

Antiproliferative Activities of Parthenolide and Golden Feverfew Extract Against Three Human Cancer Cell Lines

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ABSTRACT The medicinal herb feverfew [*Tanacetum parthenium* (L.) Schultz-Bip.] has long been used as a folk remedy for the treatment of migraine and arthritis. Parthenolide, a sesquiterpene lactone, is considered to be the primary bioactive compound in feverfew having anti-migraine, anti-tumor, and anti-inflammatory properties. In this study we determined, through *in vitro* bioassays, the inhibitory activity of parthenolide and golden feverfew extract against two human breast cancer cell lines (Hs605T and MCF-7) and one human cervical cancer cell line (SiHa). Feverfew ethanolic extract inhibited the growth of all three types of cancer cells with a half-effective concentration (EC₅₀) of 1.5 mg/mL against Hs605T, 2.1 mg/mL against MCF-7, and 0.6 mg/mL against SiHa. Among the tested constituents of feverfew (*i.e.*, parthenolide, camphor, luteolin, and apigenin), parthenolide showed the highest inhibitory effect with an EC₅₀ against Hs605T, MCF-7, and SiHa of 2.6 μg/mL, 2.8 μg/mL, and 2.7 μg/mL, respectively. Interactions between parthenolide and flavonoids (apigenin and luteolin) in feverfew extract also were investigated to elucidate possible synergistic or antagonistic effects. The results revealed that apigenin and luteolin might have moderate to weak synergistic effects with parthenolide on the inhibition of cancer cell growth of Hs605T, MCF-7, and SiHa.

KEY WORDS: • antiproliferative activity • apigenin • breast cancer • feverfew • flavonoid • Hs605T • luteolin • MCF-7 • parthenolide • SiHa • *Tanacetum parthenium*

INTRODUCTION

SESQUITERPENE LACTONES (SQLs) are secondary metabolites found in most species of Compositae (Asteraceae) and in at least 14 angiosperm families. There are more than 4,000 SQLs with known structures.¹ In the 1960s, parthenolide was first reported as a new SQL from feverfew (*Tanacetum parthenium*), with the initial structure later revised as shown in Figure 1.² Parthenolide was believed to be the primary bioactive compound in feverfew,^{3–7} a medicinal herb used in prophylactic treatment for migraine with positive therapeutic effects in clinical trials.^{8–10}

One of the most important characteristics of SQLs is their anti-tumor activity.^{1,11,12} A number of naturally occurring SQLs with the α -methylene- γ -lactone (α -methylenenbutyrolactone) structure, such as parthenolide and helenalin, were reported to have potent cytotoxic activity towards nor-

mal human fibroblast, human laryngeal carcinoma, and human cells transformed with Simian Virus 40.¹³ Specifically, parthenolide at a concentration of 2.3 μg/mL had cytotoxic activity against the human epidermoid carcinoma of the nasopharynx.¹⁴ At concentrations above 5.0 μM and an exposure time of 24 hours, parthenolide irreversibly inhibited cell growth of mouse fibrosarcoma (MN-11) and human lymphoma (TK6) cell lines, but the effect was reversible at lower concentrations.¹⁵ In addition, parthenolide triggered apoptosis in invasive sarcomatoid hepatocellular carcinoma cells (SH-J1) and in ordinary hepatoma cells at 5–10 μM. Cell growth was arrested at sublethal concentrations (1–3 μM).¹⁶ However, hydrogenation of the conjugated α -methylene- γ -lactones, including parthenolide, produced inactive compounds.¹³ Moreover, further studies proposed that the anti-tumor activity of SQLs might occur at the DNA replication level.^{17–20}

Recent studies have revealed that the anti-tumor activity of parthenolide may be related to the suppression of T-cells from activation-induced cell death,²¹ or the inhibition of the transcription factor, nuclear factor- κ B (NF- κ B), that plays a pivotal role in regulating genes important for tumor invasion, metastasis, and chemoresistance.^{22,23} NF- κ B is com-

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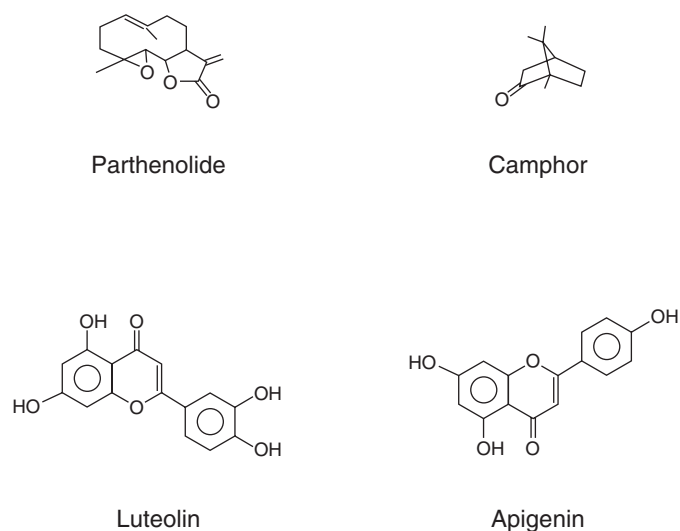


FIG. 1. Structure of parthenolide, camphor, apigenin, and luteolin.

only a powerful target for drugs used in cancer treatment.²⁴ Inhibition of NF- κ B upon treatment with ethanolic extracts of leaves from Asteraceae plants such as *Artemisia ludoviciana* ssp. *mexicana*, *Calea zacatechichi*, and *Polymnia maculate*, all rich in SQLs, has been shown to occur at a concentration of 25 μ g/mL.²⁵ These results implied that parthenolide or feverfew extract might be an effective treatment for breast cancer.

Although considerable research has been focused on anti-migraine properties of parthenolide,^{3,26,27} attempts to investigate the antiproliferative activity, especially to important human cancer cells, by parthenolide or feverfew extract are limited. In addition, some metabolites in feverfew extract other than parthenolide, such as apigenin and luteolin,²⁸ that may also have inhibitory activities against human cancers have not been adequately investigated. For example, apigenin was reported to significantly inhibit ultraviolet-induced mouse skin tumorigenesis,^{29,30} and luteolin has shown strong growth inhibition of MCF-7³¹ and other tumors.³²

In this study, we examined the effects of parthenolide, apigenin, luteolin, and golden feverfew extract on three malignant human cancer cell lines, including breast cancer (MCF-7 and Hs605T) and cervical cancer (SiHa). Antiproliferative activities of parthenolide, flavonoids, and feverfew extracts were determined using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) bioassay. Interaction between the pure chemicals and feverfew extract was investigated to elucidate possible synergistic or antagonistic effects.

MATERIALS AND METHODS

Materials and chemicals

Dried golden feverfew powders were obtained from Clemson University Coastal Research and Education Cen-

ter, Charleston, SC. Chemical standards parthenolide (99% purity) and camphor (98% purity) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Apigenin (95% purity) and luteolin were purchased from Sigma Chemical Co. (St. Louis, MO). Ethanol (high-performance liquid chromatography grade) was obtained from Fisher Scientific (Fairlawn, NJ).

Preparation of golden feverfew extract

Feverfew powder (1.5 g) was weighed into a 50-mL glass bottle containing a magnetic stirring bar. Ethanol (15 mL) was added. The bottle was sealed with Parafilm, and vigorous stirring was done for 10 minutes. Clear extract was obtained by filtration through Whatman No. 1 filter paper, and concentrated under nitrogen gas to 1 mL.

Cell culture

GIBCO[®] Dulbecco's modified Eagle's medium and Dulbecco's phosphate-buffered saline were obtained from Invitrogen[™] (Carlsbad, CA). RPMI 1640 medium, penicillin/streptomycin, sodium bicarbonate, trypsin EDTA solution, and fetal bovine serum were obtained from Sigma.

The Hs605T and MCF-7 human breast cancer cell lines and the SiHa human cervical cancer cell line purchased from American Type Culture Collection (Rockville, MD) were cultured according to a modified published procedure.³³ MCF-7 and SiHa were grown in Dulbecco's modified Eagle's medium, while Hs605T was grown in RPMI 1640 with 1% penicillin/streptomycin, 1% sodium bicarbonate, and 10% fetal bovine serum. Cells were grown in the flasks in a CO₂ incubator supplied with 5% CO₂ at 37°C. Flasks were seeded with 2×10^6 cells per flask. Medium renewal was carried out two times a week until confluence was reached. A hemocytometer was used for the counting of cells.

The cell suspensions were diluted to a final concentration of 5×10^4 cells/mL. Cell suspension (200 μ L) was placed in each well of a 96-well flat-bottom microplate. Parthenolide, camphor, luteolin, and apigenin (1.2 mg/mL) and feverfew extract (1,500 mg/mL) were dissolved in ethanol to desired concentrations, and then 2 μ L of each sample was added to the cell suspensions. To test the interactions among parthenolide, flavonoids, and feverfew extract, each treatment was prepared by combination of two solutions in equivalent volumes from 100 μ L of feverfew extract (3 mg/mL), 100 μ L of pure parthenolide (5.2 μ g/mL), or 100 μ L of flavonoids in a selected concentration. Ethanol was used as the solvent control. Four replications were performed for each sample per treatment. After a 24-hour incubation, MTS assay (CellTiter 96[®] aqueous nonradioactivity cell proliferation assay, Promega, Madison, WI) was used to test cancer cell viability on a Bio-Rad (Hercules, CA) microplate reader at 490 nm. Control cultures were prepared with ethanol-containing growth medium and performed on each test.

The cancer cell viability was calculated by the following equation:

$$\text{cell viability (\%)} = \frac{\text{absorbance of sample at 490 nm}}{\text{absorbance of solvent control at 490 nm}} \times 100$$

The half-effective concentration (EC_{50}) was used to compare the inhibitive activity of the tested chemicals and feverfew extract. This was calculated as the effective concentration that inhibited the net cell growth to 50% of control growth. The lower the EC_{50} , the higher was inhibition ability.

Statistical analysis

Each treatment was repeated a minimum of three times with four replications of each sampling. A 4×4 full factorial experiment in randomized complete block design was used to test inhibitory activity of four standard chemicals with four concentrations against three human cancer cell lines. Statistical analysis was conducted using SAS System for Windows version 8 (SAS Institute Inc., Cary, NC). For the interaction test, Dunnett's test and linear contrasts were used to compare treatment effects. Difference among all sample means were determined by analysis of variance at $P < .05$.

RESULTS

Inhibitory activity of standard chemicals against three human cancer cell lines

Parthenolide, camphor, apigenin, and luteolin, the major bioactive constituents in feverfew (Fig. 1), were detected in golden feverfew extract (data not shown). Parthenolide showed the highest inhibitory capacity against three cancer cell lines among the four tested standard chemicals at concentrations in the range of 0.012–12 $\mu\text{g/mL}$ (Fig. 2). Parthenolide at the concentration of 12 $\mu\text{g/mL}$ caused the lowest cancer cell viability for the cell lines of Hs605T, MCF-7, and SiHa with $29.4 \pm 1.4\%$, $33.5 \pm 9.6\%$, and $18.6 \pm 7.3\%$, respectively, which were significantly lower than those caused by other chemicals ($P < .0001$).

In contrast, only weak inhibitory activities of both apigenin and luteolin at the highest concentration of 12 $\mu\text{g/mL}$ were observed against Hs605T and SiHa with about 80% cancer cell viability. Similar weak inhibitory activities of all chemicals were recorded at the concentration equal and lower than 1.2 $\mu\text{g/mL}$ except for parthenolide against the Hs605T cells. No inhibitory activity of camphor was observed (Fig. 2).

Since parthenolide was observed to inhibit the growth of all three cancer cell lines by nearly 40% within the range of concentrations from 1.2 $\mu\text{g/mL}$ to 12 $\mu\text{g/mL}$ ($P < .0001$), an investigation with a narrower concentration range of parthenolide was considered necessary for calculating an accurate EC_{50} . The antiproliferative ability of parthenolide within the range of 0.4–12 $\mu\text{g/mL}$ was determined (Fig. 3).

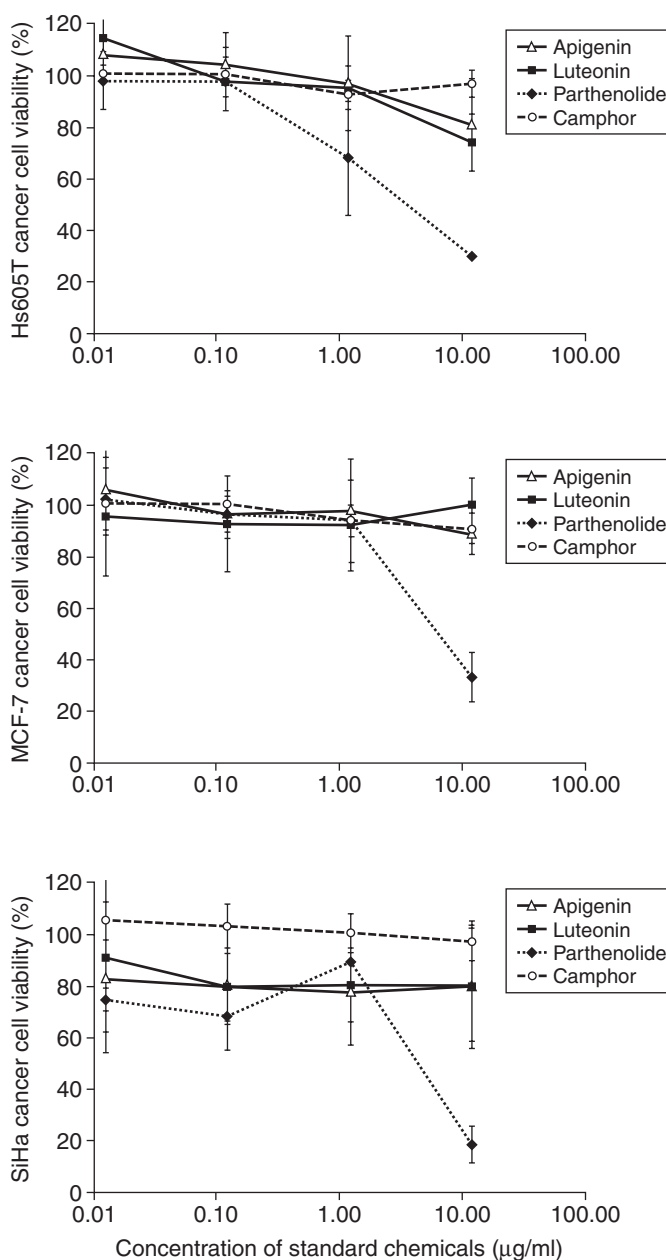


FIG. 2. Dose–response curves of antiproliferative activity of four chemicals against Hs605T, SiHa, and MCF-7 cancer cell lines. Data are mean \pm standard deviation values ($n = 12$).

At 3.6 $\mu\text{g/mL}$, the cancer cell viabilities of Hs605T, MCF-7, and SiHa were $22.5 \pm 9.5\%$, $27.2 \pm 8.6\%$, and $26.3 \pm 7.1\%$, respectively. Similar low cancer cell viability was observed among the three cancer cell lines at higher parthenolide concentrations (5.2 $\mu\text{g/mL}$ and 12 $\mu\text{g/mL}$).

Inhibitory activity of golden feverfew extract against three human cancer cell lines

At a feverfew extract concentration of 3 mg/mL based on dry weight (DW), the cancer cell viability decreased to

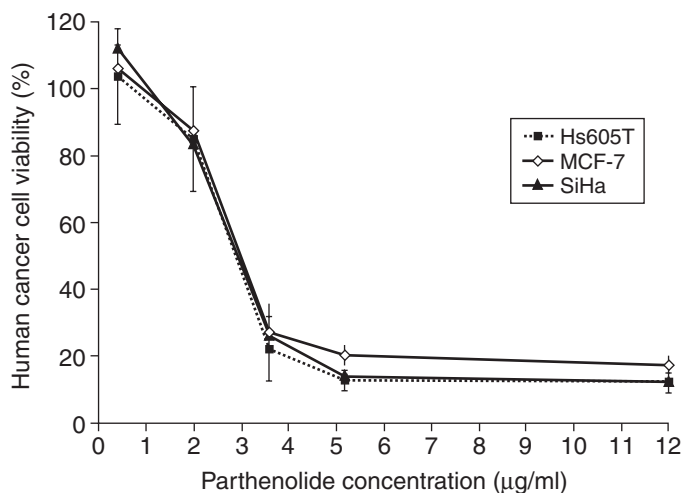


FIG. 3. Dose–response curves of antiproliferative activity of parthenolide against Hs605T, SiHa, and MCF-7 cancer cell lines. Data are mean \pm standard deviation values ($n = 12$).

14.8 \pm 4.1%, 25.2 \pm 12.2%, and 21.7 \pm 8.0% for Hs605T, MCF-7, and SiHa, respectively (Fig. 4). Low cell viability (approximately 20%) was observed among all three cancer cell lines at higher feverfew extract concentrations (7.5 mg DW/mL and 15 mg DW/mL).

Additionally, feverfew extract inhibited the growth of the three cancer cell lines with cell and dose dependency (Table 1 and Fig. 4). The lowest EC_{50} (0.6 mg DW/mL of feverfew extract) was obtained against SiHa, suggesting an increased sensitivity of SiHa cancer cells to the golden feverfew extract when compared with that of Hs605T and MCF-7 cancer cell lines. A higher EC_{50} (1.5 mg DW/ml of feverfew extract) against Hs605T and the highest EC_{50} (2.1 mg DW/ml of feverfew extract) against MCF-7 were noted.

Interactions among parthenolide, flavonoids, and feverfew extract

Because parthenolide was found in our previous tests to be possibly associated, synergistically and/or antagonistically, with other chemicals in the golden feverfew extract, we conducted further studies on interactions among parthenolide, flavonoids (apigenin or luteolin), and the feverfew extract, for which the result is shown in Figure 5. Combination of the golden feverfew extract (3 mg/mL) and parthenolide (5.2 μ g/mL) in an equivalent volumetric ratio (treatment B) exhibited the strongest inhibitory capacity against the three human cancer cell lines, with less than 20% cancer cell viability (Fig. 5). This suggested that antiproliferative activities of parthenolide and golden feverfew extract were synergistic because the results previously shown (Figs. 3 and 4) indicated that both 2.6 μ g/mL parthenolide and 1.5 mg/mL golden feverfew extract, separately, only allowed cancer cell viability of approximately 50%. Moreover, the inhibitory activity of parthenolide was confirmed

by the remarkably low cancer cell viabilities observed in treatment D (parthenolide and apigenin combination) and treatment G (parthenolide and luteolin combination). Both of these inhibitory activities were significantly stronger ($P < .001$) than those of the counterparts without parthenolide, treatment E (apigenin and ethanol combination) and treatment I (luteolin and ethanol combination). Therefore, the lowest cancer cell viability in treatment B (equivalent to 5.6 μ g/mL of parthenolide) could be attributed to the high content of parthenolide in the experimental bioassay. All the experimental treatments have produced significantly lower cancer cell viabilities compared with the control ($P < .0001$) except treatment I, which had no readily observable antiproliferative activities against the tested cancer cells ($P = .14$ for Hs605T, $P = .91$ for MCF-7, $P = .94$ for SiHa).

DISCUSSION

Our results were contrary to the previous report that strong inhibitory activity of luteolin at concentrations of 1 nM–1 μ M was observed against MCF-7 cell lines.³¹ The reason for the activity difference may be attributed to the difference in bioassays in terms of cell culture time (5–6 days vs. 1 day) used in the two experiments.

Parthenolide exhibited a dose-dependent, cell-dependent inhibitory activity against the three cancer cell lines. The lowest EC_{50} (2.6 μ g/mL) was obtained for parthenolide against Hs605T, with comparable EC_{50} values against SiHa and MCF-7 (2.7 μ g/mL and 2.8 μ g/mL, respectively). These data were in agreement with the previous report²³ that the half-maximal growth-inhibitory concentration (IC_{50}) of parthenolide against two human breast cancer cell lines was 0.8 μ M for HBL100 cells and 2 μ M for MDA-MB-231 cells. In that study, Patel *et al.*²³ found that parthenolide could inhibit the NF- κ B DNA binding in MDA-MB-231 and

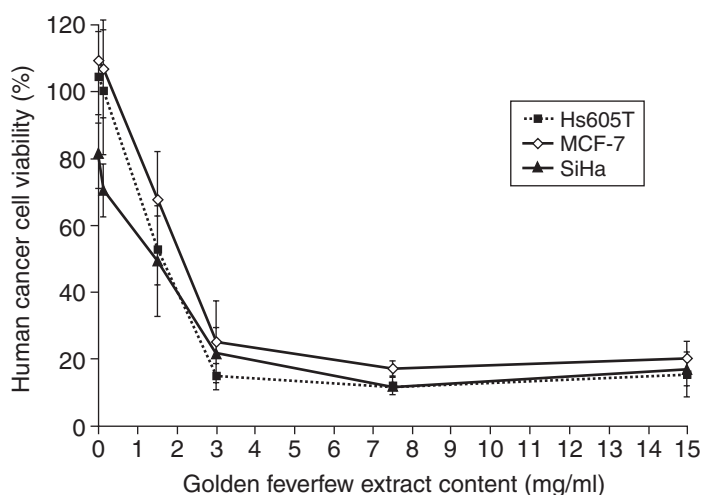


FIG. 4. Dose–response curve of antiproliferative activity of golden feverfew extract against Hs605T, SiHa and MCF-7 cancer cell lines. Data are mean \pm standard deviation values ($n = 12$).

TABLE 1. EC₅₀ VALUES OF PARTHENOLIDE, GOLDEN FEVERFEW EXTRACT, APIGENIN, AND LUTEOLIN AGAINST Hs605T, MCF-7, AND SiHa CANCER CELL LINES

	EC ₅₀ in cancer cell line		
	Hs605T	MCF-7	SiHa
Parthenolide (μg/mL)	2.6	2.8	2.7
Golden feverfew (mg/mL)	1.5	2.1	0.6
Apigenin (μg/mL)	NA	>100	NA
Luteolin (μg/mL)	NA	NA	NA

NA, not applicable.

HBL100 cells. Bork *et al.*²⁵ also reported that parthenolide could prevent NF-κB activation completely at 5 μM for HeLa cells. These reports implied that parthenolide might inhibit the growth of Hs605T, MCF-7, and SiHa cancer cells via a similar mechanism by inhibiting NF-κB activation, though they did not exclude other mechanisms that had been suggested. For example, parthenolide extracted from European feverfew was reported to suppress the expression of the death reporter CD95 (APO-1/Fas) and its ligand (CD95L),²¹ aberrant expression of which may lead to severe diseases such as cancer. Additionally, parthenolide was suggested to undergo Michael addition with enzymes or proteins bearing cysteine or sulfhydryl groups.¹⁹ Although such theories existed, the true mechanism of the antiproliferative activity of parthenolide against Hs605T, MCF-7, and SiHa cancer cell lines remains for further study.

Feverfew extract, like purified parthenolide, exhibited a strong inhibitory activity against the three human cancer cell lines (Fig. 4), especially against the SiHa cell line with the lowest cell viability. Since the golden feverfew ethanolic extract contained nearly 0.20% (DW) parthenolide, which was confirmed by our gas chromatography/mass spectrometry analysis, the feverfew extract EC₅₀ value (0.6 mg DW/mL) against SiHa expressed in equivalent parthenolide content was equal to 1.2 μg/mL (0.6 mg/mL × 0.20%). This value was much lower than the single parthenolide EC₅₀ value (2.7 μg/mL) against SiHa, which suggested that other chemicals in feverfew extract might have synergistic cytotoxic effects with parthenolide against the SiHa cancer cell line. This hypothesis was supported by the previous observation that the parthenolide IC₅₀ significantly decreased to <0.1 μM for HBL100 cells and nearly 0.8 μM for MDA-MB-231 when parthenolide was combined with 1 nM microtubule-stabilizing agent paclitaxel.²³

However, the hypothesized synergistic effect on the two breast cancer cell lines was not as significant as in SiHa cells. The equivalent parthenolide content of the feverfew extract EC₅₀ value (2.1 mg DW/mL of feverfew extract) against MCF-7 was nearly 4.2 μg/mL (2.1 mg/mL × 0.20%). This value was slightly higher than the pure parthenolide EC₅₀ value (2.8 μg/mL) against MCF-7. A similar result was also observed with the Hs605T cell line. This

implied that other chemicals in feverfew extract might have no synergistic effect with or even decrease the cytotoxic activity of parthenolide against MCF-7 and Hs605T, though parthenolide might still be the main compound to inhibit the growth of cancer cells.

Interestingly, MCF-7 cells seemed more tolerant to both parthenolide and feverfew extract in the two bioassays compared with the other two cancer cell lines. It is worthy of mention that MCF-7, being an estrogen-responsive human breast cancer, was stimulated in cell growth when treated with the estrogenic hormone estradiol at 1 nM, even in the presence of cancer cell inhibitors such as the isoflavone genistein.³⁴ However, the same report indicated that there were different efficacies among different phytoestrogens with respect to both cell growth and gene activation.³⁴ Since apigenin and luteolin were detected in the golden feverfew ethanolic extract, these two flavonoids might also play roles like genistein, or they might have antagonistic influence with parthenolide to affect the cell growth of the MCF-7 cancer cells.

From the results of the interaction bioassays, all treatments were subjectively classified into two groups: one exhibiting strong inhibitory activities with average cell viabilities less than 40% for all cancer cells studied (*i.e.*, Hs605T, MCF-7, and SiHa), and another exhibiting much weaker activities (Fig. 5). The former group included treatments B (golden feverfew extract and parthenolide combination), D (parthenolide and apigenin combination), F (golden feverfew extract and apigenin combination), and G (parthenolide and luteolin combination). All members of this group had shown similar inhibitory activities without significant dif-

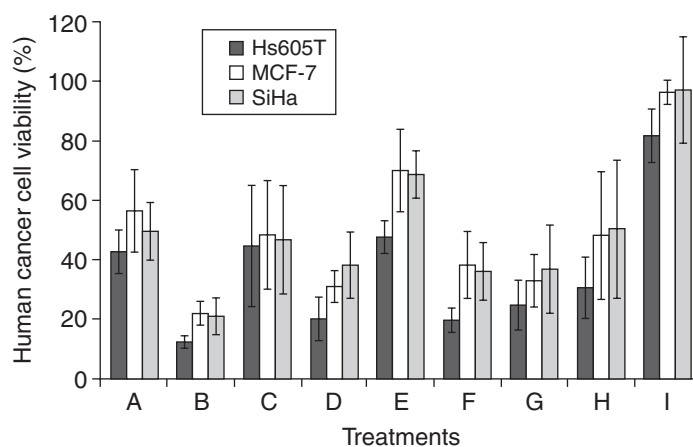


FIG. 5. Interaction between pure chemicals and golden feverfew extract (GFE). Labels A–I represent treatments as follow: A, 100 μL of GFE (3 mg/mL) + 100 μL of ethanol (ETH); B, 100 μL of GFE (3 mg/mL) + 100 μL of parthenolide (PTH) (5.2 μg/mL); C, 100 μL of PTH (5.2 μg/mL) + 100 μL of ETH; D, 100 μL of apigenin (Api) (15 μg/mL) + 100 μL of PTH (5.2 μg/mL); E, 100 μL of Api (15 μg/mL) + 100 μL of ETH; F, 100 μL of Api (15 μg/mL) + 100 μL of GFE (3 mg/mL); G, 100 μL of Lu (10 μg/mL) + 100 μL of PTH (5.2 μg/mL); H, 100 μL of luteolin (Lu) (10 μg/mL) + 100 μL of GFE (3 mg/mL); and I, 100 μL of Lu (10 μg/mL) + 100 μL of ETH.

ferences against the two breast cancer cell lines. In addition, there was marginally no significant difference between treatment B and treatment F against the SiHa cancer cell line ($P = .051$).

Interestingly, all the treatments in the highly inhibitory group (B, D, F, and G) were in the form of a binary combination with components from golden feverfew extract, parthenolide, apigenin, and luteolin. Another group with lower inhibitory capacity, including treatments A, C, E, H, and I, were combinations of solvent ethanol with parthenolide, flavonoids, or feverfew extract except for treatment H (feverfew extract with luteolin), which showed a significantly higher cancer cell viability ($P < .05$). This strongly implied the possible existence of synergistic effects among the tested chemicals in the combinations (B, D, F, and G). For example, the golden feverfew extract and apigenin (treatment F) demonstrated a relatively lower cancer cell viability of $19.5 \pm 4.3\%$, $38.4 \pm 11.5\%$, and $36.2 \pm 10.2\%$, when compared with the combination of golden feverfew extract and ethanol (treatment A) with cancer cell viability of $42.0 \pm 7.4\%$, $56.3 \pm 14.2\%$, and $49.0 \pm 10.0\%$ for Hs605T, MCF-7, and SiHa cancer cells, respectively.

Treatment F also exhibited significantly lower cancer cell viabilities than those observed in treatment E (apigenin and ethanol combination) with $47.8 \pm 5.5\%$, $70.3 \pm 14.3\%$, and $69.3 \pm 8.0\%$ for Hs605T, MCF-7, and SiHa cancer cells, respectively (all $P < .05$). Therefore, the combination of apigenin and feverfew extract was more effective in killing cancer cells than either feverfew extract or apigenin solution alone. Similar results were also observed with the combination of parthenolide with apigenin against the cancer cells.

The combination of parthenolide and apigenin (treatment D) demonstrated significantly (all $P < .05$) lower cancer cell viabilities ($19.9 \pm 7.7\%$, $30.9 \pm 5.5\%$, and $37.9 \pm 11.5\%$, respectively) than those observed in treatment E (apigenin and ethanol combination) ($47.8 \pm 5.5\%$, $70.3 \pm 14.3\%$, and $69.3 \pm 8.0\%$, respectively) for Hs605T, MCF-7, and SiHa cancer cells. Also, the data from treatment D exhibited a lower cancer cell viability for Hs605T ($P = .0039$) compared with the combination of parthenolide and ethanol (treatment C) with a higher cancer cell viability of $44.8 \pm 20.5\%$, though, in this case, no significant differences were found in cell viabilities for MCF-7 and SiHa cells.

These results suggested that parthenolide was primarily responsible for the inhibition of growth of the tested cancer cells, and apigenin might act in moderate synergism with parthenolide in the overall expressed inhibitory activities.

In contrast to apigenin, luteolin did not show antiproliferative activities against the three human cancer cell lines, which was consistent with our conclusion from the previous test. Luteolin was not found to have a synergistic effect with the golden feverfew extract. Treatment H (combination of golden feverfew extract and luteolin) had no significant difference in cancer cell viabilities compared with treatment A (combination of golden feverfew extract and ethanol) (all $P > .25$).

However, it is noteworthy that a combination of parthenolide and luteolin (treatment G) produced a seemingly contradictory result as a significantly lower cancer cell viability was recorded for Hs605T with $24.8 \pm 8.5\%$ compared with treatment C (combination of parthenolide and ethanol) with $44.8 \pm 20.5\%$ ($P < .05$). This result seemed incongruent with our conclusions about the interaction between the feverfew extract and luteolin. The data from treatment G exhibited significantly (all $P < .05$) lower cancer cell viabilities than those observed in treatment I (luteolin and ethanol combination) with $82.4 \pm 9.1\%$, $97.7 \pm 4.0\%$, and $97.0 \pm 18.5\%$ for Hs605T, MCF-7, and SiHa cancer cells, respectively. This supported our conclusion that the low cancer cell viabilities observed with treatment G could be attributed to the strong inhibitory activity of parthenolide, but the contribution of luteolin might have been hidden to some extent against all cancer cell lines.

CONCLUSIONS

Among the tested constituents of feverfew (*i.e.*, parthenolide, camphor, luteolin, and apigenin), parthenolide showed the highest inhibitory capacity against the growth of all three types of cancer cell lines with EC_{50} values against Hs605T, MCF-7, and SiHa of $2.6 \mu\text{g/mL}$, $2.8 \mu\text{g/mL}$, and $2.7 \mu\text{g/mL}$, respectively. Parthenolide was therefore considered the major cancer cell inhibitor in our tests. The feverfew extract, containing parthenolide, also exhibited antiproliferative activity against the growth of all three types of cancer cells with an EC_{50} of 1.5 mg/mL against Hs605T, 2.1 mg/mL against MCF-7, and 0.6 mg/mL against SiHa. Through the combination comparison, apigenin might have moderate synergistic effects with parthenolide on the inhibition of cancer cell growth of Hs605T, MCF-7, and SiHa, while luteolin might have weak synergistic effect with parthenolide against Hs605T cells. In general, the present study has expanded our understanding of the anticancer activity of parthenolide and feverfew extract, and their possible synergistic effects with the other bioactive chemicals such as apigenin and luteolin. However, the relationship among luteolin, parthenolide, and feverfew extracts needs more investigations.

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