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

Irmanida Batubara, Harlinda Kuspradini, and Tohru Mitsunaga

Abstract

Taxifolin (**1**) and some flavanonol rhamnosides (neoastilbin (**2**), astilbin (**3**), and isoastilbin (**4**)) have been isolated kempas (*Koompassia malaccensis*). Our previous research about antimicrobial activity against *Streptococcus* inus and glucosyltransferase inhibitory activity of these compounds have been reported. Now, we carried out the anti- and tyrosinase inhibition properties of all four compounds. Antimicrobial against *Propionibacterium acnes*, *P. acnes* e inhibitory activity and antioxidant activity were established for anti-acne activity. Tyrosinase inhibition property was sured using L-tyrosine and L-DOPA as substrate. The results for anti-acne showed that no antimicrobial activity nst *P. acnes* for all compounds, the best lipase inhibition properties showed on compound **4** with IC_{50} about 1.36 l, and % inhibition for antioxidant at concentration 10 μg/ml are 31.16, 25.64, 28.47, and 31.01% respectively. sinase inhibition of compound **1** at concentration 1 mg/ml is 24.12% for monophenolase and 5.18% for diphenolase. pound **2** has tyrosinase inhibition about 25.95% (monophenolase) and 14.18% (diphenolase) at concentration 1 l. Compound **3** has tyrosinase inhibition about 27.17% (monophenolase) and 6.23% (diphenolase) at same ntration, while compound **4** has tyrosinase inhibition about 11.17% (monophenolase) and 9.75% (diphenolase).

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

Introduction

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

Compounds targeting acne therefore should be able hibit *P. acnes* population and inhibit *P. acnes* lipase ity, as a result reduce pro-inflammatory lipids in m as well as reduce post-acne scar formation. The erials that have antioxidant activity may be useful for ing hypertrophic scars and keloid formation on the (Furakawa *et al.* 1995). In other words, compounds aterials claiming good for acne control should ess anti-bacterial, anti-lipase, anti-inflammatory, and idant activities.

Tyrosinase inhibitors have been a great concern y due to the key role of tyrosinase in both mammalian nogenesis and fruit or fungi enzymatic browning ng 2009). Melanogenesis is a principal parameter of entiation of melanocytes and melanoma cells uchi *et al.* 2005). The formation of melanin in human influenced or reduced by several mechanisms, ding anti-oxidation, direct tyrosinase inhibition, min inhibition of migration from cell to cell and onal activities etc (Pawelek and Korner 1982). sinase is responsible for pigmentation of skin, eyes hair. It made tyrosinase inhibitors have been used ently in cosmetics and depigmenting agents for rpigmentation. 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 antidysentery 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 ^{13}C-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 ^{1}H-NMR spectrum. Thus, the compound **1** was identified as a dihydroquercetin/taxifolin. The flavanonol like taxifolin signals were commonly observed in ^{1}H-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 flavanonol, which indicates that rhamnose should be connected with C-3 of taxifolin moiety (Figure 2).

Therefore Compounds **2**, **3**, and **4** are assumed as flavanonol-3-*O*-rhamnoside. As the result of comparing the ^{1}H 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, IC_{50} value of compound **4**, isoastilbin was calculated. It showed that isoastilbin has IC_{50} about 1.36 μg/ml. This IC_{50} value is lower than IC_{50} 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).

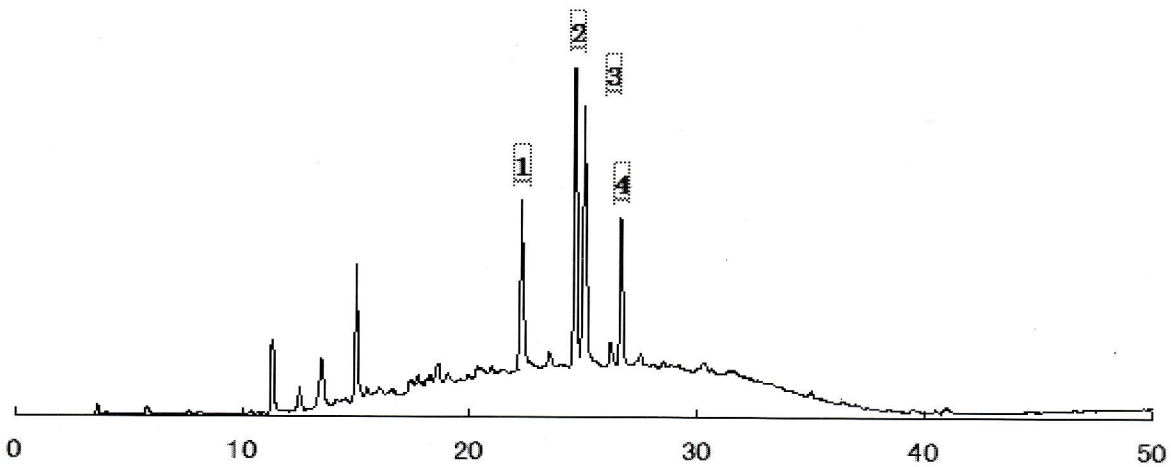

Figure 1. HPLC chromatogram of Kempas crude extract.
Column : VP-ODS (250mm x 4.6mm i.d.); gradient program : MeOH : 0.05%TFA = 10% : 90% - 80%: 20% (40 min), 100%:0% (50 min), wavelength : 280 nm; flow rate : 1 ml/min; analysis time: 50 min.

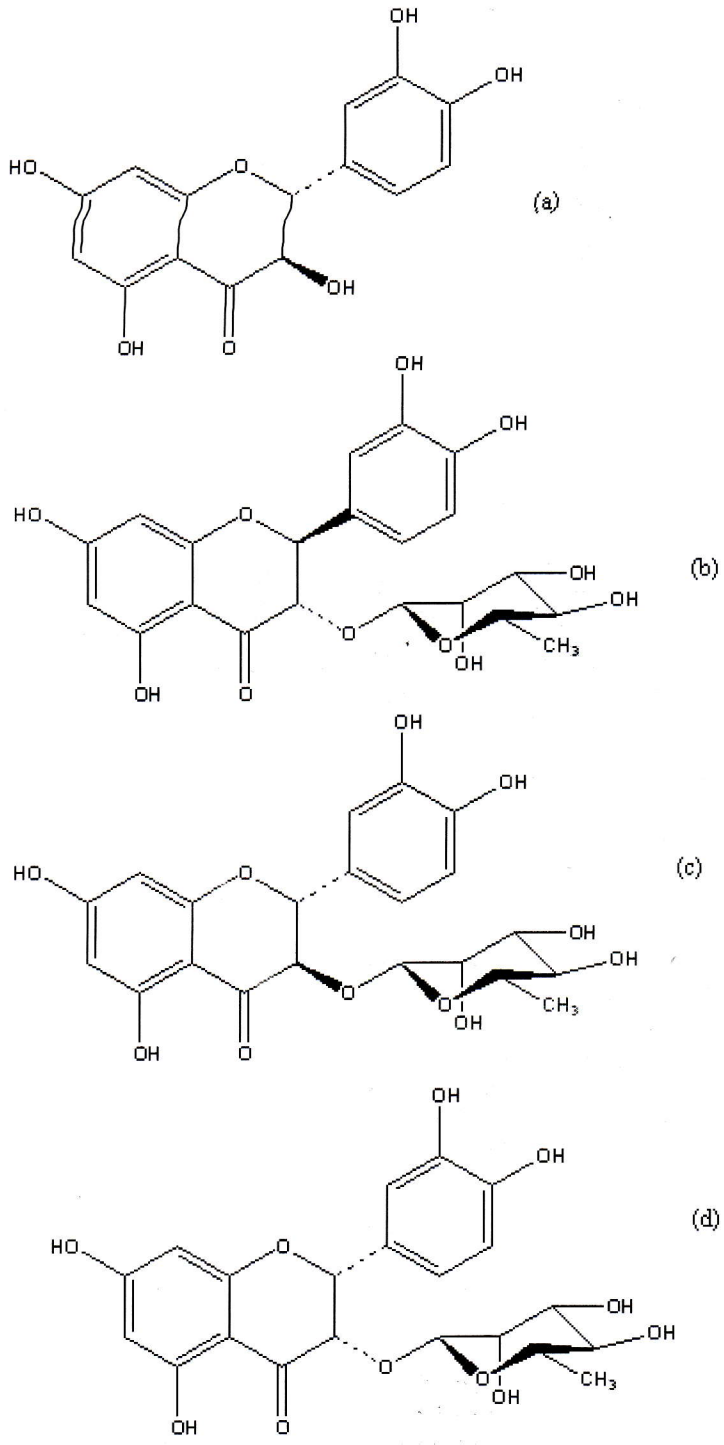

Figure 2. Structures of taxifolin and flavanonol rhamnosides isolated from *Koompassia malaccensis* wood 50% ethanol extracts. Compound **1, 2, 3,** and **4**. a) compound **1** (taxifolin), b) **2** (neoastilbin), c) **3** (astilbin), d) **4** (isoastilbin).

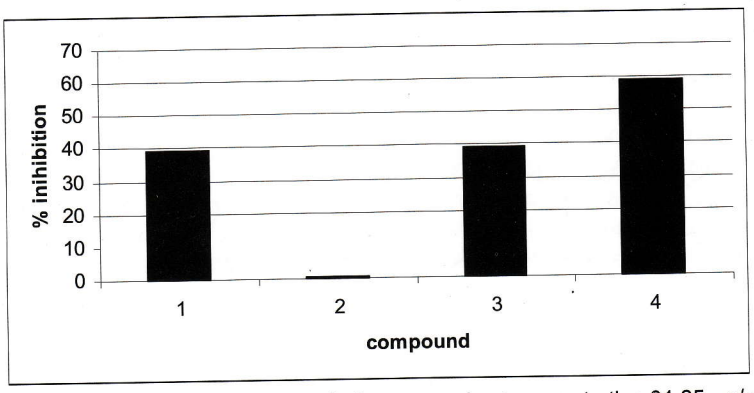

Figure 3. Lipase inhibitory activity of all compounds at concentration 31.25 μg/ml.

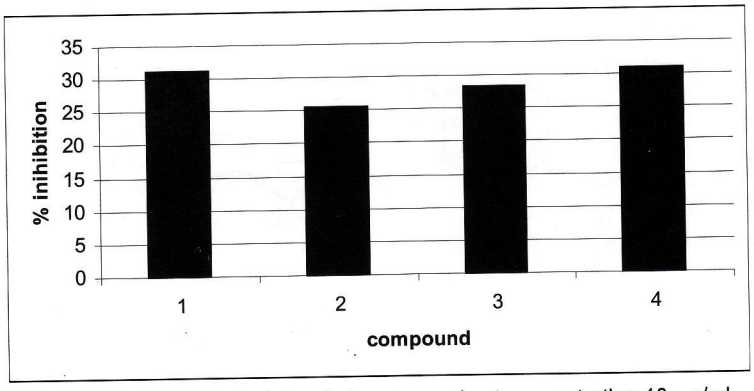

Figure 4. Antioxidant activity of all compounds at concentration 10 μg/ml.

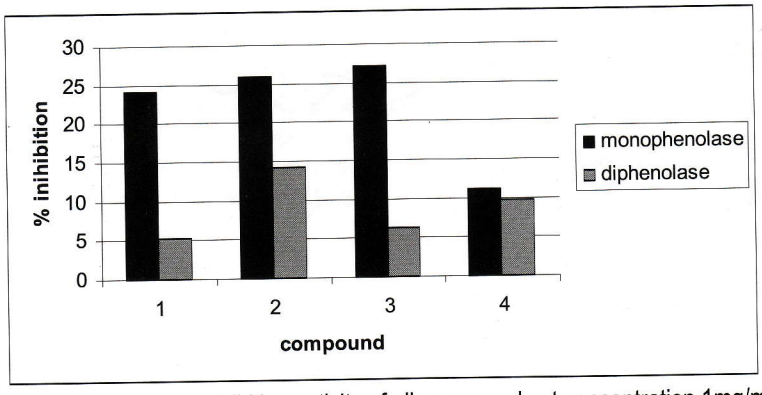

Figure 5. Tyrosinase inhibition activity of all compounds at concentration 1mg/ml.

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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Irmanida Batubara*
Department of Chemistry
Faculty of Mathematics and Natural Sciences
Bogor Agricultural University
Darmaga, Bogor 16880, Indonesia.

Biopharmaca Research Center
Bogor Agricultural University
Taman Kencana, Bogor 16151, Indonesia.

*Corresponding Author
Tel. : +62-251-8373561
Fax. : +62-251-8347525
E-mail address : ime@ipb.ac.id

Harlinda Kuspradini
Department of Forest Product Technology
Faculty of Forestry, Mulawarman University
Samarinda, Indonesia.

Tohru Mitsunaga
Department of Applied Biological Science
Faculty of Applied Biological Sciences
Gifu University
1-1 Yanagido, Gifu 501-1193, Japan.