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Flower Bracts of Temulawak (*Curcuma xanthorrhiza*) for Skin Care: Anti-Acne and Whitening Agents

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Abstract

This research aims to explore the skin care potency of temulawak flower bract. The dried flower bract was extracted using *n*-hexane, ethyl acetate (EtOAc), and methanol (MeOH). Its essential oil was separated by distillation. The anti-acne potency was determined by antioxidant, anti-bacteria against *Propionibacterium acnes* and lipase inhibition activities, while whitening was determined by tyrosinase inhibition. The results showed that MeOH extract was the most potent as antioxidant and EtOAc extract was the most potent as tyrosinase and lipase inhibitors. α-curcumene is responsible for inhibition of lipase activity while xanthorizol is responsible for inhibition of *P. acnes* growth.

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Keywords: antioxidant; flower bract; *Curcuma xanthorrhiza;* lipase inhibitor; *Propionibacterium acnes*; tyrosinase inhibitor

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1. Introduction

Temulawak (*Curcuma xanthorrhiza*) is a Zingiberaceae species which is empirically widely used as a traditional medicine especially its rhizome. Traditionally, it is widely used to treat kidney stones, fever, high-cholesterol level, joint pain, and hepatitis. Many research studies have been conducted on the active component of the temulawak rhizome for its antioxidant, antilipidemia, antibacterial, and antifungal activities[1–3]. The utilization of temulawak rhizome is causing a voluminous amount of wasted agricultural byproduct wasted. One of these byproducts is the flower bract part. It is interesting to explore the potency of temulawak flower bract for other purposes to decrease the volume of agricultural waste.

Natural skin-care product remains an important theme on the research of natural products since skin-care could affect self-confidence. Anti-acne and whitening agents are interesting subject on natural skin care. On this research, the anti-acne potency was determined by antioxidant using diphenylpicryl hydrazyl (DPPH) method, antibacteria against *Propionibacterium acnes* and *P. acnes* lipase inhibition activities, while whitening potency was determined by tyrosinase inhibition (monophenolase and diphenolase).

On the other hand, the information on the active compounds in temulawak flower bract is still limited. Therefore, this research aimed to explore the potency and identify the active compounds of temulawak flower bract for skin care: namely antibacterial against *P. acnes*, lipase inhibition, antioxidant and tyrosinase inhibition activities.

2. Materials and methods

2.1 Plant materials and extraction methods

The flower bract of temulawak was collected from Sukabumi, West Java, Indonesia. The sample was oven dried before extraction. About 15 g dried temulawak flower bract was extracted by increasing the polarity of solvents (150 mL). First, *n*-hexane was used as solvent, then extraction was continued to the residue using ethyl acetate (EtOAc), and finally using methanol (MeOH). The essential oil of flower bract of temulawak (1 kg) was separated by distillation method. The most potent extract was separated by silica gel column chromatography (CC) and preparative thin-layer chromatography (TLC).

2.2 Assay methods

The anti-acne potency was determined by UV-Vis spectrometric method for antioxidant, antibacterial, lipase inhibition and tyrosinase inhibition activities. Antioxidant activity was determined using DPPH method with (+)-cathechin (Tokyo Chemical Industry, Japan) and ascorbic acid as positive controls and ethanol as blank[4]. For antioxidant activity test, a 100 µL sample, 100 µL 2(N-morpholino)ethanesulfonic acid (MES) buffer pH 7.4, and 100 µL DPPH (11.8 mg DPPH in 100 mL ethanol) were added in each well of a 96 well plate. After 30 min, the absorbance of the mixture was measured at 514 nm. The inhibitory activity was calculated.

Antibacterial activity against *P. acnes* was performed by determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). They are concentrations by which there was no visually detectable bacterial growth and all the bacteria were dead, respectively[4]. Briefly the medium consisted of GAM Broth Nissui 0.5 %, glucose 1 % (Wako Pure Chemical Industries, Ltd., Japan), yeast extract 0.3 % (Difco Laboratories, France), Nutrient Broth 0.5% (Difco Laboratories, France), and 0.2 % Tween-80 (MP Biomedical, Japan). After addition of Tween-80, the medium was sterilized in an autoclave. To each well of the 96 well plate, a 100 µL sample (serial concentration, diluted in DMSO 20 %) consisting of 95 µL medium and 5 µL inoculums was added. The inoculum was prepared at a concentration of 10^{-2} CFU/mL. *P. acnes* and incubated in the medium for 72 h under anaerobic conditions. The negative control for antibacterial activity was dimethyl sulfoxide (DMSO), while the positive controls were tetracycline (MP Biomedical, Japan), and 3-methyl 4-isopropylphenol (IPMP) (TCI, Japan).

P. acnes lipase inhibition activity test was conducted using the 2,3-dimercapto-1-propanol tributyrate (BALB) method as described before[4]. Briefly, all reagents (Dainippon Sumitomo Pharma Co, Ltd, Japan) were put in tubes 1

and 2. The reagents were 390 μL DTNB in tris buffer (coloring agent), 10 μL PMSF (esterase inhibitor), 25 μL lipase, and 25 μL sample or solvent (DMSO). Both tubes were incubated at 30 °C for 5 min after which 50 μL of the BALB solution (substrate) was added to tube 1, then the tubes were mixed well and incubated at 30 °C for 30 min. After 30 min, the reaction in the tube was stopped by adding 500 μL stopping reagent to both tubes. In tube 2, 50 μL substrate solution was added and then both tubes were shaken to mix the solution well and then centrifuged to remove the insoluble materials. The absorbance of each tube was measured at 414 nm. Tetracycline was used as the positive control based on its activity to inhibit the lipase production of *P.acnes*[5].

Tyrosinase inhibition (monophenolase and diphenolase) was determined by previous methods and kojic acid was used as positive control[6]. Briefly, about 70 μL of extract diluted in DMSO was combined with 30 μL of tyrosinase (Sigma, 333 Unit mL^{-1} in phosphate buffer). After incubation at room temperature for 5 min, 110 μL of the substrate (2 mM L-tyrosine or 12 mM L-DOPA) was added. Incubation commenced for 30 min at room temperature and the absorbance was measured at 510 nm. The concentration of the plant extract at which half of the original tyrosinase activity was inhibited was determined.

2.3 Silica gel column chromatography (CC), preparative thin-layer chromatography (TLC), gas chromatography-mass spectrometer (GC-MS) and Fourier transform-infrared spectrometer (FT-IR) conditions

The most potent extract was separated first by open CC. Silica gel 60 with 75 μm particle size was used as the stationary phase with diethyl ether, dichloromethane and methanol as the mobile phase (step gradient method). The fraction was grouped by TLC. The TLC plate was an aluminum plate of silica gel $G_{60}F_{254}$ with 0.25 mm thickness from Merck. The eluent was dichlorometane:diethyl ether (1:1), and detection was through ultraviolet light on 254 nm.

The component of temulawak flower bract essential oils was determined by GC-MS, while the functional groups in the active fraction was determined by FT-IR. Conditions for GC-MS analysis consisted of using electron impact ionization (EI) method on GC-17A gas chromatograph (Shimadzu, Kyoto, Japan) coupled to a GC-MS QP 5050A mass spectrometer (Shimadzu); fused silica capillary column (30 m × 2.5 mm; 0.25 mm film thickness), coated with DB-5 (Agilent J&W, Canada); column temperature at 100 °C (2 min) to 250 °C at the rate of 3 °C per min; carrier gas, helium at constant pressure of 7.59 psi (1 psi equals 6 894.76 Pascals)**.**. Acquisition mode was scan. Compound identification was performed by comparing the mass spectra of the peaks with library data. Percentage composition was computed from GC peak areas.

Functional groups in active fraction was analyzed by FT-IR Alpha (Bruker, UK). About 2 mg active fraction was mixed with potassium bromide (KBr) and was made as pellet by hand press for 10 min. The transmittance was measured at 4 000 cm^{-1} to 400 cm^{-1} wavenumber.

3. Results and discussion

3.1 Potency of crude extract and essential oil as anti-acne

Extraction of compounds from temulawak flower bract gave different yields, 1.92 % for *n*-hexane extract, 0.80 % for EtOAc extract, 11.47 % for MeOH extract, and 0.01 % for essential oil. This result showed that the major compound in flower bract of temulawak is a polar compound which can be extracted in methanol. Based on the phytochemical assay test, the MeOH extract consisted of saponin, flavonoid, tannin and alkaloid.

The potency of each extract and essential oil as anti-acne, antibacterial against *P. acnes,* lipase inhibition, and antioxidant agent is shown in Table 1. The results showed that temulawak flower bract essential oil had the lowest MIC and MBC values which mean that the oil is the most active in inhibiting the *P. acnes* growth. *P. acnes* is the bacteria living in the skin and responsible for the inflammation process in the acne formation. Compared to the activities of the positive control, the essential oil had almost the same activity with IPMP, but not better than tetracycline. This oil also inhibited 50 % of the *P. acnes* lipase activity at the concentration of 500 $\mu g \cdot mL^{-1}$, almost the same potency with tetracycline as the positive control.

Lipase produced by *P. acnes* is also included on the acne formation[7]. Inhibiting the lipase activities could decrease the inflammation during acne formation. EtOAc extract was found to be the most active extract to inhibit

the lipase activity (Table 1). It was almost the same with IPMP as the positive control and more active compared to tetracycline. This extracts also had potency as antioxidant.

Table 1. The antibacterial, lipase inhibition, and antioxidant activities of temulawak flower bract crude extracts and essential oil

Extract name	Antibacteria (mg · mL^{-1})		Lipase inhibition at 500 μg · mL^{-1} (%) *)	Antioxidant IC$_{50}$ (μg · mL^{-1})
	MIC	MBC		
n-hexane	> 2	> 2	36.09 ± 1.5	> 200.00
EtOAc	> 2	> 2	80.50 ± 0.9	12.68
MeOH	2.00	> 2	5.90 ± 0.5	6.60
Essential oil	1.00	2.00	49.01 ± 1.1	> 200.00
Tetracycline	0.03	0.03	47.3 ± 5.5	-
IPMP	1.00	1.00	81.4 ± 2.5	-
Catechin	-	-	-	2.94 ± 0.03 *)
Ascorbic acid				3.82 ± 0.03 *)

Noted : IC$_{50}$ is the concentration which shows 50 % inhibition of activity.
*) (value ± se) μg · mL^{-1}

Antioxidant potency test of extracts showed that MeOH extract was the most active extract to inhibit DPPH radical. The activity of MeOH extract was lower compared to catechin and ascorbic acid as the positive controls (Table 1). Based on our previous report, antioxidant activity of MeOH and EtOAc extracts of temulawak flower bract are better compared to temulawak rhizome which had IC$_{50}$ value of 80.72 μg · mL^{-1} [8].

3.1 Potency of crude extracts and essential oils as whitening agent

The whitening potency is related to tyrosinase inhibition on monophenolase and diphenolase reaction. Tyrosinase is the enzyme which is responsible for the conversion of L-tyrosine to DOPA and also converted DOPA to DOPA quinone[9]. By inhibiting the tyrosinase activity, the melanin formation which is responsible for skin color could be inhibited.

The activity of crude extract and essential oil as tyrosinase inhibitor is shown in Table 2. EtOAc extract was the most potent extract as tyrosinase inhibitor since it has the lowest IC$_{50}$ value compared to other extracts. Based on our previous report on the temulawak rhizome which has IC$_{50}$ of 0.27 mg · mL^{-1} for monophenolase and 0.90 mg · mL^{-1} for diphenolase, temulawak flower bract is not as good as the temulawak rhizome as whitening agent[6].

Table 2. The tyrosinase inhibition activity of temulawak flower bract extracts and essential oil

Extract name	IC$_{50}$ (mg · mL^{-1})	
	Monophenolase	Diphenolase
n-hexane	> 2.00	> 2.00
EtOAc	1.97	1.57
MeOH	> 2.00	1.71
Essential oils	> 2.00	> 2.00
Kojic acid	0.02	0.16

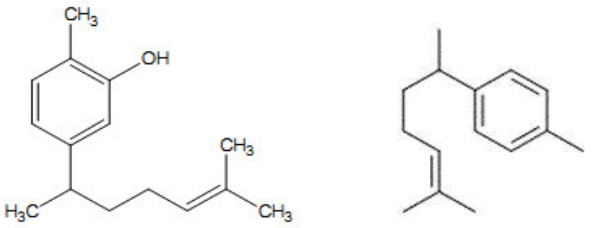
3.2 Compounds in temulawak flower bract essential oils

The essential oil of temulawak flower bract had potency as antibacteria and lipase inhibitor. It is interesting to identify the compounds present in essential oils which is responsible for the activity. Chemical constituents present in the temulawak flowers bract is determined by GC-MS and the results are shown in Table 3. The major

components in the oil are xanthorrhizol and α-curcumene (Fig 1). These major components belong to the sesquiterpene group with bisabolane type structure.

Table 3. The compounds in temulawak flower bract essential oils

Group		Compound name	Content (%)
monoterpene		Isoborneol	0.04
		Camphor	0.21
sesquiterpene	bisabolene	α-curcumene	15.12
		Xanthorrhizol	16.13
	elemene	β-elemene	4.60
	others	β-farnesene	0.29
		Trans-caryophyllene	3.48



(a) (b)

Fig. 1. Structure of (a) xanthorrhizol and (b) α-curcumene

The activities of the two major components for anti-acne are shown in Table 4. The results showed that xanthorrhizol inhibit *P. acnes* growth at the concentration of 0.5 mg · mL^{-1}. The antibacterial activity of xanthorrhizol was better than IPMP as the positive control, but xanthorrhizol had no lipase inhibition activity and not good as antioxidant. Unlike xanthorrhizol, α-curcumene did not have good antibacterial and antioxidant activities, but had lipase inhibition activity. Xanthorrhizol is reported as antinociceptive[10], antimetastatic[11], and anti-fungi[3]. Hwang also reported that xanthorrhizol is an antibacterial with broad spectrum activity, stable from heat and safe for human skin[12].

Table 4. Anti-acne activities of xanthorrhizol and α-curcumene

Compounds name	Antibacterial against *P. acnes* (mg ·mL^{-1})		Lipase inhibition at 500 μg · mL^{-1} (%) *)	Antioxidant IC_{50} (μg · mL^{-1})
	MIC	MBC		
Xanthorrhizol	0.50	1.00	9.1 ± 1.1	> 16.7
α—curcumene	2.00	> 2.00	57.0 ± 4.5	> 16.7
Tetracycline	0.03	0.03	47.3 ± 5.5	-
IPMP	1.00	1.00	81.4 ± 2.5	-
Catechin	-	-	-	2.94 ± 0.03*)
Ascorbic acid				3.82 ± 0.03*)

Noted : IC_{50} is the concentration which shows 50 % inhibition of activity.
*) (value ± se) μg · mL^{-1}

3.3 Separation of temulawak flower bract EtOAc extracts

EtOAc extract of temulawak flower bract was separated by silica gel column chromatography based on its tyrosinase inhibition, lipase inhibition and antioxidant activities. Phytochemical analysis test showed that EtOAc extract consisted of an alkaloid and a flavonoid. The separation yielded 12 fractions. The activities of each fraction are shown in Table 5.

Fraction F1, F3, F10, and F12 are active as tyrosinase and lipase inhibitors as well as an antioxidant. Fraction F12 is the most active fractions because it has the lowest IC_{50} value for tyrosinase inhibition, low IC_{50} value for antioxidant and high lipase inhibition activity.

Between the four active fractions, F1 was separated further because it has the highest volume compared to the other fractions. Separation was continued by preparative thin layer chromatography. This separation gave 2 fractions, namely F1.1 and F1.2. The activities of F1.1 and F1.2 are shown in Table 5. The activities test results showed that F1.2 is an active fraction compared with F1.1. Fraction F1.2 has tyrosinase and lipase inhibition activities as well as antioxidant activity. To understand the functional groups in F1.2 as well as in F12, the IR spectrum measurement was performed.

The infrared spectrum showed that the F12 which is the most active fraction has the functional groups of hydroxyl, aromatics and C–O–C. It means that this fraction is consists of flavonol group compounds. Phytochemical test analysis showed that F12 is consisted of flavonol. Flavonol compounds reported to have an activity against *P. acnes* lipase activity are myricetin (IC_{50}: 0.38 mM) and quercetin (IC_{50}: 0.42 mM)[13]. But robinetin which also belongs to the flavonol group does not inhibit but accelerates the lipase activity. Some of the reported flavonols also has whitening activity[14]. It means that further research is needed to identify the active component from F12. Distinct from fraction F12, fraction F1.2 had a carbonyl group which was not found in F12. Based on this result the component in F1.2 could be flavonoid. Phytochemical test analysis showed that F1.2 consisted of auron.

Table 5. Tyrosinase inhibition, lipase inhibition and antioxidant activities of fractions from EtOAc extracts of temulawak flower bract

Fractions	Tyrosinase inhibition IC_{50} (mg· mL^{-1})		Lipase inhibition at	Antioxidant
	Monophenolase	Diphenolase	500 μg · mL^{-1} (%)	IC_{50} (μg ·mL^{-1})
F1	1.51	1.74	81.30	281.08
F2	> 2.0	> 2.0	9.54	189.36
F3	1.54	1.49	75.43	71.93
F4	> 2.0	> 2.0	8.88	100.89
F5	> 2.0	> 2.0	9.84	146.98
F6	1.68	1.85	50.36	> 400
F7	> 2.0	> 2.0	11.65	> 400
F8	> 2.0	> 2.0	12.44	> 400
F9	> 2.0	> 2.0	9.95	114.23
F10	1.33	1.74	16.81	298.38
F11	1.17	1.48	31.83	> 400
F12	0.97	1.08	70.58	92.28
F1.1	> 2.00	> 2.00	15.27	184.80
F1.2	0.97	1.70	80.33	199.97
Kojic acid	0.02	0.16	-	-
Tetracycline	-	-	47.3	-
IPMP	-	-	81.4	-
Catechin	-	-	-	2.94
Ascorbic acid				3.82

Infrared spectrum of F12 and F1.2 are shown in Fig 2. The vibration types from the functional group in F12 and F12 are listed in Table 6.

Table 6. Vibration types of functional group in fraction F12 and F1.2

Wavenumber (cm^{-1})		Functional group
F12	F1.2	
3429	3421	stretch O–H
2912	2921	stretch C–H
-	1715	stretch C=O
1634	1459	stretch C=C aromatic
1383	-	Bend C–H
1274	1263	Stretch C–O–C vinyl ether
1093	1122	C–O–C ether
618	899, 815, 723, 566	Fingerprint area

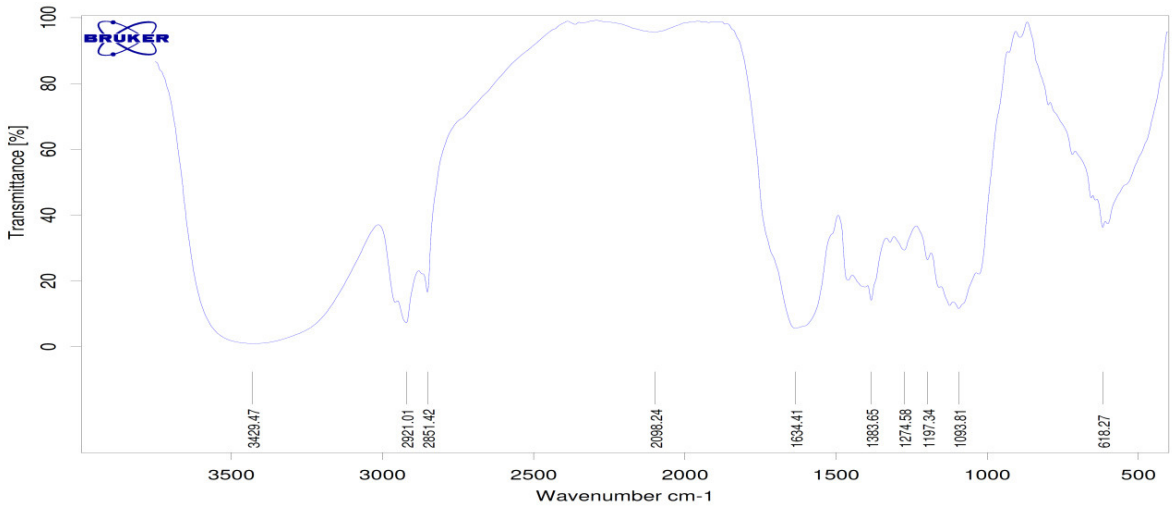


Fig. 2 (a)

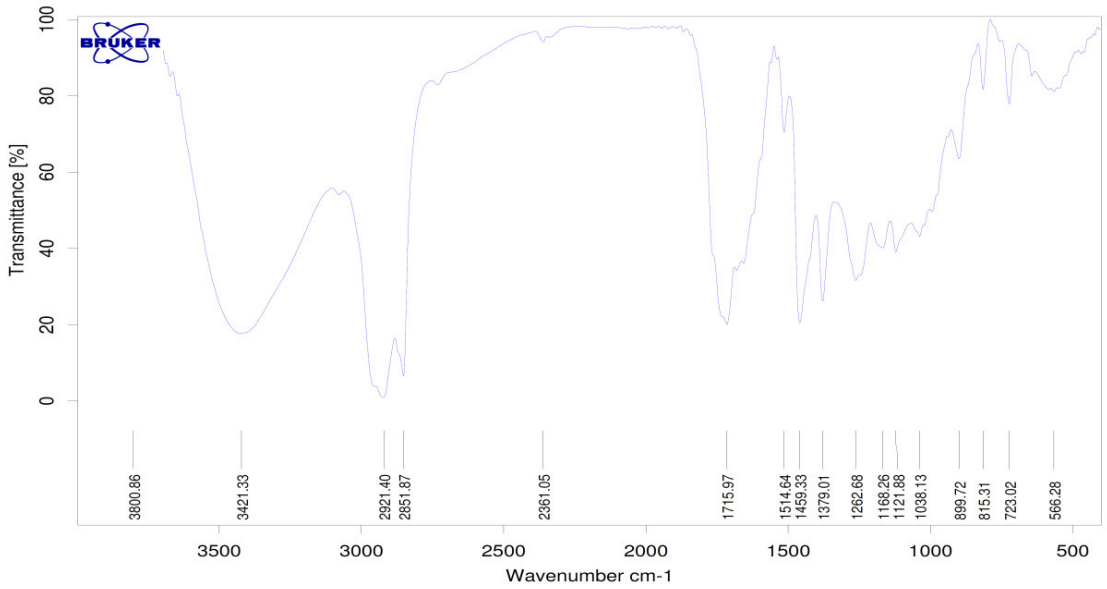


Fig. 2 (b)

Fig. 2. Infrared spectra of (a) fraction F12 and (b) fraction F1.2

4. Conclusion

Temulawak flower bract has a good potential to be developed as a skin care product. The MeOH extract was the most potent extract for antioxidant activity followed by the EtOAc extract. The best extract as tyrosinase and lipase inhibitors was EtOAc extract. Fraction 12 of EtOAc extracts contained flavonol and fraction F1.2 contained auron based on the infrared spectrometry and phytochemical assay. The α-curcumene in the flower bract essential oil is responsible for the inhibition of lipase activity while xanthorizol is responsible for the inhibition of growth of *P. acnes*.

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