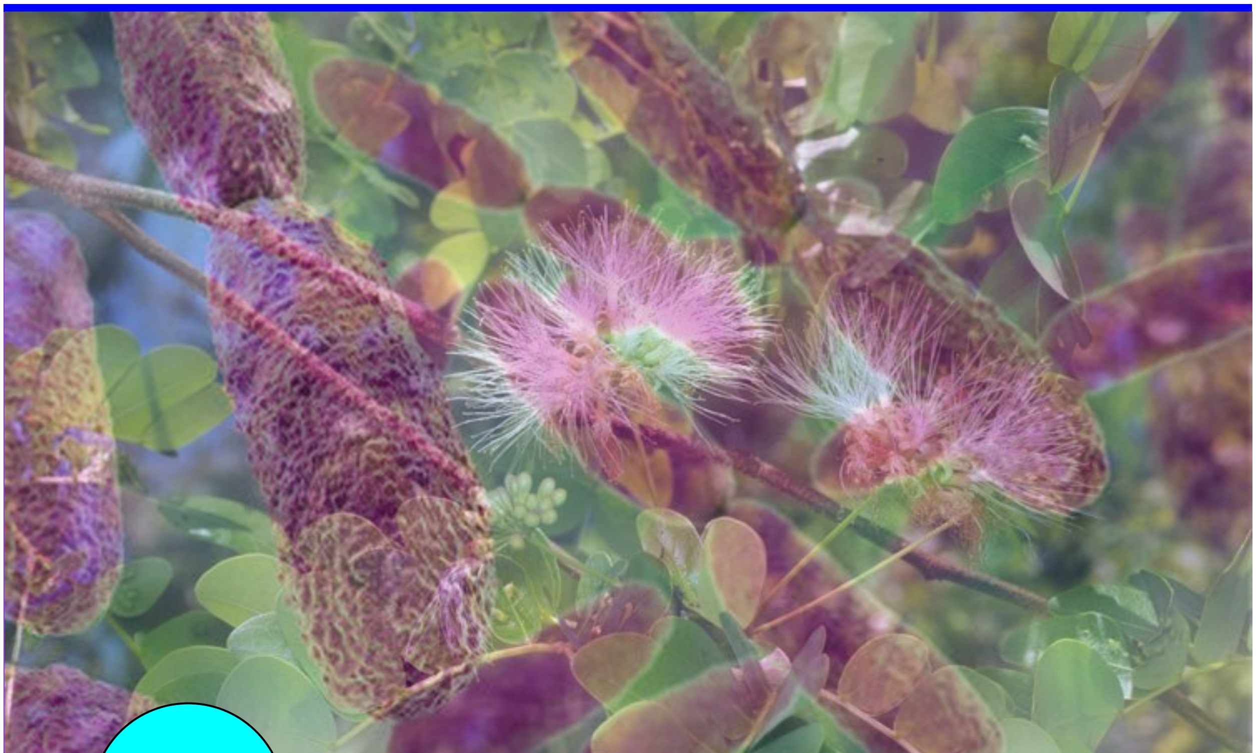

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Antibacterial activity of *Cathormion umbellatum*

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Abstract

The aim of this study was to determine the antibacterial activity of *Cathormion umbellatum* extracts against seven antibiotic-resistant bacteria. The pods, leaves and branches of *C. umbellatum* were extracted with ethanol and methanol. The disc diffusion assay was used to screen the antibacterial activity and broth microdilution and colorimetric assay were used to measure the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. The result indicated that the highest inhibition zone (11 mm) was presented in ethanolic pods extract against multidrug resistance *Klebsiella pneumoniae*. The lowest MIC value of 0.1 mg/mL was obtained from branch extracted with ethanol against colistin resistant *Pseudomonas aeruginosa*. The lowest MBC values of 1.6 mg/mL were obtained when using *C. umbellatum* leaves extracted with methanol against all test antibiotic-resistant bacteria. This is the first report presented *C. umbellatum* extracts have the potential to eliminate antibiotic-resistant bacteria in patients. These findings show the antibacterial effect of *C. umbellatum*.

Introduction

Antibiotic-resistant bacteria are a consequence of improper and/or overuse of antibiotics (Naeim et al., 2020) which as the main causes of human death worldwide in the hospital (Abadi et al., 2019). Many antibiotic groups that bacteria were resisted such as β-lactams, aminoglycosides, ulfonamides, and fluoroquinolones (Huai et al., 2019). Some of the most life-threatening antibiotic-resistant bacterial strains with severe human implications worldwide are *Pseudomonas aeruginosa*, *Acinetobacter baumannii* (Abadi et al., 2019), *Klebsiella pneumonia*, *Escherichia coli* (Gregova and Kmet, 2020), *Staphylococcus aureus* (MRSA) (Bhattacharya, 2014), non-typhoidal *Salmonella*, *Mycobacterium tuberculosis* (Prestinaci et al., 2015), *Stenotrophomonas maltophilia* (Çıkman et al., 2016), *Enterococcus faecalis* (Miller et al., 2014), *Proteus mirabilis* (Tumbarello et al., 2012), and *Burkholderia pseudomallei* (Bugrysheva et al., 2017). The finding for new drug sources to treat disease infected by antibiotic-resistant bacteria is required.

Medicinal plants are rich in a numerous variety of active compounds which have as antimicrobial properties such as berberine, piperine, eugenol, alicin, catechin, curcumin, saponins, tannins, alkaloids, alkenyl phenols, glycoalkaloids, flavonoids, sesquiterpenes lactones, terpenoids, and phorbol esters (Abdallah, 2011; Khameneh et al., 2019). The quinones from *Lawsonia inermis* had an antimicrobial activity against *P. aeruginosa* (Habbal et al., 2011). Hypericin from *Hypericum perforatum*, had general antimicrobial properties activity against methicillin-resistant and methicillin-sensitive *Staphylococcus* (Bahmani et al., 2019). PLR9 isolated from endophytic fungus *Aspergillus neobridgeri* shows antimicrobial activity against multi-drug resistant bacteria (Sadrati et al., 2020). Tannins isolated from the *Pimenta dioica* leaves show antimicrobial activity against methicillin resistant *S. aureus* (Al-Harbi et al., 2017). The aqueous extract of

Lannea fruticosa showed the highest inhibition zone activity against both *P. aeruginosa* and *P. mirabilis* which was 20 mm and 19.5 mm, respectively (Kidane et al., 2019).

Cathormion umbellatum (Vahl) Kosterm is a flowering plant in the legume family, Fabaceae which belongs to the mimosoid clade of the subfamily Caesalpinioideae. *C. umbellatum* is Thai mimosaceous plants that contained high antioxidant activity and can be stimulated white blood cell proliferation (Tunsaringkarn et al., 2014). Only antibacterial activity of *C. umbellatum* extracted with ethanol against *E. coli* was reported (Ramli, 2010). The determination of antibacterial activity against antibiotic-resistant bacteria has still lacked. Therefore, the aim of this study was to determine the antibacterial activity of *C. umbellatum* extracts against seven antibiotic-resistant bacteria collected from the Roi Et Hospital, Thailand.

Materials and Methods

Chemicals and reagents

Ethanol and methanol were purchased from QRëC™ (New Zealand). Dimethyl sulfoxide was purchased from Sigma-Aldrich (USA). Nutrient broth and bacterial agar were purchased from HiMedia (India). Iodonitrotetrazolium chloride was purchased from G-Biosciences (USA).

Plant materials and extraction

The fresh branch, leaves and pods of *C. umbellatum* were collected from Tha Muang Community, Tha Muang sub district, Selaphum District, Roi Et Province, Thailand. All plant samples were dried using hot air oven (POL-EKO-APARATURA company, Wodzisław Śląski, Poland) at 50°C for 48 hours before were grounded into powder. The plant powder was extracted with ethanol and methanol with shaking for 3 hours and then filtered and evaporated using a rotary vacuum evaporator (BÜCHI Labortechnik AG, Switzerland). The percent yield was calculated (Rattanasuk and Phiwthong, 2021). The plant extracts were adjusted the final concentration to 500 mg/mL using dimethyl sulfoxide.

Antibacterial activity determination

The antibacterial activity of the *C. umbellatum* extracts was tested against seven antibiotic-resistant bacteria including *A. baumannii*, *S. maltophilia*, *E. faecalis*, *B. pseudomallei*, *P. mirabilis*, multidrug resistance *K. pneumoniae*, colistin resistant *P. aeruginosa*. The active bacterial cultures were adjusted the cell concentration at OD_{600} to 0.1 before used.

The antibacterial activity of *C. umbellatum* extract was primary determined using disc diffusion assay (Boongapim et al., 2021; Malaka et al., 2018). Ten microliters of each *C. umbellatum* extract (500 mg/mL) was dropped onto the center of the paper disc. The dimethyl sulfoxide was used as a negative control. The bacterial culture plates were incubated at 37°C for 24 hours. The inhibition zone formation around the paper disc indicated as antibacterial activity of *C. umbellatum* extracts were measured.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of *C. umbellatum* extracts were determined using a broth microdilution and colorimetric assay (Rattanasuk and Phiwthong, 2020). The *C. umbellatum* extracts which presented the inhibition zone from the previous part were 2-fold serial diluted in a 96-well plate containing NB. The 96-well bacterial culture plates were incubated at 37°C for 24 hours. The iodonitrotetrazolium chloride (4 mg/mL) solution was added into each well of the 96-well bacterial culture plate and then incubated at 37°C for 1 hour. The MIC was referred to as the lowest concentration of the *C. umbellatum* extract that can inhibit bacterial growth. The MBC was considered as the lowest concentration of *C. umbellatum* extract that can eliminate the bacteria that did not produce a color change after the addition of iodonitrotetrazolium chloride (Dzotam et al., 2016).

Results

Percent yield and inhibition zone

The result of percent yield indicated that the highest percent yields at 15.9% was obtained when used the *C. umbellatum* leaves extracted with methanol, followed by pods extracted with methanol (13.3%) and leaves extracted with ethanol (11.7%), respectively. The lowest percent yields at 4.9% was found in branch extracted with ethanol.

The result of disc diffusion assay indicated that the highest inhibition zone at 11 mm was presented in ethanolic pods extract against multidrug resistance *K. pneumoniae*, followed by pods extracted with ethanol (10 mm), branch extracted with ethanol (9.5 mm) and leave extracted with methanol (9 mm) against *B. pseudomallei*, *P. mirabilis* and colistin resistant *P. aeruginosa*, respectively (Table I).

MIC and MBC values

The results indicated that the lowest MIC value of 0.1 mg/mL against colistin resistant *P. aeruginosa* was obtained from branch extracted with ethanol followed by 0.1 mg/mL was obtained from leave extracted with methanol against *P. mirabilis*, pods and leave extracted with methanol against *B. pseudomallei* (0.4 mg/mL), respectively (Table II). The lowest MBC values of 1.6 mg/mL were obtained when using *C. umbellatum* leaves

Table I

Inhibition zone (mm)

	Pod		Leaf		Branch	
	Ethanol	Methanol	Ethanol	Methanol	Ethanol	Methanol
A. baumannii	7	7	6.5	7	7.5	6.6
S. maltophilia	7.5	7.5	6.5	7	7	7.5
E. faecalis	7	8	8	7	8.5	7
B. pseudomallei	10	7.5	6.5	7.5	7.5	6.5
P. mirabilis	7	8	8	8	9.5	6.5
Multidrug resistant *K. pneumoniae*	11	7.5	7.5	8	8	7
Colistin resistant *P. aeruginosa*	7.5	7.5	8	9	7.5	8

Table II

Minimum inhibitory concentrations and minimal bactericidal concentration

Minimum inhibitory concentrations (mg/mL)						
	Pod		Leaf		Branch	
	Ethanol	Methanol	Ethanol	Methanol	Ethanol	Methanol
A. baumannii	0.8	0.8	0.8	0.8	1.6	1.6
S. maltophilia	0.8	0.8	0.8	0.8	3.1	3.1
E. faecalis	0.8	0.8	1.6	0.2	3.1	3.1
B. pseudomallei	0.4	0.2	0.4	0.8	1.6	1.6
P. mirabilis	0.8	0.8	0.8	0.1	1.6	3.1
Multidrug resistant *K. pneumoniae*	0.8	1.6	1.6	0.8	3.1	3.1
Colistin resistant *P. aeruginosa*	1.6	1.6	0.8	0.8	0.1	1.6
Minimal bactericidal concentration (mg/mL)						
A. baumannii	1.6	3.1	3.1	1.6	3.1	3.1
S. maltophilia	1.6	1.6	1.6	1.6	6.3	6.3
E. faecalis	1.6	1.6	3.1	1.6	6.3	12.5
B. pseudomallei	1.6	1.6	1.6	1.6	3.1	3.1
P. mirabilis	3.1	1.6	1.6	1.6	3.1	6.3
Multidrug resistant *K. pneumoniae*	3.1	6.3	3.1	1.6	6.3	6.3
Colistin resistant *P. aeruginosa*	3.1	3.1	1.6	1.6	3.1	3.1

extracted with methanol against all test antibiotic-resistant bacteria, leaves extracted with ethanol against *S. maltophilia*, *B. pseudomallei P. mirabilis* and colistin resistant *P. aeruginosa*, pods extracted with ethanol against *A. baumannii*, *S. maltophilia*, *E. faecalis* and *B. pseudomallei*, pods extracted with methanol against *S. maltophilia*, *E. faecalis*, *B. pseudomallei* and *P. mirabilis*. The highest MBC value of 12.5 mg/mL was found in branch extracted with methanol against *E. faecalis*.

Discussion

C. umbellatum shows antibacterial and antioxidant activity (Tunsaringkarn et al., 2014). It has been found that the ethanolic branch extract was presented that the lowest MIC value of 0.1 mg/mL against CoR-PA and methanolic leave extract was showed the lowest MBC values of 1.6 mg/mL against all test antibiotic-resistant bacteria. The mechanism of action is not clear.

The are no reports about that antibacterial activity *C. umbellatum* extract against antibiotic-resistant bacteria. Only a report about the ethanolic extract of *C. umbellatum* leaves has a MIC value of 0.8 mg/mL against *S. aureus*, *B. subtilis* and *E. coli* is presented (Ramli, 2010). The *C. umbellatum* extract is presented high antibiotic potential activity due to lower MIC values compared with using antibiotics (Kawamura-Sato et al., 2000) or *Lannea fruticose* (Kidane et al., 2019), *Tanacetum vulgare* and *Bidens sulphurea* extract (Chiavari-Frederico et al., 2020). The present study indicates that the MIC values of *C. umbellatum* pods and leave extracted with methanol against *B. pseudomallei* are lower than MIC values of amoxicillin-clavulanic acid (8 mg/mL), ceftazidime (8 mg/mL), imipenem (2 mg/mL), meropenem (2 mg/mL), doxycycline (2 mg/mL), tetracycline (8 mg/mL), chloramphenicol (8 mg/mL) and trimethoprim-sulfamethoxazole (4 mg/mL) (Karatuna et al., 2020).

The MBC values of *C. umbellatum* extract against *A.*

baumannii from this research are higher than MBC of colistin (0.5 mg/L) and sulbactam (32 mg/L) (Thamlikitkul and Tiengrim, 2014). The *Litsea cubeba* oil exhibits a strong inhibitory effect with MBC value of 0.1% (v/v) against *S. maltophilia* (Zhang et al., 2020). Red honey and white honey have MBC values of 30.4-62.5% and 60.7-75% (v/v) against multidrug resistant bacteria (Wasihun and Kasa, 2016). Proanthocyanidins and flavonoids glycoside are potential phytochemical groups content of *C. umbellatum* extracts which act as an antibacterial reagent (Ramli, 2010). Proanthocyanidins are phytochemicals found from *C. umbellatum* which synthesized from tannin and are presented various biological activities including antioxidant, anticancer, antidiabetic, neuroprotective, and antimicrobial activity (Rauf et al., 2019). The proanthocyanidins of *Dalbergia monetaria* extracts present the antibacterial activity against methicillin sensitive *S. aureus*, methicillin-resistant *S. aureus* and *P. aeruginosa* with MIC values of 64, 64 and 32 µg/mL, respectively (de Moura et al., 2020). The curcuminoids have an antimicrobial activity against *E. faecalis* with MBC of 50 µg/mL (Suttipalin et al., 2014).

Conclusion

The present study shows the antimicrobial activity of *C. umbellatum* against antibiotic-resistant bacteria.

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Conflict of Interest

Authors declare no conflict of interest

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