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RESEARCH ARTICLE

Aqueous Edible Flower Extracts as Functional Agents: Synergistic Chemosensitization of *Clitoria ternatea* and Multifunctional Bioactivity of *Nelumbo nucifera*

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Abstract

Edible flowers are increasingly recognized as rich sources of bioactive compounds; however, research on their aqueous extracts, particularly regarding therapeutic synergy, remains limited. This study aimed to evaluate the phytochemical profiles, antioxidant capacities, antidiabetic potential, and cytotoxic effects of aqueous extracts from 4 Thai edible flowers: *Clitoria ternatea* L. (butterfly pea), *Nelumbo nucifera* Gaertn. (lotus), and 2 cultivars of *Ixora* sp. (pink and red ixora). Total phenolic (TPC) and flavonoid (TFC) contents were determined, alongside DPPH, ABTS, and GC-MS profiling. Antidiabetic potential was assessed via α-glucosidase inhibition. Furthermore, cytotoxicity against HT-29 colorectal cancer cells was evaluated over 24 - 72 h, and synergistic interactions with 5-fluorouracil (5-FU) were quantified using the Combination Index (CI). Results revealed that lotus extract possessed the highest TPC and TFC, correlating with its superior antioxidant activity and potent α-glucosidase inhibition. Lotus extract also showed the highest long-term cytotoxicity against HT-29 cells at 72 h. Conversely, butterfly pea extract showed lower initial bioactivity but demonstrated a remarkably rapid onset of cytotoxicity and a significant synergism with 5-FU, effectively achieving a substantial 31-fold dose reduction of the chemotherapeutic drug. Interestingly, lotus extract exhibited antagonism when combined with 5-FU, suggesting mechanistic divergence in drug-extract interactions. GC-MS profiling revealed that acetic acid and DDMP predominated in the butterfly pea extract, whereas tyrosol and DDMP were the major components in the lotus extract. These findings highlight lotus as a promising agent for oxidative stress and glycemic control, while introducing butterfly pea as a potential chemosensitizing adjuvant to enhance 5-FU efficacy for colorectal cancer therapy. Collectively, this study supports the utilization of Thai edible flowers as prospective functional ingredients and multitarget nutraceuticals.

Keywords: Butterfly pea, Lotus, α-Glucosidase, Colorectal cancer, 5-Fluorouracil

Introduction

Edible flowers have recently emerged as a novel and promising category of functional foods, moving beyond their traditional role as culinary decoration. Recent literature has recognized them as valuable sources of bioactive metabolites, with specific focus on their diverse phytochemical profiles [1-3]. Comprehensive screening across various species has revealed that these flowers are particularly abundant in phytochemicals—such as polyphenols, flavonoids, and terpenoids [4-8]. These dietary nutraceuticals serve not only as nutritional components but also demonstrate distinct pharmacological properties, including anti-

inflammatory, antioxidant, antidiabetic, and anticancer activities, which vary significantly depending on the specific floral genus and chemical composition [9-12].

Among global health concerns, cancer remains one of the leading causes of illness and death, highlighting the urgent need for safe, accessible, and effective preventive strategies. In particular, colorectal cancer represents a major global health burden with high incidence and mortality rates [13]. To evaluate potential therapeutic interventions, the HT-29 human colorectal adenocarcinoma cell line is widely utilized as a well-established *in vitro* model due to its high relevance in colorectal cancer progression and chemotherapeutic drug screening [14,15]. Interestingly, plant-derived nutraceuticals such as curcumin, resveratrol, and various flavonoids have demonstrated the ability to act synergistically with conventional chemotherapeutic agents like 5-fluorouracil (5-FU) [16,17]. This combination strategy offers a promising avenue for enhancing therapeutic efficacy while potentially reducing adverse clinical side effects [18-20].

Despite their health potential, the majority of research on edible flowers employs organic solvents (such as ethanol or methanol) to enhance phytochemical yield [4,21,22]. Such extraction methods, however, do not reflect the actual dietary consumption patterns of these flowers, which are generally consumed fresh or prepared in aqueous-based culinary dishes. Importantly, no study has systematically evaluated the biological activities and the specific bioactive profiling of these aqueous extracts alongside their potential to function as chemosensitizers. Consequently, the exact role of these water-soluble compounds remains an unaddressed gap in functional food research.

Therefore, this study aims to investigate the multifunctional biological profiles of aqueous extracts from 4 edible flowers: *Clitoria ternatea* L. (butterfly pea), *Nelumbo nucifera* Gaertn. (lotus), and 2 cultivars of *Ixora* sp. (red and pink ixora). These species were selected because they are commonly found and easily accessible plants in Thailand. Specifically, we evaluated their antioxidant, antidiabetic, and cytotoxic activities, as well as their synergistic chemotherapeutic potential when combined with 5-FU against HT-29 colorectal cancer cells. We hypothesized that the aqueous extracts of these edible flowers would possess distinct bioactive profiles, offering specific metabolic benefits and exhibiting cytotoxicity as well as potential chemosensitization with 5-FU. This research emphasizes aqueous extraction to reflect common daily consumption, offering essential scientific evidence to support the classification of these flowers as functional ingredients and reliable nutraceuticals for enhancing cancer therapy and metabolic health.

Materials and methods

Preparation of edible flower extracts

Four fresh edible flowers were used in this study: *Clitoria ternatea* L. (butterfly pea), *Nelumbo nucifera* Gaertn. (lotus), and 2 cultivars of *Ixora* sp. (red and pink ixora). All samples were collected from Ramkhamhaeng University and its vicinity in Bangkok, Thailand. The petals were separated, rinsed with distilled water, and air-dried to remove surface moisture. Subsequently, the samples were dried in a hot-air oven at 45 °C until a constant weight was achieved. To simulate the common daily consumption of fresh edible flowers, aqueous extracts were prepared using a modified cold-maceration method [23,24]. Briefly, 125 g of each sample was mixed with 250 mL of distilled water and ground using a blender. The mixtures were then incubated at 4 °C for 1 h to enhance extraction while minimizing the risk of thermal degradation of bioactive compounds. The supernatants were separated by cheesecloth and then centrifuged at 5,000 rpm for 15 min at 4 °C. The collected supernatants were subsequently lyophilized to obtain a dry extract powder, which was stored at −20 °C. For further investigation, the lyophilized powder was reconstituted in distilled water to the desired concentrations.

Determination of total phenolic content (TPC)

Total phenolic content was measured using the Folin–Ciocalteu reagent according to a modified version of the procedure in [25]. Briefly, 20 µL of the extract was mixed with 500 µL of 10% (v/v) Folin–Ciocalteu reagent and allowed to react for 10 min at room temperature. Subsequently, 1 mL of 7.5% (w/v) sodium carbonate solution was added, and the mixture was incubated for 60 min under ambient conditions. The absorbance at 765 nm was determined via UV-Vis spectrophotometry. Results were calculated against a gallic acid standard (**Figure S1**) and reported as

milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract).

Determination of total flavonoid content (TFC)

Total flavonoid content was determined using an aluminum chloride colorimetric method adapted from procedure described in Wolfe *et al.* [26] with slight modifications. An aliquot of 200 µL of extract was mixed with 150 µL of 5% (w/v) sodium nitrite and incubated for 5 min. Then 150 µL of 10% (w/v) aluminum chloride was added, followed by a 6 min reaction period. Subsequently, 500 µL of 1 M sodium hydroxide was introduced, and the total volume was adjusted to 1.5 mL with distilled water. The absorbance was measured at 510 nm. Quantification was performed using a rutin standard curve (**Figure S2**), and results were expressed as milligrams of rutin equivalents per gram of extract (mg RE/g extract).

Determination of antioxidant activities

DPPH radical scavenging assay

The free radical scavenging activity of the extracts was evaluated using the DPPH assay following a modified procedure described in [27]. Briefly, 10 mg/mL of the sample was combined with 1.5 mL of 0.1 mM DPPH in ethanol. This mixture was then kept in the dark at room temperature for 10 min, followed by absorbance measurement at 517 nm.The percentage of DPPH radical scavenging activity was calculated as:

$$DPPH\ scavenging\ activity\ (\%) = \left[\frac{(A-B)}{A}\right] \times 100$$

where A and B represent the absorbance of the control and the sample, respectively.

ABTS radical scavenging assay

ABTS radical scavenging activity was determined according to a modified protocol described in [28]. The generation of ABTS radical cation ($ABTS^{+}$) was performed by combining 7 mM ABTS with 2.45 mM potassium persulfate in a 1:1 ratio. This solution was left to react for 16 h at room temperature in the dark. Before use, the $ABTS^{+}$ stock was diluted with distilled water until an absorbance of 0.80 ± 0.02 at 734 nm was achieved. Subsequently, 10 mg/mL of the sample was mixed with 1.5 mL of $ABTS^{+}$ working solution and kept in darkness for 10 min at room temperature. Absorbance was measured at 734 nm. The scavenging activity was calculated using:

$$ABTS\ scavenging\ activity\ (\%) = \left[\frac{(A-B)}{A}\right] \times 100$$

where A and B represent the absorbance of the control and the sample, respectively.

Determination of IC_{50} towards α-glucosidase

The inhibitory activity against α-glucosidase was evaluated following a modified method described in Kim *et al.* [29]. Briefly, varying concentrations of the extract were pre-incubated with α-glucosidase enzyme in 0.1 M phosphate buffer (pH 6.8) for 10 min. Subsequently, *p*-nitrophenyl-α-D-glucopyranoside (pNPG) was added as a substrate, and the reaction mixture was incubated at 37 °C for an additional 10 min. The reaction was terminated by adding 0.1 M sodium carbonate. The release of *p*-nitrophenol was monitored by measuring absorbance at 405 nm. The percentage of enzyme inhibition was calculated as:

$$Percentage\ of\ inhibition\ (\%) = \left[\frac{(A-B)}{A}\right] \times 100$$

where A is the absorbance of the control and B is the absorbance in the presence of the extract. The IC_{50} value, defined as the concentration required to inhibit 50% of enzyme activity, was determined from the dose–response curve by plotting percentage inhibition against extract concentration.

Cell culture

The HT-29 human colon cancer cell line (HT-29 cells) was purchased from iCell Bioscience Inc. (China). Cells were propagated and maintained in DMEM enriched with 10% FBS and 1% (v/v) antibiotic-antimycotic solution (all from Gibco, Thermo Fisher Scientific Inc. (USA)). The incubator provided a humidified 5% CO_2 atmosphere and a temperature of 37 °C.

Evaluation of cytotoxic effects

The cytotoxic effects of the aqueous edible flower extracts against HT-29 cells were assessed by the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl

tetrazolium bromide (MTT, Sigma-Aldrich, Saint Louis, USA) assay. The experiment included untreated HT-29 cells as a control and culture medium as a blank control. HT-29 cells were seeded in triplicate into 96-well plates at a density of 5×10^4 cells/well and incubated for 24 h to allow for cell adherence. Subsequently, these cells were treated with the extracts for 24, 48, and 72 h. The extracts were prepared across 6 concentrations (50 to 3.13 mg/mL) using a 2-fold serial dilution method. After treatment at each timepoint, the MTT solution was put into the wells, and the plates were incubated for 4 h. Afterward, the solution was taken out, and 100% DMSO was added to dissolve the formazan crystals. Cell cytotoxicity was assessed by measuring absorbance at 570 nm with a microplate reader. The percentage of viable cells was then calculated based on the following equation:

$$Cell\ viability\ (\%) = \frac{Mean\ OD_{sample}}{Mean\ OD_{control}} \times 100$$

The percentage of cell viability was determined by normalizing the data against the untreated control (defined as 100% viability). The half-maximal inhibitory concentration (IC_{50}) was then determined using a nonlinear regression equation.

During the initial phase of the MTT assay, early-phase morphological observations were performed using an inverted light microscope. Although the standard endpoints were 24 - 72 h, significant early-stage cellular alterations were observed within the first 6 h of exposure. Consequently, the morphology of HT-29 cells treated with 50 mg/mL of the extracts was recorded and compared against untreated control cells to show the rapid phenotypic changes.

Determination of synergistic activity from a combination of 5-FU and the extracts

The mode of interaction between the aqueous edible flower extracts and 5-fluorouracil (5-FU, Sigma-Aldrich, Saint Louis, USA) on the inhibition of HT-29 cell proliferation was determined using the isobolographic approach as modified from [30]. HT-29 cells were treated with various concentrations of the samples and 5-FU for 24 h. The key combination points chosen for analysis were those that achieved the IC_{50} of the extracts combined with the IC_{25} of 5-FU. The degree of interaction was quantified by calculating the Combination Index (CI) according to the following equation:

$$CI = \frac{D_1}{Dx_1} + \frac{D_2}{Dx_2} + a\frac{D_1D_2}{Dx_1Dx_2}$$

where $(Dx)_1$ is the IC_{50} of 5-FU alone, $(Dx)_2$ is the IC_{50} of the extracts alone, $(D)_1$ is the IC_{50} of 5-FU in combination with the extracts, $(D)_2$ is the IC_{50} of the extracts in combination with 5-FU, and a is set at 0, assuming a mutually exclusive mode of drug action because 5-FU and the multi-constituent crude extracts are expected to possess different mechanisms of action or target distinct pathways.

CI values were interpreted as antagonism (CI > 1.1), additive effect (CI = 0.9 - 1.1), or synergism (CI < 0.9) based on the Chou and Talalay model [31].

GC-MS analysis and compound identification

Bioactive components were identified using a Gas Chromatograph-Mass Spectrometer (GC-MS) Agilent 7890B GC system coupled with a 5977B MSD single quadrupole mass spectrometer (Agilent Technologies, USA). The samples were dissolved sequentially in 150 µL of deionized water and 150 µL of methanol and then centrifuged at 10,000 rpm at 4 °C for 5 min. A 1 µL volume of the supernatant was injected into the system. Separation was achieved using a capillary column (30 m length × 250 µm internal diameter, phase thickness 0.25 µm) with helium as the carrier gas at a constant flow rate of 1 mL/min. The front inlet was operated in split mode at 250 °C. The oven temperature was programmed at an initial temperature of 50 °C, held for 5 min, ramped at 5 °C/min to 160 °C, held for 4 min, and ramped at 10 °C/min to 250 °C, held for 10 min. The GC-MS was used to gather the data at an ionization voltage of 70 eV and an ionization mode of electron ionization (EI). The ion source and quadrupole temperatures were set at 230 and 150 °C, respectively. The mass spectrum was recorded in scan mode from m/z 35 to 500. Compound identification was identified by comparing the mass spectra with the Wiley23 and the National Institute of Standards and Technology (NIST) 23 mass spectral libraries. Only compounds with a math score of more than 90% were accepted and reported.

Statistical analysis

The data, reported as mean ± standard deviation (SD) from 3 independent biological replicates, were statistically analyzed using SPSS for Windows V.21 (SPSS, Chicago, IL, USA). A completely randomized design (CRD) was employed. Normally distributed data were examined using one-way analysis of variance (ANOVA), with statistical significance set at $p < 0.05$. Differences between individual group means were subsequently determined using Duncan's multiple range test as the post hoc analysis.

Results and discussion

Total phenolic and flavonoid content of aqueous edible flower extracts

Initial screening of the 4 edible flower extracts involved the quantification of TPC and TFC. As shown in **Table 1**, significant variations in TPC and TFC were observed among the 4 edible flowers. Lotus exhibited the highest concentrations of both TPC (2.72 mg GAE/g extract) and TFC (1.76 mg RE/g extract), while red ixora showed the lowest concentrations of both TPC (1.22 GAE/g extract) and TFC (0.68 mg RE/g extract).

Table 1 Total phenolic and flavonoid content of edible flower aqueous extracts.

Samples	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg RE/g extract)
Butterfly pea	1.39 ± 0.00^{c}	0.77 ± 0.04^{c}
Lotus	2.72 ± 0.03^{a}	1.76 ± 0.02^{a}
Red ixora	1.22 ± 0.01^{d}	0.68 ± 0.00^{d}
Pink ixora	1.80 ± 0.01^{b}	1.56 ± 0.02^{b}

Data were expressed as mean ± SD.

^{a,b,c,d} Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$).

Interestingly, this trend differs from the findings of Kaisoon *et al.* [33], who studied Thai edible flower teas and reported that red ixora tea had a higher TPC (approx. 25.59 mg GAE/g DW) compared to lotus tea (approx. 17.85 mg GAE/g DW) when using a high-temperature extraction (100 °C for 10 min). This difference may be due to variations in sample preparation and extraction conditions. The use of dried tea products and heat in previous studies contrasts with the room-temperature aqueous extraction used in this study, which may enhance the recovery of different phenolic fractions in lotus [32]. Furthermore, while the previous study only focused on TPC, our findings fill a gap by providing the TFC for these species, highlighting lotus as a superior source of flavonoids in aqueous extraction. Additionally, an evaluation of 12 Thai edible flower species using methanol reported that ixora had a higher TPC than lotus, whereas lotus was found to have a higher TFC. They concluded that extracts with higher flavonoid content do not always possess a higher phenolic content, suggesting that the composition of phytochemicals varies across floral species [33]. In contrast, our findings showed a consistent trend between TPC and TFC across all tested species, where lotus remained the highest in both parameters. Collectively, these differences highlight the significant impact of extraction temperature, solvent polarity, and sample preparation on the detectable phytochemical yields of edible flowers.

Antioxidant capacities of aqueous edible flower extracts

The antioxidant activity of the 4 edible flower extracts was assessed by DPPH and ABTS radical scavenging assays. The results showed a similar trend observed for TPC and TFC. As shown in **Table 2**, the lotus extract had the greatest antioxidant activity, with scavenging percentages of 79.86% for DPPH and 85.30% for ABTS. In contrast, butterfly pea exhibited the lowest DPPH scavenging capacity (28.6%), whereas red ixora showed the lowest activity in the ABTS assay (38.46%). These findings suggest a positive relationship between phenolic content and antioxidant efficacy, consistent with reports highlighting the significant role of flavonoids and phenolics in driving the antioxidant potential of lotus [34]. Consequently, this finding suggests that the antioxidant properties of these flowers

are primarily influenced by their phenolic and flavonoid components.

Our findings are consistent with a previous report [33], which evaluated 12 Thai edible flowers using methanol as an extraction solvent. They reported that lotus (96.9%) exhibited a higher DPPH scavenging activity compared to ixora (62.52%) and butterfly pea (32.7%), respectively. These results further emphasize the significant antioxidant potential of lotus in aqueous systems; as demonstrated in our study, its bioactive constituents remain highly effective even when extracted using water-based methods without organic solvents.

Table 2 Antioxidant activities of edible flower aqueous extracts.

Samples	DPPH-scavenging activity (%)	ABTS-scavenging activity (%)
Butterfly pea	28.60 ± 0.21^{d}	40.64 ± 0.10^{c}
Lotus	79.86 ± 0.55^{a}	85.30 ± 0.83^{a}
Red ixora	39.31 ± 0.15^{c}	38.46 ± 0.35^{d}
Pink ixora	48.49 ± 0.35^{b}	53.83 ± 0.57^{b}

Data were expressed as mean ± SD.

^{a,b,c,d} Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$).

***In vitro* antidiabetic activity of aqueous edible flower extracts**

The antidiabetic potential of the edible flower extracts was investigated via their ability to inhibit the α-glucosidase enzyme. Significant variations in inhibitory efficacy were observed across the extracts (**Table 3**). Remarkably, the lotus extract displayed the most potent antidiabetic activity, with the lowest IC_{50} of 1.27 μg/mL, followed by pink and red ixora, which also showed effective inhibition in the microgram range. In contrast, butterfly pea exhibited the lowest inhibitory effect, with a significantly higher IC_{50} of 21.18 mg/mL. This trend parallels the results obtained from the phytochemical and antioxidant assays, suggesting a strong positive relationship between the concentration of TPC and TFC and the observed antidiabetic potential.

Table 3 *In vitro* antidiabetic activity of edible flower aqueous extracts.

Samples	IC_{50} towards α-glucosidase
Butterfly pea	21.18 ± 0.12 mg/mL^{d}
Lotus	1.27 ± 0.03 μg/mL^{a}
Red ixora	3.86 ± 0.05 μg/mL^{c}
Pink ixora	2.21 ± 0.06 μg/mL^{b}

Data were expressed as mean ± SD.

^{a,b,c,d} Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$).

Notably, our aqueous lotus extract exhibited significantly higher potency compared to the ethyl acetate fraction of the lotus flower, which was reported to have an IC_{50} of 29.06 mg/mL [35]. This suggests that our aqueous extraction method is highly effective in recovering the potent antidiabetic constituents from lotus. In contrast, the lower efficacy of our butterfly pea extract differs from previous findings using ethanolic extraction (IC_{50} = 39.77 mg/mL) [36] and aqueous extraction at 90 °C (IC_{50} = 3.15 mg/mL) [37]. These discrepancies could be attributed to the variation in extraction solvent, temperature, and method, which likely results in distinct bioactive profiles [38]. However, it is worth noting that a commercial standard reference drug (such as acarbose) was not included in this preliminary screening phase, and the specific enzyme inhibition kinetics were not evaluated. Consequently, establishing comparative kinetic models

remains a limitation of the present study. Further comprehensive investigations utilizing isolated pure compounds alongside standard reference drugs are warranted to fully elucidate these enzymatic parameters.

To conclude, the superior inhibitory activity of lotus can be attributed to its enrichment of phenolic and flavonoid constituents. These compounds are known to interact with the active sites of α-glucosidase through hydrogen bonding and hydrophobic interactions, effectively reducing its catalytic activity [39-41]. Furthermore, the strong antioxidant activity of the lotus extract may provide a synergistic advantage in reducing oxidative stress associated with diabetes. [42]. These findings highlight the potential of lotus extract as a promising natural agent for postprandial blood glucose management.

Cytotoxic effects of aqueous edible flower extracts

The cytotoxic effects of the 4 aqueous edible flower extracts were evaluated against HT-29 cells using the MTT assay. Cells were treated with varying concentrations of each extract, and cell viability was then assessed following 24, 48, and 72 h incubation periods. As shown in **Figure 1** and **Table 4**, the inhibitory activities of all extracts exhibited a clear time- and dose-dependent manner. Among the tested samples, lotus and butterfly pea extracts demonstrated a significant antiproliferative potential compared to the *Ixora* sp. At 24 h, butterfly pea showed an IC_{50} of 39.04 mg/mL, while lotus required a slightly higher concentration (47 mg/mL). However, by 72 h, the lotus extract (IC_{50} = 13.49 mg/mL) exhibited significantly stronger cytotoxic activity compared to the butterfly pea extract (IC_{50} = 22.63 mg/mL). In contrast, both red and pink ixora exhibited no detectable activity at 24 h and maintained significantly higher IC_{50} values throughout the study (37.23 and 34.86 mg/mL at 72 h, respectively).

Previous studies have documented the cytotoxic potential of both butterfly pea and lotus, although research has predominantly focused on organic solvent extractions. Regarding butterfly pea, aqueous extracts have shown inhibition against breast, ovarian, and liver cancer lines with IC_{50} values ranging from 175.3 to 304.7 mg/mL [43], while organic extracts (ethanol and methanol) exhibited higher potency across various lines, such as in HCT116 colorectal cells (IC_{50} = 29.2 mg/mL), medullary thyroid cancer cell lines (IC_{50} = 61.6 mg/mL), and MCF-7 cells (IC_{50} = 17.46 mg/mL) [44,45]. Similarly, anticancer investigations of lotus have primarily utilized methanolic and ethanolic extracts from seeds, leaves, and roots, showing significant cytotoxicity against breast, ovarian, and osteosarcoma cells with IC_{50} values ranging from 79.73 to 100.18 mg/mL [46-49]. However, there is currently no reported data regarding the antiproliferative efficacy of aqueous flower extracts against HT-29 cells until the present study.

Table 4 IC_{50} of the edible flower aqueous extracts against HT-29 cells at various times.

Samples	IC_{50} (mg/ml)		
	24 h	48 h	72 h
Butterfly pea	39.04 ± 4.61[a]	24.54 ± 0.83[a]	22.63 ± 4.27[b]
Lotus	47.00 ± 4.25[a]	18.97 ± 6.35[a]	13.49 ± 4.07[a]
Red ixora	N/A	51.89 ± 2.77[b]	37.23 ± 4.41[c]
Pink ixora	N/A	44.26 ± 3.94[c]	34.86 ± 4.86[c]

Data were expressed as mean ± SD.

[a,b,c,d] Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$).

Interestingly, while the primary focus of this study was on long-term cytotoxicity (24 - 72 h), early-phase microscopic morphological changes revealed that the butterfly pea extract induced rapid and progressive morphological changes in HT-29 cells. Initial cellular alterations were detectable as early as 2 h post-exposure, becoming increasingly pronounced at 4 and 6 h. As shown in **Figure 2**, HT-29 cells treated with 50 mg/mL of butterfly pea extract exhibited clear signs of cytotoxicity, characterized by significant cellular shrinkage and exfoliation, whereas untreated control cells remained confluent and healthy. In contrast, such rapid morphological shifts were not observed in cells treated with the lotus extract at the same concentration and time points. This early-onset, time-dependent cytotoxicity is consistent with the IC_{50} results at 24 h, where butterfly pea demonstrated slightly more potent initial activity than lotus, suggesting a faster and more immediate mechanism of action for the butterfly pea aqueous extract.

Collectively, these findings reveal a significant difference between the extracts. Butterfly pea demonstrates a rapid cytotoxic effect despite its relatively lower TPC and TFC values, whereas lotus, with the highest TPC and TFC, acts more gradually but achieves superior long-term efficacy. This suggests that the cytotoxic effects are determined not only by the quantity of polyphenols but also by the qualitative nature of the bioactive compounds. The early-stage potency of butterfly pea suggests a possibility that it may be partly associated with cyclotides—small, cysteine-rich proteins known for their potent membrane-disrupting and pro-apoptotic properties—which could potentially elicit immediate cellular responses even at low concentrations [50]. These specialized metabolites, alongside specific anthocyanins, may possess enhanced cellular permeability or a specific affinity for immediate cell death pathways, such as the induction of apoptosis or cell cycle arrest [51]. In contrast, the delayed but robust response of lotus suggests a cumulative effect, where its abundant phenolic profile requires longer exposure to achieve its maximum efficacy. This suggests that the cytotoxic effects of edible flowers are often a result of phytochemical quality and structural specificity rather than only quantitative abundance. Although the observed IC_{50} values indicate that these aqueous extracts possess relatively weak cytotoxicity compared to commercial purified drugs, these findings highlight the potential of edible flowers—particularly lotus—as dietary sources of functional constituents that exert moderate antiproliferative effects against colorectal cancer cells. Hence, the biological significance of these findings should be interpreted with caution, as these crude extracts function primarily as preventive dietary components rather than potent therapeutic agents.

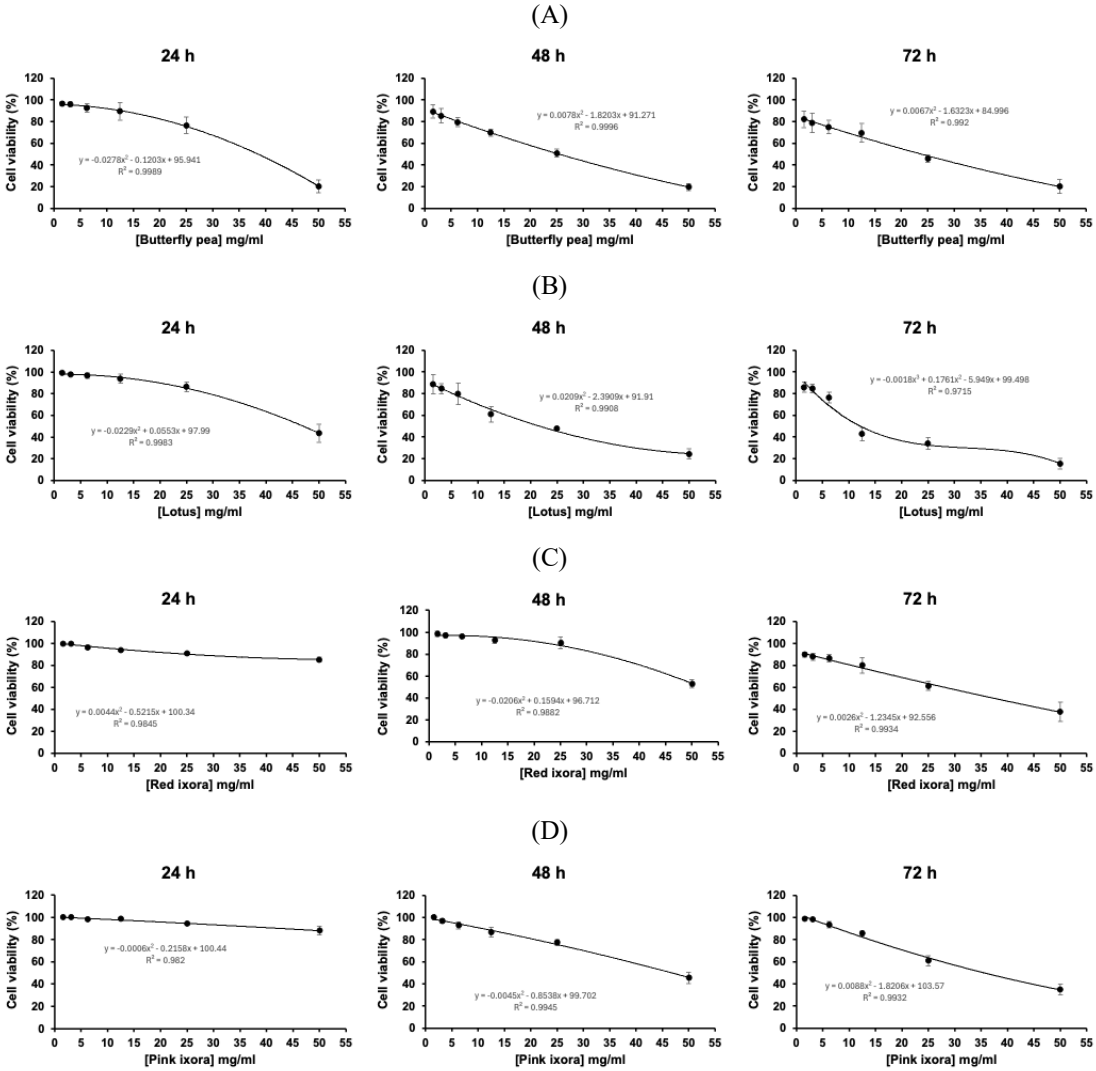


Figure 1 Effects of edible flower aqueous extracts on HT-29 cell viability**.** HT-29 cells were treated with varying concentrations of (A) butterfly pea, (B) lotus, (C) red ixora, and (D) pink ixora for 24, 48, and 72 h. Cell viability was determined using the MTT assay. Values represented are mean ± SD from 3 independent biological replicates.

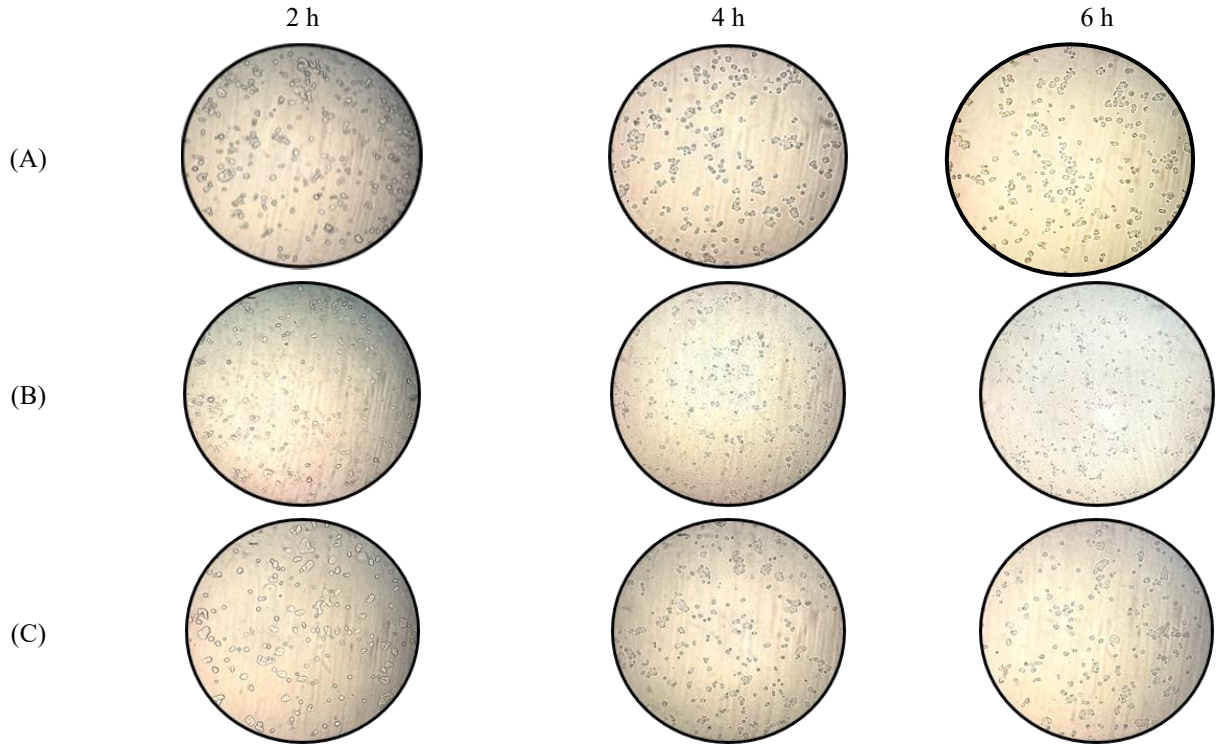


Figure 2 Early-phase morphological observation of HT-29 cells treated with edible flower extracts. Microscopic images of HT-29 cells at 2, 4, and 6 h post-exposure. (A) Untreated control cells. (B) Cells treated with 50 mg/mL of aqueous butterfly pea extract. (C) Cells treated with 50 mg/mL of aqueous lotus extract.

Despite the promising findings, several limitations of the current study must be acknowledged. First, this screening phase utilized a single colorectal cancer cell line (HT-29); therefore, further validation across a broader panel of other colorectal cancer cell lines (such as HCT116, Caco-2, or SW480) as well as other cancer types (such as MCF-7 breast or A549 lung cancer cells) is warranted to confirm the generalizability of these effects. Second, the cytotoxicity of the extracts toward normal cells was not evaluated, which is a critical step for establishing their selective toxicity and safety profiles. Third, while these *in vitro* results provide a valuable baseline, the lack of *in vivo* animal models limits our immediate understanding of the bioavailability, metabolic transformation, and systemic efficacy of these edible flower extracts. Lastly, advanced molecular mechanistic assays, such as apoptosis markers, cell cycle arrest, or specific signaling pathway analyses, were not performed in this preliminary study.

Synergistic interactions of 5-FU combined with aqueous butterfly pea and lotus extracts

Based on the potent cytotoxic effects of butterfly pea and lotus extract, the mode of interaction between these 2 extracts and 5-FU was further investigated to assess potential synergistic effects against HT-29 cells using the Combination Index (CI). The dose-response curves for each combination are illustrated in **Figures 3** and **4**, and the derived IC_{50} values for CI calculation are summarized in **Table 5**. The results showed a significant distinction in interaction modes. The combination of butterfly pea extract with 5-FU exhibited a synergistic effect, as indicated by a CI value of 0.64. This result suggests that the butterfly pea extract can significantly enhance the cytotoxicity of 5-FU against HT-29 cells at lower doses. Interestingly, this synergism may be closely linked to the rapid cytotoxic onset observed in the butterfly pea extract. This early-phase action indicates that constituents of butterfly pea extract may function as immediate chemosensitizers, possibly by enhancing membrane permeability or interfering with initial survival signaling, which could improve the uptake and effectiveness of 5-FU. In contrast, the lotus

extract demonstrated an antagonistic interaction with a CI value of 1.13. This indicates that components in the aqueous lotus extract may partially interfere with the antiproliferative mechanisms of 5-FU. The observed interference is further supported by the lack of rapid initial activity in the lotus extract, suggesting that the timing of bioactive onset is a crucial factor in either enhancing or preventing the efficacy of chemotherapeutic agents.

Table 5 Combination index (CI) value of edible flower aqueous extracts combined with 5-FU against HT-29 cells at 24 h.

Samples	IC_{50} of 5-FU (mg/mL)	IC_{50} of samples (mg/mL)	IC_{50} of 5-FU in combination (mg/mL)	IC_{50} of samples in combination (mg/mL)	CI (24 h)	Interpretation
Butterfly pea	0.31	39.04	0.01	22.86	0.64	Synergism
Lotus	0.31	47.00	0.06	36.68	1.13	Antagonism

To our knowledge, this is the first study to explore the synergistic potential of aqueous butterfly pea extract in combination with 5-FU. Our findings are consistent with the previous research on the biological effects of butterfly pea; for instance, cyclotides from this plant have been shown to act as chemosensitizers when combined with paclitaxel in resistant lung cancer cells. This effect is primarily driven by membrane permeabilization, which facilitates increased drug uptake into the cells [52]. This rapid physical interaction with the cell membrane may explain the early morphological alterations observed in our study as early as 4 h post-treatment. It is possible that by disrupting membrane integrity, these metabolites accelerate intracellular 5-FU accumulation, thereby maximizing its capacity to inhibit thymidylate synthase and disrupt DNA synthesis even at sub-lethal concentrations [18]. However, it is important to note that these potential synergistic mechanisms discussed herein remain preliminary and conceptual. Consequently, the synergism between the aqueous butterfly pea extract and 5-FU observed in this study is hypothesized to be supported by the combined mechanistic principles of the bioactive compounds, which enhance cellular susceptibility and promote intracellular interactions. On the contrary, the antagonism observed with the lotus extract contrasts with previous reports where isolated compounds like rutin showed synergistic efficacy with 5-FU in prostate cancer (PC3 cells) and colorectal cancer (Caco-2 cells) [53]. This difference may be attributed to the complex composition of the crude lotus extract. The high abundance of specific phenolic acids or other cumulative compounds in lotus, which contributed to its high TPC, might interfere with the metabolic pathways or compete for the same molecular targets as 5-FU, resulting in decreased efficacy of the medicine [54,55]. Furthermore, because 5-FU-mediated cytotoxicity partially relies on the intracellular generation of reactive oxygen species (ROS) to trigger apoptosis [56], the exceptional radical-scavenging capacity of the lotus extract, owing to its abundant polyphenols, could potentially exert an unintended cytoprotective effect on HT-29 cells. It is possible that this antioxidant rescue mechanism counteracts the oxidative stress induced by 5-FU, thereby potentially mitigating its chemotherapeutic efficacy [56] and leading to the observed antagonistic interaction. This interpretation is strongly supported by recent pharmacodynamic interaction studies in colon cancer cell lines (HCT-116 and DLD-1 cells), which demonstrated that concomitant agents can significantly antagonize 5-FU-induced apoptosis and cell cycle arrest, even when their intracellular uptake is highly limited [57]. This suggests that extracellular or membrane-level interferences by co-administered components could be critical, yet underestimated, factors that may mitigate 5-FU-based chemotherapeutic outcomes. Nevertheless, since the scope of the present study focused on the preliminary screening of cytotoxic and synergistic trends, specific mechanistic validations—such as apoptosis assays, cell cycle analysis, direct intracellular ROS determination,

mitochondrial membrane potential assessment, or signaling pathway investigations—were not explicitly conducted at this stage. Therefore, the exact molecular pathways underlying the chemosensitization by butterfly pea remain speculative and must be interpreted with caution. These crucial mechanistic parameters provide excellent opportunities for future comprehensive investigations.

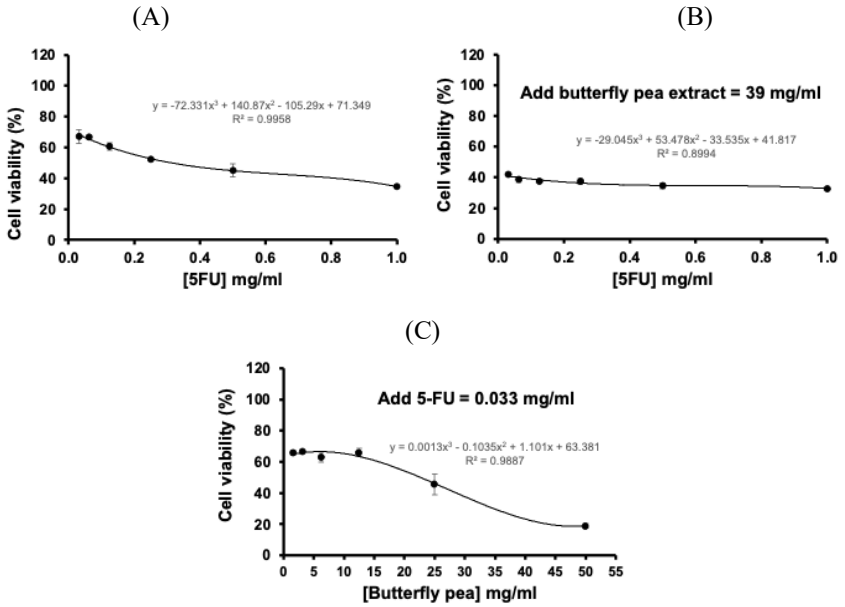


Figure 3 Antiproliferative effect of 5-FU and their combination with butterfly pea extract against HT-29 cells at 24 h. HT-29 cells were treated with (A) 5-FU, (B) 5-FU combined with fixed concentration of butterfly pea extract, and (C) butterfly pea extract combined with fixed concentration of 5-FU. Values presented are mean± SD derived from 3 independent biological replicates.

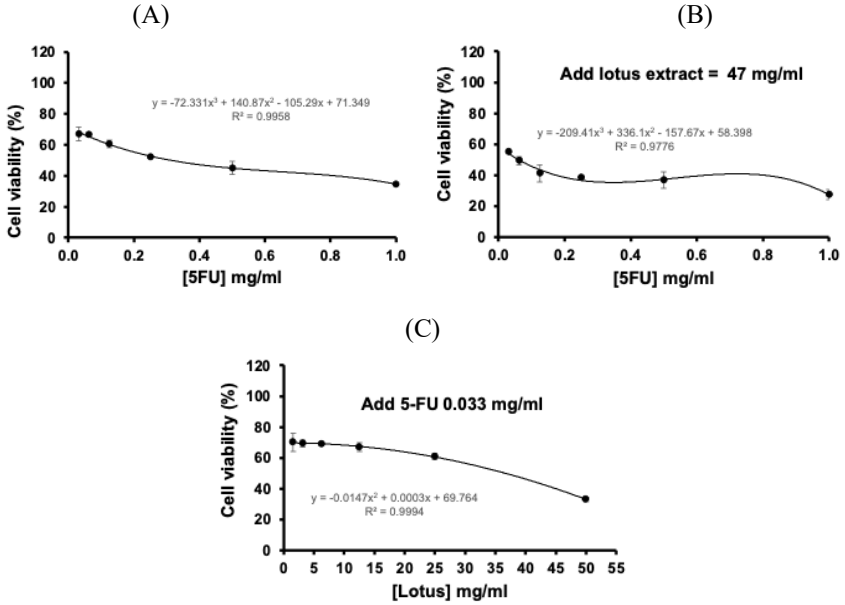


Figure 4 Antiproliferative effect of 5-FU and their combination with lotus extract against HT-29 cells at 24 h. HT-29 cells were treated with (A) 5-FU, (B) 5-FU combined with fixed concentration of lotus extract, and (C) lotus extract combined with a fixed concentration of 5-FU. Values presented are mean ± SD derived from 3 independent biological replicates.

Chemical composition of aqueous butterfly pea and lotus extracts

The bioactive components of the aqueous butterfly pea and lotus extracts were analyzed via GC-MS, with peak identification conducted through comparison with established compounds in the NIST library.

For the aqueous butterfly pea extract, a total of 28 peaks were identified (**Table 6** and **Figure 5**). The identified compounds were classified into several chemical classes, including carboxylic acid, pyranone derivatives, ketone, furan, and fatty acids. Carboxylic acid was the most common type of chemical, with acetic acid (31.27%) being the single most common. The next most common compounds were 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP) (11.78%), and dihydroxyacetone (7.95%). In comparison, the aqueous lotus extract showed a different but related profile with 24 identified peaks (**Table 7** and **Figure 6**). The major chemical groups included phenols, pyranone derivatives, amides, and carboxylic acids. Benzeneethanol, 4-hydroxy- (tyrosol), was the primary compound at 17.31%, followed by DDMP (12.57%) and 2-propenamide (7.92%).

Crucially, the high quantitative presence of DDMP in both extracts, along with the high concentration of acetic acid in the aqueous butterfly pea extract, suggests that these molecules may be sugar decomposition products resulting from the extraction of highly water-soluble polar molecules at room temperature [58]. Similarly, the GC-MS profile of the lotus extract highlighted a high proportion of tyrosol, which is likely associated with its high TPC and antioxidant capacity. Although DDMP [59-61] and tyrosol [62-65] have been reported to possess antioxidant and anticancer properties, their standalone potencies are typically insufficient to explain the strong cytotoxic effects observed in these extracts. Therefore, to avoid overinterpretation of GC-MS data, these volatile profiles should not be viewed as standalone therapeutic indicators; instead, the potent cytotoxicity is likely attributed to non-volatile compounds such as anthocyanins and flavonoids, which are abundant in these flowers [66-68] but remain undetectable by GC-MS [69-71]. Consequently, the direct association proposed between the identified volatile compounds via GC-MS and the observed biological activities remains speculative and should be regarded as a preliminary screening indicator rather than a definitive causal relationship. However, specific minor compounds identified via GC-MS may still play a critical role through synergistic interactions. Notably, the butterfly pea extract had a specific profile of fatty acids, including n-hexadecanoic acid (0.58%) and 9,12-octadecadienoic acid (Z,Z)- (0.42%). These fatty acids are known to modulate cell membrane fluidity, potentially acting as chemosensitizers that facilitate the uptake of other bioactive molecules or co-administered drugs like 5-FU [72-74]. This may explain why the butterfly pea extract exhibited superior synergistic activity with 5-FU, aligning with morphological observations of early cell membrane disruption within 4 h post-treatment. Beyond the anticancer potential, the major volatile constituents identified via GC-MS also hint at promising anti-diabetic implications. Notably, tyrosol (the primary compound in the lotus extract) has been well-documented to protect pancreatic beta cells from oxidative damage and improve insulin sensitivity [75]. Therefore, the high total phenolic content and the distinctive chemical profile of the lotus extract not only govern its cytotoxicity but also suggest its broader therapeutic value in managing oxidative stress-related metabolic disorders, such as diabetes.

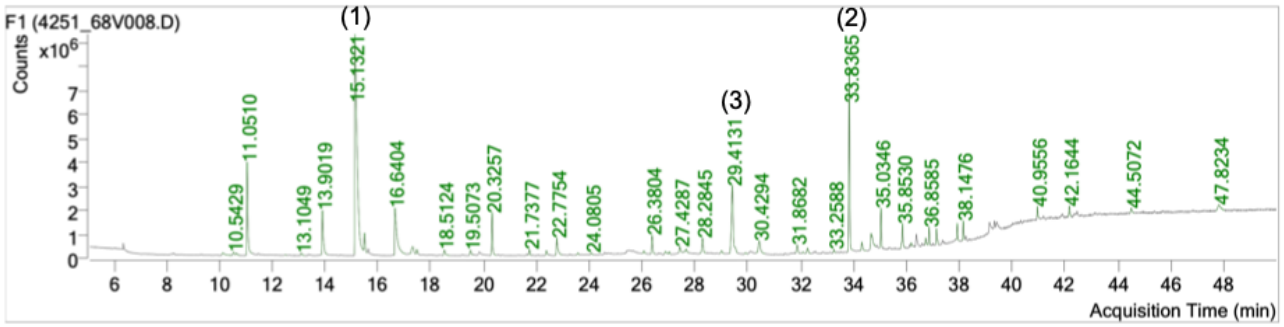


Figure 5 GC-MS chromatograms of the aqueous butterfly pea extract. Each detected spectral peak corresponds to a particular identified compound, with the peak intensity representing the relative abundance of the compounds. Key peaks are identified as follows: (1) acetic acid, (2) 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), and (3) dihydroxyacetone.

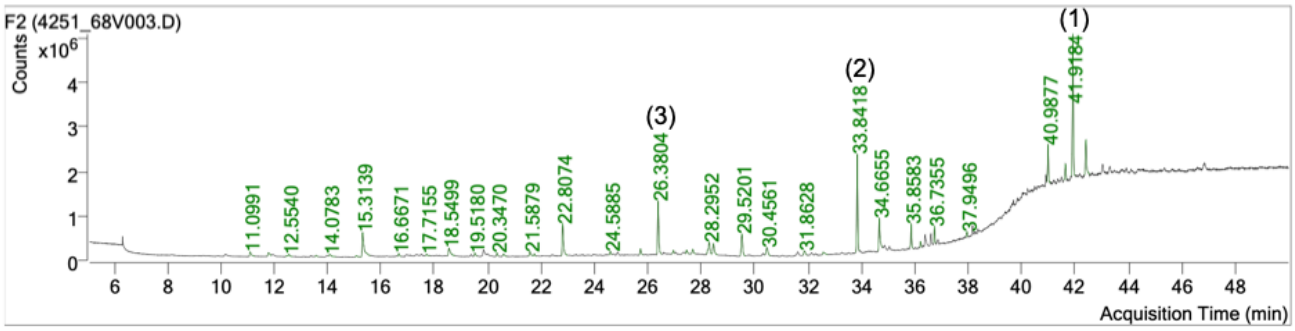


Figure 6 GC-MS chromatograms of the aqueous lotus extract. Each detected spectral peak corresponds to a particular identified compound, with the peak intensity representing the relative abundance of the compounds. Key peaks are identified as follows: (1) benzeneethanol, 4-hydroxy- (tyrosol), (2) 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), and (3) 2-propenamide.

When comparing our GC-MS results with previous studies, significant variability was observed dependent on the extraction solvent and the specific plant part used [45,46,76-78]. Notably, even among studies utilizing water as the main solvent, the resulting GC-MS profiles still differed. For instance, specific esters or alkaloids have been reported as the primary components of aqueous butterfly pea extract [43]; however, our analysis highlighted a predominance of organic acids and pyranone derivatives such as DDMP. These discrepancies in phytochemical profiles can be due to several factors. Apart from the geographical origin and environmental conditions of the samples, minor variations in extraction parameters, such as temperature and time, as well as analytical techniques, can significantly alter the profile of identified volatile compounds [70,71,79,80].

Table 6 Phytochemical compounds identified in aqueous butterfly pea extract by GC-MS analysis.

Peak	RT	Peak area	Estimated concentration (%)	Name	MF	MW	Classification
1	10.5429	458745.2	0.25	Dioxene (1,4-Dioxin, 2,3-dihydro-)	$C_4H_6O_2$	86.09	Ether
2	11.0510	12518296.4	6.78	Hydroxyacetone (2-Propanone, 1-hydroxy-)	$C_3H_6O_2$	74.08	Propanone
3	13.1049	334483.4	0.18	1-Hydroxy-2-butanone	$C_4H_8O_2$	88.11	Ketone
4	13.9019	6771496.2	3.67	1-Hydroxybut-3-en-2-one	$C_4H_6O_2$	86.09	Ketone
5	15.1321	57714492.7	31.27	Acetic acid	$C_2H_4O_2$	60.05	Carboxylic acid
6	16.6404	12318783.7	7	Formic acid	CH_2O_2	46.03	Carboxylic acid
7	18.5124	935669.4	0.51	4-Cyclopentene-1,3-dione	$C_5H_4O_2$	96.08	Ketone
8	19.5073	755017.5	1	2(3H)-Furanone, dihydro-	$C_4H_6O_3$	102.09	Lactone
9	20.3257	5014360.4	2.72	3-Furanmethanol	$C_5H_6O_2$	98.10	Furan
10	21.7377	696411.1	0.38	2-Furanmethanol, 5-methyl-	$C_6H_8O_2$	112.13	Furan
11	22.7754	2997513.7	1.62	1,2-Cyclopentanedione	$C_5H_6O_2$	98.10	Ketone
12	24.0805	232193.0	0.13	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	$C_6H_8O_2$	112.13	Ketone
13	26.3804	2359150.4	1.28	2-Propenamide	C_3H_5NO	78.08	Amide
14	27.4287	884040.4	0.48	3-Hydroxy-2H-pyran-2-one	$C_5H_4O_3$	112.08	Pyrone
15	28.2845	2382434.7	1.29	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	$C_6H_8O_3$	128.13	Furan
16	29.4131	14666700.1	7.95	Dihydroxyacetone	$C_3H_6O_3$	90.08	Ketone
17	30.4294	3019299.7	1.64	3(2H)-Furanone, 4-hydroxy-5-methyl-	$C_5H_6O_3$	114.10	Ketone
18	31.8682	1310285.0	0.71	2-Hydroxy-gamma-butyrolactone	$C_4H_6O_3$	102.09	Lactone
19	33.2588	434379.1	0.24	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	Fatty acid methyl ester
20	33.8365	21742372.7	11.78	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.12	Pyranone derivative
21	35.0346	3997909.3	2.17	2-Acetyl-2-hydroxy-.gamma.-butyrolactone	$C_6H_8O_4$	102.09	Lactone
22	35.8530	2969582.5	1.61	4-(1-Aminoethyl)phenol	$C_8H_{11}NO$	137.18	Phenol
23	36.8585	1697201.9	0.92	5-(Hydroxymethyl)dihydrofuran-2(3H)-one	$C_5H_8O_3$	116.11	Lactone
24	38.1476	1839868.6	1	2(3H)-Furanone, dihydro-4-hydroxy-	$C_4H_6O_3$	102.09	Lactone
25	40.9556	1078772.8	0.58	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Fatty acid
26	42.1644	1240381.8	0.67	2,5-Cyclohexadiene-1,4-dione, compd. with 1,4-benzenediol (1:1)	$C_{12}H_{10}O_4$	232.23	Quinhydrone
27	44.5072	770432.4	0.42	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.40	Fatty acid
28	47.8234	2119557.4	1.15	Lactone G	$C_5H_8O_4$	156.22	Lactone

Table 7 Phytochemical compounds identified in aqueous lotus extract by GC-MS analysis.

Peak	RT	Peak area	Estimated concentration (%)	Name	MF	MW	Classification
1	11.0991	465512.4	0.99	2-Propanone, 1-hydroxy-	$C_3H_6O_2$	74.08	Ketone
2	12.5540	289109.1	0.61	3-Hexanone, 2,5-dimethyl-4-nitro-	$C_8H_{15}NO_3$	173.21	Ketone
3	14.0783	341744.1	0.73	Pyrazine, trimethyl-	$C_7H_{10}N_2$	122.17	Pyrazine
4	15.3139	2979433.8	6.33	Acetic acid	$C_2H_4O_2$	60.05	Carboxylic acid
5	16.6671	220345.8	0.47	Pyrrole	C_4H_5N	67.09	Pyrrole
6	17.7155	132201.2	0.28	1H-Pyrrole, 2-methyl-	C_5H_7N	81.12	Pyrrole
7	18.5499	916050.5	2	Dimethyl Sulfoxide	C_2H_6OS	78.13	Sulfoxide
8	19.5180	242100.8	0.51	4-Butanolide	$C_4H_6O_2$	86.04	Lactone
9	20.3470	202524.8	0.43	2-Furanmethanol	$C_5H_6O_2$	98.10	Alcohol
10	21.5879	411478.3	0.87	3-Methyl-6,7-dihydro-5H-cyclopenta[b]pyrazine	$C_8H_{10}N_2$	134.18	Pyrazine
11	22.8074	2663638.2	5.66	Acetamide	C_2H_5NO	59.07	Amide
12	24.5885	281757.7	0.6	2,4(1H,3H)-Pyrimidinedione, 5-hydroxy-	$C_4H_4N_2O_3$	128.09	Uracil
13	26.3804	3726576.7	7.92	2-Propenamide	C_3H_5NO	71.08	Amide
14	28.2952	1311162.7	2.79	Furaneol	$C_6H_8O_3$	128.11	Ketone
15	29.5201	1902850.3	4.04	p-Cresol	C_7H_8O	108.14	Phenol
16	30.4561	964802.8	2.05	3(2H)-Furanone, 4-hydroxy-5-methyl-	$C_5H_6O_3$	114.10	Ketone
17	31.8628	447385.6	0.95	Butanoic acid, 3-hydroxy-3-methyl-	$C_5H_{10}O_3$	118.13	Carboxylic acid
18	33.8418	5917122.0	12.57	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.13	Pyranone derivative
19	34.6655	2763000.1	5.87	Glycerin	$C_3H_8O_3$	92.09	Polyol
20	35.8583	1363919.3	2.90	4-(1-Aminoethyl)phenol	$C_8H_{11}NO$	137.18	Phenol
21	36.7355	870257.1	1.85	Succinimide	$C_4H_5NO_2$	99.99	Amide
22	37.9496	284447.4	0.60	4-Hydroxy-3,5-dimethoxystyrene	$C_{10}H_{12}O_3$	180.20	Amide
23	40.9877	2360427.8	5.01	3,4-Dihydroxy-5-methyl-dihydrofuran-2-one	$C_5H_8O_4$	132.11	Lactone
24	41.9184	8151622.0	17.31	Benzeneethanol, 4-hydroxy-	$C_8H_{10}O_2$	138.16	Phenol

Conclusions

In conclusion, this study demonstrated that the biological efficacy of edible flower aqueous extracts is influenced by a complex interaction between phytochemical quantity and molecular specificity. The findings highlight 2 distinct therapeutic profiles among the tested edible flowers.

The aqueous extract of lotus emerges as the most effective multi-target candidate for overall health enhancement. It demonstrated the highest TPC and TFC, which were directly linked to its exceptional antioxidant capacities and remarkable α-glucosidase inhibitory activity. Furthermore, lotus demonstrated the highest cytotoxicity against HT-29 cancer cells after 72 h. These findings highlight lotus as a promising functional ingredient, particularly for postprandial glycemic management and oxidative stress reduction.

In contrast, the aqueous extract of butterfly pea demonstrated a unique profile characterized by molecular specificity rather than quantity. Despite its lower phytochemical content and weaker antidiabetic activity, butterfly pea exhibited the most rapid cytotoxic effects response and showed significant synergism with 5-FU. This rapid onset suggests that specific components act as early-phase chemosensitizers, enhancing 5-FU efficacy through kinetic-driven mechanisms rather than total phenolic abundance. Although these specific compounds were not fully identified via GC-MS in this study, they likely modulate cellular pathways that enhance cancer cell sensitivity to 5-FU. This highlights the promising capability of butterfly pea as a potential chemosensitizing candidate warranting further mechanistic validation in colorectal cancer.

Overall, while all tested flowers possess bioactivity, lotus stands out as a multitarget candidate for general health—particularly for diabetes management—whereas butterfly pea presents distinct potential as a synergistic adjuvant to conventional chemotherapy. These findings provide a scientific basis for utilizing Thai edible flowers as functional ingredients and as a source for developing novel multitarget nutraceuticals. Future research should incorporate in vitro gastrointestinal digestion models to assess bioactive compound stability and subsequent cytotoxicity. Additionally, expanding into comprehensive multi-dose and time-point profiling is warranted to fully characterize the synergistic dynamics with 5-FU. To pinpoint the specific bioactive constituents and underlying mechanisms, targeted metabolomics with proper validation, compound isolation, and deeper molecular signaling pathway analyses are required. Finally, evaluating selective toxicity against normal cell lines and validating these findings in *in vivo* models are necessary to confirm their multi-target nutraceutical potentials.

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Declaration of generative AI in scientific writing

The authors acknowledge the use of generative AI writing assistance tools (e.g., Gemini and QuillBot) in the preparation of this manuscript, specifically for language editing and grammar correction. No content generation or data interpretation was performed by AI. The authors take full responsibility for the content and conclusions of this work.

CRediT author statement

Chompoonuth Porncharoennop: Conceptualization; Methodology; Formal analysis; Investigation; Resources; Writing-Original draft preparation; Writing-Reviewing and Editing; Project administration. **Apatsara Truadnok**: Investigation. **Manit Srising**: Investigation. **Khakhanang Ratananikom**: Methodology; Validation; Investigation; Resources; Writing-Reviewing and Editing; Supervision.

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Supplementary Material

TPC calibration curve

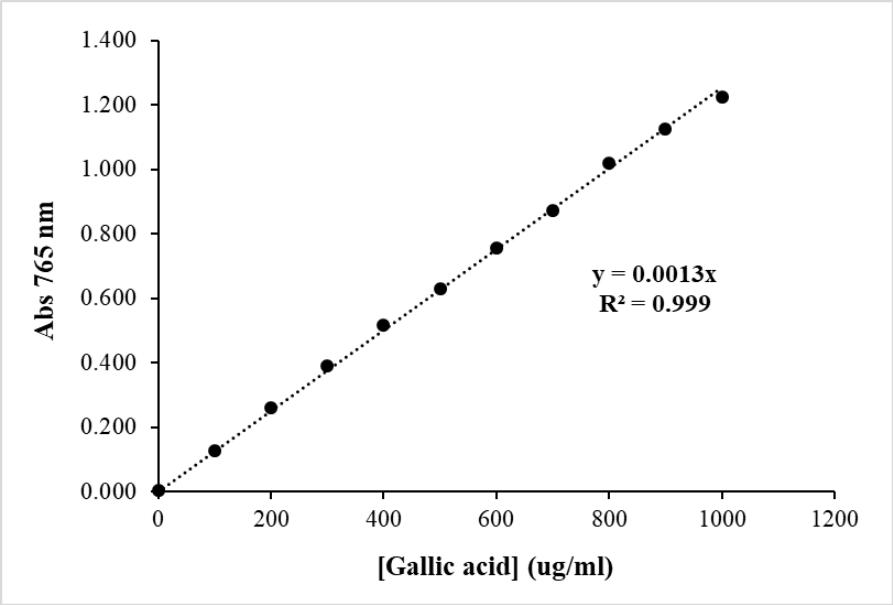


Figure S1 Calibration curve of gallic acid standard for total phenolic content (TPC) assay. This calibration curve was utilized to determine and quantify the total phenolic concentration in the aqueous edible flower extracts based on absorbance measured at 765 nm.

TFC calibration curve

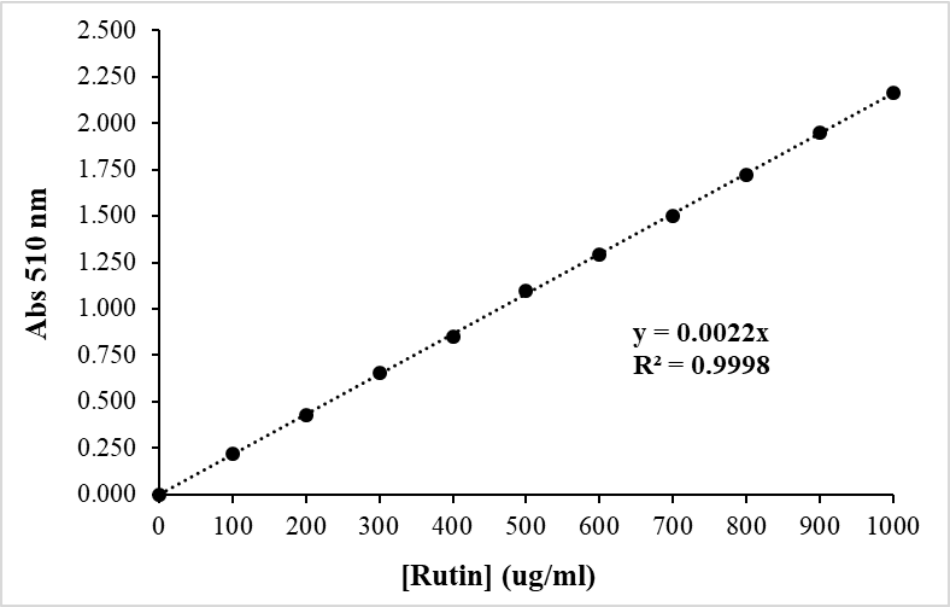


Figure S2 Calibration curve of rutin standard for total flavonoid content (TFC) assay. This calibration curve was utilized to calculate and quantify the total flavonoid concentration in the aqueous edible flower extracts based on absorbance measured at 510 nm.