 cells harbor-partum, 15%) there was a slight increase, but at a concentration of 250 µg/mL (mean 64.93% cell growth) the decreased were more, and the maximum peak decreased at a concentration of 500 µg/mL (median 49 cells harbor-partum, 66%), whereas at a concentration of 1000 µg/mL (average growth of 53.69% of cells) a decline in average growth of cells smaller than 500 µg/mL concentration.

Figure 2 showed the average growth of tongue cancer cells after incubation with ethanol extract of mangosteen peel for 48 hours. The average growth of tongue cancer cells in zero concentrations after 48 hour of incubation was 100% and used as controls. Average reduction of cell growth started in the concentration of 62.5 µg/mL (mean of cell growth was 74.18%) and continues to decrease with increasing concentrations of ethanol extract of mangosteen peel. Peak reduction was at a concentration of 500 µg/mL (mean cell growth, 52.34%) while the concentration 1000 µg/mL (mean cell growth, 58.52%) occurs on average slightly increased cell growth compared to the concentration of 500 µg/mL.

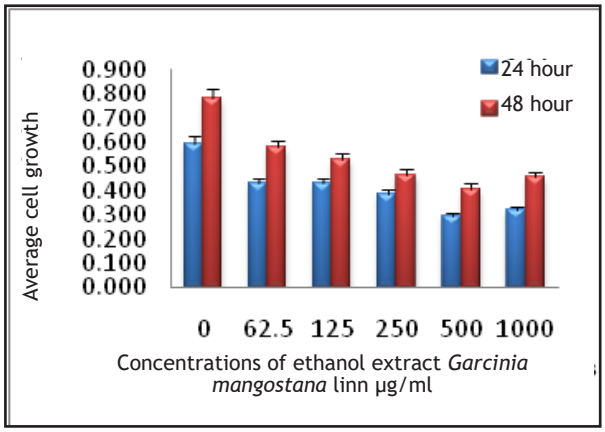

Figure 3. Comparison of average tongue cancer cells proliferation between 24-hour and 48-hour incubation periods

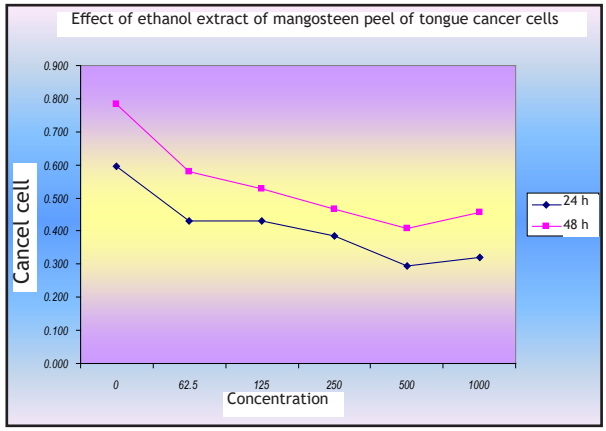

Figure 4. Average cancer cells proliferation in 24-hour and 48-hour incubation periods

Figure 3 shows the comparison of SP-C1 tongue cancer cells between the 24 hour incubation and the 48 hour incubation within various concentration of ethanol extract of mangosteen peels. The average of cancer cells proliferation in both incubation time begans to decrease in the 62.5 µg/ml concentrate, and continuously decreased as the concentration of ethanol extract of mangosteen peels increases. The average cells proliferation peak was reached in the 500 µg/ml concentrate with 24 hours of incubation as much as 49.66%, and as much as 52.34% in the 48 hours of incubation period. Therefore, the 500 µg/ml concentrate of ethanol extract of mangosteen peels was more effective to inhibit the average cells proliferation. The 24-hour incubation period for the 500 µg/ml ethanol extract of mangosteen peels concentrate had the most effective inhibition cells proliferation score as much as 50.30%. The average cells proliferation increased in 1000 µg/ml concentrate with both the 24-hour-incubation period (cells proliferation average was 53.69%) and the 48-hour-incubation periods (cells proliferation average was 58.52%).

Figure 4 shows the effect of ethanol extract of mangosteen peels towards the reduction of the average cancer cells growth as well as the inhibition of human tongue cancer cells proliferation with 24-hour and 48-hour incubation periods. It also shows that the most effective reduction of the average cancer cells growth and the maximum inhibition of cells proliferation occur in the 500 µg/ml concentrate in both 24-hour and 48-hour incubation periods.

DISCUSSION

There have been studies performed to analyze anticancer effect of Xanton isolated from mangosteen (*Garcinia mangostana* Linn.) peel, for instance towards the line hepatocellular cells[6] towards SKBR3 human breast cancer[8] and towards human leukemia. Ho *et al.*[6] studied the cytotoxicity effect of 6 Xantons isolated from mangosteen peel and found out that E garcinone has cytotoxicity effect towards the cell lines in liver cancer.[6] Matsumoto et al.[8] studied the effect of 6 Xantons (α, ß, dan γ mangostin, mangostinon, garcinon E dan 2-isoprenyl-1.7-dihidroxy-3-metoxy Xanton) isolated from mangosteen peel towards the leukemic cells HL60 inhibition. The result shows that all types of Xanton present significant inhibitory action, especially the α, ß, and γ mangostin.[6,9]

Moongkarndi *et al.* assessed the cell proliferation inhibitory action, apoptosis, as well as antioxidant of the methanol extract of mangosteen peels using the breast cancer cell line SKBR3. The result showed that mangosteen peels has the most significant cell proliferation inhibitory effect (ED_{50} = 9.25 ± 0.64 µg/ml) as well as inducts apoptosis process.[6,10]

Suksamrarn *et al.* isolated three new Xantons of the mangosteen peels, mangostenon C, D and E, that were as beneficial as 16 other known Xantons. The cytotoxicity effect of these Xanton was tested on three different cancer cell lines; oral epidermoid carcinoma (KB), breast cancer (SM-1), and the lung cancer cell lines (NCI-H187). Mangostenon C showed cytotoxic effects against

all three cell lines with IC50 values respectively 2.8, 3.53, and 3.72 µg/ml. The results of this study also show that α-mangostin has cytotoxic effects on oral epidermoid carcinoma cells (KB) (IC_{50} =2.08 µg/ml).[6]

This study showed that ethanol extract of mangosteen peel achieves minimal inhibitory action (IC_{50}) in inhibiting the *Supri's Clone 1* (SP-C1) tongue cancer cells proliferation. The first graph showed an average growth of SP-C1 human tongue cancer after being incubated with various concentrations of ethanol extract of mangosteen peel (0/control, 62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml, and 1000 µg/ml) for 24 hours. Concentration of control was considered to have an average rate of cell growth by 100%. The 62.5 µg/ml concentrate had given decreasing effect of the average cell growth with the average value of cell proliferation by 72.3%. It showe that the inhibitory action of the SP-C1 human tongue cancer cells after the 24-hour of incubation period had an inhibitory value of 27.68%. In the concentration of 125 µg/ml, the average cell growth decrease as much as 72.15% with the inhibitory value of 27.85%. The 250 µg/ml concentrate presents greater average cell growth reduction as much as 64.93% which means the greater inhibitory value, as much as 35.07%. The greater the concentration of ethanol extract of mangosteen peel, the greater tongue cancer cell growth inhibitory value produced.

At a concentration of 500 µg/ml, the average cell growth decreased maximally with the rate of 49.66%, and the cell growth inhibition showed the value of 50.3%. It means that at a concentration of 500 µg/ml with 24-hour incubation period, the ethanol extract of mangosteen peel achieves IC50 with the cell growth inhibition value of 50.3%. At the 1000 µg/ml concentrate, the value of average cell growth decreased, however the value was lower than the value at the 500 µg/ml concentrate, that was as much as 53.69% and the cell growth inhibition value was 46.3%. From this result, we can conclude that at the concentration of 500 µg/ml the inhibition reaches its effective peak, however at higher concentration which is at the 1000 µg/ml concentrate, SP-C1 tongue cancer cells' receptors started to saturate. Therefore they cannot grasp the active molecule in the ethanol extract of mangosteen peel. Besides, at the higher the concentration of ethanol extract of mangosteen peel, collisions occured between higher active compounds molecules that were expected to stimulate cell growth.

Cell proliferation is a process of increasing the number of cells that involves the cell cycle. Normal cell cycle is strictly regulated by Cyclin, Cyclin Dependent Kinase (CDK) dan Cyclin Dependent Kinase Inhibitor (CKI) that work together continuously and jointly responsible for controlling the various phases of cell cycle control mechanisms in the check point.[1,2]

In cancer cells, damage occurs to the basic regulatory mechanism of the cell behavior and the control of normal cell growth is impaired, resulting in uncontrolled cell growth. Ethanol extract of mangosteen peel affects as an inhibitor of SP-C1 tongue cancer cells growth by creating intervention in the cell cycle.[1,2]

The result of previous research reports that the inhibition of cell cycle showed by α-mangostin and ß-mangostin inhibit the G1 phase, whereas the S phase is inhibited by γ-mangostin. It is related to the modulation of expression of Cyclin, CDK2, and p27 in human colon cancer cells DLD-1. Moreover, α-mangostin increases the microRNA-143 level that negatively regulates Erk5 in translation phase as well as increases the inhibitory activity while the supplementary therapy using 5-fluorouracil.[6]

The inhibition of SP-C1 human tongue cancer cells growth by the ethanol extract of mangosteen peel occurs between G1 to S and between phase S to G2, by inhibiting the signal from cMyc so that the sinthesis between Cyclin D and CDK4/6 occurs and lead to the phosphorilation of Rb protein binding with E2F. It results in separation of E2F so that E2F is able to activate the beginning of cell cycle in phase G1 to pass through the restriction point and start the cell proliferation process.[4,11]

The inhibitory of cell growth also occurs at phase S by inhibiting the signal from cMyc within synthesis between Cyclin E and CDK 2 that results in phosphorilating protein binding of Rb and E2F. The phosphorilation results in separation of Rb and E2F and lead to activation of cell cycle to continue to the next phase of the cell cycle. Thus, ethanol extract of mangosteen peel plays role in inhibiting the signal between cMyc towards Cyclin D with CDK 4/6, as well as between Cyclin E with CDK2 so that the growth signal could not cause

phosphorilation of protein Rb and E2F binding, and the cell cycle cannot be activated.[6,11.]

CONCLUSION

Ethanol extract of mangosteen peel accomplishes its minimum inhibition (IC_{50}) in inhibiting Supri's Clone 1 (SP-C1) human tongue cancer cells proliferation in vitro with the inhibitory value of 50.3% at the concentration of 500 µg/ml. Minimum inhibition of cancer cell proliferation (IC_{50}) of ethanol extract of mangosteen peel was accomplished in the 24-hour incubation period.

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