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# Studies on Active Substances in Herbs Used for Hair Treatment. I. Effects of Herb Extracts on Hair Growth and Isolation of an Active Substance from *Polyporus umbellatus* F.[1)]

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**The effects of methanol extracts of 80 herbs on hair growth were investigated, using normal $C_3H$/He mice from which telogen hair on the back had been removed. Eighteen of the extracts apparently promoted hair regrowth on the mice. As one of active principles in *Polyporus umbellatus* F., 3,4-dihydroxybenzaldehyde was isolated by column chromatography on Amberlite XAD-2, Sephadex LH-20 and silica gel.**

**Keywords** herb; *Polyporus umbellatus*; 3,4-dihydroxybenzaldehyde; $C_3H$/He mice

Many herbal extracts are used as hair tonics, usually on the basis of long tradition, rather than scientifically proven effectiveness.[2)] Some of them have been studied in attempts to find a good hair growth promoter,[3)] and recently lansic acid contained in *Lansium domesticum*, was shown to be effective.[4)]

As a part of our studies aimed at identifying active constituents of herbs that promote hair growth,[1)] we have examined methanol extracts of 80 herbs, which were selected on the basis of a literature survey.[2)] Various methods have been reported for evaluating hair growth-promoting ability,[3,5)] but all of them have disadvantages relating to the species, age, body region or condition of the test animal. In this study, pharmacological hair growth-promoting activity was measured by Ogawa's method using 9-week-old $C_3H$/He mice,[5a)] in which the anagen hair regrowth after removal of back telogen hair is evaluated, because this method is considered to be reliable for judging the ability of test samples to promote hair regrowth.

This paper deals with the effects of various herb extracts on hair regrowth and describes the isolation of one of the active principles in *Polyporus umbellatus* F.

## Results and Discussion

In relation to hair regrowth, it is well known that 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and all-*trans*-retinoic acid (RA) alter cell proliferation and differentiation in the epithelium and it has also been suggested that they promote vascular proliferation.[6,7)] As positive controls for hair regrowth on $C_3H$/He mice, TPA and RA were used, according to Ogawa and Hattori.[5a)] On the 8th day after topical application, the regrowth of anagen hair first became morphologically apparent in the control group. At this time, as shown in Table I, the luminance (L) value of the TPA-administered group decreased slightly in comparison with that of the control.[1)] TPA showed significant stimulation of hair regrowth on days 10, 13 and 15. RA showed a similar activity to that of TPA, but its effect was more variable. Thus, this method was employed for the first screening of the effective herbs on hair regrowth and for the isolation study on active substances in them.

In this experiment, on days 13 and 15 after TPA application anagen hair regrowth was almost completed and on day 17 hair growth was observed visually. But it was technically difficult to evaluate the effectiveness for hair growth by colorimetry after day 15. Thus, in the following study, measurement was carried out on days 10 and 13, when the condition of regrown hair was suitable for observation.

Accordingly, we examined the effect of methanol extracts of 80 herbs on hair regrowth using this bioassay. As shown in Table II, relative hair growth activity (G.A.) showed that about half of the herb extracts had no apparent effects, while the following results were obtained with the others.

On the 10th day, only 4 of the methanol extracts, from

TABLE I. Effect of TPA and RA on Hair Regrowth in Normal $C_3H$/He Mice

| | Dose (μg/mouse) | 0d | | 3d | | 6d | | 8d | | 10d | | 13d | | 15d | | 17d | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | L | G.A. | L | G.A. | L | G.A. | L | G.A. | L | G.A. | L | G.A. | L | G.A. | L | G.A. |
| TPA | 12.3 | 54.6 (1.3) | 1.3 | 57.0 (1.3) | −1.8 | 55.8 (1.1) | 0 | 51.4 (1.5) | 3.4 | 46.7 (1.8)[a)] | 9.0 | 36.4 (1.2)[a)] | 15.2 | 27.1 (1.4)[a)] | 20.8 | 25.2 (0.7) | 3.8 |
| RA | 4.0 | 54.8 (1.3) | 0.9 | 56.7 (0.7) | −1.3 | 55.2 (1.4) | −1.1 | 51.8 (1.6) | 2.6 | 47.6 (2.6) | 7.2 | 36.3 (6.3) | 15.4 | 26.4 (5.0) | 22.8 | 19.1 (1.3)[b)] | 27.1 |
| EtOH | | 55.3 (0.3) | 0 | 56.0 (1.1) | 0 | 55.8 (0.4) | 0 | 53.2 (2.0) | 0 | 51.3 (2.0) | 0 | 42.9 (4.1) | 0 | 34.2 (4.8) | 0 | 26.2 (1.6) | 0 |

L value indicates luminance and each value is the mean (±S.D.). The G.A. value was calculated according to Eq. 1. *a*) and *b*) indicate significant differences from the control (EtOH), $p<0.05$ and $p<0.01$, respectively.

TABLE II. Effect of Herb Extracts and Catechol on Hair Regrowth in Normal $C_3H$/He Mice[a)]

| Herb | Family | Yield (%) | n | 10d G.A. | 13d G.A. |
|---|---|---|---|---|---|
| *Crataegus cuneata* S. *et* Z. | Rosaceae | 10.5 | 4 | 7.9[c)] | 19.7[c)] |
| *Ligustrum lucidum* A. | Oleaceae | 28.0 | 4 | 7.0[c)] | 15.7[c)] |
| *Polyporus umbellatus* F. | Polyporaceae | 1.0 | 4 | 8.3[b)] | 14.3[b)] |
| *Brassica hirta* M. | Cruciferae | 27.0 | 5 | 2.7 | 13.2[b)] |
| *Vitex rotundifolia* L. fil. | Vervenaceae | 10.5 | 5 | 2.1 | 13.2 |
| *Pachyma hoelen* R. | Polyporaceae | 2.0 | 4 | 3.8 | 11.6 |
| *Bletilla striata* (T.) R. fil. | Orchidaceae | 5.5 | 4 | 4.5[b)] | 10.9[b)] |
| *Polygala senega* L. var. *latifolia* T. *et* G. | Polygalaceae | 24.8 | 4 | 2.9 | 9.0 |
| *Magnolia kobus* DC. | Magnoliaceae | 13.8 | 5 | −0.2 | 7.6 |
| *Dictamnus albus* L. subsp. *dasycarpus* (T.) K. | Rutaceae | 4.0 | 4 | 2.3 | 7.0 |
| *Valeriana officinalis* L. var. *latifolia* M. | Valerianaceae | 11.5 | 4 | 2.8 | 6.6 |
| *Phyllostachys nigra* M. var. *henosis* S. | Bambusaceae | 16.0 | 4 | 5.4 | 6.0 |
| *Ampelopsis japonica* M. | Vitaceae | 3.5 | 4 | 2.9 | 6.0 |
| *Daphne genkwa* S. *et* Z. | Thymelaeaceae | 8.5 | 4 | 2.8 | 6.0 |
| *Artemisia gigantea* K. | Carduaceae | 10.0 | 3 | 2.1 | 5.9 |
| *Glehnia littoralis* F. S. | Glehnia | 6.5 | 5 | 3.5 | 5.6 |
| *Leonurus heterophyllus* S. | Labiatae | 2.0 | 4 | 2.0 | 5.3 |
| *Benincasa cerifera* S. | Cucurbitaceae | 8.5 | 4 | −0.2 | 5.3 |
| *Pulsatilla cernua* (T.) S. | Ranunculaceae | 11.0 | 4 | 1.7 | 4.8 |
| *Asparagus cochinchinensis* M. | Liliaceae | 15.0 | 4 | 2.8 | 4.6 |
| *Laminaria japonica* A. | Laminariaceae | 12.5 | 4 | 3.3 | 4.2 |
| *Akebia quinata* (T.) D. | Lardizabalaceae | 5.0 | 3 | 0.9 | 4.1 |
| *Aquillaria sinensis* (L.) G. | Thymelaeaceae | 34.5 | 4 | 2.3 | 3.9 |
| *Plantago major* L. var. *asiatica* D. | Plantaginaceae | 9.0 | 4 | 0.7 | 2.9 |
| *Nardostachys jatamansi* DC. | Valeriaceae | 21.5 | 4 | −0.5 | 2.9 |
| *Dianthus superbus* L. | Caryophyllaceae | 10.0 | 4 | −1.3 | 2.4 |
| *Paeonia lactiflora* P. | Ranunculaceae | 36.5 | 3 | 1.1 | 2.4 |
| *Curcuma zedoaria* R. | Zingiberaceae | 4.5 | 4 | −0.6 | 2.1 |
| *Dioscorea tokoro* M. | Dioscoreaceae | 6.0 | 4 | 1.4 | 1.7 |
| *Cannabis sativa* L. | Cannabinaceae | 11.0 | 4 | −0.2 | 1.6 |
| *Coix lachryma-Jobi* L. | Gramineae | 7.5 | 4 | −1.6 | 1.5 |
| *Eclipta prostrata* L. | Carduaceae | 4.5 | 4 | 2.0 | 1.5 |
| *Ligusticum acutilobum* S. *et* Z. | Umbelliferae | 22.9 | 5 | −2.7 | 1.2 |
| *Angelica dahurica var. pai-chi* K. H. *et* Y. | Apiaceae | 21.5 | 3 | 0.4 | 1.1 |
| *Polygonum aviculare* L. | Polygonaceae | 8.5 | 4 | 1.6 | 1.0 |
| *Gardenia jasminoides* E. var. *grandiflora* N. | Rubiaceae | 21.0 | 4 | 0.7 | 0.7 |
| *Citrus aurantium* L. | Rutaceae | 28.6 | 5 | −1.3 | 0.6 |
| *Cassia angustifolia* V. | Leguminosae | 18.0 | 4 | −3.1 | 0.5 |
| *Kochia scoparia* (L.) S. | Chenopodiaceae | 6.0 | 4 | −1.1 | 0.2 |
| *Phytolacca esculenta* van H. | Phytolaccaceae | 9.5 | 4 | −1.1 | 0 |
| *Juncus decipiens* N. | Juncaceae | 7.5 | 4 | −0.2 | 0 |
| *Loranthus parasiticus* (L.) M. | Loranthaceae | 11.0 | 4 | 1.6 | 0 |
| *Ligusticum sinense* O. | Apiaceae | 16.0 | 4 | 1.0 | −0.2 |
| *Prunus ansu* K. | Rosaceae | 29.5 | 4 | −1.5 | −0.3 |
| *Sanguisorba officinalis* L. var. *carnea* R. | Rosaceae | 24.5 | 4 | 0 | −0.4 |
| *Pharbitis nil* (L.) C. | Convolvulaceae | 21.0 | 4 | 0.2 | −0.7 |
| *Asiasarum sieboldii* (M.) F. M. | Asaraceae | 11.7 | 5 | 0.2 | −0.9 |
| *Punnica granatum* L. | Punicaceae | 32.0 | 4 | −3.8[b)] | −1.0 |
| *Prunus japonica* T. | Rosaceae | 33.5 | 4 | 0.2 | −1.2 |
| *Rosa polyantha* S. *et* Z. var. *genuina* N. | Rosaceae | 21.0 | 4 | −3.3 | −1.4 |
| *Zea mays* L. | Gramineae | 3.0 | 4 | −1.1 | −1.5 |
| *Cocculus trilobus* A. P. DC. | Stephania | 3.0 | 3 | −0.4 | −1.6 |
| *Alisma orientale* J. | Alismataceae | 13.0 | 3 | 0 | −1.7 |
| *Selinum monnieri* L. | Apiaceae | 9.0 | 4 | −0.2 | −2.2 |
| *Pinus densiflora* S. *et* Z. | Pinaceae | 25.5 | 4 | 1.1 | −2.4 |
| *Terminalia chebula* R. | Combretaceae | 24.0 | 4 | −1.9 | −2.9 |
| *Juglans regia* L. var. *sinensis* DC. | Juglandaceae | 9.5 | 4 | −2.4 | −3.6 |
| *Curcuma longa* L. | Zingiberaceae | 15.0 | 4 | 0.2 | −3.7 |
| *Lepidium apetalum* W. | Cruciferae | 12.0 | 4 | −2.0 | −3.9 |
| *Sophora anguistifolia* S. *et* Z. | Leguminosae | 13.5 | 4 | −1.9 | −3.9 |
| *Dipsacus asper* W. | Dipsacaceae | 27.5 | 4 | −1.7 | −4.2 |
| *Coptis japonica* M. | Ranunculaceae | 13.0 | 4 | −0.7 | −4.6 |
| *Cassia tora* L. | Cassiaceae | 12.7 | 5 | −3.1 | −4.6 |
| *Scutellaria baicalensis* G. | Labiatae | 14.0 | 4 | −0.5 | −4.8 |
| *Cnidium officinale* M. | Umbelliferae | 14.0 | 4 | −1.1 | −5.0 |
| *Psoralea corylifolia* L. | Leguminosae | 27.5 | 4 | −1.9 | −5.5 |
| *Rhus javanica* L. | Anacardiaceae | 75.0 | 4 | −0.6 | −5.8 |
| *Broussonetia papyrifera* (L.) V. | Moraceae | 14.0 | 4 | −1.6 | −7.3 |
| *Aconitum japonicum* T. var. *genuinum* N. | Ranunculaceae | 8.5 | 4 | −3.1 | −7.7 |
| *Rehmannia glutinosa* L. | Scrophulariaceae | 52.5 | 4 | −2.8 | −8.7 |
| *Crocus sativus* L. | Iridaceae | 56.0 | 3 | −2.7 | −10.4 |
| *Agastache rugosa* O. K. | Lamiaceae | 11.0 | 4 | −3.4[b)] | −11.6[b)] |
| *Biota orientalis* E. | Cupressaceae | 14.0 | 4 | −6.0 | −11.9 |
| *Boswellia carterii* B. | Burseraceae | 53.5 | 4 | −5.4 | −12.7[b)] |
| *Cimicifuga foetida* L. | Ranunculaceae | 8.0 | 4 | −3.9 | −13.2 |
| *Santalum album* L. | Santalaceae | 3.5 | 4 | −4.3 | −13.3 |
| *Chrysanthemum morifolium* R. | Compositae | 17.5 | 4 | −3.8 | −14.8 |
| *Rheum palmatum* L. var. *tanguticum* M. | Polygonaceae | 23.0 | 4 | −4.0 | −15.0[b)] |
| *Zingiber officinale* R. | Zingiberaceae | 9.5 | 4 | −4.6[c)] | −16.9[c)] |
| *Abutilon avicennae* G. | Malvaceae | 13.5 | 4 | −9.0[b)] | −17.2 |
| Catechol | | | | | 27.2[c)] |

The G.A. value was calculated according to Eq. 1. *a*) The doses of herb extract and catechol were 2 mg/mouse and 1.1 mg/mouse, respectively. *b*) and *c*) indicate significant differences from the control, $p<0.05$ and $p<0.01$, respectively.

*Crataegus cuneata* S. *et* Z., *Ligustrum lucidum* A., *Polyporus umbellatus* F. and *Bletilla striata* (T.) R. fil., significantly promoted hair regrowth. On the 13th day, 18 kinds of herb extracts showed apparent hair regrowth promotion (G.A. $>5.0$) visually. In particular, the extracts of the 4 herbs mentioned above and *Brassica hirta* M. showed significant activities almost equal to those of the positive controls, TPA and RA. There have been previous reports on 4 herbs, *Magnolia salicifolia* M., *Vitex rotundifolia* L. fil., *Brassica hirta* M. and *Dictamnus albus* L. subsp. *dasycarpus* (T.) K., of the 18 which stimulated hair growth.[3a,b)] There is no apparent relation between their families and activities.

On the other hand, clear inhibition of hair regrowth was observed with 16 kinds of herb extracts (G.A. $<-5.0$), among which the extracts of *Abutilon avicennae* G., *Zingiber officinale* R., *Rheum palmatum* L. var. *tanguticum* M., *Boswellia carterii* B. and *Agastache rugosa* O. K., showed significant effects. It has been reported that *Zingiber officinale* R. inhibited hair growth in mice.[3a)] The inhibitions by *Rheum palmatum* L. var. *tanguticum* M. and *Boswellia carterii* B. may be derived from their anti-inflammatory effects, as betamethasone valerate completely inhibits hair regrowth in $C_3H$/He mice.[5a)]

TABLE III. Materials with Potent Hair Regrowth-Promoting Activity in Normal Mice

| Name | Part |
|---|---|
| *Crataegus cuneata* S. *et* Z. (Sanzashi) | Fruit |
| *Ligustrum lucidum* A. (Joteishi ) | Fruit |
| *Polyporus umbellatus* F. (Chyorei) | Fungal body |
| *Brassica hirta* M. (Hakugaishi) | Seed |
| *Vitex rotundifolia* L. fil. (Mankeishi) | Fruit |
| *Pachyma hoelen* R. (Bukuryou) | Fungal body |
| *Bletilla striata* (T.) R. fil. (Byakkyuu) | Tuber |

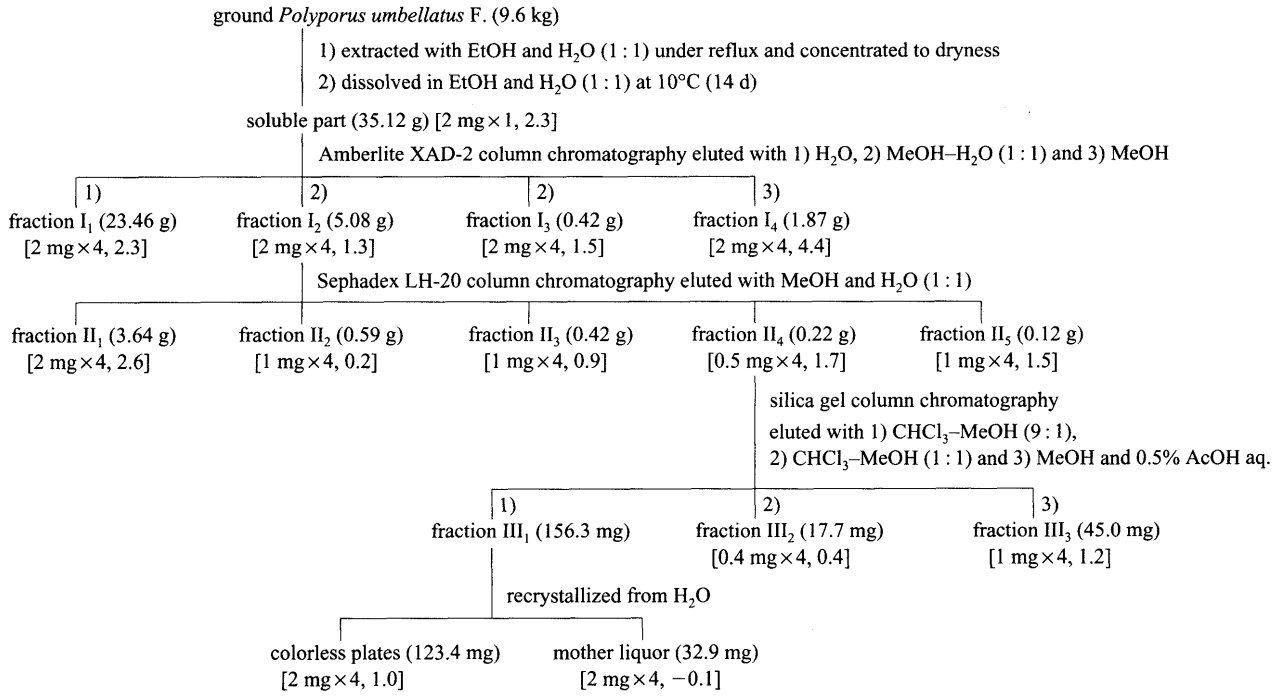


Chart 1. Isolation of Active Constituents from *Polyporus umbellatus* F.
( ) indicates yield. [ , ] indicates daily application dose (/mouse) and time (d), and the average hair growth score.

TABLE IV. Relationship between G.A. and Scoring of Hair Regrowth of Mice after Removal of Normal Hair

| G.A. | ——5.0 | −4.9——1.0 | −0.9—3.0 | 3.1—10.0 | 10.1—16.0 | 16.1—21.0 | 21.1—25.0 | 25.1—29.0 | 29.1— |
|---|---|---|---|---|---|---|---|---|---|
| Score | −2 | −1 | 0 | 1 | 2 | 3 | 4 | 5 | 6 |

The G.A. value was calculated according to Eq. 1.

As shown in Table III, about half of the 7 highly potent herbs with G.A. values over 10.0 were fruits or seed, which contain relatively high contents of fatty acids. It is known that lansic acid and pentadecanoic acid triglyceride promote hair growth.[4,8] Speculating that lipophilic acids in those herbs might be active components, we have studied the active principle in *Vitex rotundifolia* L. fil. and found that a mixture of fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid and eicosanoic acid) showed the hair regrowth activity.[9] Studies on the other fruits or seed are in progress.

We were particularly interested in *Polyporus umbellatus* F. and *Pachyma hoelen* R. among the 7 active herbs, as the contents of fatty acids in the fungal bodies are very low, and there has been no previous report on their hair regrowth-promoting activities. We have therefore studied the active components in *Polyporus umbellatus* F.

Bioassay-guided fractionation of *Polyporus umbellatus* F., as shown in Chart 1, afforded 3,4-dihydroxybenzaldehyde as an active compound after 3 steps of column chromatography on Amberlite XAD-2, Sephadex LH-20 and silica gel. The activity during purification is presented in Chart 1 as the average scores for simplicity, according to the hair regrowth scoring system shown in Table IV.

As shown in the chart, ground fungal body of *Polyporus umbellatus* F. was extracted with ethanol and water (1 : 1) under reflux. The extract, which showed potent activity, was dissolved in the same solvent at 10 °C, and subjected to Amberlite XAD-2 column chromatography using methanol and water as an eluent. The activity emerged in all fractions. In this paper, we will deal with fraction $I_2$. Column chromatography of active fraction $I_2$ on Sephadex LH-20 gave five fractions, $II_1$ to $II_5$, using MeOH–$H_2O$ as an eluate. The most potent activity was found in fraction $II_4$. The final purification of the active compound in fraction $II_4$ was achieved by silica gel column chromatography. Recrystallization of the product $III_1$ eluted with $CHCl_3$–MeOH (9 : 1) from $H_2O$ afforded the compound as colorless plates, which showed activity. Work on the constituents of the other active fractions is in progress.

The spectral (IR, UV, NMR and MS) data of the isolated active principle suggested it to be 3,4-dihydroxybenzaldehyde. This conclusion was confirmed by direct comparison of the spectral data with those of authentic 3,4-dihydroxybenzaldehyde obtained from Wako Co. The activity of the authentic sample was equal to that of the isolated compound, as shown in Table V.

3,4-Dihydroxybenzaldehyde is known to be a component of *Pinellia ternata*.[10] It has anti-arthritic and anti-inflammatory activities,[11] and inhibits cancer cell proliferation,[12] but this is the first report that the compound also promotes hair regrowth. It is of interest to consider the relationship between the chemical structure and biological activity of 3,4-dihydroxybenzaldehyde and catechol. 3,4-Dihydroxybenzaldehyde is fairly stable in

TABLE V. Effect of 3,4-Dihydroxybenzaldehyde on Hair Regrowth in Normal $C_3H$/He Mice

| Dose (mg)[a] | 0.5 | 1.0 | 2.0 |
|---|---|---|---|
| G.A. | 0.5 | 0.7 | 5.4 |

*a*) It indicates daily application dose (/mouse) 4 times (d). The G.A. value was calculated according to Eq. 1 ($n=7$ or 8).

aqueous solution and has anti-inflammatory activity, but catechol is very unstable and causes irritation,[13] although it has potent hair regrowth activity, as shown in Table II. The fact that both compounds promote hair regrowth, suggests that the catechol moiety is the key structure for inducing the cell proliferation and differentiation required for hair regrowth.[12,14]

In conclusion, it was found that Ogawa's method using the back hair of $C_3H$/He mice is effective for evaluating pharmacological activity of test materials to promote hair regrowth, and several herb extracts were found to promote hair regrowth significantly. In view of the stability and anti-inflammatory activity of 3,4-dihydroxybenzaldehyde, an active ingredient isolated from *Polyporus umbellatus* F., it appears to be a good candidate for use in hair growth preparations. Studies on the mechanism of hair growth promotion by 3,4-dihydroxybenzaldehyde are under way.

## Experimental

**Hair Regrowth-Promoting Activity of 80 Herb Extracts** Materials: Commercial herb products were used in this study. TPA and RA were purchased from Wako Co.

Preparation of Test Samples: Samples of pulverized herbs (20 g) were extracted with MeOH (200 ml) under reflux for 2 h. Each extract was evaporated *in vacuo*, and 2 mg of the residue was dissolved in 20 $\mu$l of EtOH for use as a test sample. The yields (%) of the extracts are shown in Table I. TPA and RA were dissolved in EtOH at concentrations of 1.0 mM and 0.02% (w/v), respectively. RA was used together with vitamin E (1.0% (w/v)) acording to Ogawa and Hattori.[5a] EtOH was used as the control.

Measurement of Hair Regrowth Activity in Mice: The colorimeter used for the measurement of luminance was a Minolta Chroma meter CR-100 (Minolta Camera Co.) or a DS-506 (Keihin Densoku Co.).

Four 9-week-old male $C_3H$/He mice (age at the initial application of test material) were used per group in general. The hair on the back was clipped off with hair clippers (*ca.* $3 \times 4\,cm^2$). The clipped area was treated with softening cream (Jully, Kowa Co. or Simfree for etiquette, Pola Co.) for 5 or 10 min then the cream was washed off with warm water. After 24 h, the test sample was applied topically once. The luminance (L) of the depilated area was measured on various days (shown in Tables I and II) after application with a colorimeter and the G.A. was calculated by use of the averaged luminance (L) value of each group, according to the following equation. Statistical significance of differences between groups was assessed by using Student's $t$ test.

$$\text{G.A. (\%)} = \left(1 - \frac{\text{mean luminance of administered group}}{\text{mean luminance of control group}}\right) \times 100 \quad (1)$$

**Isolation of Active Principle from *Polyporus umbellatus* F.** Instruments Used Were as Follows: mass spectrum (MS), JEOL DX-300 spectrometer; infrared (IR) spectrum, JASCO IR-700 spectrophotometer; nuclear magnetic resonance (NMR) spectra, JEOL GSX-270 and JEOL GSX-500 Fourier-transform spectrometer; UV spectrum, Hitachi model 200-20 spectrophotometer.

Measurement of Hair Regrowth Activity of Each Fraction in Mice: The method was as described above, with 8 or 9 animals per group. Test material was dissolved in 30 $\mu$l of EtOH–$H_2O$ (1 : 1), and applied once a day for 4 d. EtOH–$H_2O$ (1 : 1) was used as the control. The effect of the material on hair regrowth was scored (see Table IV) on the 13th day after the first application.

Extraction: Ground *Polyporus umbellatus* F. (9.6 kg) was extracted with EtOH–$H_2O$ (1 : 1, 96 l) under reflux for 3 h. The solution was filtered, and the filtrate was evaporated *in vacuo* to give the crude extract (76.8 g). The extract was exposed to EtOH–$H_2O$ (1 : 1, 920 ml) at 10 °C for 14 d. The soluble part was concentrated in an evaporator to afford the extract (35.12 g).

Amberlite XAD-2 Column Chromatography of the Extract: The extract (35.12 g) was dissolved in $H_2O$ (1 l), and applied to an Amberlite XAD-2 column ($5 \times 60$ cm). Elution with water (2.5 l), MeOH–$H_2O$ (1 : 1, 6 and 9 l) and MeOH (5 l) afforded fractions $I_1$ (23.46 g) (water effluent), $I_2$ (5.08 g) (MeOH–$H_2O$), $I_3$ (0.42 g, MeOH–$H_2O$) and $I_4$ (1.87 g, MeOH).

Chromatography of Fraction $I_2$ on Sephadex LH-20: Fraction $I_2$ (5.08 g) was subjected to gel filtration on a Sephadex LH-20 column (980 ml, $4.5 \times 62$ cm). Elution with MeOH–$H_2O$ (1 : 1) gave Fraction $II_1$ (3.64 g), $II_2$ (0.59 g), $II_3$ (0.42 g), $II_4$ (0.22 g) and $II_5$ (0.12 g) ($K_d$ value = 0 to 0.65, 0.65 to 1.14, 1.14 to 2.24, 2.24 to 2.89 and 2.89 to 5.00, respectively).

Silica Gel Column Chromatography of $II_4$: Fraction $II_4$ (0.22 g) was subjected to column chromatography on a silica gel column ($1.5 \times 33$ cm). Elution with $CHCl_3$–MeOH (9 : 1, 100 ml), $CHCl_3$–MeOH (1 : 1, 100 ml), MeOH (100 ml) and AcOH–$H_2O$ (0.5 : 99.5, 100 ml) afforded $III_1$ (156.3 mg) ($CHCl_3$–MeOH (9 : 1)), $III_2$ (17.7 mg) ($CHCl_3$–MeOH (1 : 1)) and $III_3$ (45.0 mg) (MeOH and AcOH–$H_2O$). Recrystallization of $III_1$ from $H_2O$ gave colorless plates (123.4 mg) of the active compound.

Identification of the Active Compound: The active compound (dec. 152—153 °C (dec. 153—154 °C))[15] was identified as 3,4-dihydroxybenzaldehyde by direct comparison of its physical data (IR, UV, MS and NMR) with those of an authentic sample.

## References and Notes


1) *a*) H. Horikawa, S. Shimada, M. Fukushima, *J. Soc. Cosmet. Chem. Jpn.*, **19**, 99 (1983); *b*) Some of the results were reported in *Journal of Medical and Pharmaceutical Society for WAKAN-YAKU*, **6**, 362 (1989); Y. Inaoka, H. Kuroda, M. Nishino, M. Fukushima, S. Zheng.
2) Li Wen Liag, "Qian Jia Miao Fang," the last volume, Bei Jing Publications, Beijing, 1985, p. 155; Jin Li Weng, "Yi Fang Lei Ju," Vol. 4, Ren Ming Wei Sheng Publications, Beijing, 1981, p. 566; Wu Quian, "Yi Zong Jin Jian," Vol. 4, Ren Ming Wei Sheng Publications, Beijing, 1973, p. 108; Zhao Bing Nan and Zhang Zhi Li, "Jian Min Zhong Yi Pi Fu Bing Xue," Zhong Guo Zan Wang Pablications, Beijing, 1983, p. 241.
3) *a*) S. Tanaka, M. Saito, M. Tabata, *Planta Medica*, Suppl., **1980**, 84; *b*) K. Kaname, *Fragrance J.*, **57**, 71 (1985); *c*) M. Kubo, H. Matsuda, M. Fukui, Y. Nakai, *Yakugaku Zasshi*, **108**, 971 (1988); *d*) K. Toda, M. Ohta, Y. Ishibasi, T. Nokita, M. Ohkido, Y. Suzuki, S. Nishiwaki, F. Yamaguchi, T. Takemura, H. Nagai, N. Takada, K. Ohkuma, S. Harada, A. Igarashi, H. Nakanishi, T. Tomisawa, H. Itoh, T. Nagashima, Y. Hori, K. Nakayama, S. Ritani, H. Urabe, K. Minami, *Yakuri To Chiryou*, **16**, 4721 (1988).
4) Y. Kobayashi, Y. Nogawa, H. Nishimura, K. Seimoto, *Yakuri To Chiryou*, **17**, 4223 (1989); K. Habaguchi, M. Watanabe, Y. Nakadaira, K. Nakanishi, A. K. Kiang, F. Y. Lim, *Tetrahedron Lett.*, **34**, 3731 (1968).
5) *a*) H. Ogawa, M. Hattori, *Current Problems in Dermatology*, **11**, 159 (1983); *b*) M. Kamimura, N. Sasaki, *J. Vitaminology*, **11**, 1 (1965); *c*) N. Okuyama, *Nippon Seirigaku Zasshi*, **19**, 292 (1957).
6) G. S. Bazzano, N. Terezakis, W. Galen, *J. Am. Acad. Dermatology*, **15**, 880 (1986).
7) S. Inohara, H. Tateishi, Y. Takeda, Y. Tanaka, S. Sagami, *Arch. Dermatol. Res.*, **280**, 182 (1988).
8) K. Ohba, *Fragrance J.*, **80**, 109 (1986); K. Nagashima, *Yakuri To Chiryou*, **17**, 2363 (1989).
9) Y. Inaoka, H. Kuroda, M. Fukushima, in preparation.
10) T. Hasegawa, *Proc. Jpn. Acad.*, **35**, 246 (1956).
11) K. Watanabe, F. Kimura, M. Shinmei, *J. Pharmacobio-Dyn.*, **15**, 239 (1992).
12) A. J. Lin, J. S. Driscoll, *J. Pharm. Sci.*, **70**, 806 (1981); M. M. Wick, G. B. FitzGerald, *ibid.*, **76**, 513 (1987).
13) B. L. Van Duuren, "Chemical Carcinogens," ed. by Searle, C. E., Am. Chem. Soc., Washington, D. C., 1976, p. 24.
14) M. Shibata, M. Hirose, M. Yamada, M. Takematsu, S. Uwagawa, N. Ito, *Carcinogenesis*, **11**, 997 (1990).
15) M. Windholz, "The 11th Merck Index," Merck & Co., Inc., Rahway, N.Y., 1989, p. 1254.