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6,8-Dibromo- and 6,8-Diiodo-5,7-dihydroxyflavones as New Potent Antibacterial Agents

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Thirteen flavones including chrysin, three natural and nine synthesized compounds, were examined for antibacterial activity. 6,8-Dibromo- (**11**) and 6,8-diiodo-5,7-dihydroxyflavone (**12**) exhibited the highest activity against all bacteria with MIC 31.25–62.5 μM. For *Propionibacterium acnes* and *Staphylococcus aureus*, these compounds were bacteriostatic agents, while for *Streptococcus sobrinus*, *S. mutans*, and *Salmonella typhi*, they were bactericidal agents. The combination of **11** and **12** with known antibiotics displayed synergistic effect. The combination of **11** with streptomycin (**16**) revealed the most synergistic effect with rate in increasing antibacterial activity of streptomycin in combination was sixteen folds against *P. acnes*, *S. sobrinus*, and *S. mutans*.

Keywords: Flavone | Antibacterial agent | Synergistic effect

Increasing resistance of pathogenic bacteria against available antibacterial agents is a major concern among scientists and clinicians worldwide. Nowadays, pathogenic microorganisms are more difficult to treat with existing drugs.[1] Numerous efforts have been made to find new and potent antibacterial compounds from various sources including nature and synthesis with expectation to decrease the resistance of pathogenic bacteria.[2] Several studies demonstrated that certain compounds revealed promising antibacterial activity such as carnosic acid,[3] α-mangostin,[4] epigallocatechin gallate,[5] and erybraedin A.[6] In addition, synergistic effect of natural and synthesized compounds with commercial antibiotics in combination could contribute to increase the antibacterial activity of either antibiotics or compounds. The potential chalcone analogues (2′-bromo-2-hydroxychalcone and 4-hydroxychalcone) in combination with non-β-lactam antibiotic (ciprofloxacin) were previously reported to possess synergistic effects against methicillin-resistant *Staphylococcus aureus* (MRSA) at very low MICs for ciproflaxocin in both combination with eight-fold increased susceptibility of MRSA.[7] Another report demonstrated the synergistic effect of the combination between tetrandrine (*bis*benzylisoquinoline alkaloid) and cefazolin reduced 75–94%/75–88% compared with the agents alone against 90% of the tested pathogenic strains (SCCmec III type MRSA isolates).[8]

Flavones belong to a class of flavonoid widely distributed in leaves, flowers, and fruits as aglycones or their glycosides. Celery, parsley, and ginkgo biloba are among the major sources of flavones. There have been a lot of studies concerning the inhibitory activities of flavones. Natural flavones such as chrysin (5,7-dihydroxyflavone), apigenin (4′,5,7-trihydroxyflavone), luteolin (3′,4′,5,7-tetrahydroxyflavone) have been reported to possess various biological activities as anti-inflammatory,[9] antitumor,[10] anticancer,[11] antibacteria,[12] and antipruritic.[13] Furthermore, the structural modification of flavones has been investigated in order to enhance their biological activities. The ether and halogenated derivatives of chrysin have been evaluated for their biological activities as hypoglycemic,[14] anticancer,[15] and anti-inflammatory agents,[16] respectively. Moreover, chrysin derivatives were synthesized by alkylation and acetylation to evaluate their cytotoxicity,[17] antitumor activity,[10] and inhibitory activity of prostaglandin production.[18]

Thirteen flavones were collected including 5-hydroxy-7-methoxyflavone (**1**), 5-hydroxy-3,7-dimethoxyflavone (**2**), and 5-hydroxy-3,7,4′-trimethoxyflavone (**3**) from the rhizomes of *Kaempferia parviflora* Wall. Ex Baker (Thai name: Kra-chai-dum, Zingiberaceae). The others with different substituents were synthesized by functionalization of chrysin (**13**) (Figure 1). 5,7-Dimethoxyflavone (**4**) was prepared by refluxing **13** with $(CH_3)_2SO_4$ in the presence of K_2CO_3 in acetone.[19] Six ether analogues of flavones (**5**–**10**) were synthesized by reacting **13** with selected bromoalkane in the presence of K_2CO_3.[20] 6,8-Dibromo- (**11**) and 6,8-diiodo-5,7-dihydroxychrysin (**12**) were manipulated from the reaction of **13** in acetone with NaBr/oxone,[16] and that in acetic acid with I_2/CH_2Cl_2.[19] All compounds were purified by silica gel column and characterized by ^{1}H NMR.

The preliminary screening of antibacterial activity of thirteen flavones against 5 pathogenic bacteria including *P. acnes* (KCCM41747), *S. aureus* (ATCC25923), *S. sobrinus* (KCCM11898), *S. mutans* (ATCC25175), and *S. typhi* (ATCC

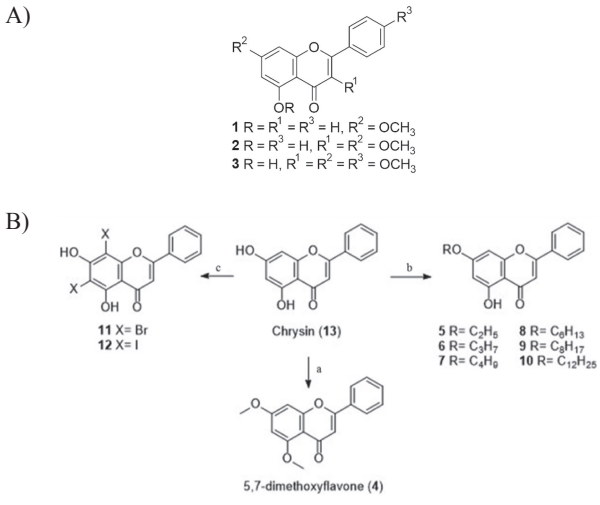

Figure 1. A) Structures of natural flavones isolated from the rhizomes of *K. parviflora*. B) Synthesis of flavone analogues: a) dimethyl sulfate, K_2CO_3, acetone, reflux, 24 h; b) RBr, K_2CO_3, acetone, reflux, overnight; c) NaBr, oxone, acetone or I_2, acetic acid.

Table 1. Inhibition zone (mm) of thirteen flavone analogues (**1**–**13**)

Compound[a]	Inhibition zone average (mm) ± SD				
	P. acnes KCCM41747	*S. aureus* ATCC25923	*S. sobrinus* KCCM11898	*S. mutans* ATCC25175	*S. typhi* ATCC442
1	8.67 ± 0.47	10.67 ± 0.47	9.67 ± 0.47	10.67 ± 0.94	8.00 ± 0.00
2	8.00 ± 0.00	9.00 ± 0.00	11.00 ± 0.82	11.67 ± 0.47	11.00 ± 0.82
3	9.00 ± 0.00	9.00 ± 0.00	11.00 ± 0.00	9.33 ± 0.47	9.67 ± 0.47
4	9.33 ± 0.47	9.33 ± 0.94	12.33 ± 0.47	11.33 ± 0.47	8.00 ± 0.00
5	7.00 ± 0.00	≤6.00	8.67 ± 0.47	7.00 ± 0.00	≤6.00
6	7.00 ± 0.00	≤6.00	9.00 ± 0.82	7.00 ± 0.00	7.00 ± 0.00
7	8.67 ± 0.47	9.67 ± 0.47	10.67 ± 0.47	9.67 ± 0.47	10.67 ± 0.47
8	9.00 ± 0.00	9.33 ± 0.94	11.67 ± 0.47	13.00 ± 0.00	9.00 ± 0.00
9	9.00 ± 0.00	10.33 ± 0.47	13.33 ± 0.47	11.33 ± 0.47	8.67 ± 0.94
10	9.67 ± 0.47	9.00 ± 0.00	8.00 ± 0.00	10.33 ± 0.47	8.67 ± 0.47
11	21.00 ± 0.82	20.00 ± 0.82	19.67 ± 0.47	19.67 ± 0.47	19.67 ± 0.47
12	20.33 ± 0.94	20.67 ± 0.47	18.67 ± 0.47	18.67 ± 1.25	18.33 ± 1.25
13	10.33 ± 0.47	11.00 ± 0.82	13.67 ± 0.94	15.33 ± 0.47	11.67 ± 0.47

[a]1 mM; criteria of inhibition zone activity (mm): inhibition zone >15.0: excellent, 13.1–15.0: very good, 10.1–13.0: good, 8.1–10.0: moderate, 6.1–8.0: weak, ≤6.0: no activity.

Table 2. MICs and MBCs of **11**, **12** and known antibiotics (**14**–**17**)[a]

Compound	*P. acnes* KCCM 41747		*S. aureus* ATCC 25923		*S. sobrinus* KCCM 11898		*S. mutans* ATCC 25175		*S. typhi* ATCC 477	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
11	31.25	250	31.25	250	62.50	250	62.5	250	62.5	250
12	31.25	250	31.25	250	62.50	250	62.5	250	62.5	250
14	15.62	250	62.50	500	15.62	250	15.62	250	15.62	250
15	7.81	125	3.91	250	1.95	250	0.98	250	0.49	125
16	15.62	125	15.62	125	3.91	125	1.95	15.625	3.91	250
17	62.50	1000	125	>1000	125	>1000	125	>1000	125	>1000

[a]Serial concentration of compounds (1000–0.488 μM) by two-fold serial dilution. MIC and MBC (μM).

442) was conducted by diffusion method (Table 1).[21] Among the tested compounds, halogen-substituted flavones (**11** and **12**) displayed the strongest activity against all bacteria with an excellent inhibition zone followed by **1**, **2**, **4**, **8**, **9**, and **10** with good activity against *S. mutans*. Between bromo- and iodo-substituents of 5,7-dihydroxyflavone, **11** showed slightly higher antibacterial activity than **12** except for *S. aureus*. Comparing between **13** with 5,7-dihydroxy group and 5,7-dimethoxyflavone (**4**), **13** exhibited higher activity than **4**. The former exhibited good to excellent activity against five bacteria, especially *S. mutans*, with inhibition zone about 15 mm while **4** showed weak activity against *S. typhi*. For these flavones, the hydroxy groups at C-5 and C-7 played an important role for this activity.

In addition, **9** revealed a very good activity while **2**, **3**, **4**, **7**, and **8** showed good inhibitory activity against *S. sobrinus*. Good activity was also observed by **2** and **7** against *S. typhi*, whereas **1** and **9** exhibited good activity against *S. aureus*. **11** and **12** were selected to further examine their potent growth inhibition by determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Antibacterial tests of **11** and **12** with four antibiotics (**14**–**17**) were conducted by determining MIC and MBC using colorimetric assay according to Sarker et al.[22] and Koochak et al.[23] Determination of antibacterial susceptibility with two-fold serial dilution (250–7.81 μM) disclosed the MIC of **11** and **12** at 31.25 and 31.25 μM against *P. acne* and *S. aureus*, respectively, while their MBCs were 250 μM.

Both compounds showed the same activity against *S. sobrinus*, *S. mutans*, and *S. typhi* with MIC 62.5 μM and MBC 250 μM. MIC and MBC of four antibiotics were found to be in the range of 125–0.488 μM. The MIC index was calculated by MBC/MIC to determine if the compounds possessed bactericidal or bacteriostatic properties.[24] When MIC index is ≤4, the compound is bactericidal while when the MIC index >4, the compound is bacteriostatic. For *P. acnes* and *S. aureus*, the MIC index of **11** and **12** were 8. Thus, for these bacteria, both compounds were bacteriostatic agents. Whereas their MIC index on *S. sobrinus*, *S. mutans*, and *S. typhi* were 4 revealing that these compounds were bactericidal agent. The results of these compounds are presented in Table 2.

In certain cases, the use of some antibiotics alone is not effective to inhibit pathogenic bacteria even some bacteria become resistance. In addition, the use of antibiotics at high doses may affect normal cells in humans. Therefore, there is a need to search for new antibacterial agents and test their activity alone and in combination. Generally, the antibacterial activity of

Table 3. MICs of **11** and **12** in combination with known antibiotics (**14**–**17**)[a]

Antibiotics	*P. acnes* KCCM 41747				*S. aureus* ATCC 25923				*S. sobrinus* KCCM 11898				*S. mutans* ATCC 25175				*S. typhi* ATCC 477			
	11		**12**		**11**		**12**		**11**		**12**		**11**		**12**		**11**		**12**	
	MIC[b]	Act[c]	MIC[b]	Act[c]	MIC[b]	Act[c]	MIC[b]	Act[c]	MIC[b]	Act[c]	MIC[b]	Act[c]	MIC[b]	Act[c]	MIC[b]	Act[c]	MIC[b]	Act[c]	MIC[b]	Act[c]
14	1.953	S	1.953	S	7.8125	S	3.906	S	1.953	S	1.953	S	1.953	S	1.953	S	1.953	S	1.953	S
15	0.977	S	0.977	S	0.488	S	0.488	S	0.244	S	0.244	S	0.122	S	0.122	S	0.122	S	0.122	S
16	0.977	S	0.977	S	1.953	S	1.953	S	0.244	S	0.488	S	0.122	S	0.244	S	0.488	S	0.488	S
17	7.813	S	7.813	S	15.625	S	15.625	S	15.625	S	15.625	S	15.625	S	15.625	S	15.625	S	15.625	S

[a]Serial concentration of compounds (125–0.488 μM) by two-fold serial dilution. [b]Combination. [c]Activity. S (Synergis); The FICI was interpreted as follows: synergy, FICI ≤ 0.5; additive, 0.5 < FICI 1; indifference, 1 < FICI ≤ 2; antagonism, FICI > 2. Each test was run in duplicate.

flavonoid derivatives against Methicillin-resistant *Staphylococcus aureus* (MRSA) is weak. Nonetheless, they could contribute to enhance antibacterial activity of antibiotics when they are in combination.[7] In this research, halogenated flavones (**11** and **12**) were selected for testing their antibacterial activity in combination with known antibiotics namely chloramphenicol (**14**), tetracycline (**15**), streptomycin (**16**), and ampicillin (**17**) using broth checkerboard method.[25] The range of tested compounds and antibiotics in combination was started from a half of MIC of each compound in two serial dilutions. Synergistic was determined by measuring the fractional inhibitory concentration index (FICI).[7]

According to Table 3, all combinations between halogenated flavones (**11** and **12**) and antibiotics against all bacteria were interpreted to be synergistic. The combination of **11** with streptomycin (**16**) exhibited the most synergistic effect against *P. acnes*, *S. sobrinus*, and *S. mutans*.

The rate in increasing antibacterial activity of streptomycin in combination was sixteen fold. However, **12** in combination with streptomycin (**16**) revealed the most synergistic effect only against *P. acnes*. In the combinations, the activity of streptomycin (**16**) against *P. acnes*, *S. sobrinus*, and *S. mutans* was increased 16-fold, lowering MICs from 15.625 to 0.977, 3.906 to 0.244, and 1.953 to 0.122 μM, respectively.

In conclusion, thirteen flavones were evaluated for their antibacterial activity. The hydroxy groups at C-5 and C-7 play an important role in this activity. 6,8-Dibromo- (**11**) and 6,8-diiodo-5,7-dihydroxyflavone (**12**) exhibited the highest antibacterial activity with MICs 31.25 μM against *P. acnes* and *S. aureus*, and 62.5 μM against *S. sobrinus*, *S. mutans*, and *S. typhi*, respectively. While their MBCs of 250 μM against all bacteria. These compounds were bacteriostatic agents for *P. acnes* and *S. aureus*, while for *S. sobrinus*, *S. mutans*, and *S. typhi*, they were bactericidal agents. All of the combinations between halogenated flavones (**11** and **12**) and antibiotics against all bacteria showed synergistic effect. Pairing **11** with streptomycin (**16**) had the most synergistic effect against *P. acnes*, *S. sobrinus*, and *S. mutans* with rate in increasing antibacterial activity of streptomycin in combination was sixteen fold compared with antibiotic tested alone.

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