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Cytotoxicity of quercetin and curcumin combination against HeLa cells line

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ABSTRACT

Curcumin and quercetin combination are potential candidates for anticancer. Previous studies reported this combination active against several cancer cells including breast cancer, myeloid leukemia, and melanoma. However, the activity of this combination against cervical cancer has not been reported, yet. This study aimed to evaluate the cytotoxicity of the combination of curcumin and quercetin against HeLa and Vero cells. MTT assay was applied to evaluate cell growth inhibition. The inhibitory 50% concentration (IC_{50}) of curcumin and quercetin on HeLa and Vero cells was determined and the selectivity index (SI) was then calculated. Combination index (CI) was calculated after evaluation of the cytotoxicity of the curcumin and quercetin combination at various concentration of 1 IC_{50}, ½ IC_{50}, and ¼ IC_{50}. The IC_{50} of the curcumin against the HeLa and Vero cells were 26.57 ± 2.00 µM and 73.89 ± 12.93 µM with a SI of 2.78. Whereas the IC_{50} of the quercetin against the HeLa and Vero cells were 149.52 ± 21.09 µM and 1094.47 ± 15.68 µM with a SI of 7.32. The CI of combinations of curcumin and quercetin at concentrations of ¼ IC_{50} was 0.78 indicating a mild-moderate synergistic effect, whereas the combination at a concentration of 1 IC_{50} or ½ IC_{50}, the CI value was >0.9 indicating an antagonistic effect.

ABSTRAK

Kombinasi kurkumin dan kuersetin merupakan kandidat potensial antikanker. Penelitian sebelumnya melaporkan kombinasi ini aktif melawan beberapa sel kanker termasuk kanker payudara, leukemia myeloid, dan melanoma. Namun, aktivitas kombinasi ini melawan kanker serviks belum dilaporkan. Penelitian ini bertujuan untuk mengevaluasi sitotoksisitas kombinasi kurkumin dan kuersetin terhadap sel HeLa dan Vero. Uji MTT dilakukan untuk mengevaluasi penghambatan pertumbuhan sel. Konsentrasi penghambatan 50% (IC_{50}) kurkumin dan kuersetin pada sel HeLa dan Vero ditetapkan untuk menghitung indeks selektivitas (SI). Indeks kombinasi (CI) dihitung setelah evaluasi sitotoksisitas kombinasi kurkumin dan kuersetin pada berbagai konsentrasi yaitu 1 IC_{50}, ½ IC_{50}, dan ¼ IC_{50}. Nilai IC_{50} kurkumin terhadap sel HeLa dan Vero adalah 26,57 ± 2,00 µM dan 73,89 ± 12,93 µM dengan nilai SI sebesar 2,78. Sedangkan IC_{50} kuersetin terhadap sel HeLa dan Vero adalah 149,52 ± 21,09 µM dan 1094,47 ± 15,68 µM dengan nilai SI sebesar 7,32. Nilai CI kombinasi kurkumin dan kuersetin pada konsentrasi ¼ IC50 sebesar 0,78 menunjukkan efek sinergis ringan-sedang, sedangkan kombinasi pada konsentrasi 1 IC50 atau ½ IC50 nilai CI >0,9 menunjukkan adanya efek antagonis.

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INTRODUCTION

Cervical cancer has the highest mortality rate among cancers, accounting for 60% of all reported cases. In 2018, the incidence of cervical cancer ranked second only to breast cancer, with 348,809 reported cases and 207,210 casualties. Indonesia sees an estimated 180,000 new cancer cases annually, with a staggering 75% mortality rate.[1] Various treatments have been implemented for cancer patients, including surgery, radiation, chemotherapy, and the use of herbal medicine. The advancement of herbal remedies involves rigorous testing through *in vitro, in vivo,* and *in silico* methods to scrutinize active compounds and their respective mechanisms of action.[2]

Secondary metabolites of medicinal plants have been widely reported to have cytotoxic activity, namely quercetin, curcumin,[3] and rutin.[4] Even some of them have been successfully developed as anticancer and used in clinics such as vincristine, and vinblastine.[5] Curcumin and quercetin were combined against breast cancer cells due to their synergistic effect.[6] Moreover, through its antioxidant effects, this combination can modulate several inflammation-mediated signaling pathways (reactive oxygen species/ROS, nitric oxide, and pro-inflammatory cytokines) thereby helping to protect cells from molecular changes that promote HDFB cell migration.[7] However, the combination of curcumin and quercetin has not been studied for its cytotoxicity against HeLa cells.

The anticancer activity of curcumin and quercetin compounds individually is based on their activity as antioxidants, immune system enhancers, as well as antiproliferation and apoptosis promoters. Curcumin is able to inhibit metastasis and invasion of cervical cancer cells so that it can prevent spread to other organs.[8] However, curcumin is also reported to be toxic to normal cells. Therefore, its combination with quercetin could reduce its concentration and the toxic effect on normal cells.

Based on their IC_{50} value against cancer cells, the cytotoxic activity of a compound can be categorized into 4 groups. A compound can be categorized to have high cytotoxic activity if it has IC_{50} value of ≤ 20 µg/mL, moderate cytotoxic activity if it has an IC_{50} value between 21-200 µg/mL, weak cytotoxic activity if it has an IC_{50} value between 201-500 µg/mL, and no cytotoxic activity if it has an IC_{50} value of >501 µg/mL.[9]

The effectiveness of a combination of compounds as an anticancer can be evaluated based on its combination index (CI) value. A combination of two compounds can produce synergistic or antagonistic effects depending on the CI value obtained in an experiment. The combination is considered as synergism potential if the CI value of <1, additive effect if the CI value of 1, and antagonism if the CI value of >1.[10]

The combination of quercetin and curcumin has been reported in previous studies. This combination proved can reduce the proliferation of breast cancer cells,[3] myeloid leukemia cells,[11] and melanoma cells.[12] However, the effect of the combination of curcumin and quercetin on cervical cancer has not been studied. This study aimed to evaluate the effect of the combination of quercetin and curcumin on HeLa cells. Furthermore, the selectivity index (SI) of this combination was also evaluated.[13]

MATERIALS AND METHODS

Materials

This study was conducted after approval by the Ahmad Dahlan University Research Ethics Committee (Ethical Clearance number 012212203). The ingredients in this study were curcumin (MERCK-Schuchardt), quercetin (Sigma-Aldrich), FBS (Fetal Bovine Serume, Gibco), stopper solution in the form of SDS (UltraPure®) 10% in 0.1 N HCl, Trypsin-EDTA 0, 25% (Gibco),

MTT reagent [3-94,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] (Vybrant®), PBS solution pH 7.4 (Gibco), DMEM, Dimethyl Sulfoxide (DMSO), Penstrep, MTT, and HeLa cells obtained from the Faculty of Medicine and Health Sciences, Universitas Muhammadiyah, Yogyakarta.

Equipment

The tools in this research were LAF (Labconco®), ELISA reader (Bio-Rad®), CO_2 incubator (Heraceus®), blue tip, inverted microscope (Zeiss®), conical (Nunclone®), analytical balance (Sartorius®), yellow tip, micropipette (Gilson®), glassware (Pyrex®), 96-well plate (Nunc®), centrifuge (Sorvall®), tissue culture dish, Eppendorf tube, vortex (Labinco® L46), and hemocytometer (Nebauer®).

Cytotoxic test against HeLa cells

The 3.68 mg of curcumin was dissolved in 1 mL of DMSO in an Eppendorf tube with the help of a vortex to obtain a concentration of 10,000 μM. A total of 3.02 mg of quercetin was dissolved in 1 mL of DMSO in another Eppendorf tube with the help of a vortex to obtain a concentration of 10,000 μM.[14]

The cytotoxic test was carried out by inserting 100 μL of $5x10^3$ HeLa cells per plate into DMEM media and then incubating at 37 °C for 24 hr. Curcumin and quercetin with different concentrations were added 100 μL to each well in the plate and incubated at 37°C overnight in humidified conditions of 5% CO_2/95% atmospheric air. The final concentrations of curcumin in the plate were 50, 25, and 12.5 μM. The final concentrations of quercetin were 400, 100, and 50 μM. The plate was then removed from the incubator and washed with PBS. 100 μl of 0.5 mg/mL MTT reagent was added to each well containing the treatment sample and incubated at 37°C for 2-4 hr. Living cells will show a purple color because they react with MTT. The condition of the cells was observed using an inverted microscope, to stop the MTT reaction, 100 μL of 10% SDS in 0.01 N HCl was added as a stopper reagent to each well. The plate was incubated at room temperature overnight, and wrapped in aluminum foil. Cell absorbance was read using an ELISA reader and the IC_{50} value was calculated as a cytotoxic parameter.[15]

Cytotoxic test against Vero cells

Determination of cytotoxic activity was carried out by transferring 100 μL of Vero cells into wells so that there were $5x10^3$ in each well and then incubating for 24 hr at 37°C. The condition of cell distribution was checked using an inverted microscope. If the cells were 80% confluent, remove the medium from each well and wash with PBS. Curcumin and quercetin at different concentration series were added 100 μL to each well in the plate and incubated at 37°C overnight in humidified conditions of 5% CO2/95% atmospheric air. Testing on Vero cells with the final concentration on the plate for curcumin and quercetin at a concentration of 25; 50; 100; and 200 μM. The plate was then removed from the incubator and washed with PBS.[15]

The plate was then removed from the incubator and washed with PBS. 100 μL of MTT reagent was added to each well containing the treatment sample and incubated at 37 °C for 2-4 hr. Living cells will show a purple color because they react with MTT. To stop the reaction, 100 μL of SDS 10% stopper reagent was added to each well. The plate was incubated at room temperature overnight, and wrapped in aluminum foil. Cell absorbance is read using an ELISA reader, and the selectivity index value is calculated as a parameter for the safety level of an anticancer agent.[16] The cytotoxic test parameter in the form of IC_{50} is obtained from the relationship equation between log concentration vs percent cell viability, by entering the value 50 in y from this equation to get the x value. Next, the value of x is

determined by its antilog to obtain a concentration that inhibits cell death by 50% (IC$_{50}$).

Determination of selectivity index (SI)

The SI value reflects how optimal the sample frequency is for the test cell. If the SI value exceeds 2, it indicates that the substance has a high level of selectivity against cancer cells.[13] The SI is calculated by the following equation:

$$SI = \frac{IC_{50}\,normal\;cell}{IC_{50}\,cancer\;cell} \quad (1)$$

Determination of combination index (CI)

Determination of the CI was carried out by transferring 100 µl of HeLa cells into 5x10^3 wells. Next, the cells were incubated at 37 °C for 24 hr. When the cells reached 80% confluence, the medium in each well was discarded and the cells were washed with PBS. Sample treatment in the CI test was carried out by adding 50 µL of curcumin and quercetin each with a concentration of 1 IC$_{50}$, ½ IC$_{50}$, ¼ IC$_{50}$ per well (TABLE 1). The next steps were the same as the cytotoxic test.

The CI is determined from a formula that contains the combination concentration, which produces a cell viability value produced by a concentration that is equivalent to a single concentration. The data started from the absorbance of the test results for a combination of curcumin and quercetin in TABLE 1 of 50 µL each. The formula used to find the CI is:

$$CI = \frac{(C)_1}{(Cx)_1} + \frac{(C)_2}{(Cx)_2} \quad (2)$$

$(Cx)_1$ and $(Cx)_2$ are the combined concentrations that provide the same effect as single concentrations, namely curcumin and quercetin, and C_1, C_2 are the concentrations of the combination of the two compounds to provide the same effect.[13]

TABLE 1. Concentrations of curcumin and quercetin added to the combination test with a volume 50 µL each

Value of IC$_{50}$	Concentration (µM)	
	Curcumin	Quercetin
1 IC$_{50}$	26	150
½ IC$_{50}$	13	75
¼ IC$_{50}$	6.5	37.5

RESULTS

Cytotoxicity single curcumin and quercetin on HeLa and Vero cells

The cytotoxicity test of curcumin and quercetin against HeLa and Vero cells was performed using a MTT assay. The morphological changes of the HeLa and Vero cells after 24 h incubation with curcumin and quercetin under microscopic examination are shown in FIGURE 1. The relationship between the log concentration of curcumin or quercetin vs. % viability of the HeLa and Vero cells is shown in FIGURE 2.
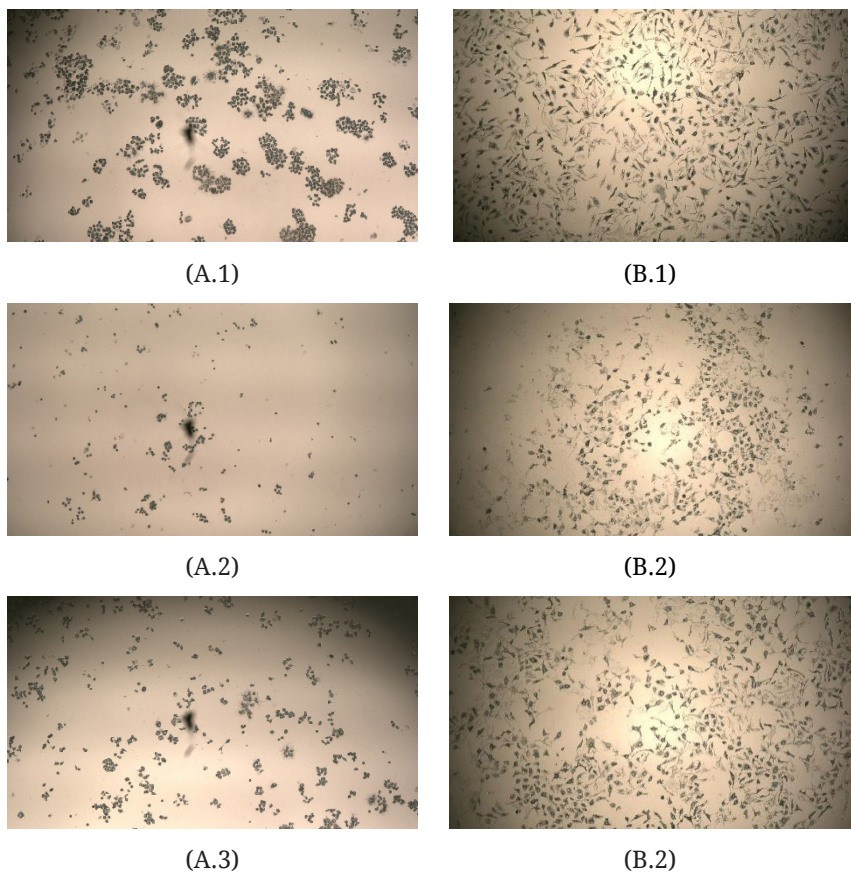
(A.1)

(B.1)

(A.2)

(B.2)

(A.3)

(B.2)

FIGURE 1. Microscopic photographs of the morphological changes of cells induced by curcumin and quercetin after 24 hr treatment and comparison with untreated controls. A. HeLa cells and B. Vero cells: 1. Control; 2. Curcumin 50 μM; 3. Quercetin 50 μM.
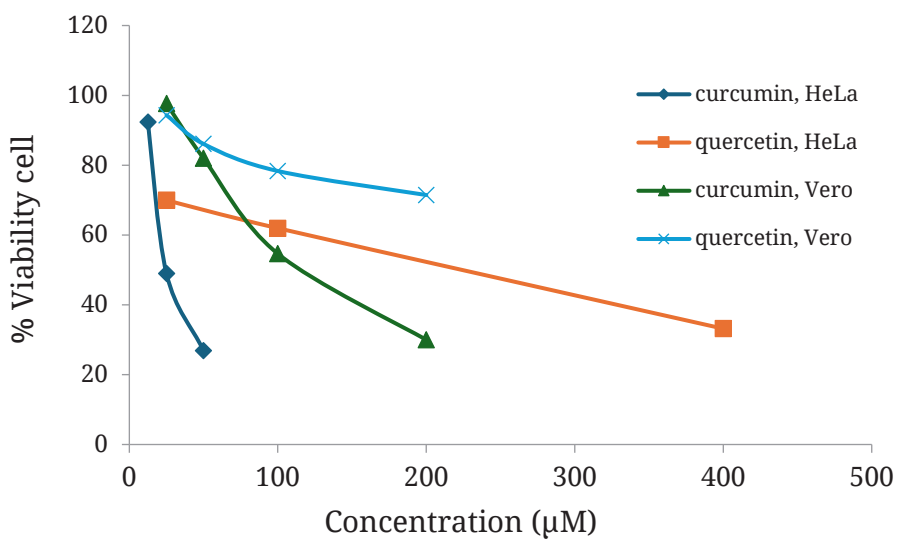

FIGURE 2. Viability of HeLa and Vero cells after 24 hr incubation with curcumin and quercetin in various concentration.

Based on the relationship between the log concentration vs. % of the cell viability, the IC_{50} of curcumin and quercetin was calculated. The IC_{50} values of the curcumin against the HeLa and Vero cells were 26.57 ± 2.00 μM and 73.89 ± 12.93 μM, respectively, whereas the IC_{50} values of the quercetin against the HeLa and Vero cells were 149.52 ± 21.09 μM and 1094.47 ± 15.68 μM, respectively (TABLE 2).

TABLE 2. IC_{50} of quercetin and curcumin against HeLa and Vero cells

Sample	Cells	IC_{50} (mean ±SD **μM**)	SI
Curcumin	HeLa	26.57 ± 2.00	2.78
Curcumin	Vero	73.89 ±12.93	
Quercetin	HeLa	149.52 ± 21.09	7.32
Quercetin	Vero	1094.47 ±15.68	

Selectivity index assay

The IC_{50} value of the tested compounds was then used to determine their SI. The selectivity index results for curcumin and quercetin in HeLa cells and Vero cells were 2.78 and 7.32 (TABLE 2).

Combination test

The CI determination was carried out at concentrations of 1 IC_{50}, ½ IC_{50}, and ¼ IC_{50} with a volume of 50uL each of curcumin and quercetin (TABLE 1). The results showed changes in the morphology of HeLa cells due to changes in concentration, namely that they appeared rounded and separated from the tissue culture dish at a ratio of ¼ IC_{50}. Percent of cell viability was calculated using the MTT method based on the uptake of living cells by the influence of combinations of curcumin and quercetin at various concentrations at IC_{50} and below IC_{50} (TABLE 3). The CI values obtained from 9 combinations of curcumin and quercetin in HeLa cells at concentrations of ¼ IC_{50} curcumin (6.5 μM) and quercetin (32.5 μM) was 0.78 indicating a mild-moderate synergistic effect. Whereas the combination of curcumin and quercetin at a concentration of IC_{50} or ½ IC_{50}, the CI value was >0.9 indicating an antagonistic effect.

TABLE 3. The CI of curcumin and quercetin

Compound		Quercetin consentration (μM)		
		150	75	32.5
Curcumin consentration (μM)	26	1.52	1.51	1.25
	13	1.3	1.2	1.08
	6.5	1.33	1.08	0.78

DISCUSSION

Cytotoxicity single curcumin and quercetin on HeLa and Vero cells

The IC_{50} value of curcumin and quercetin a HeLa cells and in normal cells (Vero cells) was carried out to calculate the SI. The results showed that curcumin has moderate cytotoxicity on both HeLa cells (IC_{50} of 26.57 ± 2.00 μM) and Vero cells (IC_{50} of 73.89 ± 12.93 μM),[9] although curcumin is more toxic on HeLa cells compared to Vero cells (TABLE 2). The cytotoxicity of curcumin is due to active α-β unsaturated carbonyl bonds in MCF-7, HeLa, and CEMss cells. Curcumin can reduce cell proliferation by regulating the cell cycle in the mitosis phase.[16] In line with curcumin, quercetin also has unsaturated α-β bonds carbonyl, which has activity in MCF-7 cells and HeLa cells. Quercetin has anticancer activity by reducing metastasis and inhibiting proliferation. This is because quercetin has $C_{3'4'}$ dihydroxy, C_{2-3} unsaturated carbonyl, as well as C_3 and C_5 hydroxy, which have antioxidant activity. The result of this activity is inhibition of telomerase, thereby reducing cell proliferation.[17]

The cytotoxic activity of curcumin and quercetin through induction of apoptosis by damaging cancer cell DNA encourages research on the synergy of this combination.[6] This is in line with the

potential of the flavonoid in this research, namely quercetin, to be combined with cytotoxic compounds. Another flavonoid, namely naringenin, has potential as an adjuvant because its structure is rich in chromophores.[18] Likewise, curcumin has the potential to act as an anti-cancer agent when combined with other cytotoxic compounds, which are synergistic.[19]

Selectivity index assay

These results indicate that curcumin and quercetin have a high level of selectivity against HeLa cells (TABLE 2).[20] Curcumin has high safety in Vero cells, as indicated by a SI value most 3 (2.78). The IC_{50} results of curcumin and quercetin on Vero cells show that although single curcumin has an effect on HeLa cells, it also has an IC_{50} that is toxic to Vero (normal) cells. This supports the need to combine it with co-chemotherapy, in this case, quercetin. The CI is used to evaluate whether the combination is synergistic, additive, or antagonistic at a certain concentration.

Combination test

The CI value provides information concerning the interaction of two compounds as synergic, additive, or antagonistic. This study showed that the interaction of curcumin and quercetin at small concentration (¼ IC_{50}) provide a synergistic effect. In contrast, at higher concentration (1 IC_{50}, ½ IC_{50}) provide an antagonistic effect (TABLE 3).

Curcumin and quercetin single have various pathways in induce of apoptosis in cancer cells.[21,22] The molecular mechanism of anticancer combination of curcumin and quercetin is through the Wnt/β-catenin pathway, which can produce various types of genes, some of which are cyclooxygenase 2 (COX_2) and cyclin D1. In various types of tumors, it is found that cyclin D1 is overexpressed so that it can facilitate cells from the Gap1 phase to the synthesis (S) phase. Curcumin and quercetin are known to

influence the regulation of cyclin D1 and Axin2, which can then reduce β-catenin, thereby suppressing cell proliferation genes.[11]

Combination of quercetin and curcumin increased BRCA1 histone H3K9 promoter acetylation. Quercetin regulates the expression levels of proinflammatory agents such as COX_2 and controls histone acetylation. Quercetin can also inhibit β-catenin expression. Cell death via apoptosis can be triggered via receptor-mediated signaling pathways or via mitochondria. Drugs with chemotherapy or other stimulant effects can change the inner properties of the mitochondrial membrane and cause the mitochondrial membrane to lose its potential and release cytochrome c from the mitochondria into the cytosol.[6]

The combination of curcumin and quercetin induces apoptosis through the mitochondrial pathway and inhibits AKT and ERK phosphorylation.[23] Similar research shows that quercetin and curcumin can trigger apoptosis through the mitochondrial pathway, making them potentially anticancer. Further research can be carried out to determine the mechanism of cell death by the combination of curcumin and quercetin at a ratio of 6.5 µM curcumin and 32.5 µM quercetin, as well as track the IC_{50} of the combination on the synergistic effect.

A limitation of this study is that the IC_{50} of the combination of curcumin and quercetin was not determined after determining the CI. It is necessary to determine the IC_{50} of the combination to determine the level of toxicity obtained after the combination. To find out more about the mechanisms and pathways of cell death, it is also necessary to test using immunocytochemical or flow cytometer methods.

CONCLUSION

The combination of curcumin and quercetin higher than ¼ IC_{50} has antagonistic activity with a CI value >0.9, whereas at concentrations lower than ¼

IC$_{50}$, has a mild to moderate synergistic level with a CI value of 0.78. The curcumin and quercetin combination at this concentration have cytotoxic activity against HeLa cells with high SI.

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REFERENCES

1. Agustiansyah P, Sanif R, Nurmaini S, Irfannuddin, Legiran. Epidemiology and risk factors for cervical cancer. Bioscientia Medicina: J Biomed Trans Res 2021; 5(7):624-31.
   https://doi.org/10.32539/bsm.v5i7.326

2. Rani DM, Wongso H, Purwoko RY, Winarto NB, Shalas AF, Triatmoko B, *et al.* Anti-cancer bioprospecting on medicinal plants from Indonesia: A review. Phytochemistry 2023; 216,113881.
   https://doi.org/10.1016/j.phytochem.2023.113881

3. Mansourizadeh F, Alberti D, Bitonto V, Tripepi M, Sepehri H, Khoee S, *et al.* Efficient synergistic combination effect of quercetin with curcumin on breast cancer cell apoptosis through their loading into Apo ferritin cavity. Colloids Surf B Biointerfaces 2020; 191:110982.
   https://doi.org/10.1016/j.colsurfb.2020.110982

4. Farha AK, Gan RY, Li HB, Wu DT, Atanasov AG, Gul K, *et al.* The anticancer potential of the dietary polyphenol rutin: Current status, challenges, and perspectives. Crit Rev Food Sci Nutr 2018; 62(3):832–59.
   https://doi.org/10.1080/10408398.2020.1829541

5. Lichota A, Gwozdzinski K. Aktivitas antikanker senyawa alam dari lingkungan tumbuhan dan laut. Int J Mol Sci 2018;19(11):3533.
   https://doi.org/10.3390/ijms19113533

6. Kundur S, Prayag A, Selvakumar P, Nguyen H, McKee L, Cruz C, *et al.* Synergistic anticancer action of quercetin and curcumin against triple-negative breast cancer cell lines. J Cellular Physiol 2018; 234(7):11103-18.
   https://doi.org/10.1002/jcp.27761

7. Chittasupho C, Manthaisong A, Okonogi S, Tadtong S, Samee W. Effects of quercetin and curcumin combination on antibacterial, antioxidant, *in vitro* wound healing and migration of human dermal fibroblast cells. Int J Mol Sci 2022; 23(1):142.
   https://doi.org/10.3390/ijms23010142

8. Zhang X, Zhu L, Wang X, Zhang H, Wang L, Xia L. Basic research on curcumin in cervical cancer: Progress and perspectives. Biomed Pharmacother 2023;162:114590.
   https://doi.org/10.1016/j.biopha.2023.114590

9. Sajjadi SE, Mustafa G, Mehrangiz H, Leili M. Cytotoxic effect of *Cousinia verbascifolia* Bunge against OVCAR-3 and HT-29 cancer cells. J Herb Med Pharmacol 2015; 4(1):15-9.

10. Zhang N, Fu JN, Chou TC. Synergistic combination of microtubule targeting anticancer fludelone with cytoprotective panaxytriol derived from panax ginseng against MX-1 cells *in vitro*: experimental design and data analysis using the combination index method. Am J Cancer Res 2016; 6(1):97-104.

11. Altundag EM, Yilmaz AM, Serdar BS, Jannuzzi AT, Koçtürk S, Yalçın AC. Synergistic induction of apoptosis by quercetin and curcumin in chronic myeloid leukemia (K562) cells: II. Signal transduction pathways involved. Nutr Cancer 2021; 73(4):703–12.
    https://doi.org/10.1080/01635581.2020.1767167

12. Srivastava NS, Srivastava RAK. Curcumin and quercetin synergistically inhibit cancer cell

proliferation in multiple cancer cells and modulate Wnt/β-catenin signaling and apoptotic pathways in A375 cells. Phytomedicine 2019; 52:117–28. https://doi.org/10.1016/j.phymed.2018.09.224

13. López-Lázaro M. A simple and reliable approach for assessing anticancer activity *in vitro.* Curr Med Chem 2015; 22(11):1324-34. https://doi.org/10.2174/09298673226 66150209150639

14. Purba AKRP, Mustofa, Astuti I. Synergistic interaction between quercetin and doxorubicin on MCF-7 human breast cancer cell line. J Med Sci 2013; 45(3):120-6. https://doi.org/10.19106/JMedScie004503201303

15. Mutiah R, Suryadinata A, Nurani PS. Uji sitotoksik kombinasi cisplatin dengan ekstrak etanol benalu alpukat (*Dendrophthoe pentandra* (L) Miq.) pada hela. Majalah Kesehatan 2018; 5(3):133-43. https://doi.org/10.21776/ub.majalahkesehatan.005.03.2

16. Hossain M, Das U, Dimmock JR. Recent advances in α,β-unsaturated carbonyl compounds as mitochondrial toxins. Eur J Med Chem 2019; 183:111687. https://doi.org/10.1016/j.ejmech.2019.111687

17. Jaco, VR. Structure-activity and mechanistic investigations into the cytotoxicity of flavonols in cancer cells. [Thesis]. Stellenbosch: Stellenbosch University 2022; 1-232.

18. Memariani Z, Abbas SQ, ul Hassan SS, Ahmadi A, Chabra A. Naringin and naringenin as anticancer agents and adjuvants in cancer combination therapy: efficacy and molecular mechanisms of action, a comprehensive narrative review. Pharmacol Res 2021; 171:105264. https://doi.org/10.1016/j.phrs.2020.105264

19. Paul S, Sa G. Curcumin as an adjuvant to cancer immunotherapy. Front Oncol 2021; 11:675923. https://doi.org/10.3389/fonc.2021.675923

20. Septembre-Malaterre A, Boumendjel A, Seteyen AS, Boina C, Gasque P, Guiraud P, Sélambarom J. Focus on the high therapeutic potentials of quercetin and its derivatives. Phytomed Plus 2022; 2(1):100220. https://doi.org/10.1016/j.phyplu.2022.100220

21. Mortezaee K, Salehi E, Mirtavoos-Mahyari H, Motevaseli E, Najafi M, Farhood B, Rosengren RJ, Sahebkar A. Mechanisms of apoptosis modulation by curcumin: implications for cancer therapy. J Cell Physiol 2019; 234(8):12537-50. https://doi.org/10.1002/jcp.28122

22. Hashemzaei M, Delarami Far A, Yari A, Heravi RE, Tabrizian K, Taghdisi SM, *et al.* Anticancer and apoptosis-inducing effects of quercetin *in vitro* and *in vivo.* Oncol Rep 2017; 38(2):819-28. https://doi.org/10.3892/or.2017.5766

23. Zhang JY, Lin MT, Zhou MJ, Yi T, Tang YN, Tang SL, *et al.* Combinational treatment of curcumin and quercetin against gastric cancer MGC-803 cells *in vitro.* Molecules 2015; 20(6):11524-34. https://doi.org/10.3390/molecules200611524