

Anti-acne and Tyrosinase Inhibition Properties of Taxifolin and Some Flavanonol Rhamnosides from Kempas (*Koompassia malaccensis*)

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Abstract

Taxifolin (**1**) and some flavanonol rhamnosides (neoastilbin (**2**), astilbin (**3**), and isoastilbin (**4**)) have been isolated from kempas (*Koompassia malaccensis*). Our previous research about antimicrobial activity against *Streptococcus sobrinus* and glucosyltransferase inhibitory activity of these compounds have been reported. Now, we carried out the anti-acne and tyrosinase inhibition properties of all four compounds. Antimicrobial against *Propionibacterium acnes*, *P. acnes* lipase inhibitory activity and antioxidant activity were established for anti-acne activity. Tyrosinase inhibition property was measured using L-tyrosine and L-DOPA as substrate. The results for anti-acne showed that no antimicrobial activity against *P. acnes* for all compounds, the best lipase inhibition properties showed on compound **4** with IC₅₀ about 1.36 µg/ml, and % inhibition for antioxidant at concentration 10 µg/ml are 31.16, 25.64, 28.47, and 31.01% respectively. Tyrosinase inhibition of compound **1** at concentration 1 mg/ml is 24.12% for monophenolase and 5.18% for diphenolase. Compound **2** has tyrosinase inhibition about 25.95% (monophenolase) and 14.18% (diphenolase) at concentration 1 mg/ml. Compound **3** has tyrosinase inhibition about 27.17% (monophenolase) and 6.23% (diphenolase) at same concentration, while compound **4** has tyrosinase inhibition about 11.17% (monophenolase) and 9.75% (diphenolase).

Key words: taxifolin, flavanonol rhamnosides, anti-acne, tyrosinase inhibition, *Koompassia malaccensis*.

Introduction

Acne is a very common skin disease characterized by pimples on the face, chest, and back. It occurs when the pores of the skin become clogged with oil, dead skin cells, and bacteria. Acne is not a simple disease; it may sometimes lead to social phobia, lowered self-image, and depression (Koo and Smith 1991).

Compounds targeting acne therefore should be able to inhibit *P. acnes* population and inhibit *P. acnes* lipase activity, as a result reduce pro-inflammatory lipids in sebum as well as reduce post-acne scar formation. The materials that have antioxidant activity may be useful for relieving hypertrophic scars and keloid formation on the skin (Furakawa *et al.* 1995). In other words, compounds or materials claiming good for acne control should possess anti-bacterial, anti-lipase, anti-inflammatory, and antioxidant activities.

Tyrosinase inhibitors have been a great concern solely due to the key role of tyrosinase in both mammalian melanogenesis and fruit or fungi enzymatic browning (Chang 2009). Melanogenesis is a principal parameter of differentiation of melanocytes and melanoma cells (Ohguchi *et al.* 2005). The formation of melanin in human body influenced or reduced by several mechanisms, including anti-oxidation, direct tyrosinase inhibition, melanin inhibition of migration from cell to cell and hormonal activities etc (Pawelek and Korner 1982). Tyrosinase is responsible for pigmentation of skin, eyes and hair. It made tyrosinase inhibitors have been used frequently in cosmetics and depigmenting agents for hyperpigmentation. Investigation of inhibitors of this

enzyme may lead to development of novel skin whitening agents.

Kempas (*Koompassia malaccensis*) is an important and valuable wood because it is used as flooring, moldings, furniture, and veneer in Indonesia. It belongs to the Leguminosae family and widely distributed in Sumatera and Kalimantan. Many plants in Leguminosae show bioactivities such as antidiabetic, antimalaria, and antioxidant. Traditionally, the bark of kempas is used to prepare medicinal baths because of its antifever and antidyentery activities (Kobayashi *et al.* 1996).

In previous report (Kuspradini *et al.* 2009), taxifolin (**1**) and some flavanonol rhamnosides (neoastilbin (**2**), astilbin (**3**), and isoastilbin (**4**)) have been isolated from kempas (*K. malaccensis*). Some activities of taxifolin have been reported but not many activities report for flavanonol rhamnosides. On this paper we performed antimicrobial activity against *P. acnes*, *P. acnes* lipase inhibitory activity, antioxidant activity and tyrosinase inhibitory activity of taxifolin and some flavanonol rhamnosides isolated from kempas.

Materials and Methods

Plant Material

K. malaccensis was provided by the Department of Forest Product Technology, Mulawarman University, Kalimantan. Its voucher specimen (FHT.LA.13.11m) was deposited at the Wood Anatomy Laboratory of Mulawarman University, Indonesia.

Fractionation, Isolation, and Identification of Compounds 1, 2, 3, and 4.

The fractionation, isolation and identification of compounds 1, 2, 3, and 4 were performed the same with our previous report (Kuspradini *et al.* 2009).

Anti-acne Activity Assay

Anti-acne activity assay was performed based antimicrobial against *P. acnes*, *P. acnes* lipase inhibitory activity, and antioxidant activity. All of these activities were performed like methods in Batubara *et al.* (2009).

Tyrosinase Inhibitory Activity

Inhibition of tyrosinase activity (monophenolase) and DOPA auto-oxidation (diphenolase). This assay was performed using methods as described earlier (Curto *et al.* 1999; Nerya *et al.* 2003; Batubara *et al.* 2010).

Results and Discussion

Figure 1 shows HPLC of crude extract of Kempas. It was purified by p-HPLC to isolate the four compounds, and identified by NMR. The UV-Vis spectra of some major peaks, 1, 2, 3, and 4 were similar to that of taxifolin. By analysis of the NMR data that was generated in this study, and by comparison of the physical and spectral data with those reported in the literature, Compound 1 was identified as taxifolin (Lee *et al.* 2003).

The ¹³C-NMR data of Compound 1 exhibited a typical signal of flavonol-type skeleton: 83 ppm at C-2 and 77 ppm at C-3. The saturated bond between C-2 and C-3 was confirmed by the presence of doublets at 4.90 ppm (H-2) and 4.49 ppm (H-3) in ¹H-NMR spectrum. Thus, the compound 1 was identified as a dihydroquercetin/taxifolin. The flavanone like taxifolin signals were commonly observed in ¹H-NMR spectroscopies of Compound 2, 3,

and 4. Additionally, a glycoside signals were observed at 4.02~5.40 ppm, 3.50~4.16 ppm, 3.32~3.65 ppm, 3.17~3.57 ppm, 2.27~4.22 ppm, and 0.88~1.15 ppm, which indicates rhamnose residue. Furthermore, the heteronuclear multiple bond coherence (HMBC) of these compounds showed a correlation between the anomeric proton of rhamnose and C-3 carbon of flavanone, which indicates that rhamnose should be connected with C-3 of taxifolin moiety (Figure 2).

Therefore Compounds 2, 3, and 4 are assumed as flavanone-3-O-rhamnoside. As the result of comparing the ¹H NMR data of references Compound B, C, and D were identified as neoastilbin (De Britto *et al.* 1995), astilbin (Guo *et al.* 2007), and isoastilbin (Du *et al.* 2005), respectively, as illustrated in Figure 2.

The anti-acne properties of the 4 compounds start with antimicrobial test against *P. acnes*. The results showed that all compounds have no antimicrobial properties. This result is not the same with our finding before that all of these compounds have antimicrobial activity against *S. sobrinus* (Kuspradini *et al.* 2009).

Other anti-acne property tested is *P. acnes* lipase inhibition activity. The result of this test is shown in Figure 3. Inhibition properties for lipase of compound 1, 2, 3, and 4 at concentration 31.25 µg/ml are 39.18, 0.31, 39.24, and 59.19% respectively. Since compound 4 is the best compound for lipase inhibitor, IC₅₀ value of compound 4, isoastilbin was calculated. It showed that isoastilbin has IC₅₀ about 1.36 µg/ml. This IC₅₀ value is lower than IC₅₀ value of IPMP as positive control (166.4 µg/ml). The last activity for anti-acne is antioxidant property. The % inhibition for antioxidant of all compounds at concentration 10 µg/ml are 31.16, 25.64, 28.47, and 31.01% respectively (Figure 4).

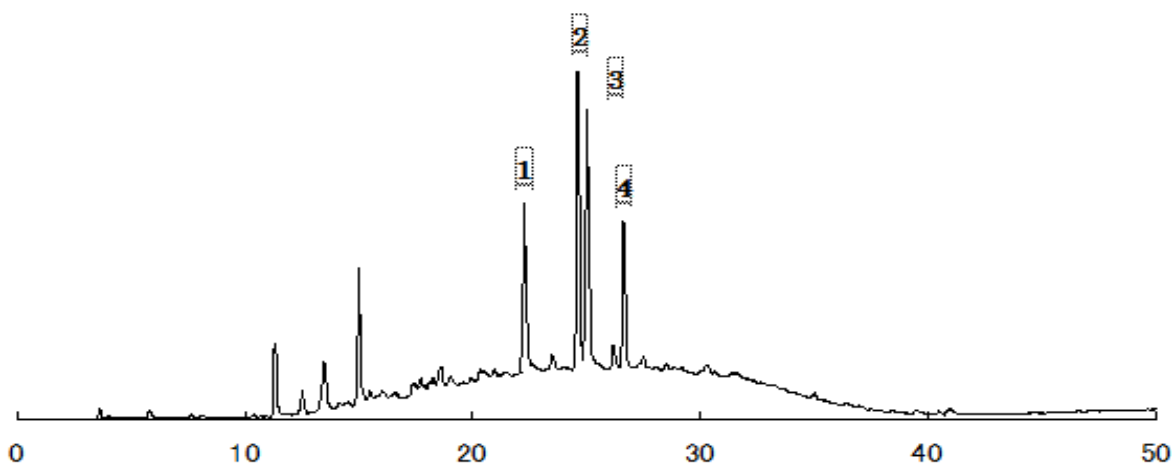


Figure 1. HPLC chromatogram of Kempas crude extract.

Column : VP-ODS (250mm x 4.6mm i.d.); gradient program : MeOH : 0.05%TFA = 10% : 90% - 80% : 20% (40 min), 100%:0% (50 min), wavelength : 280 nm; flow rate : 1 ml/min; analysis time: 50 min.

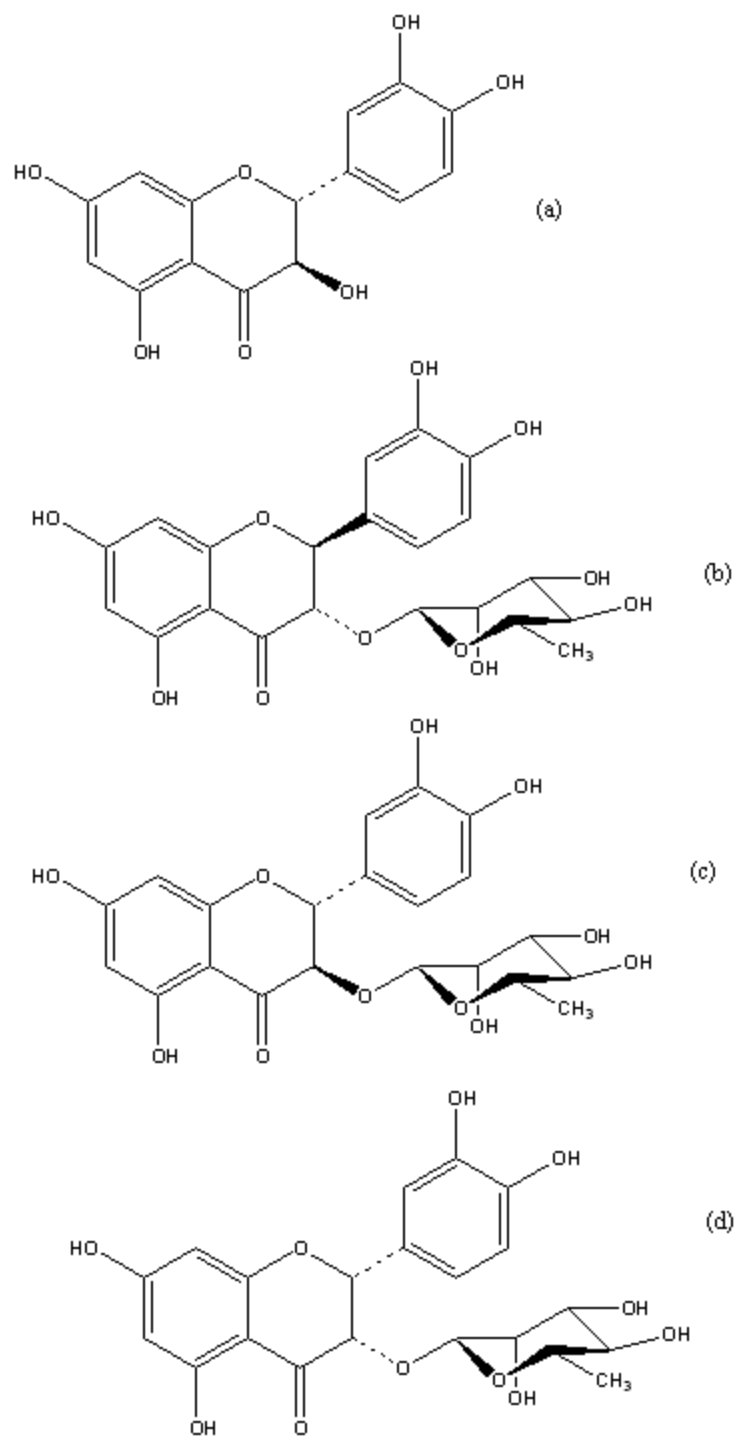


Figure 2. Structures of taxifolin and flavanone rhamnosides isolated from *Koompassia malaccensis* wood 50% ethanol extracts. Compound 1, 2, 3, and 4. a) compound 1 (taxifolin), b) 2 (neoastilbin), c) 3 (astilbin), d) 4 (isoastilbin).

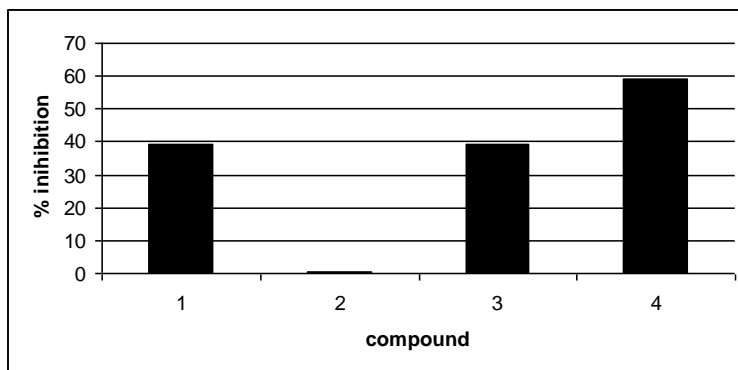


Figure 3. Lipase inhibitory activity of all compounds at concentration 31.25 µg/ml.

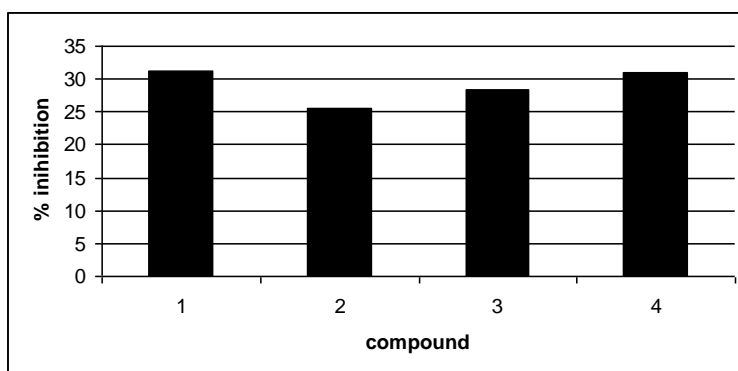


Figure 4. Antioxidant activity of all compounds at concentration 10 µg/ml.

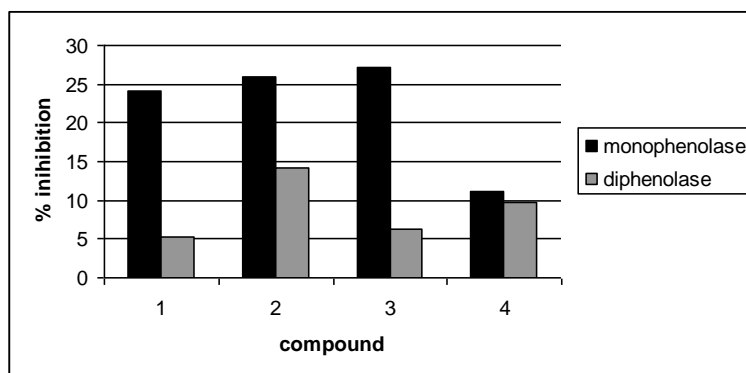


Figure 5. Tyrosinase inhibition activity of all compounds at concentration 1 mg/ml.

Tyrosinase activity of all compounds are not good. It only showed that tyrosinase inhibition of compound 1 at concentration 1 mg/ml is 24.12% for monophenolase and 5.18% for diphenolase. Compound 2 has tyrosinase inhibition about 25.95% (monophenolase) and 14.18% (diphenolase) at concentration 1 mg/ml. Compound 3 has tyrosinase inhibition about 27.17% (monophenolase) and 6.23% (diphenolase) at same concentration, while compound 4 has tyrosinase inhibition about 11.17% (monophenolase) and 9.75% (diphenolase).

Conclusions

The results for anti-acne showed that no antimicrobial activity against *P. acnes* for all compounds. The tyrosinase activities are also not good from all compounds.

Only isoastilbin showed the best lipase inhibition properties with IC_{50} about 1.36 µg/ml, and % inhibition for antioxidant of taxifolin, neoastilbin, astilbin, and isoastilbin at concentration 10 µg/ml are 31.16, 25.64, 28.47, and 31.01% respectively.

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