

Anti-androgenic Activity of Myricae Cortex—Isolation of Active Constituents from Bark of *Myrica rubra*

Hideaki MATSUDA,^{*,a} Miho YAMAZAKI,^a Keizo MATSUO,^a Yusuke ASANUMA,^b and Michinori KUBO^a

Faculty of Pharmaceutical Sciences, Kinki University,^a 3–4–1 Kowakae, Higashiosaka, Osaka 577–8502, Japan and Daiichi Pharmaceutical Co., Ltd.,^b 3–14–10, Nihonbashi, Chuo-ku, Tokyo 103–8234, Japan.

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The aqueous ethanol extract of Myricae Cortex (bark of *Myrica rubra* SIEB. et ZUCC., Myricaceae) showed *in vitro* testosterone 5 α -reductase inhibitory activity and *in vivo* anti-androgenic activity using growth of flank organ in castrated Syrian hamsters and/or hair regrowth after shaving in testosterone-treated C57Black/6CrSlc mice. Three constituents, myricanone, myricanol, and myricetin were identified as the main active principles.

Key words *Myrica rubra*; testosterone 5 α -reductase; anti-androgenic activity; hair regrowth

Androgens have been indicated to be factors inciting common baldness and both androgenic action and a genetic predisposition to be prerequisite to development of alopecia.¹⁾ Testosterone is metabolized intracellularly by the enzyme testosterone 5 α -reductase to an active androgen, dihydrotestosterone, which binds to androgen receptors and shows various hormonal actions.²⁾ Thus the anti-androgenic drugs which exhibited inhibitory activity in testosterone 5 α -reductase and/or in the successive biological processes, e.g. binding between dihydrotestosterone and androgen receptor, and protein synthesis may be useful for protection from alopecia.

Many crude drugs have been used for treatment of thermal burn in traditional Chinese medicine. From these natural Chinese drugs, we selected the twenty crude drugs cited in Table 1 and assayed their 50% ethanol extracts for testosterone 5 α -reductase inhibitory activity. Among them, 50% ethanol extract of Myricae Cortex (bark of *Myrica rubra* SIEB. et ZUCC., Myricaceae) showed a remarkable activity similar to that of Rosae Rugosae Flos (buds of *Rosa rugosa* THUNB. var. *plena* REGL., Rosaceae) whose anti-androgenic activity based on the testosterone 5 α -reductase inhibitory effect has been suggested.³⁾ In the Japanese cosmetic market, an extract obtained from Rosae Rugosae Flos is used to promote hair growth. In this paper, we describe the comparison of anti-androgenic activity *in vitro* and *in vivo* of Myricae Cortex it with that of Rosae Rugosae Flos, and also the testosterone 5 α -reductase inhibitory activity guided fractionation of Myricae Cortex extract leading to isolate active constituents.

MATERIALS AND METHODS

Reagents The following chemicals were used in this study: tris(hydroxymethyl)aminomethane, testosterone, β -nicotinamide adenine dinucleotide phosphate tetrasodium salt (reduced form, NADPH), dichloromethane (Nacalai Tesque), hexyl *p*-hydroxybenzoate (Tokyo Kasei), ethinylestradiol (Wako) and Prostetone[®] (oxendolone, Takeda). All chemical reagents were of reagent grade and were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), unless otherwise noted.

Preparation of 50% Ethanol Extracts from Chinese Crude Drugs All crude drugs (1 g) were powdered and ex-

tracted with 50% ethanol (5 ml) at room temperature for 24 h. After filtration, the fresh filtrates were used for *in vitro* assay of inhibition of testosterone 5 α -reductase activity and for an *in vivo* anti-androgenic experiment using testosterone-treated C57Black/6CrSlc mice. Myricae Cortex (100 g) and Rosae Rugosae Flos (100 g) were crushed and extracted with 50% ethanol (500 ml, each) at room temperature for 24 h. Each extract was evaporated and lyophilized to give a 50% ethanol extract; the yields (%) were 20.4 and 16.3%, respectively. These 50% ethanol extracts were used for *in vivo* anti-androgenic experiment using the flank organ in castrated Syrian hamsters at an appropriate concentration in 50% ethanol.

Isolation of Myricanone, Myricanone, Myricetin, and Myricitrin from Myricae Cortex The crushed cortex (3 kg) was extracted with 50% ethanol (30 l) at room temperature for 3 d. After filtration, the extract was concentrated under reduced pressure to a volume of 4 l. The concentrated solution was extracted successively with hexane (4 l \times 2) and EtOAc (4 l \times 2). The combined hexane and EtOAc layers were dried over anhydrous sodium sulfate and evaporated to dryness to give a more active hexane soluble fraction (4.92 g) and a less active EtOAc soluble fraction, respectively. The active hexane soluble fraction (4.9 g) was chromatographed over silica gel (Fuji Chemicals, BW-350, 300 g) eluted with hexane–EtOAc (7 : 3 v/v) to give 14 fractions and the testosterone 5 α -reductase inhibitory activity of each fraction was examined. The active fraction 9 was purified by recrystallization from MeOH to give myricanone, colorless prisms, mp 186–189 °C (68 mg). Recrystallization of another active fraction 12 from diethyl ether gave myricanol, colorless needles, mp 95–105 °C (1.10 g). The structures of these two known compounds were deduced from the comparison of their NMR spectral data with those of the reported data (the chemical structures are shown in Fig. 1).

The combined EtOAc layer was dried over anhydrous sodium sulfate and evaporated to dryness to give an EtOAc soluble fraction from which myricetin (1.88 g) and myricitrin (640 mg, myricetin-3-*O*-rhamnoside) were isolated and identified in a similar manner as described in our previous report.⁴⁾ Melting points were determined on a Yanagimoto micro melting point apparatus without correction. Optical rotations were measured on a JASCO DIP-370. ¹H- and ¹³C-NMR spectra were taken on JOEL JNM-GSX500 at 500 and 125 MHz, respectively, in CDCl₃ with tetramethylsilane as an

* To whom correspondence should be addressed. e-mail: matsuda@phar.kindai.ac.jp

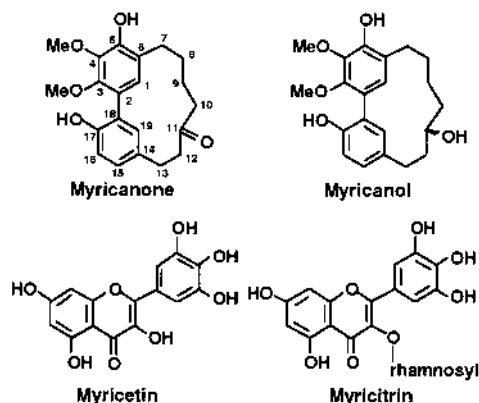


Fig. 1. Chemical Structures of Myricanone, Myricanol, Myricetin, and Myricitrin Isolated from Myrica Cortex

internal standard.

Myricanol Colorless needles, mp 95–106 °C, $[\alpha]_D^{20}$ –62.8°, ($c=1.0$, CHCl_3). $^{13}\text{C-NMR}$: 22.9 (C-8), 25.4 (C-9), 25.8 (C-10), 26.9 (C-12), 34.7 (C-7), 39.4 (C-13), 61.4 (2× OMe), 68.5 (C-11), 116.8 (C-16), 122.6 (C-2), 123.4 (C-18), 124.6 (C-6), 129.4 (C-19), 129.9 (C-1), 130.7 (C-14), 133.1 (C-15), 138.6 (C-4), 145.9 (C-5), 147.6 (C-3), 151.3 (C-17). a Interchangeable. These $^{13}\text{C-NMR}$ spectral data and $^1\text{H-NMR}$ spectral data were in good accordance with the reported data.^{5,6)}

Myricanone Colorless prisms, mp 188–191°. $^{13}\text{C-NMR}$: 22.0 (C-8), 24.7 (C-9), 27.0 (C-7), 28.9 (C-13), 42.7 (C-10), 46.1 (C-12), 61.4 (OMe), 61.5 (OMe), 117.0 (C-14), 123.1 (C-18), 123.2 (C-2), 125.8 (C-6), 129.1 (C-1, C-14, C-19), 132.6 (C-15), 139.0 (C-4), 146.2 (C-5), 148.0 (C-3), 152.0 (C-17), 213.5 (C-11). a Interchangeable. These $^{13}\text{C-NMR}$ spectral data and $^1\text{H-NMR}$ spectral data were in good accordance with the reported data.^{5,6)}

Animals Male SD strain rat (6 weeks of age), male Syrian strain golden hamsters (4 weeks of age), and male C57Black/6CrSlc strain mice (7 weeks of age) were provided by Japan SLC (Shizuoka, Japan). They were maintained in an air-conditioned room with lighting from 7 a.m. to 7 p.m. The room temperature (about 23 °C) and humidity (about 60%) were controlled automatically. Laboratory pellet chows (Labo MR Stock and Labo R stock, Nihon Nosan Kogyo Co., Ltd., Tokyo, Japan) and water were freely available.

Assay for Inhibition of Testosterone 5 α -Reductase Activity Testosterone 5 α -reductase was prepared according to the method of Imai.⁷⁾ From twenty-four hour-fasted rat (male, Slc-SD strain, aged 6 weeks), the liver was isolated after circulation with ice-cold Krebs–Ringer phosphate buffer (pH 7.4). The liver was homogenized with 5 volumes of ice-cold Tris–HCl buffer (10 mM, pH 7.2) and centrifuged at 9000×*g* for 10 min. The supernatant was stored at –80 °C until use. Inhibition assay of 5 α -reductase was performed according to the method described by Iyata⁸⁾ with minor modification. The reaction solution contained 50% ethanol extract solution (0.2 ml) obtained from Chinese crude drug or 50% ethanol solution (0.2 ml) of various concentrations of test compound: 1.0 ml of Tris–HCl buffer pH 7.2, 0.3 ml of testosterone (500 $\mu\text{g}/\text{ml}$ in propylene glycol–Tris–HCl buffer pH 7.2 (1 : 1v/v)), and 1.0 ml of the enzyme solution. The reactions were started by the addition of 0.5 ml of NADPH

(0.77 mg/ml in Tris–HCl buffer pH 7.2). The mixture was incubated at 37 °C for 30 min, and the reaction was stopped by addition of 5.0 ml of dichloromethane. After addition of 0.5 ml of hexyl *p*-hydroxybenzoate (0.1 mg/ml in methanol, an internal standard (I.S.) for HPLC), the tube was shaken for 10 min and centrifuged at 900×*g* for 10 min. The organic layer (4 ml) was transferred to another tube and evaporated to dryness. The residue was dissolved in 5.0 ml of methanol, and an aliquot of 10 μl was injected into the HPLC system (Jasco 880-PU, 875-UV). HPLC conditions: column; YMC-Pak ODS-AM302 (150×4.6 mm I.D.) at 40 °C, mobile phase; methanol–H₂O (65 : 35 v/v), flow rate; 1.0 ml/min, detection; UV 254 nm, t_R ; testosterone, 7 min, I.S., 14 min. The control-0 min tube received 5.0 ml of dichloromethane before addition of NADPH, while the control-30 min tube received 0.2 ml of 50% ethanol instead of the test sample. The procedure described above was then carried out for these control tubes. 5 α -Reductase inhibitory activity was determined from the following equation using the peak-area ratios ($r=T/I.S.$).

Inhibition (%) = $100 \times (r \text{ of test sample} - r \text{ of control-30 min}) / (r \text{ of control-0 min} - r \text{ of control-30 min})$. IC₅₀ values were graphically calculated from the inhibition percent values at several concentrations.

Growth Suppression of Hamster Flank Organs An anti-androgenic assay using hamster flank organ growth measured by the increase in area of pigmented macule was performed according to the method described by Liang and Liao⁹⁾ with minor modification. Pre-pubertal male Syrian golden hamsters were castrated at 3 weeks of age by excision of the testicles. Intact hamsters were not castrated. After 1 week, hamsters were divided into 7–8 animals per group in order to have similar weight in each group. Hair on the lower back of each animal was shaved with an electric hair clipper weekly to expose the flank organ. A testosterone solution 5 μl (0.01% in ethanol) was applied topically to the right flank organ once a day for 21 d with a pipette (GILSON, France) and a polypropylene disposable tip. After 30 min of testosterone treatment, 5 μl of one of the following sample solutions was applied topically to the right flank organ once a day for 21 d in a similar way. Sample solutions: (1) 50% ethanol as control, (2) 2% oxendolone as positive control (3) 50% ethanol extract of Myrica Cortex, (4) 50% ethanol extract of Rosae Rugosae Flos, and (5) myricanol, myricetin, and myricitrin in an appropriate concentration. As vehicle, 5 μl of ethanol was applied topically to the left flank organ. In the intact group, 5 μl of ethanol was applied on both flank organs, and after 30 min, 5 μl of 50% ethanol was applied on both flank organs. The surface of the flank organ was wiped with an ethanol pad before each treatment. On the day after the last treatment, animals were sacrificed by an intraperitoneal injection of pentobarbital. Growth of both flank organs, the treated (right side) and vehicle (left side) was determined by measuring the length of the long axis and the short axis of the pigmented spot (pigmented macule) with calipers. The surface area (mm²) of the spot was calculated by multiplication of length of the two axis.

Hair Regrowth after Shaving in Testosterone-Treated C57Black/6CrSlc Mice According to the method described by Yokoyama¹⁰⁾ with minor modification, the dorsal hairs of C57Black/6CrSlc mice (7 weeks of age, one group using 10–11 mice) were shaved with an electric hair clipper.

Table 1. Inhibitory Effects of 50% Ethanol Extract Solutions from Various Crude Drugs on *in Vitro* Testosterone 5 α -Reductase Activity

Japanese name	Samples		Inhibition (%)
	Botanical origin	Family name	
楊梅皮	<i>Myrica rubra</i> SIEB. et ZUCC.	Myricaceae	80.7
玫瑰花	<i>Rosa rugosa</i> THUNB. var. <i>plena</i> REGEL	Rosaceae	79.0
鷄血藤	<i>Mucuna birdwoodiana</i> TUTCHER	Leguminosae	58.4
拳參	<i>Polygonum bistorta</i> L.	Polygonaceae	58.2
何首烏	<i>Polygonum multiflorum</i> THUNB.	Polygonaceae	57.4
補骨脂	<i>Psoralea corylifolia</i> L.	Leguminosae	57.1
牡丹皮	<i>Paeonia moutan</i> SIMS.	Paeoniaceae	52.7
桂皮	<i>Cinnamomum cassia</i> BLUME	Lauraceae	38.4
竹節人參	<i>Panax japonicum</i> C. A. MEYER	Araliaceae	31.9
吳茱萸	<i>Evodia bodinieri</i> DODE	Rutaceae	25.9
丹參	<i>Salvia miltiorrhiza</i> BUNGE	Labiatae	22.7
半枝蓮	<i>Scutellaria barbata</i> D. DON	Labiatae	20.2
覆盆子	<i>Rubus chingii</i> HU	Rosaceae	14.4
紅花	<i>Carthamus tinctorius</i> L.	Compositae	9.9
地膚子	<i>Kochia scoparia</i> SCHRAD.	Chenopodiaceae	5.1
當歸	<i>Angelica sinensis</i> DIELS	Umbelliferae	2.8
桃仁	<i>Prunus persica</i> BATSCH.	Rosaceae	2.1
升麻	<i>Cimicifuga simplex</i> WORSKARL	Ranunculaceae	-1.1
川芎	<i>Cnidium officinale</i> MAKINO	Umbelliferae	-2.4
芍藥	<i>Paeonia lactiflora</i> PALL.	Paeoniaceae	-3.9

Beginning the next day, 100 μ l of testosterone solution (0.05% in ethanol) was applied topically to the shaved dorsum once a day for 25 d with a pipette and a polypropylene disposable tip. After 30 min of testosterone treatment, 100 μ l of one of the following sample solutions was applied topically to the shaved dorsum once a day for 25 d in a similar way. Sample solutions: (1) 50% ethanol as control, (2) 50% ethanol extract solution of Myricae Cortex, (3) 50% ethanol extract solution of Rosae Rugosae Flos, (4) 2% oxendolone solution.

The hair regrowth at 7, 14, 18, 21, and 25 d after beginning topical application was calculated using the following score: 0: no hair growth was observed; 1: less than 20% growth; 2: 20% to less than 40% growth; 3: 40% to less than 60% growth; 4: 60% to less than 80% growth; 5: 80% to 100% growth was observed.

Statistical Analysis The experimental data were tested for statistically significance using Bonferroni/Dunn's multiple range test method.

RESULTS

Testosterone 5 α -reductase inhibitory activities of 50% ethanol extract solutions obtained from several Chinese crude drugs are shown in Table 1. The extract from Myricae Cortex showed the strongest inhibition of the enzyme activity, and was comparable to the strong inhibitor of Rosae Rugosae Flos.

The IC₅₀ values on testosterone 5 α -reductase of some constituents of Myricae Cortex and ethinylestradiol as a reference compound are listed in Table 2. The values of myricanol and myricanone were 3.7 and 3.8 mM, respectively, but those of myricetin and myricitrin were weaker and ineffective.

Results of an *in vivo* anti-androgenic activity assay using male Syrian strain golden hamsters are depicted in Tables 3, 4, and 5. Table 3 shows the growth of right and left pigment macules of intact and castrated male hamsters, and the stimu-

Table 2. IC₅₀ Values of Inhibitory Effects of Myricanone, Myricanol, Myricetin, and Myricitrin from Myricae Cortex and Ethinylestradiol on *in Vitro* Testosterone 5 α -Reductase Activity

Sample	IC ₅₀ (mM)
Myricanone	3.8
Myricanol	3.7
Myricetin	8.0
Myricitrin	No effect
Ethinylestradiol	0.81

Table 3. Stimulation of Testosterone (T) to Flank Organ in Castrated Hamsters

	Treatment of flank organ		Pigment macule area (mm ²)	
	Left	Right	Left	Right
Intact	Vehicle	Vehicle	32.7 \pm 1.6	30.6 \pm 2.4
Castrated	Vehicle	Vehicle	9.8 \pm 1.1	9.1 \pm 1.1
Castrated+T	Vehicle	Testosterone	9.5 \pm 1.4	22.1 \pm 1.1*

In intact or castrated groups, 5 μ l ethanol was applied on both flank organs. In control (Castrated+T) group, 5 μ l ethanol was applied on the left organ, and testosterone 0.5 μ g per 5 μ l ethanol (0.01%) was applied on the right organ. Treatment was carried out daily for 21 d. At the end of treatment period, the area of pigmented macules was determined. Each value represents the mean \pm S.E. of 7–8 hamsters. Significantly different from the castrated group, *: $p < 0.01$.

lation of left pigment macules in castrated hamsters by testosterone treatment. The pigmented macules of the intact 3 week old Syrian hamsters apparently grew in response to increased production of endogenous androgen. The pigmented macules of castrated Syrian hamsters did not grow, but testosterone obviously stimulated the growth of the pigmented macules on the right side in castrated hamsters.

The effects of oxendolone, 50% ethanol extract of Myricae Cortex and 50% ethanol extract of Rosae Rugosae Flos on testosterone stimulated growth of pigment macules are shown in Table 4. Fifty percent ethanol extracts of both drugs

Table 4. Effects of 50% Ethanol Extract from Myricae Cortex (ME-ext), Rosae Rugosae Flos (RE-ext) and Oxendolone on Testosterone-Stimulated Growth of Pigmented Macules of Hamster Flank Organ

Treatment of right flank organ Testosterone (T; 0.5 μ g/5 μ l ethanol) + sample solution	Pigment macule area (mm ²)		
	Left (untreated)	Right (treated)	Inhibition (%)
T+50% ethanol [Control]	7.2 \pm 1.0	19.4 \pm 1.8	—
T+ME-ext 0.2 mg/5 μ l 50% ethanol	7.1 \pm 0.6	12.0 \pm 1.3*	38
T+ME-ext 0.5 mg	6.0 \pm 0.7	11.8 \pm 1.3*	39
T+RE-ext 0.2 mg	6.4 \pm 1.0	11.8 \pm 0.8*	39
T+RE-ext 0.5 mg	6.1 \pm 1.2	10.1 \pm 1.4*	48
T+Oxendolone 0.33 μ mol	4.8 \pm 1.1	12.8 \pm 1.1*	34

A testosterone (T) solution 5 μ l (0.01% in ethanol) was applied topically to the right flank organ once a day for 21 d. After 30 min of T treatment, 5 μ l of the sample solution was applied topically to that flank organ. As vehicle, 5 μ l of ethanol was applied on the left flank organ, and after 30 min, 5 μ l of 50% ethanol was applied. At the end of the treatment period, the area of pigmented macules was determined. Each value represents the mean \pm S.E. of 7–8 hamsters. Significantly different from the castrated group, *: p <0.01.

Table 5. Effects of Myricanol, Myricetin, and Myricitrin from Myricae Cortex and Oxendolone on Testosterone-Stimulated Growth of Pigmented Macules of Hamster Flank Organ

Treatment of right flank organ Testosterone (T; 0.5 μ g/5 μ l ethanol) + sample solution	Pigment macule area (mm ²)		
	Left (untreated)	Right (treated)	Inhibition (%)
T+50% ethanol (Control)	1.9 \pm 1.3	19.8 \pm 0.4	—
T+Myricanol 0.015 μ mol/5 μ l 50% ethanol	2.1 \pm 0.3	16.5 \pm 1.8*	17
T+Myricanol 0.05 μ mol	1.5 \pm 0.6	12.4 \pm 0.6**	37
T+Myricetin 0.015 μ mol	2.7 \pm 0.5	15.3 \pm 1.4**	23
T+Myricetin 0.05 μ mol	2.1 \pm 0.4	13.7 \pm 0.8**	31
T+Myricitrin 0.015 μ mol	1.8 \pm 0.3	19.0 \pm 1.4	4
T+Myricitrin 0.05 μ mol	1.9 \pm 0.4	15.0 \pm 1.2**	24
T+Oxendolone 0.33 μ mol	1.9 \pm 0.4	10.7 \pm 0.5**	46

A testosterone (T) solution 5 μ l (0.01% in ethanol) was applied topically to the right flank organ once a day for 21 d. After 30 min of T treatment, 5 μ l of the sample solution was applied topically to the same organ. As vehicle, 5 μ l of ethanol was applied on the left flank organ, and after 30 min, 5 μ l of 50% ethanol was applied. At the end of the treatment period, the area of pigmented macules was determined. Each value represents the mean \pm S.E. of 7–8 hamsters. Significantly different from castrated group, *: p <0.05, **: p <0.01.

showed a significant anti-androgenic activity as did oxendolone, an anti-androgenic drug.

The results of another run of a similar experiment using oxendolone, myricanol, myricetin and myricitrin are depicted in Table 5. Both myricanol and myricetin which exhibited testosterone 5 α -reductase inhibitory activity also had a significant anti-androgenic activity. Although myricitrin lacked testosterone 5 α -reductase inhibitory activity, this compound showed a similar anti-androgenic activity.

Another anti-androgenic assay *in vivo* was performed using a hair regrowth assay in testosterone sensitive male C57Black/6CrSlc strain mice. As shown in Fig. 2, testosterone treatment remarkably suppressed hair regrowth in these strain mice. Fifty percent ethanol extract solution of Myricae Cortex showed a significant anti-androgenic activity as did oxendolone. However, in this experimental model, the anti-androgenic activity of a 50% ethanol extract solution of Rosae Rugosae Flos was less effective than that of Myricae Cortex.

DISCUSSION

Myricae Cortex is prescribed as an anti-diarrhetic and anti-microbiotic in Chinese medicine and has been shown to contain tannins,¹¹⁾ the flavonols⁴⁾ myricetin, myricitrin and quercetin, and the diarylheptanoids¹²⁾ myricanol and myricanone. The inhibitory effect of Myricae Cortex on melanin biosynthesis was described in our previous paper,⁴⁾ however,

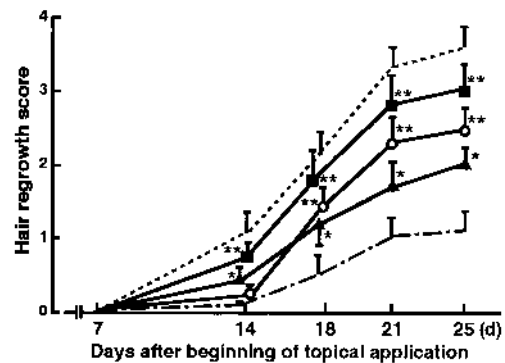


Fig. 2. Effects of 50% Ethanol Extracts from Myricae Cortex and Rosae Rugosae Flos and Oxendolone on Hair Regrowth after Shaving in Testosterone-Treated C57Black Mice

The dorsal hairs of C57Black/6CrSlc mice (7 weeks of age) were shaved. Beginning the next day, 100 μ l of testosterone solution (0.05% in ethanol) was applied topically to the shaved dorsum once a day for 25 d. After 30 min of testosterone treatment, 100 μ l of the following sample solutions were also applied topically to the shaved dorsum once a day for 25 d. Control and testosterone-treated control were applied with 50% ethanol alone or 50% ethanol together with 0.05% testosterone, respectively. The regrowth after beginning of topical application was calculated by scoring. - - -, control; - - -, testosterone-treated control, \circ ; 50% ethanol extract solution of Myricae Cortex, \blacktriangle ; 50% ethanol extract solution of Rosae Rugosae Flos, \blacksquare ; 2% oxendolone solution. Each point represents the mean \pm S.E. of 10–11 mice. Significantly different from the control group, *: p <0.05, **: p <0.01.

its testosterone 5 α -reductase inhibitory effect has not previously been reported. The anti-androgenic drugs which exhibited inhibitory activity in testosterone 5 α -reductase and/or the successive biological processes may be useful as hair

growth promoting agents and also to protect against alopecia, thus the present study was targeted to examine the anti-androgenic activities of the extract obtained from Myrica Cortex, to characterize the active components, and then to apply the extract to a hair growth promoting agent originated from a natural resource.

The first screening for testosterone 5α -reductase inhibitory activity of 50% ethanol extracts of about twenty Chinese crude drugs found those of Myrica Cortex and Rosae Rugosae Flos showed a remarkable activity. A search for the constituents of the activity of Myrica Cortex using bioassay-guided chromatography of the active hexane soluble fraction led to the isolation of two active compounds, myricanol and myricanone. Myricetin, a major flavonol of the bark, was weakly active, but myricitrin, a flavonol-glycoside, was inactive. Thus the testosterone 5α -reductase inhibitory activity of Myrica Cortex can be partially attributed to myricanol, myricanone, and myricetin.

An anti-androgenic assay *in vivo* was performed using flank organ growth measured by increase in the area of pigmented macule in castrated Syrian hamsters. Fifty percent ethanol extracts of Myrica Cortex and Rosae Rugosae Flos obviously inhibited the growth of pigmented macules treated by testosterone. These samples showed the significant anti-androgenic activity as did oxendolone, an anti-androgenic drug. Both myricanol and myricetin which exhibited testosterone 5α -reductase inhibitory activity also had a significant anti-androgenic activity. Although myricitrin lacked testosterone 5α -reductase inhibitory activity, this compound showed a similar anti-androgenic activity. This result can be explained by assuming that the anti-androgenic activity acts to inhibit binding between dihydrotestosterone and androgen receptor, and/or protein synthesis inhibition.

Another anti-androgenic assay *in vivo* was performed using hair regrowth assay in testosterone sensitive male C57Black/6CrSlc strain mice. In this experimental model animal, it has been noted that testosterone caused a disorder in the hair growth cycle in dermal papilla cells.²⁾ Fifty percent ethanol extract of Myrica Cortex showed a significant anti-androgenic activity as did oxendolone.

Based on the above results, it was found that 50% ethanol extract of Myrica Cortex showed a significant anti-androgenic activity in both these *in vivo* assays, and the bark contained three testosterone 5α -reductase inhibitory active constituents, myricanol, myricanone, and myricetin.

In conclusion, Myrica Cortex may be useful to protect against alopecia, and may be a candidate as a hair growth promoting agent originating from a natural resource.

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