NOTE**Isolation and Synthesis of Cosmetic Substances from African Dietary Leaves, *Celosia argentea* L. for Skin Depigmentation**

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Abstract: Six compounds were isolated from the leaves of *Celosia argentea* L.. Tyrosinase inhibitory and superoxide scavenging activity of the compounds was examined so as to obtain a skin depigmentation agent for cosmetic application. Eugenyl *O*- β -D-glucopyranoside (citrusin C, **1**) showed strong tyrosinase inhibitory activity. Two-step inhibitory effects of successive tyrosine and DOPA oxidation for tyrosinase were stronger compared to arbutin used commercially at present (47.20% inhibition of tyrosine oxidation and 87.93%, DOPA oxidation, compared to 63.00% for tyrosine and 7.70% for DOPA oxidation in the case of arbutin). Moreover, we succeed in providing compound **1** abundantly in the synthetic study which used acetobromo- α -D-glucose and Sn(OTf)₂.

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Key words: citrusin C, eugenyl *O*- β -D-glucopyranoside, skin depigmentation effect, tyrosinase inhibitory activity, superoxide scavenging activity

1 Introduction

Sierra Leone is situated in Middle West Africa and its people enjoy longevity. Assuming foods may be related to the longevity, we investigated the constituents in the leaves of *Celosia argentea* L., used for food preparation. *Celosia argentea* L. grows in the torrid zone of Africa, and is also found in warm places of west Japan. Some terpenoids and saponins in roots and flavonoids in leaves and stems may possibly be active constituents in *Celosia argentea* L.. Shah *et al.* (1) reported alcohol extracts of the leaves of *Celosia argen-*

tea L. to have an antibacterial activity and the seeds, diuretic activity in albino rats and human volunteers.

In a bioassay with germinating seeds, Pandya (2) found aqueous extracts of fresh leaves, stem, and roots of *Celosia argentea* L. to inhibit growth of shoots and roots of bajra (*Pennisetum americanum*). Ashraf and Sen (3) presented evidence for the inhibitory effects of aqueous extracts of *Celosia argentea* L. on bajra and similar inhibitory effects on til (*Sesamun indicum*).

We isolated five compounds from the leaves of *Celosia argentea* L. and investigated their effects on the germination of lettuce seeds (4). We investigated skin depigmentation effects of these compounds for cosmetic use (tyrosinase inhibitory activity and superoxide scavenging activity).

2 Experimental

2.1 Materials

Commercially available dry powders of the leaves of African *Celosia argentea* L. were used.

2.2 Separation of Crude Functional Molecules

The dry powder of leaves of *Celosia argentea* L. (789.9 g) were extracted with 70% EtOH-H₂O (3 L) in the dark for 1 day. A 70% EtOH-H₂O extract (100.8 g) was concentrated to 700 mL *in vacuo*, and extracted with hexane and 1-BuOH. Saturated aqueous solution of lead subacetate was added to the 1-BuOH extract and the precipitates were removed by filtration. The filtrate was adjusted to pH 9.0 and extracted with 1-BuOH to obtain a mixture of crude glycoside (neutral components, 3.75 g) from 1-BuOH solution.

2.3 Gel Filtration

Gel filtration was carried out in a prepacked column (5 cm × 1 m) of HW-40F TSK gel (Tosoh Co., Ltd.) under medium pressure and absorption of the eluates was measured at 280 nm with an UVICON 750 (Advantec Toyo Co., Ltd.). Neutral components were eluted with 20 L distilled H₂O and then 10 L 50% MeOH-H₂O. The eluate was collected in every min and each fraction was concentrated *in vacuo* and freeze-dried.

2.4 Column Chromatography on Silica Gel

Each fraction obtained by gel filtration was chromatographed on silica gel (C-300 Wako gel, Wako Pure Chemical Industries Ltd.) with CHCl₃-MeOH (5:1 or 8:1) as the eluent.

2.5 Analysis

Melting point (mp) was corrected on Yanagimoto micro melting point apparatus. Optical rotation was measured with a JASCO DIP-1000. UV and IR spectra were recorded on Shimadzu UV-160A spectrometer and a Perkin-Elmer 1760-X infrared fourier transform spectrometer, respectively. MS spectra were obtained with JEOL JMS-HX 100 and JMA-DA 5000 spectrometers under Xe bombardment (6.0 keV). NMR spectra were measured with a JEOL EX-270 instrument in CD₃OD with TMS as an internal standard (270 MHz for ¹H and 67.5 MHz for ¹³C).

2.6 Bioassay

2.6.1 Inhibition of tyrosinase activity [5, 6]

Tyrosinase activity was determined by the dopachrome method with L-tyrosine or L-DOPA as substrate. (a) With L-tyrosine substrate: A mixture (3 mL) of 0.55 mM L-tyrosine, a 10 mM phosphate buffer (pH 6.8), 30 units mushroom tyrosinase [Sigma Chemical Co. (EC 1.14.18.1)] and sample (a 15 mM Me₂SO solution; 0.1 mL) was incubated at 37°C for 10 min. The absorbance of the mixture was measured at 475 nm. A control reaction was carried out without test sample. Percentage inhibition of tyrosinase was calculated as,

$$\text{Tyrosinase inhibition (\%)} = \{[T_2 - (T_1 - T_3)] / T_2\} \times 100$$

Where T_1 and T_2 are absorbance of the test solution and control, respectively, and T_3 is sample absorbance.

(b) With L-DOPA substrate: A mixture (3 mL) of 0.85 mM L-DOPA, 19mM phosphate buffer (pH 6.8), 30 units mushroom tyrosinase and sample (a 15 mM Me₂SO solution; 0.1 mL) was incubated at 25°C for 1.75 - 2.75 min. The control reaction was carried out without test sample. Absorbance of the mixture was measured at 475 nm before and after incubation. Percentage inhibition of tyrosinase was calculated as,

$$\text{Tyrosinase inhibition (\%)} = [(T_1 - T_2) / T_1] \times 100$$

Where T_1 is difference in absorbance of the control before and after incubation and T_2 is difference in absorbance before and after incubation.

2.6.2 Superoxide scavenging activity [7, 8]

Superoxide scavenging activity was determined by NBT reduction with a Wako SOD test kit (Wako Pure Chemical Ind. Ltd.). A mixture (2.1 mL) of sample (a 15 mM Me₂SO solution; 0.1 mL), nitroblue tetrazonium (NO₂-TB) solution, xanthin solution, xanthinoxidase and superoxide dismutase was incubated at 37°C for 20 min. Sodium dodecyl sulfate was then added to the mixture and absorbance was measured at 560 nm.

2.7 Enzymatic Hydrolysis [9]

Sample (5 mg) solution in a Na₂HPO₄-citric acid buffer (pH 4.0, 2 mL) was treated with a β -glucosidase (Wako Pure Chemical Industries Ltd.; 3 mg) and stirred for 3 days at 37°C. The reaction mixture was extracted with Et₂O, and the extract was analyzed by EI MS and GLC [Shimadzu GC-14B gaschromatograph equipped with FID; column, Carbowax 20M, 0.25 mm × 20 m, temp., 80-180°C (2°C/min); carrier gas, N₂, 1.0

2.8 Synthesis

3 Results and Discussion

1 **2** **3** : R=Glc⁶-Rham
4 : R=Glc
5 **6**

Compounds **2**, **6**, and **1a** possessed no tyrosinase inhibitory activity, while for compound **1**, good inhibition of 47.20% of tyrosine and 87.93% of DOPA oxidation were noted, as shown in **Table 1**. This compound inhibited tyrosinase activity more than arbutin (63.00% of tyrosine oxidation and 7.70% of DOPA oxidation). Compound **1** showed two-step inhibition of tyrosine and DOPA oxidation, and inhibited tyrosinase activity with phased efficiency. Compound **1** is thus shown to be a

Compound	Tyrosinase (%)		S. S ^{b)} (%)
	Tyrosine	DOPA	
1	47.20	87.93	4.35
2	3.29	— 18.83	27.52
6	36.03	24.62	45.85
1a ^{a)}	34.31	15.75	43.48
Arbutin	63.00	7.70	0.80

b) Scavenging activity of superoxide.

highly effective inhibitor of tyrosinase activity, and thus should serve as a good skin depigmentation agent for cosmetic use. Moreover, we succeed in providing compound **1** abundantly in the synthetic study which used acetobromo- α -D-glucose and $\text{Sn}(\text{OTf})_2$.

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