

# Efficacy of Anthocyanin extracts from butterfly pea flowers for inhibition of skin pathogenic bacteria and free radicals

Nitchakamol Byunheang and Yingmanee Tragoolpua\*  
 Division of Microbiology, Department of Biology Faculty of Science Chiang Mai University  
 \*Email: yingmanee.t@cmu.ac.th



## Abstract

Butterfly pea (*Clitoria ternatea*) is a flowering plant commonly found in tropical Asia, including Thailand. It has a unique flower appearance, especially the purple-blue color of the flower, which is rich in anthocyanin. In this study, dried butterfly pea flowers were extracted using water and 95% ethanol as a solvent for extraction. Moreover, the anthocyanin from butterfly pea flowers were extracted using 1% acetic acid and 1% acetic acid in 50% ethanol. The extracts were tested for anti-free radical properties and inhibitory activities against growth of bacteria; *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis* and *Streptococcus agalactiae*. It was found that the crude aqueous extracts demonstrated the highest antioxidant activity of 13.10±1.07 mg gallic/g extract by the DPPH assay. In addition, the anthocyanin extracted with 1% acetic acid showed the highest anti-free radical activity of 47.40±8.68 mg trolox/g extract by ABTS assay. The highest antibacterial activity was found from anthocyanin from butterfly pea extract with 1% acetic acid in 50% ethanol, which was able to inhibit the growth of all tested bacteria at the minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of 62.5 mg/ml. However, the crude ethanolic extract of butterfly pea demonstrated MIC and MBC of 125 mg/ml. Therefore, anthocyanin and crude extract of butterfly pea flowers demonstrated anti-free radical and anti-bacterial activity against skin pathogenic bacteria.

## Introduction

Medicinal plants present a promising alternative for the treatment of skin disease. The butterfly pea flower (*Clitoria ternatea*) is traditional use in health promotion. It contains bioactive compounds such as anthocyanins, which have demonstrated the ability to inhibit various pathogenic bacteria and anti-free radical activity.

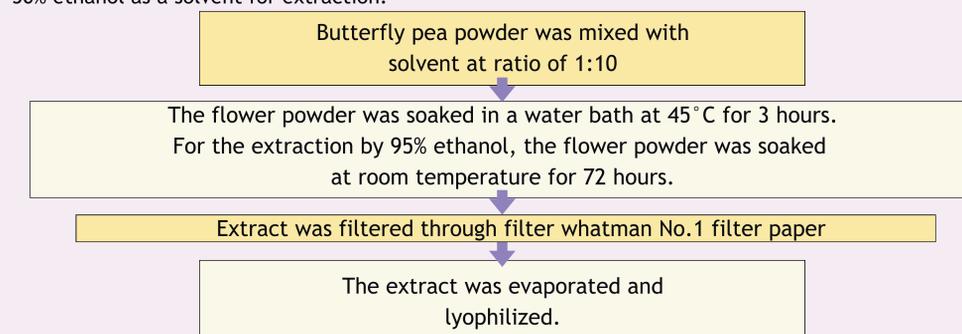
## Objective

To determine anti-free radical and anti-bacterial activities of anthocyanin from butterfly pea extracts obtained from different solvents including DI water, 95% ethanol, 1% acetic acid, and 1% acetic acid in 50% ethanol.

## Method

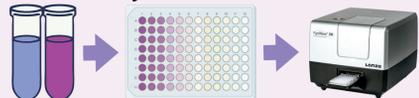
### Extraction methods

Butterfly pea powder was extracted using DI water, 95% ethanol, 1% acetic acid and 1% acetic acid in 50% ethanol as a solvent for extraction.



### Antioxidant activity

#### • DPPH assay



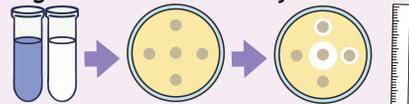
#### • ABTS assay



Anti-free radical activities of the extracts and anthocyanin were determined by DPPH and ABTS assays.

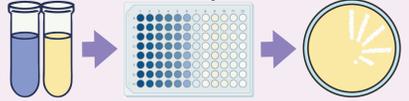
### Antibacterial activity

#### • Agar well diffusion assay



Extracts and gentamicin were tested against pathogenic bacteria on agar plate, incubated 24 hr 37°C and inhibition zones were measured.

#### • MIC and MBC analysis



The extracts were prepared and mixed with tested bacteria in a 96-well plate. Wells with no visible bacterial growth (MIC) were streaked onto agar plates to verify the MBC, defined as the lowest extract concentration that can inhibit bacterial growth.

## Results

### • Yield (%) of butterfly pea extracts

Table 1 Yield (%) of butterfly pea extracts

Extracts	Yield (%)
DI water	39.75
Ethanol	15
1% acetic acid	37.83
1% acetic acid in 50% ethanol	41.58

### • DPPH and ABTS activity

#### DPPH

#### ABTS

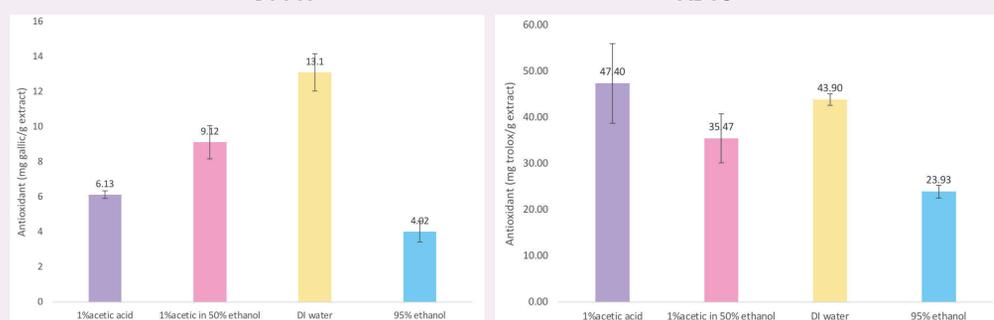


Fig 1 Antioxidant activity of extracts

Table 2 Antioxidant activity of butterfly pea extracts

Extracts from different solvent	Antioxidant activity			
	DPPH		ABTS	
	IC <sub>50</sub> (mg/ml)	Antioxidant (mg gallic acid/g extract)	IC <sub>50</sub> (mg/ml)	Antioxidant (mg trolox/g extract)
DI water	0.42±0.04	13.10±1.07	4.34±0.04	43.90±1.23
Ethanol	1.39±0.21	4.20±0.58	7.97±0.31	23.93±1.36
1% acetic acid	0.90±0.03	6.13±0.21	4.08±0.68	47.40±8.68
1% acetic acid in 50% ethanol	0.61±0.065	9.12±0.95	5.43±0.72	35.47±5.32

### • Agar well diffusion assay

Table 3 Inhibition zone (mm) of butterfly pea extracts for against pathogenic bacterial growth

Extracts from different solvent	Inhibition zone (mm)			
	Bacteria tested			
	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. epidermidis</i>	<i>S. agalactiae</i>
DI water	0.00	0.00	0.00	0.00
Ethanol	12.67±2.08	16.67±0.57	12.33±0.57	14.33±2.08
1% acetic acid	0.00	0.00	0.00	0.00
1% acetic acid in 50% ethanol	19.67±2.30	0.00	16.00±1.00	16.33±0.57
Gentamicin 1 mg/ml	34.00±0.00	37.00±0.00	33.00±0.00	32.00±0.00

### • MIC and MBC analysis

Table 4 MIC and MBC (mg/ml) of butterfly pea extracts for inhibitor pathogenic bacterial growth

Extracts from different solvent	MIC and MBC (mg/ml)							
	Bacteria tested							
	<i>S. aureus</i>		<i>P. aeruginosa</i>		<i>S. epidermidis</i>		<i>S. agalactiae</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
DI water	250	250	250	250	>250	>250	>250	>250
Ethanol	125	125	125	125	125	125	125	125
1% acetic acid	250	250	125	125	250	250	>250	>250
1% acetic acid in 50% ethanol	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
Gentamicin 1 mg/ml	0.008	0.008	0.008	0.008	0.008	0.008	0.06	0.06

## Discussion and Conclusion

The anthocyanin extract that obtained using 1% acetic acid in 50% ethanol, showed the highest yield and greater antioxidant activity than extracts from other solvents. Moreover, the anthocyanin extract that obtained using 1% acetic acid in 50% ethanol also effectively inhibited bacterial growth at the lowest concentration of 62.5 mg/ml, followed by the ethanolic extract, extract from 1% acetic acid, and aqueous extract.

The Butterfly Pea flowers comprise various anthocyanin, including cyanidin-3-glucoside, delphinidin-3-glucoside, and ternatin A1. These pigments are water-soluble but exhibit greater stability in acidic environments and enhances their stability. Furthermore, anthocyanin derivatives contain a diverse range of organic and inorganic compounds, which can be more effectively extracted using 50% ethanol. Moreover, the anthocyanin can disrupt bacterial cell membranes, leading to leakage of proteins and nucleic acids, lose their ability to synthesize essential proteins and enzymes, and intracellular metabolic processes.

## References

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