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RESEARCH ARTICLE

Invitro Antioxidant and Cytotoxic Potential of Ficus carica L. and Olea europeae L. Against Cervical Cancer

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ABSTRACT

Introduction: *Cervical cancer is a malignant infectious disease caused by Human Papilloma Virus (HPV) in the cervix. Fig and olive oil containing flavonoid has been shown to have antioxidant and anticancer activity.* **Objective:** *This study aimed to evaluate antioxidant and cytotoxic activity of combined Ficus carica L. and Olea europeae L. against HeLa cervical cancer cells.*

Methods: *This is an experimental study with a post-test only control group design. The HeLa cells were divided into 5 groups: fig extract, olive oil, combined fig extract and olive oil (at the ratio of 1:3, 1:1, 3:1), positive control (doxorubicin). The cytotoxic and antioxidant activity were evaluated by using MTT Assay and DPPH, respectively. The cytotoxic results were analyzed using probit and antioxidant activity was analyzed by using linear regression to obtain IC_{50} values.*

Results: *The IC_{50} cytotoxic of fig extract, olive oil, combined fig extract and olive oil (at the ratio of 1:3, 1:1, 3:1) with positive control (doxorubicin) were 13063,915 µg/mL, 679,593 µg/mL, 1562,356 µg/mL, 746,923µg/mL, 563,626 µg/mL and 13,707 µg/mL respectively. The IC_{50} antioxidant of fig extract, olive oil, and combination of fig extract and olive oil (3:1) was 105.9272 ppm, 23.1276 ppm, and 21,0689 ppm respectively.*

Conclusion: *The combination of fig extract and olive oil (3:1) was shown to have the highest antioxidant and cytotoxic activity against HeLa cervical cancer cells.*

Keywords: *Cytotoxic, Antioxidant, Fig Extract (Ficus carica L.), Olive Oil (Olea europeae L.), HeLa cells.*

ABSTRAK

Pendahuluan: Kanker serviks adalah penyakit menular ganas yang disebabkan oleh *Human Papilloma Virus* (HPV) di leher Rahim. Buah tin dan minyak zaitun yang mengandung flavonoid telah terbukti memiliki aktivitas antioksidan dan antikanker. **Tujuan:** Penelitian ini bertujuan untuk mengevaluasi aktivitas antioksidan dan sitotoksik kombinasi Ficus carica L. dan Olea europeae L. terhadap sel kanker serviks HeLa.

Metode: Penelitian ini merupakan penelitian eksperimental dengan desain *post-test control group.* Sel HeLa dibagi menjadi 5 kelompok: ekstrak buah tin, minyak zaitun, ekstrak buah tin gabungan dan minyak zaitun (dengan perbandingan 1: 3, 1: 1, 3: 1), kontrol positif (doxorubicin). Aktivitas sitotoksik dan antioksidan masing-masing dievaluasi dengan menggunakan MTT Assay dan DPPH. Hasil sitotoksik dianalisis menggunakan probit dan aktivitas antioksidan dianalisis dengan menggunakan regresi linier untuk mendapatkan nilai IC_{50}.

Hasil: Sitotoksik IC_{50} dari ekstrak buah tin, minyak zaitun, kombinasi buah tin dan minyak zaitun (dengan perbandingan 1:3, 1:1, 3:1) dengan kontrol positif (doxorubicin) adalah 13063.915 µg/mL, 679.593 µg/mL, 1562.356 µg/mL, 746.923µg/mL, 563.626 µg/mL dan 13.707 µg/mL. IC_{50} antioksidan ekstrak tin, minyak zaitun, dan kombinasi ekstrak tin dan minyak zaitun (3:1) masing-masing adalah 105,9272 ppm, 23,1276 ppm, dan 21,0689 ppm.

Kesimpulan: Kombinasi ekstrak buah tin dan minyak zaitun (3:1) terbukti memiliki aktivitas antioksidan dan sitotoksik tertinggi terhadap sel kanker serviks HeLa.

Kata Kunci: Sitotoksik, antioksidan, Buah Tin (Ficus carica L.), Olive Oil (Olea europeae L.), Sel Hela

INTRODUCTION

Figs (Ficus carica L.) containing active compounds such as phenols, terpenoids, flavonoids and alkaloids in vitro have shown to have effect in inhibiting the proliferation of various cancer cells and antioxidant properties (Joseph and Justin Raj, 2011). Research was conducted by Ghanbari et al., (2012) show that olive oil has the main class content of phenol compounds namely phenolic acids, flavonoids, lignans and biophenol which have potential as antioxidants and anticancer.

Cancer is one of the life-threatening diseases in the world. In Indonesia, the prevalence of cancer is the number seven cause of death (Wahyuni et al.,

2015). The prevalence of cervical cancer in Indonesia is in the first rank, followed by breast cancer (Dasar, 2013). Cervical cancer is a malignant disease caused by infection from Human Papilloma Virus (HPV) that occurs around the cervical tissue (Fitriana and Ambarini, 2012). The prevalence of HPV infection as many as 75% occurs in women who have had free sex before marriage (Cancer Foundation of Indonesia, 2017).

Patients infected with HPV are treated with chemotherapy, radiation therapy, or even to undergo surgery (Lui and Grandis, 2012). Chemotherapy can produce very strong toxic effects to cancer cells and can attack normal cells so that they cause adverse side effects (Baqutayan, 2012). Therefore, there is a need for alternative treatments for cervical cancer, one of which uses medicinal plants mentioned in the Qur'an, in the first and second verse of At-Tin which means "For the sake of figs and olives", these figs and olive plants are mentioned together (Ministry of Religion of the Republic of Indonesia, 2014).

In the results of a study by Kichaoui et al., (2016) it was shown that fig fruit (Ficus carica L.) extract contains the compound of Quercetin-3-O-glucoside which has been proven to have an intrinsic mechanism that is capable of reducing HeLa cell proliferation. Likewise, in the study of Yao et al., (2014), it was stated that olive oil extract (Olea europaea L.) contains Oleuropein compounds and intrinsically has a cytotoxic effect on HeLa cells. Because both can potentially inhibit the proliferation of HeLa cervical cancer cells, a research related to the combination of figs and olive oil as anticancer agents is needed, so that they can be used as effective and maximum treatment and expected to have a synergistic and mutual reinforcing mechanism to inhibit proliferation of HeLa cervix cancer cell (Yao et al., 2014). Threfore, aim of the study to evaluate antioxidant and cytotoxic activity of combined Ficus carica L. and Olea europeae L. against HeLa cervical cancer cells

METHODS

Figs Extraction

Extraction was carried out through maceration method using 70% ethanol with a ratio of 1:4 for 5 days, stirred every day. Extracts were separated from the solution by evaporation with temperatures below 50^{o}C until thick extracts were formed and stored at 4^{o}C (Jasmine et al., 2015).

Flavonoid Identification

As many as 1 ml of ethanol extract samples of figs and olive oil were put into the test tube, then added sulfuric acid (H2SO4) 2N as many as 2 drops and strongly shaken. Samples positively contain flavonoids when the solution undergoes a very striking color change to yellow, red or brown (Lisi, Runtuwene and Wewengkang, 2017).

Total Flavonoid Level Test

a. Preparation of Quercetin Raw Curves

As many as 10.0 mg quercetin raw was dissolved using aquadest solvent until the volume limit of the pumpkin volume was 10.0 mL (quercetin level to 1000 µg / mL or 1 mg / mL). Then, standardized curves from mother liquor 1000 µg / mL with 5 different concentrations (20, 40, 60, 80 and 100 µg / mL) were made, added 0.3 ml of 5% NaNO2, added 0.3 ml of AlCl3 10% which was left to stand for 5 minutes, plus 2 ml of 1M NaOH and aquadest until the pumpkin volume limit was 10.0 ml. The raw series is left to the appropriate operating time and measured at maximum wavelength (John et al., 2013).

b. Determination of Total Flavonoid Levels on Fig Extract Fruit (Ficus carica L.) and Olive Oil (Olea europaea L.)

10.0 mg of fig extract (Ficus carica L.) and olive oil (Olea europaea L.) were dissolved with distilled water, then pipetted by 1 ml and added solvents until the volume flask was 10.0 ml (extract content became 1 mg / mL or 1000 µg / mL). The next treatment was the same as the treatment on standard quercetin carried out by triplo method and the total flavonoid content was expressed in units of mg equivalent to quercetin in each gram of extract (John et al., 2014).

Cytotoxic Test

Cells were planted on 96-well plates with 5x103 cells/wells incubated for 24 hours. Cells were treated through samples with a ratio of 1:0; 1:3; 1:1; 3:1; 0:1 for 24 hours. The media were removed and washed with 100µl PBS (Sigma), adding MTT reagent 5 mg / mL to each well and incubated at 37^{o}C for 4 hours. Alive cells can convert MTT reagent to purple formazan after incubation by adding SDS 10% HCL 0.01 N reagent stopper and incubating it for 24 hours. To read absorbance with an ELISA reader is at λ 595 nm (Cancer Chemoprevention Research Center, 2015).

Test of antioxidant activity using the DPPH method

To make a DPPH (1.1 diphenyl-2-picrylhydrazyl) solution of 1000 ppm is done by weighing DPPH 10

mg diluted with ethanol in a 10 mL volumetric flask. Measuring flasks are coated with aluminum foil and stored in a closed cupboard to avoid light. Fig extract, olive oil and a combination of both with comparisons of 3:1 are dissolved using ethanol in a 10 mL volumetric flask, then made in concentration series of 5, 10, 15, 20, 25 mg/ L. It was taken 2 mL from each concentration and added with 2 mL DPPH solution which was placed in a test tube and vortexed for 2 minutes then incubated for 30 minutes. Absorbance was measured at a wavelength of 516 nm with UV-Vis spectrophotometry. Then it is calculated as the percentage of DPPH color reduction by using the equation:

Antidote to free radical activity (%) = $\frac{1-\textit{Sample Absorbamce}}{\textit{Control Absorbance}}$x100% (Munte, Runtuwene and Citraningtyas, 2015)

Data analysis of cytotoxic test was using probit analysis to obtain IC_{50} values of samples that showed a percentage of culture death cell as much as 50%. Then, Data analysis of antioxidant test was using linear regression.

RESULTS

The results of the determination showed that the fig was a family of Moraceae and a species of Ficus

Table 1. Phytochemical Screening Test of Fig Fruit Extract (Ficus carica L.) and Olive Oil (Olea europeae L.)

No	Parameter Test	Reagent	Color	Method	Description
1.	Flavonoid of Fig Fruit extract	H_2SO_4 2N	Dark Brown	Tube Test	Positive 
2.	Flavonoid of Olive Oil	H_2SO_4 2N	Dark Brown	Tube Test	Positive 

Table 2. Total Flavonoid Content of Fig Fruit Extract and Olive Oil

Sample	Replication	Absorbance	Flavonoid Substance (mg/g QE)	Mean (mg/g QE)	SD
Fig Fruit Extract	1	0.3911	177.9661	179.3220	1.2222
	2	0.3921	179.6610		
	3	0.3925	180.3390		
Olive Oil	1	0.4204	227.6271	231.1864	11.6851
	2	0.4169	221.6949		
	3	0.4302	244.2373		

Tabel 3. IC50 value of figs extract, olive oil and a combination of both by comparison (1:3, 1:1, 3:1) to HeLa cervical cancer cells.

Concentration Comparison (Fig Fruit Extract : Olive Oil)	IC_{50} Cytotoxic (µg/mL)
1 : 0	13063.915
0 : 1	679.593
1 : 3	1562.356
1 : 1	746.923
3 : 1	563.626
Doxorubicin Positive Control	13.707

Tabel 4. IC_{50} value of figs eact, olive oil and combination of both with a ratio of 3:1

Replication	IC_{50} (ppm)		
	Fig Fruit Extract	Olive Oil	Fig Fruit Extract : Olive Oil (3:1)
I	106.4570	23.1326	21.0963
II	106.5558	23.2721	21.0642
III	104.7688	22.9780	21.0462
Mean	105.9272	23.1276	21.0689
SD	1.0045	0.1471	0.0254
Mean ± SD	105.9272 ± 1.0045	23.1276 ± 0.1471	21.0689 ± 0.0254

carica L. and olive oil was a family of Oleaceae and a species of Olea europeae L.

The results of figs extraction were obtained as many as 318.70 grams with extract water content values of 6.08%. The results of phytochemical test analysis of figs and olive oil extracts have flavonoids. The phytochemical test results are presented in the Table 1. Test results for total flavonoids levels are presented in Table 2.

Cytotoxic activity tests of figs and olive oil extracts and a combination of both were carried out to determine the inhibitory activity of HeLa cervical cancer cells using the MTT assay method. The cytotoxic test results are presented in table 3.

Determination of antioxidant activity of figs, olive oil and combination of both extracts with a ratio of 3:1 was carried out by measuring IC50 values using the DPPH method. The IC50 test results of antioxidant activity are presented in Table 4.

Determination of the combination index of fig fruit and olive oil with a ratio of 1: 3, 1: 1, and 3: 1 was carried out to see the potential synergistic effects of a combination using the IK = (D) 1 / (Dx) 1 + (D) equation 2/(Dx) 2. The results of the combination of figs and olive oil extracts with a ratio of 1: 3, 1: 1, and 3: 1 were 2.42; 1.16; and 0.87 respectively.

DISCUSSIONS

The results of the determination showed that the samples of figs from the species of Ficus carica L. and olive oil from the type of Olea europaea L contribute thick extract as many as 318.70 grams, so that the percentage of yield was 58.34%. The fig extract was obtained with a moisture content of 6.08% with a requirement of 5-30% thick extract of water content (Voigt, 1994).

The qualitative test of flavonoids was carried out using a tube method which aims to ensure the presence of flavonoids contained in figs and olive oil extract. Figs and olive oil extracts showed positive flavonoids with striking color changes from orange to brown (Puspa et al., 2017).

The average of total flavonoid content of olive oil is 231.19 mg/g QE and the results of the total flavonoid content of fig extract is 179.32 mg/g QE. From the results of the two samples, the results of the total flavonoid levels above can be seen that the highest total flavonoid levels are found in olive oil.

According to (Tiwary et al., 2015), the potential of an extract is said to have cytotoxic activity if the IC_{50} value <1000 µg / mL and no cytotoxic activity if the IC_{50} value is> 1000 µg/mL. Cytotoxic tests on HeLa cervical cancer cells obtained IC_{50} values in figs, olive oil and combination of both had significantly different results. In the cytotoxic test results, it was obtained from a combination of fig and olive oil in a ratio of 3:1 and it had cytotoxic activity when compared to extracts of figs which did not have cytotoxic activity. This is believed to be related to antioxidant activity from a combination of figs extract and olive oil.

Antioxidant activity showed that the combination of figs and olive oil with a ratio of 3:1 had the highest antioxidant activity because the IC_{50} values obtained belonging to a very strong antioxidant and if the IC_{50} value is <50 ppm (Garcia-Davis et al., 2019). Antioxidant activity in fig extract belongs to moderate antioxidants because the IC_{50} value is in the range of 101-150 ppm, and the IC_{50} value of olive oil has antioxidant activity which is classified as very strong because it has an IC_{50} value of <50 ppm (Garcia-Davis et al., 2019). This is in line with the research conducted by Ouchemouck et al., (2017) which stated that the flavonoid compounds contained in Ficus carica have no effect on cytotoxic tests in concentrations tested but have antioxidant activity. This is supported by the results of the test for total flavonoid content of olive oil of 231.18 mg/g QE and fig extract of 179.32 mg/g QE.

The combined index value of a combination

sample of fig extract and olive oil in a ratio of 1:3 has an antagonistic effect because it enters into a combination index range of 1.45-3.3. At a ratio of 1:1, it has a mild to moderate antagonistic effect because it enters the combination index range from 1.1 to 1.45. At a ratio of 3:1, it has a mild to moderate synergistic effect because it enters into a combination index range of 0.7-0.9 (Suttirak and Manurakchinakorn, 2014), so it can be interpreted that the combination of fig and olive oil extract in a ratio of 3:1 has synergistic effects and increase cytotoxic activity against HeLa cervical cancer cells when combined.

CONCLUSIONS

This study showed the presence of antioxidant activity and cytotoxic power on a combination of fig extract (Ficus carica L.) and olive oil (Olea europaea L.) on HeLa cervical cancer cells (21,0689 ppm and 563,626 µg / mL). The combination of fig extract (Ficus carica L.) and olive oil (Olea europaea L.) in a ratio of 3: 1 has a synergistic effect, cytotoxic and a very strong antioxidant activity in inhibiting the proliferation of HeLa cervical cancer cell.

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