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NOTE

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Brazilin from *Caesalpinia sappan* wood as an antiacne agent

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Abstract In screening experiments for antiacne activity, methanolic and 50% ethanolic extracts of *Caesalpinia sappan* wood showed the most potent activity out of 28 species of plants extracts. These extracts showed inhibition of *Propionibacterium acnes* growth, lipase inhibitory activity, and antioxidant activity. In order to isolate the active compound from *C. sappan*, separation of the extract components was performed by column chromatography and preparative high-performance liquid chromatography (HPLC). Brazilin, protosappanin A, and sappanone B were isolated from methanolic extracts. Brazilin showed better antibacterial activity [minimum inhibitory concentration (MIC) = minimum bactericidal concentration (MBC) = 0.50 mg/ml] than protosappanin A (MIC = MBC = 1.00 mg/ml) and sappanone B (MIC = MBC > 2.00 mg/ml). The 50% inhibitory concentration (IC_{50}) for lipase inhibition was lowest for brazilin (6 μM), which showed strong inhibition compared with protosappanin A (100 μM) and chloramphenicol (677 μM, positive control). The antioxidant activity of brazilin (IC_{50} 8.8 μM) was not significantly different from protosappanin A (9.1 μM) and (+)-catechin (10.2 μM). The antioxidant activity of brazilin and protosappanin A were higher than sappanone B (IC_{50} 14.5 μM). Brazilin is considered to have sufficiently potent activity for use as an antiacne agent.

Key words *Caesalpinia sappan* · Brazilin · Protosappanin A · *Propionibacterium acnes* · Lipase inhibitor

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Introduction

Caesalpinia sappan L. (Leguminosae) is distributed and cultivated in Southeast Asia as well as in Africa and the Americas.[1] Many biological activities of *C. sappan* have been reported, such as hepatoprotection,[2] immunomodulation,[3] hypoglycemic agent activity,[4] anticomplementary,[5] anticonvulsant,[6] anti-inflammatory, and antibacterial activity,[7,8] xanthin oxidase inhibition,[9] aldose reductase inhibition,[10] antioxidant activity,[11] and protection of the brain.[12]

In Indonesia, *C. sappan* is traditionally used for skin care, especially on Sumbawa Island,[13] and the wood of *C. sappan* is used to obtain pink pigment for drinks such as Bir Pletok, a Batavian spice drink. *Caesalpinia sappan* has already been reported as a good source of material for skin care, especially against skin photocarcinogenesis and it could be developed as a skin-whitening component for cosmetics.[14] According to the patent, the pigment of *C. sappan* has already been utilized as an antioxidant in cosmetics by a cosmetic company in Japan.[15] Based on our previous screening data, we found that *C. sappan* methanolic extracts and 50% ethanolic extracts have potential as antiacne agents.[16]

Many compounds have already been isolated from the wood of *C. sappan.* Flavonoids and phenolics[17] such as 4-*O*-methylsappanol, protosappanin A,[18] protosappanin B,[19] protosappanin E, brazilin,[20] brazilein, caesalpin J,[21] brazilide A,[22] neosappanone A,[9] caesalpin P, sappanchalcone, 3-deoxysappanone,[10] 7,3′,4′-trihydroxy-3-benzyl-2H-chromene,[23] and others. Some of the biological activities of the isolated compounds from *C. sappan* have already been reported. For example, brazilin, brazilein, and sappanchalcone showed significant inhibition of lipopolysaccharide (LPS)-induced NO production by J774.1 cell line and were found to almost completely suppress iNOS gene expres-

sion.[24] Caesalpin P, sappanchalcone, 3-deoxysappanone, brazilin, and protosappanin A were identified as aldose reductase inhibitors and are useful in the treatment of diabetic complications.[10] Brazilin has antibacterial character and has the potency to be developed into an antibiotic.[7] Sappanone A, 7-hydroxy-3-[(3,4,5-trihydroxyphenyl)methylene] chroman-4-one, 1′,4′-dihydro-spiro[benzofuran-3(2H),3′-(3H-2)benzopyran]-1′,6′,6′,7′-tetrol, and 3-{[4,5-dihydroxy-2(hydroxymethyl)phenyl]1-methyl}-2,3-dihydro-3,6-benzofurandiol were reported to have antioxidant activities.[25,26] Other compounds such as 7-hydroxy-3-[(3,4,5-trihydroxyphenyl)methylene]chroman-4-one have antioxidant and 5-lipoxigenase (5-LOX) inhibitory activities that can be useful for asthma and inflammatory diseases.[25]

Although the antibacterial, antioxidant, and anti-inflammatory effects of *C. sappan* extracts are known, the compound responsible for good antiacne control, especially the antibacterial activity against *Propionibacterium acnes* and *P. acnes* lipase inhibitory activity, have not yet been investigated. In order to identify the active compound from *C. sappan* wood conferring its good antiacne control, we performed tests on the antibacterial properties against *P. acnes*, lipase inhibitor, and antioxidant assays.

Experimental

Plant materials

Caesalpinia sappan wood was purchased from the market in Semarang, Indonesia. The identification and voucher specimens (No. 06001) were deposited in the Biopharmaca Research Center, Bogor Agricultural University, Bogor, Indonesia.

Extraction and isolation of brazilin, protosappanin A, and sappanone B

Caesalpinia sappan was dried and ground before methanol extraction. Briefly, 500 g of *C. sappan* wood meal was macerated in 5 l of methanol for 12 h and the process was repeated twice. The extracts were filtered (Whatman No. 2) and concentrated in vacuo at 30°C using a rotary evaporator to obtain 43.0 g of extract (yield 8.63% based on dried sample).

Part of the extract (10 g) was separated by column chromatography on silica gel by elution with hexane, ethyl acetate, and methanol to give 30 fractions. Some fractions eluted with ethyl acetate gave a mixture of brazilin (Fr. 4), protosappanin A (Fr. 5), sappanone B (Fr. 6–8), and some other compounds. Further purification was conducted using preparative high-performance liquid chromatography (HPLC) with a reversed-phase Inertsil ODS-3 column (TOSOH TSK Gel 21.5 mm i.d. × 300 mm) monitored at 280 nm. The solvent system used was as follows: a gradient program for 45 min from 5% to 100% methanol in 0.05% aqueous trifluoroacetic acid at a flow rate of 10 ml/min. This separation step gave crude brazilin, protosappanin A, and sappanone B. Repeated preparative HPLC gave brazilin (45.0 mg, Fig 1a), protosappanin A (27.4 mg, Fig 1b), and sappanone B (20.5 mg, Fig 1c). The structures of the compounds were determined by comparison of their spectroscopic data with those reported in the literature. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded with a JEOL ECP 600 MHz spectrometer with tetramethylsilane as internal reference, and chemical shifts were expressed in δ (ppm). Mass data were measured by gas chromatography-mass spectrometry (GC-MS) by direct injection on a Shimadzu GCMS-QP5050A instrument.

Identification of compounds

Brazilin. Amber-yellow crystals, $[\alpha]^{20}_{D}$ +118.8° (c = 1.9, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 2.73 (1H, d, J = 15.8 Hz, H-7), 2.97 (1H, d, J = 15.8 Hz, H-7), 3.67 (1H, d, J = 10.9 Hz, H-6), 3.89 (1H, d, J = 10.9 Hz, H-6), 3.93 (1H,

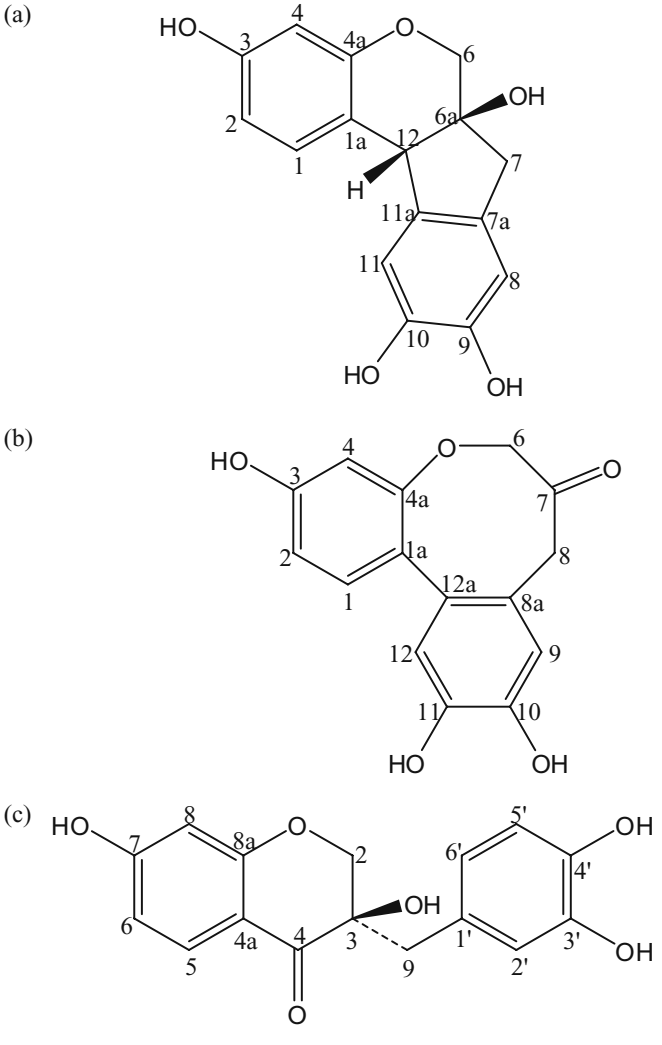

Fig. 1a–c. Structures of **a** brazilin, **b** protosappanin A, and **c** sappanone B

s, H-12), 6.26 (1H, d, J = 2.7 Hz, H-4), 6.44 (1H, dd, J = 8.2, 2.7 Hz, H-2), 6.58 (1H, s, H-11), 6.68 (1H, s, H-8), 7.15 (1H, d, J = 8.2 Hz, H-1); ^{13}C NMR (150 MHz, CD_3OD): 41.5 (C-7), 49.7 (C-12), 69.5 (C-6), 76.8 (C-6a), 102.9 (C-4), 108.7 (C-2), 111.1 (C-11), 111.6 (C-8), 114.2 (C-1a), 130.0 (C-7a), 130.9 (C-1), 136.1 (C-11a), 143.9 (C-10), 144.3 (C-9), 154.4 (C-3), 156.5 (C-4a); electron-impact mass spectrometry (EIMS) *m/z*: 286 [M^+]. The NMR data were compared with the report of Xie et al.[27]

Protosappanin A. Colorless needles, ^{1}H NMR (600 MHz, CD_3OD): δ 3.43 (2H, s, H-8), 4.45 (2H, s, H-6), 6.63 (1H, d, J = 2.1 Hz, H-4), 6.67 (1H, dd, J = 2.1, 8.2 Hz, H-2), 6.69 (2H, s, H-12 and H-9), 7.11 (1H, d, J = 8.2 Hz, H-1); ^{13}C NMR (150 MHz, CD_3OD): 45.1 (C-8), 77.6 (C-6), 108.0 (C-4), 112.2 (C-2), 116.4 (C-12), 116.5 (C-9), 124.1 (C-1a), 126.0 (C-8a), 129.9 (C-1), 130.7 (C-12a), 144.2 (C-11), 144.4 (C-10), 158.1 (C-3), 158.5 (C-4a), 204.6 (C-7); EIMS *m/z:* 278 [M^+]. The NMR data of protosappanin A were compared with the report of Nagai et al.[18]

Sappanone B. White powder, $[\alpha]^{20}_D$ +53.1° (c = 0.32, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ 2.71 (1H, d, J = 13.7 Hz, H-9), 2.79 (1H, d, J = 13.7 Hz, H-9), 3.98 (1H, d, J = 11.0 Hz, H-2), 4.09 (1H, d, J = 11.0 Hz, H-2), 6.39 (1H, d, J = 2.1 Hz, H-8), 6.53 (1H, dd, J = 2.1, 8.2 Hz, H-6′), 6.57 (1H, dd, J = 2.1, 8.2 Hz, H-6), 6.68 (1H, d, J = 8.2 Hz, H-5′), 6.74 (1H, d, J = 2.1 Hz, H-2′), 7.66 (1H, d, J = 8.2 Hz, H-5); ^{13}C NMR (150 MHz, CD_3OD): 39.5 (C-9), 72.0 (C-2), 72.8 (C-3), 102.2 (C-8), 110.9 (C-6′), 111.9 (C-4a), 114.6 (C-6), 117.6 (C-5′), 121.9 (C-2′), 126.4 (C-1′), 129.1 (C-5), 143.9 (C-4′), 144.5 (C-3′), 163.6 (C-8a), 165.4 (C-7), 194.5 (C-4); EIMS *m/z*: 302 [M^+]. The NMR data of sappanone B were compared with the report of Namikoshi et al.[28]

Bioactivity tests

The bioactivity tests (antimicrobial activity against *Propionibacterium acnes*, lipase inhibitory activity, and antioxidant activity) were performed as described in our previous report.[16]

Results and discussion

The prevalent bacterium implicated in the clinical course of acne is *Propionibacterium acnes*, a gram-positive anaerobe that normally inhabits the skin. It is implicated in the inflammatory phase of acne.[29] The bacterium *P. acnes* plays a central role in the current concept of acne pathogenesis[30] and appears to be the target of oral and topical antibiotic usage. The reduction in numbers of *P. acnes* is an indicator of the therapeutic effectiveness of an antibiotic.[31]

The antibacterial effects of brazilin, protosappanin A, and sappanone B against *P. acnes* are shown in Table 1. The results showed that brazilin isolated from methanolic extracts of *Caesalpinia sappan* wood has the same minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of about 0.50 mg/ml. Brazilin showed the best antibacterial activity when compared with protosappanin A, sappanone B, and 3-methyl 4-isopropylphenol (IPMP) as the positive control. Protosappanin A, sappanone B, and IPMP all shared the same MIC and MBC values (1.00 mg/ml). The antibacterial effects of the three compounds are lower than tetracycline and chloramphenicol. However, the effectiveness of brazilin is better than IPMP, while protosappanin A and sappanone B show the same effectiveness as IPMP.

Propionibacterium acnes secretes several metabolites that cause proinflammation, and these play an important role in the development of the inflammation of acne. Lipase, the extracellular enzyme derived from *P. acnes*, is reported to be responsible for the hydrolysis of sebum triglycerides to free fatty acids (FFAs). FFAs are implicated as irritants and comedogenic agents that lead to intensification of the inflammatory process.[32] Natural substances such as glycyrrhizic acid, (±)-catechin, and kaempferol are reported as promising candidates for the treatment of acne due to their strong inhibitory activity on GehA lipase.[33]

Based on the ability of tetracycline as an antimicrobial agent and its inhibitory activity against *P. acnes* lipase,[34] antibiotic agents were used as positive controls. It was reported that tetracycline shows complete lipolytic inhibition at a concentration of 1 mM.[32] Our results showed that

Table 1. Antibacterial activities against *Propionibacterium acnes*, and lipase inhibitory and antioxidant activities of brazilin, protosappanin A, and sappanone B

Sample	MIC (mg/ml)	MBC (mg/ml)	Lipase inhibitory IC_{50}		Antioxidant IC_{50} (μM)
			(μM)	(μg/ml)	
Brazilin	0.50	0.50	6	1.8	8.8 ± 0.2
Protosappanin A	1.00	1.00	100	27.3	9.1 ± 0.4
Sappanone B	1.00	1.00	>1650	>500	14.5 ± 0.9*
Tetracycline	0.03	0.03	1060	471.3	nd
Chloramphenicol	0.13	0.13	677	218.8	nd
IPMP	1.00	1.00	1109	166.4	nd
(+)-Catechin	nd	nd	>1750	>500	10.2 ± 0.1

MIC, Minimum inhibitory concentration; MBC, minimum bactericidal concentration; IC_{50}, 50% inhibitory concentration; IPMP, 3-methyl 4-isopropylphenol; nd, not determined
* $P < 0.05$ vs control (+)-cathecin

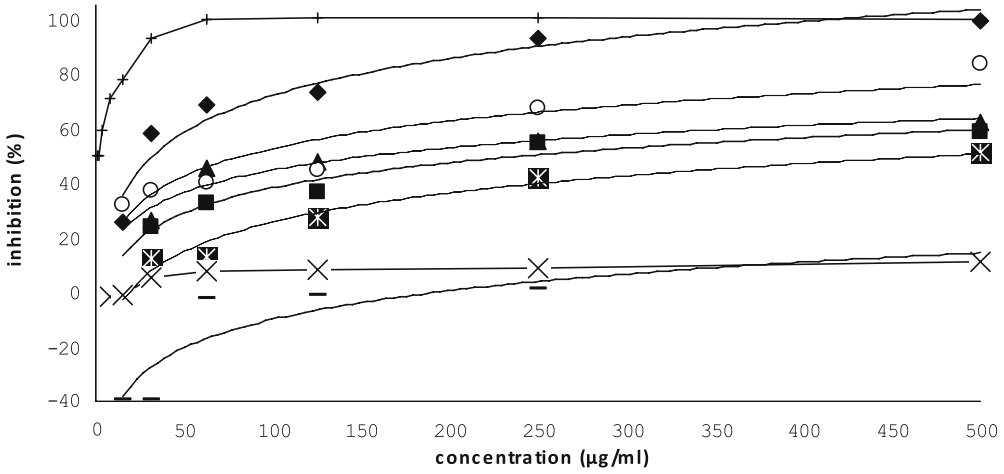

Fig. 2. Lipase inhibitory activity of brazilin (*plus signs*), protosappanin A (*filled diamonds*), and sappanone B (*bold dashes*) compared with positive controls [3-methyl 4-isopropylphenol (*filled triangles*), chloramphenicol (*filled squares*), tetracycline (*asterisks in filled squares*), (+)-catechin (*crosses*)] and *Caesalpinia sappan* methanolic extract (*open circles*)

the 50% inhibitory concentration (IC_{50}) against lipase for tetracycline is about 1 mM (Table 1).

The inhibitory effects of brazilin, protosappanin A, sappanone B, positive control, and *C. sappan* methanolic extracts in different concentrations are shown Fig 2. The results show that brazilin had the highest inhibitory effect, while protosappanin A had a stronger effect than the positive control and the *C. sappan* methanolic extracts. Conversely, sappanone B had the lowest inhibitory activity against *P. acnes* lipase.

From the data in Fig. 2, we calculated the IC_{50} of all samples and positive controls. The IC_{50} of brazilin was the lowest (6 µM), while the second most active compound was protosappanin (IC_{50} 100 µM). The IC_{50} values of chloramphenicol, IPMP, and tetracycline were 677, 1109, and 1060 µM, respectively. On the other hand, sappanone B had no activity in inhibiting lipase and at low concentration and may accelerate the lipase activity. The inhibition effect of sappanone B on lipase is only 9.45% even at a concentration of 1600 µM. Brazilin and protosappanin A, in which the catechol ring is fused to an adjacent ring, showed stronger inhibition of lipase than sappanone B or (+)-catechin, in which the catechol ring could easily rotate (Fig. 2).

It has recently been reported that, aside from inflammation, the most chronic medical condition of acne is characterized by oxidative stress. To reduce the oxidative stress of acne patients, an antioxidant compound is required. It is likely that the blood levels of antioxidants are used up rapidly in those with acne because there is a greater demand to deal with free radicals.[35] To measure the antioxidant effect of the three compounds, a scavenging test for the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical was performed.

The antioxidant test data for brazilin, protosappanin A, and sappanone B are shown in Table 1. The results show that the antioxidant effect of brazilin and protosappanin A are not significantly different with (+)-catechin used as the positive control. Protosappanin A has been reported to have high antioxidant activity compared with vitamin E in some assays of DPPH radical scavenging and reduction of ferric ion. In addition, protosappanin A also inhibited the oxidation of linoleic acid in an antioxidant assay.[24]

The antioxidant effect of sappanone B is significantly different from (+)-catechin. The IC_{50} value of sappanone B (14.5 µM) is almost twice that of brazilin (8.8 µM) and protosappanin A (9.1 µM), which means that the antioxidant effect of sappanone B is weaker than that of brazilin and protosappanin A.

Conclusions

Brazilin, protosappanin A, and sappanone B were isolated from methanolic extracts of *Caesalpinia sappan* wood. Brazilin is a potential antiacne agent because it has antibacterial activity against *Propionibacterium acnes* (MIC and MBC values of 0.50 mg/ml), good lipase inhibitory activity, and good antioxidant activity. Protosappanin A is not as effective as brazilin as an antiacne agent but is also a prospective compound.

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