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The Bioactive Composition, Antioxidant, and Antimicrobial Properties of Pink-Purple Edible Flowers in Thailand

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Abstract

Edible flowers have been traditionally consumed for their nutritional, medicinal, and culinary benefits worldwide. This study investigates the bioactive composition, antioxidant potential, and antibacterial properties of five pink-purple edible flowers in Thailand: *Antigonon leptopus* Hook. & Arn., *Plumeria obtusa* L., *Bougainvillea glabra Choisy, Ixora chinensis* Lam., and *Nelumbo nucifera* Gaertn. The total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (DPPH assay) were analyzed, along with antibacterial activity against *Salmonella typhimurium, Bacillus cereus, Staphylococcus aureus,* and *Escherichia coli* using the agar disc diffusion method. The results revealed significant variations in TPC, TFC and antioxidant activity among the tested flowers. *N. nucifera* exhibited the highest TPC (135.75 mg GAE/g dry extract), TFC (14.74 µg QE/g dry extract), and antioxidant capacity (127.37 µg Vit C/g dry extract), whereas *B. glabra* had the lowest values (12.19 mg GAE/g, 5.71 µg QE/g, and 49.44 µg Vit C/g, respectively). A similar trend was observed in antibacterial activity, where *N. nucifera* exhibited the most effective inhibition against *S. typhimurium, B. cereus, S. aureus,* and *E. coli* (1.02–1.22 cm inhibition zones), while *B. glabra* demonstrated the weakest antibacterial activity (0.00 cm against *E. coli*). Pearson correlation analysis further confirmed strong positive correlations between TPC and antioxidant activity ($r = 0.660$, $p < 0.01$), as well as TFC and antibacterial activity against *E. coli* ($r = 0.758$, $p < 0.01$). These findings indicate that phenolics and flavonoids play a major role in radical scavenging and antimicrobial mechanisms. This study provides new insights into the bioactive potential of flower extracts, highlighting *N. nucifera* as a promising natural antioxidant and antimicrobial agent for potential applications in functional foods and preservatives. Future studies should focus on identifying key bioactive compounds and optimizing extraction methods to maximize their therapeutic benefits.

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Introduction

For thousands of years, flowers have been used to enhance the appearance, color, aroma, and taste of various culinary traditions worldwide. For example, rose petals were utilized to improve the visual appeal and aesthetic quality of traditional food in ancient Greece and Rome (Melillo, 1994). In China, dried flowers were used to produce herbal tea, while fresh flowers were stir-fried, and their petals boiled to make soups (Zhang et al., 2023). In France, calendula flowers (*Calendula officinalis*) were incorporated into salads (Takahashi et al., 2020). In Europe, salads and beverages were prepared using the flowers of the dandelion (*Taraxacum officinale*) (Mlcek & Rop, 2011).

Similarly, Thailand has a rich tradition of consuming edible flowers. Ethnic groups in Northern Thailand commonly use *Cosmos bipinnatus, Bougainvillea glabra, Tagetes erecta,* and *Antigonon leptopus* to prepare floral teas and salads (Kaisoon et al., 2012). Additionally, edible flowers such as *Zingiber* (Ginger), *Hedychium* (Torch ginger), *Curcuma* (Ao), *Etlingera* (Torch ginger), *Amomum* (Chi Kuk), and *Alpinia* (Galangal) are widely incorporated into traditional Thai dishes, consumed fresh or cooked (Rachkeeree et al., 2018). Beyond their culinary uses, some Thai edible flowers are also valued for their medicinal properties in traditional medicine (Wongsa & Rattanapanone, 2021).

Edible flowers contain essential nutritional components such as proteins, lipids, saccharides, and vitamins (Pires et al., 2019), along with various bioactive substances including flavonoids, carotenoids, and phenolic acids (Mlcek & Rop, 2011; Navarro-González et al., 2014). Higher concentrations of these bioactive molecules are generally associated with increased antioxidant activity. (Ksouri et al., 2009; Moliner et al., 2018). In addition to their antioxidant properties, the phytochemicals in flowers offer significant potential health benefits for humans. Isoprenoids in lilac flowers have the potential anti-inflammatory properties (Oh et al., 2008). Arya et al. (2015) reported that flavonoids and phenolic compounds in *Woodfordia fructicosa* flowers enhance C-peptide secretion and serum insulin levels. Similarly, Kim et al. (2017) highlighted the potent anti-inflammatory and anticancer activities of fatty acids and glucosinolates derived from *Tropaeolum majus* extract. *Malva sylvestris* flowers contain rutin and quercetin, which inhibit α-amylase and α-glucosidase activity, resulting in a hypoglycemic effect (Loizzo et al., 2016). Extracts from calendula flowers have demonstrated antibacterial action against both gram-positive and gram-negative bacteria (Efstratiou et al., 2012). The antimicrobial properties of *Hibiscus rosa-sinensis* flowers have also been documented against various foodborne bacterial pathogens (Mak et al., 2013).

Nonetheless, the concentration of phytochemicals is influenced by numerous factors, including pre-harvest variables such as cultivar differences, environmental conditions, and agronomic practices, as well as post-harvest aspects like food processing and storage (Tiwari & Cummins, 2013). Additionally, Feduraev et al. (2019) reported that the phytochemical levels vary among plant organs and tissues. The highest concentration of phenolic compounds was found in the reproductive organs (seed and flower) and leaves, whereas roots and stems have the lowest concentration of phenolic chemicals. Phytochemical levels are also significantly influenced by extraction, purification, analysis, and quantification methods, each of which has varying degrees of specificity and sensitivity (Altemimi et al., 2017; Lu & Luthria, 2014). Interestingly, prior research has shown that flower pigmentation significantly affects bioactive compound content and antioxidant activity. For example, studies on *Rosa* spp. found that genotypes R4 and R10 (bright yellow) contain varying levels of carotenoids and β-carotene, while R7 (similar yellow hue) has the highest anthocyanin and betacyanin contents (Mallick et al., 2024). Popescu et al. (2025) also investigated five red flowers and found varying concentrations of total polyphenols and total anthocyanins. These findings indicate significant compositional variability among flowers of the same color.

Given this evidence, this study aims to investigate whether flowers with similar pink-purple coloration (*Antigonon leptopus* Hook. & Arn., *Plumeria obtusa* L., *Bougainvillea glabra* Choisy, *Ixora chinensis* Lam., and *Nelumbo nucifera* Gaertn.) exhibit variations in total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacity, and antibacterial properties. Understanding these differences will provide valuable insights into the relationship between flower pigmentation and bioactive properties, which may have implications for their application as natural antioxidants and antimicrobial agents in the food and pharmaceutical industries.

Materials and methods

1. Plant sample collection and preparation

Five species of edible flowers were analyzed in this study: *Antigonon leptopus* Hook. & Arn., *Plumeria obtusa* L., *Bougainvillea glabra* Choisy, *Ixora chinensis* Lam., and *Nelumbo nucifera* Gaertn., as listed in Table 1. The identified plant flower species and voucher specimens were deposited at the Department of Food Technology, Faculty of Agricultural Technology, Kalasin University. Fresh flowers were harvested in the rainy season during July to October 2023 in Kalasin Province, Thailand. The collected flowers were thoroughly washed with distilled water, and the petals were carefully separated and left at room temperature to drain. Subsequently, the petals were dried using hot air at 45°C for 20 h. The dried petals were then ground into a fine powder and stored at −20°C for subsequent experiments.

2. Chemical properties and antioxidants of extract

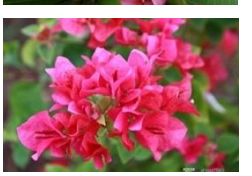
2.1 Preparation of extracts

A gram of the dried powdered samples was dissolved in 20 mL of 80% methanol. The tube was shaken at 120 rpm for 60 min in an UltraRocker™ Rocking Platform (Hercules, CA) at room temperature. Each extracted sample was then transferred into an appropriate tube and centrifuged at 9000 rpm for 15 min using a Camlab ALC 4239R high-speed refrigerated centrifuge (Camlab, Cambridge, UK). The aqueous phase was collected using a fine-needle syringe and filtered with a 0.45 µm nylon syringe.

2.2 Determination of total phenolic content (TPC)

The total phenolic content of the five flower extracts was assessed using the Folin–Ciocalteu reagent, following a slightly modified protocol as described by Salih et al. (2021). Briefly, 5 µL of the extract was combined with 1.5 mL of deionized water and 50 µL of the Folin–Ciocalteu reagent. After allowing the mixture

Table 1 Scientific name, Thai name, and morphology of five edible flowers

Scientific name	Family	Thai name	Voucher specimen	Morphology
Antigonon leptopus Hook. & Arn.	Polygonaceae	Puangchompoo	PFT202301	
Plumeria obtusa L.	Apocynaceae	Leelawadee	PFT202302	
Bougainvillea glabra Choisy	Nyctaginaceae	Fueangfa	PFT202303	
Ixora chinensis Lam.	Rubiaceae	Kem	PFT202304	
Nelumbo nucifera Gaertn.	Nelumbonaceae	Bua luang	PFT202305	

to stand at room temperature for 8 min, 50 µL of a 20% sodium carbonate solution was added to the mixture. Following a 30-min reaction period, the UV-visible spectrophotometer (Biochrom Libra S22, Cambridge, UK) was used to measure the color absorbance at 765 nm. A calibration curve was generated using varying concentrations of gallic acid (0.15 to 10 mg/mL) to determine the phenolic content. The total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per gram of dry extract.

2.3 Determination of total flavonoid content (TFC)

The total flavonoid content of five flower extracts were determined by the colorimetric method described by Nabi & Shrivastava (2016). Briefly, each extract (1.5 mL) was combined with 1.5 mL of a 10% aluminum chloride solution and incubated in the dark at room temperature for 30 min. Absorbance at 425 nm was measured using a UV-visible spectrophotometer. A calibration curve was constructed using quercetin, demonstrating linearity within the concentration range of 3 to 100 µg/mL. The total flavonoid content was expressed as micrograms of quercetin equivalent (QE) per g of dry extract.

2.4 Determination of DPPH radical scavenging capacity

The antioxidant capacity of the five flower extracts was evaluated using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, following the method described by Chaves et al. (2020). Briefly, 1 mL of each flower extract solution, ranging from 0.1 to 2 mg/mL, was mixed with 1 mL of 0.1 mM DPPH radical solution. The mixture was incubated in the dark at room temperature for 30 min, after which the absorbance was measured at 517 nm using a UV-visible spectrophotometer. A calibration curve was constructed by plotting the absorbance values of vitamin C standards (7 to 125 µg/mL) against their concentrations. The antioxidant activity of the flower extracts was expressed as µg vitamin C (Vit C)/g dry extract.

3. Antibacterial method

3.1 The Agar disc diffusion method

The antimicrobial activity of the five flower extracts was investigated against *Escherichia coli* (*E. coli*) ATCC 25922, *Bacillus cereus* (*B. cereus*) TISTR 1449, *Staphylococcus aureus* (*S. aureus*) TISTR 746 and *Salmonella typhimurium* (*S. typhimurium*) TISTR 1472 using the agar disc diffusion method, asdescribed by Heatley (1944).

The bacterial cultures were grown in nutrient broth and incubated at 37°C overnight. Each broth was adjusted to OD600 = 0.1-0.12 (10^6 CFU/mL). Each bacteria indicator (90 µL) was spread onto agar plates using a surface spread technique. Sterile filter paper discs were spotted with a diameter of 6 mm. were then positioned on the agar plates. The sterile filter paper discs were spotted with 10 µL of the flower extract (250 mg/mL) and incubated for 24 h at 37°C. The diameter of the clear zone was measured using a vernier caliper. The antibiotic vancomycin (30 µg/disc) was used as a positive control, while dimethyl sulfoxide (DMSO) and distilled water were used as negative controls.

4. Statistical analysis

The results of each experiment are presented as bar graphs, displaying the mean with standard deviations (SD), based on triplicate samples for each treatment. The collected data were analyzed using a two-way analysis of variance (ANOVA) in a completely randomized design (CRD). Post-hoc comparisons were conducted using Duncan's multiple range test to determine significant differences among samples ($p<0.05$). Pearson correlation analysis (2-tailed) was performed to evaluate the relationships between bioactive content, antioxidant capacity, and antibacterial activity. Statistical analyses were conducted using SPSS software (version 29).

Results and discussion

1. Chemical properties and antioxidants of extract

1.1 Total phenolic content (TPC)

Fig. 1A illustrates the total phenolic content (TPC) of five different flowers. The TPC of the flowers varied significantly, ranging from 12.19 mg GAE/g dry weight in *B. glabra* to 135.75 mg GAE/g dry weight in *N. nucifera*. *A. leptopus* also exhibited a notably high TPC, albeit significantly lower, at 107.64 mg GAE/g dry weight, followed by *I. chinensis* with 45.41 mg GAE/g dry weight and *P. obtusa* with 33.75 mg GAE/g dry weight.

Research findings indicate significant variation in the total phenolic contents among different flower types. Zheng et al. (2018) reported that the total phenolic content of 65 flower samples varied widely, ranging from 5.48 mg GAE/g in cucumber flowers to 312.21 mg GAE/g in roses, representing a more than 55-fold difference. Notably, the total phenolic content of Rosa species was found to be considerably higher (108.94-312.21 mg GAE/g) compared to other flowers (Zheng et al., 2018).

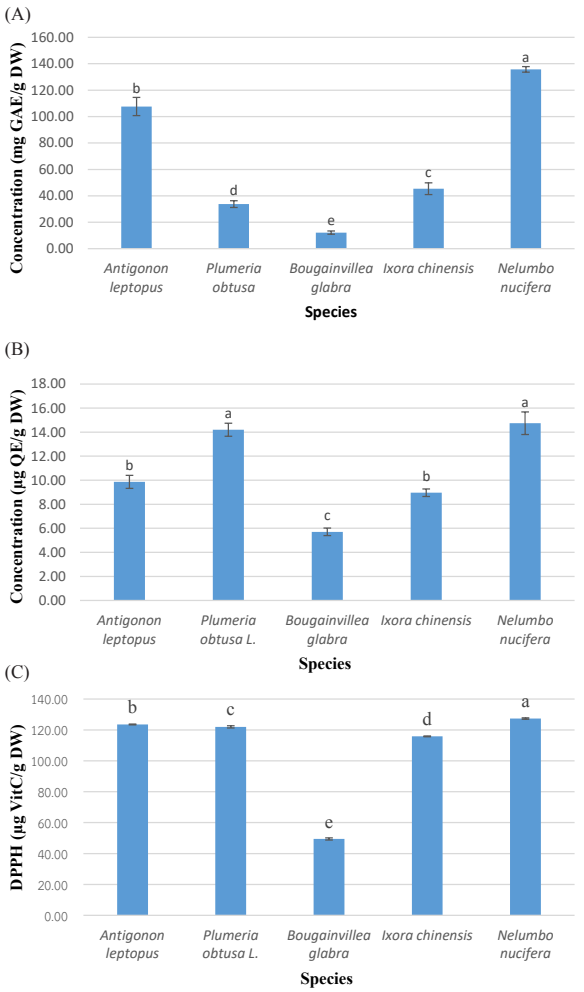


Fig. 1 Total phenolic content (A), total flavonoid content (B), and antioxidant activity (C) of five flower extracts

Additionally, remarkable differences in total phenolic content were observed among the different colors of rose. Red roses exhibited the highest total phenolic content (27.53 mg GAE/g), followed by pink roses (23.30 mg GAE/g) and white roses (17.60 mg GAE/g) (Gonçalves et al., 2020). The biosynthesis of phenolic compounds was influenced by environmental parameters such as soil type, altitude, temperature, and precipitation (Brahmi et al., 2022).

Furthermore, Feduraev et al. (2019) investigated the phenolic compounds in various plant organs and tissues, revealing that leaves and reproductive organs (flowers and seeds) exhibited the highest concentrations of phenolic compounds. Conversely, roots and stems contained the lowest concentration. Additionally, phenolic levels were significantly influenced by the methods of extraction, purification, analysis, and quantification (Dai & Mumper, 2010).

1.2 Total flavonoid content (TFC)

Fig. 1B displays the total flavonoid content of five flower samples *N. nucifera* (14.74 µg QE/g dry weight) and *P. obtusa* (14.20 µg QE/g dry weight) exhibited the highest total flavonoid content among the analyzed flowers, with no statistically significant differences between them. These were followed by *A. leptopus* (9.86 µg QE/g dry weight) and *I. chinensis* (8.96 µg QE/g dry weight), while *B. glabra* showed the lowest flavonoid content (5.71 µg QE/g dry weight). The total flavonoid content for all samples were lower than the total phenolic content values, which is expected as flavonoids are low molecular weight polyphenolic secondary metabolites (Donadio et al., 2021; Villaruz et al., 2023).

Plant organs such as leaves, seeds, bark, and flowers contain flavonoid in varying levels. According to Shi et al. (2019), the flowers of *E. bulbosa* had the greatest total flavonoid concentration, which was 2.5 times that of the bulbs and 3 times that of the leaves. The total flavonoid content was also impacted by species and cultivars. Main flavonol glycoside levels vary significantly between rose species and cultivars (Wan et al., 2019).

Flavonoids are associated with pigments of various colors and are used for a variety of purposes, including protection against abiotic stresses, UV light, bacterial and fungal phytopathogens, and auxin movement in plants (Li et al., 2018; Sandoval-Yañez et al., 2018). Many previous studies demonstrated that flavonols interact with anthocyanin to play an essential role in flower coloration (Czemmel et al., 2009; Luo et al., 2016; Tian et al., 2015). Furthermore, the stability of the flavonoids, as well as the solvents, equipment, and extraction methods used, all affect the total flavonoid concentration (Liga et al., 2023).

1.3 DPPH radical scavenging capacity

The antioxidant activity of five flower samples is presented in Fig. 1C. *N. nucifera* (127.37 µg Vit C/g dry weight), *A. leptopus* (123.54 µg Vit C/g dry weight), *P.obtusa* (121.98 µg Vit C/g dry weight), and *I. chinensis* (115.84 µg Vit C/g dry weight) exhibited the highest values of antioxidant activity, with statistically significant differences. In contrast, *B. glabra* showed the lowest antioxidant activity among the samples analyzed (49.44 µg Vit C/g dry weight). The obtained results for the antioxidant activity of five flower samples are consistent with the total phenolic content total flavonoid contents

(Fig. 1A and Fig. 1B). Many published studies indicated that antioxidant capacity shows a linear correlation with the total phenolic and flavonoid contents (Shrestha & Dhillion, 2006). The presence of other bioactive compounds, including pro-anthocyanins, tannin, anthocyanin, phenols, and alkaloids, also contributes to the antioxidant activity of flowers (Hamzah & Zubair, 2019; Mukherjee et al., 2011; Saeed et al., 2012).

The results of this study indicated that *Nelumbo nucifera* exhibits the highest TPC, TFC, and antioxidant capacity among the tested flowers. These findings are consistent with previous research, which has identified *N. nucifera* as a rich source of alkaloids, flavonoids, terpenoids, steroids, and glycosides, with kaempferol-3-O-robinobioside (Kae-3-Rob) being a key bioactive compound (Bishayee et al., 2022; Nutho & Tungmunnithum, 2023).

However, unlike most prior studies that have focused on leaves, seeds, or rhizomes, this study specifically evaluates flower extracts, offering new insights into their bioactive composition and functional properties (Ren et al., 2024; Wang et al., 2023; Ye et al., 2018; Zhang et al., 2024). The bioactive profile of *N. nucifera* varies significantly across different plant parts—leaves are rich in flavonoids and alkaloids, flowers and plumules contain flavonoids, seeds are abundant in alkaloids, and rhizomes are high in starch (Bishayee et al., 2022).

These bioactive compounds have demonstrated therapeutic potential against obesity, diabetes, neurodegeneration, cancer, and cardiovascular diseases (Bishayee et al., 2022; Wang et al., 2023). Further analysis using Pearson correlation also revealed that antioxidant activity results from complex interactions between phytochemicals rather than a single compound. Among these, flavonoids play a dominant role, primarily in quenching free radicals via the hydrogen atom transfer (HAT) mechanism (Tungmunnithum et al., 2022).

2. Antibacterial activity

The antimicrobial activity of the five tested flower extracts, as shown in Fig. 2, revealed distinct inhibition patterns against different pathogenic bacteria, including *S. typhimurium, B. cereus, S. aureus,* and *E. coli.* Among these, *N. nucifera* demonstrated the most effective antibacterial activity, forming inhibition zones ranging from 1.02 to 1.22 cm. In contrast, *B. glabra* exhibited the lowest activity, with no inhibition observed against *E. coli* (0.00 cm). Consistent with prior studies, crude plant extracts typically demonstrate stronger antibacterial activity against Gram-positive bacteria

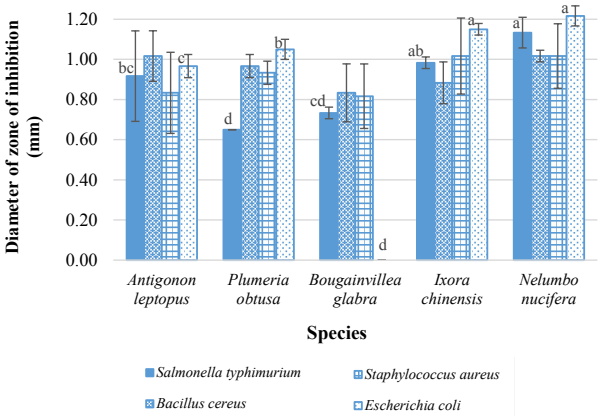


Fig. 2 Antimicrobial activity of five flower extracts against different bacterial stains

than Gram-negative bacteria. This difference is primarily due to structural variations in the bacterial cell envelope, including differences in the cytoplasmic membrane and cell wall composition (Kabuki et al., 2000; Silhavy et al., 2010; Tian et al., 2009). However, in this study, the flower extracts demonstrated inhibitory effects against both Gram-positive and Gram-negative bacteria. This aligns with the findings by Mak et al. (2013), who reported that *Cassia* flower extracts inhibited both bacterial types. Similarly, *Hibiscus rosa-sinensis* has demonstrated antimicrobial activity against a broad spectrum of Gram-positive and Gram-negative foodborne pathogens, which is attributed to its abundant polyphenols, flavonoids, and tannins (Mak et al., 2013).

The results of this study further corroborated that *N. nucifera* had the highest total phenolic and flavonoid contents among the tested flowers, which likely contributed to its superior antibacterial activity. Conversely, *B. glabra* exhibited the lowest bioactive composition, reflecting its minimal inhibition zones. Phenolic compounds such as phenolic acids, flavonoids, stilbenes, and tannins have been well-documented for their antimicrobial properties, effectively inhibiting the growth of food-borne pathogens, clinically relevant bacteria, fungi, and protozoa (Daglia, 2012; Li et al., 2014; Schmidt et al., 2012). The antibacterial mechanism of phenolics is thought to involve interactions with microbial enzymes and transport proteins, resulting in protein inactivation and subsequent inhibition of microbial growth (Scalbert, 1991).

Previous studies have highlighted that *N. nucifera* possesses strong antimicrobial properties due to its rich content of alkaloids, flavonoids, and terpenoids.

Table 2 Correlation matrix of the studied parameters

	TPC	TFC	Antioxidant activity	Inhibition of *S. typhimurium*	Inhibition of *B. cereus*	Inhibition of *S. aureus*	Inhibition of *E. coli*
TPC	1.000						
TFC	0.543*	1.000					
Antioxidant activity	0.660**	0.784**	1.000				
Inhibition of *S. typhimurium*	0.729**	0.197	0.407	1.000			
Inhibition of *B. cereus*	0.568*	0.530*	0.547*	0.319	1.000		
Inhibition of *S. aureus*	0.192	0.342	0.329	0.343	0.608*	1.000	
Inhibition of *E. coli*	0.606*	0.758**	0.972**	0.489	0.494	0.448	1.000

Remark: *Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed)

Alkaloids, such as neferine, liensinine, isoliensinine, and nuciferine, disrupt bacterial cell walls and inhibit bacterial growth (Sharma et al., 2017). Flavonoids interfere with bacterial cell membranes and essential proteins, with structural modifications like hydroxylation and prenylation enhancing their antibacterial activity (Donadio et al., 2021; Shamsudin et al., 2022). Terpenoids, including sesquiterpenoids, exhibit diverse structural mechanisms that effectively target bacterial pathogens (Li et al., 2022). In contrast, other flowers may rely on different phytochemical compositions or antibacterial mechanisms. Variations in compound structures, environmental conditions, and evolutionary adaptations influence the types and concentrations of bioactive compounds, leading to differences in antimicrobial efficacy.

The Pearson correlation analysis in Table 2 revealed significant relationships between bioactive content, antioxidant activity, and antibacterial efficacy. A strong positive correlation was observed between total phenolic content (TPC) and antioxidant activity ($r = 0.660$, $p < 0.01$), indicating that phenolics play a dominant role in radical scavenging activity, which aligns with previous findings (Platzer et al., 2022). Similarly, total flavonoid content (TFC) exhibited a significant correlation with antioxidant activity ($r = 0.784$, $p < 0.01$), reinforcing the role of flavonoids in oxidative stress reduction (Zhang et al., 2024).

In terms of antimicrobial properties, TPC showed a strong correlation with the inhibition of *S.typhimurium* ($r = 0.729$, $p < 0.01$) and *B. cereus* ($r = 0.568$, $p < 0.05$), suggested that phenolic compounds significantly contribute to bacterial inhibition through mechanisms such as membrane disruption, interference with protein synthesis, and enzyme inhibition (Ghosh et al., 2025; Todorov et al., 2025). Additionally, TFC indicated correlated significantly with *E.coli* inhibition ($r = 0.758$, $p < 0.01$), indicated that flavonoids may target Gram-negative bacteria by binding to the phospholipids of the bacterial membrane. This interaction leads to the dissipation of the proton motive force and metabolic perturbations, rather than through protein denaturation or direct cell wall interactions (Song et al., 2021).

Interestingly, while antioxidant activity showed a strong correlation with *E. coli* inhibition ($r = 0.972$, $p < 0.01$), its relationship with other bacterial strains was weaker. This suggests that antibacterial mechanisms may involve additional bioactive compounds beyond phenolics and flavonoids, such as alkaloids and terpenoids (Arrigoni et al., 2024; Bouyahya et al., 2022). The lack of significant correlation between TFC and *Salmonella typhimurium* inhibition ($r = 0.197$, $p > 0.05$). further supports the notion that different bacterial strains may exhibit varying susceptibility to plant-derived bioactive compounds (Bouyahya et al., 2022).

Conclusion

This study highlights the bioactive potential of five pink-purple edible flowers in Thailand, demonstrating significant variations in phenolic and flavonoid content, antioxidant capacity, and antimicrobial activity. *Nelumbo nucifera* exhibited the highest antioxidant and antibacterial properties, making it a promising natural source for functional foods and preservatives. The findings confirm that flower pigmentation influences bioactive composition, reinforcing the nutritional and medicinal value of edible flowers. Future research should explore compound identification, extraction optimization, and synergistic effects to enhance their application in food and health industries.

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