

Bioassay studies of 2'-O-ethylmurrangatin isolated from a medicinal plant, *Murraya paniculata*

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Abstract: A secondary metabolite, 2'-O-ethylmurrangatin (1), was isolated from the leaves of a medicinal plant, *Murraya paniculata*. The structure of compound 1 was identified with the help of spectroscopic techniques. Compound 1 was screened for its antioxidant, lipoxigenase, prolyl endopeptidase, and respiratory burst inhibitory activities. 2'-O-Ethylmurrangatin (1) was found to have significant activity against lipoxigenase enzyme and moderate respiratory burst inhibitory activity, but no activity against either DPPH radical scavenging assay or prolyl endopeptidase enzyme.

Key words: *Murraya paniculata*, Rutaceae, 2'-O-ethylmurrangatin, antioxidant, DPPH radical scavenging assay, lipoxigenase inhibition assay, prolyl endopeptidase inhibition assay, respiratory burst inhibitory assay

Introduction

Murraya paniculata (L.) Jack or Orange Jasmine belongs to the family Rutaceae, and occurs widely in India, southeast Asia, southern China, and the Malay Peninsula. The leaves are used for the treatment of diarrhea and dysentery. Leaves and root barks are also used against rheumatism, cough, and hysteria. The leaves possess antibiotic activity against *Micrococcus pyogenes* var. *aureus* and *Escherichia coli* (1,2). Previous phytochemical studies on this plant have resulted in the isolation of a new natural product, 2'-O-ethylmurrangatin (1) along with 2 known coumarins, and their anti-acetylcholinesterase and anti-butyrylcholinesterase activities were also studied (3). We now report here the antioxidant, lipoxigenase, prolyl endopeptidase, and respiratory

burst inhibitory activities of 2'-O-ethylmurrangatin (1).

Antioxidants can inhibit the oxidation of oxidizable materials in living cells. Free radicals are generated as a result of oxidation (4), and may be involved in the pathogenesis of various diseases such as cancers. They may also cause many other diseases like heart diseases, diabetes, arteriosclerosis, and arthritis (5). Antioxidants retard such processes as premature aging (6), cancers (7), and heart diseases (8) by inhibition of certain enzymes, quenching free radicals, and may result in reactive oxygen species (ROS) (4) during their catalytic reactions.

Lipoxigenases constitute a family of non-haem iron containing dioxygenases that are widely found in animals and plants. In mammalian cells,

these are the key enzymes in the biosynthesis of a variety of bioregulatory compounds such as hydroxyeicosatetraenoic acids (HETEs), leukotrienes, lipoxins, and hepoxylines (9). Lipoxygenase products play an important role in a variety of disorders such as bronchial asthma, inflammation (10), autoimmune diseases, and tumor angiogenesis (11). Lipoxygenases are therefore potential targets for the rational drug design and discovery of mechanism-based inhibitors for the treatment of these diseases.

Prolyl endopeptidase (PEP) or post-proline cleaving enzyme (EC. 3.4.21.26) catalyzes the cleavage of the peptide bonds at the carbonyl side of proline residue (12,13). This enzyme is widely distributed in various organs, particularly in the human brain. The abnormal PEP levels may be related to neuropathological disorders, such as major depression, mania, schizophrenia, and senile dementia of Alzheimer's type. PEP also plays a key role in the regulation of blood pressure by participating in the rennin-angiotensin system through metabolism of bradykinin and angiotensins II and I. Thus specific inhibitors of PEP are expected to have anti-amnesic and other effects.

Inflammation occurs as a defensive response, which induces physiological adaptations to limit tissue damage and remove the pathogenic infections. Such mechanisms involve a complex series of events including dilatation of arterioles, venules, and capillaries with increased vascular permeability and exudation of fluids including plasma proteins and leukocyte migration into the inflammatory area. For this purpose, they are equipped with various defensive mechanisms using different proteins and signaling pathways. Respiratory burst activity is one of the major molecular mechanisms of neutrophils, which play an important role in the inflammatory responses. Compounds that exhibit respiratory burst inhibitory activity may have potential as anti-inflammatory agents.

Materials and methods

General Experimental Procedures

The ^1H -NMR spectra were recorded in CDCl_3 on a Bruker AM-400 NMR spectrometer with TMS as an

internal standard using the UNIX operating system at 400 MHz. The ^{13}C -NMR spectra were recorded in CDCl_3 at 125 MHz on a Bruker AMX-500 NMR spectrometer. HREI-MS were recorded on Jeol JMS 600 and HX 110 mass spectrometers with the data system DA 5000. The IR spectra were recorded on a Jasco A-302 spectrophotometer. The UV spectra were recorded on a Hitachi U-3200 spectrophotometer. The optical rotations were measured on a JASCO DIP-360 digital polarimeter. The melting point was determined on a Buchi 510 apparatus. Column chromatography (CC) was carried out on a silica gel column (70-230 mesh). Purity of the samples was checked by TLC on pre-coated silica gel GF-254 preparative plates (20 × 20 cm, 0.25 mm thick, Merck) and were detected under UV light (254 and 366 nm), while ceric sulfate was used as spraying reagent. Lipoxygenase (EC. 1.13.11.12) (5-LOX) type I-B (isolated from Soybean) and linoleic acid were purchased from Sigma (St. Louis, MO, USA). Prolyl endopeptidase (*Flavobacterium meningosepticum* origin) was purchased from Seikagaku Corporation (Tokyo, Japan), *N*-benzyloxycarbonyl-Gly-Pro-pNA was procured from Bachem Fine Chemicals Co., and bacitracin was purchased from Sigma Co., Ltd. Dr. Hideaki Shimizu (Yakult Central Institute for Microbiological Research, Tokyo, Japan) has kindly gifted a specific inhibitor of PEP, *N*-benzyloxycarbonyl-pro-prolinal. WST-1 was purchased from Dojindo Laboratories (Kumamoto, Japan). Zymosan A was purchased from Sigma Chemicals (St. Louis, MO, USA), while Ficoll-Paque was purchased from the Pharmacia Biotech Amersham (Uppsala, Sweden). All reagents were of analytical grade. Deionized water was used in all experimental procedures.

DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging antioxidant assay

Antioxidant activity was assayed by using a non-physiological DPPH free radical scavenging assay. Different concentrations of test compounds were taken in reaction mixture, ranging from 1000 to 10 μM , while the concentration of DPPH was kept constant as 300 μM . The reaction mixture containing 5 μL of test compound in DMSO (1 mM) and 95 μL of DPPH in ethanol (300 μM) was taken in 96-well

microtiter plates (Molecular Devices, SpectraMax 340, USA) and incubated at 37 °C for 30 min. The absorbance was measured at 515 nm. Percent radical scavenging activity by test compounds was determined in comparison of the DMSO treated control group. IC₅₀ values represent the concentration of compounds required to scavenge 50% of DPPH free radicals, which were calculated by using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, MA, USA). Propyl gallate (PG) was used as a positive control (14,15).

Lipoxygenase inhibition assay

Lipoxygenase (EC. 1.13.11.12) inhibiting activity was conveniently measured by modifying the spectrophotometric method developed by Tappel (16). The reaction mixture, containing 165 mL of 100 mM sodium phosphate buffer (pH 8.0), 10 mL of test compound solution, and 20 mL of lipoxygenase solution, was incubated for 10 min at 25 °C. The reaction was then initiated by the addition of 10 mL of linoleic acid (substrate) solution. With the formation of (9Z, 11E)-(13S)-13-hydroperoxyoctadeca-9, 11-dienoate, the changes in absorbance at 234 nm were measured for 6 min. Test compounds and the control were dissolved in methanol. All the reactions were performed in triplicate in 96-well micro-plate in SpectraMax 384 Plus (Molecular Devices, USA). The IC₅₀ values were then calculated using the EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, MA, USA). The percentage (%) inhibition was calculated as follows:

$$\% \text{ Inhibition} = (E - S) / E \times 100$$

where E = activity of the enzyme without test compound

S = activity of enzyme with test compound.

Prolyl endopeptidase inhibition assay

PEP inhibition activity was assayed by a modified method of Yoshimoto et al. (17); 100 mM Tris (hydroxymethyl)-aminomethane HCl buffer containing 1 mM EDTA (pH 7.0, 247 mL), PEP (0.02 units/well) 15 mL, and a stock solution of the test compound in MeOH (8 mL, diluted to the desired range of concentrations) were mixed in a 96-well microplate and preincubated for 10 min at 30 °C. The reaction was initiated by adding 30 µL of 0.2

mM of *N*-benzyloxycarbonyl-Gly-Pro-*p*NA (in 40% 1,4-dioxane) as the substrate. The amount of released *p*-nitroaniline was measured spectrophotometrically, as increase in absorption at 410 nm with 96-well microplate reader (Molecular Devices, SpectraMax 340, USA). The percentage inhibition was calculated by the following equation:

$$\% \text{ Inhibition} = 100 - [(O.D \text{ of test compound} / O.D \text{ of control}) \times 100]$$

The potency of enzyme inhibitory activity was represented by the IC₅₀ values, which were defined as the concentration of the test compound that resulted in 50% inhibition of the enzyme with respect to the MeOH control. Z-Pro-prolinal was used as a positive control.

Respiratory burst inhibitory assay

Respiratory burst inhibitory activity of test compounds was determined by using the modified assay of Tan et al. (18). This in vitro assay is based on the reduction of highly water-soluble tetrazolium salt (WST-1) in the presence of activated human neutrophils. Heparinized fresh venous blood was drawn from healthy volunteers in a local blood bank and neutrophils were isolated by the method described in previous publications (19,20). Activity was determined in a total volume of 250 µL MHS (pH 7.4) containing 1.0×10^4 neutrophils/mL, 500 µM WST-1, and various concentrations of test compounds. The control contained buffer, neutrophils, and WST-1. All compounds were equilibrated at 37 °C and the reaction was initiated by adding opsonized Zymosan A (15 mg/mL), which was prepared by mixing with human pooled serum, followed by centrifugation at 3000 rpm and the pellet was resuspended in PBS buffer. Absorbance was measured at 450 nm. Aspirin and indomethacin were used as positive controls. IC₅₀ values were calculated by comparing with the DMSO as blank and expressed as % inhibition of superoxide anions produced. Absorbances were measured on a SpectraMax 340 microplate reader (Molecular Devices, CA, USA).

Results and discussion

Compound **1** (Figure) from the methanolic extract of the leaves of *M. paniculata* was previously reported

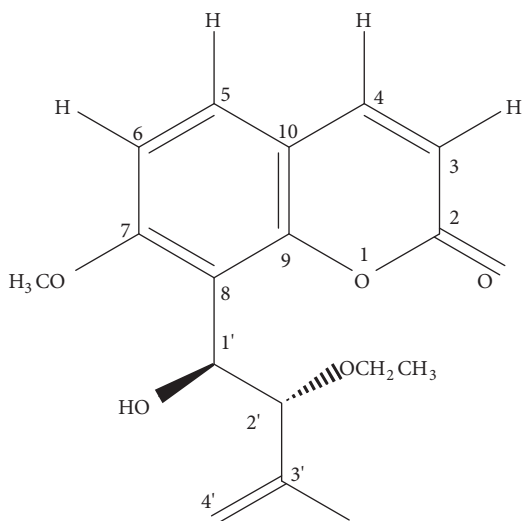


Figure. 2'-O-Ethylmurrangatin isolated from *Murraya paniculata*.

and also examined in terms of its anticholinesterase activities (3). The structure of compound **1** was identified on the basis of modern spectroscopic methods. It was found to be 2'-O-ethylmurrangatin

(**1**) (3). There was no pharmacological activity reported of compound **1**. Thus we were interested for bioassay studies of our new compound **1**.

The antioxidant activity of compound **1** was evaluated by using a DPPH radical scavenging assay. Propyl gallate was used as a positive control. Compound **1** did not show any activity against DPPH radicals.

Compound **1** was tested for lipoxygenase inhibitory activity. Baicalein was used as a positive control. Compound **1** exhibited significant activity against the enzyme (Table).

Compound **1** was also screened for its enzyme inhibitory activity against the prolyl endopeptidase (PEP). It did not display inhibitory potential against the PEP enzyme. Z-Pro-prolinal is used as a positive control.

Respiratory burst inhibitory activity of compound **1** was evaluated by using a contemporary assay (18). Indomethacine and aspirin, clinically used anti-inflammatory drugs, were used as positive controls. Compound **1** showed moderate activity, as compared to standard at 400 mg/mL concentration (Table).

Table. In vitro DPPH radical scavenging, lipoxygenase, prolyl endopeptidase, and respiratory burst inhibitory activities of compound **1**, isolated from methanolic extract of *Murraya paniculata*.

1	% Inhibition at 1 mM concentration for DPPH radical scavenging activity	Compound 1	2.65%
		Propyl gallate *	94.0%
2	IC ₅₀ (mM) ± S. E. M. for lipoxygenase inhibitory activity	Compound 1	28.1 ± 0.8
		Baicalein *	22.7 ± 0.5
3	IC ₅₀ (mM) ± S. E. M. for prolyl endopeptidase (PEP) inhibitory activity	Compound 1	>1000
		Z-Pro-prolinal *	880 ± 0.001
4	IC ₅₀ (mM) ± S. E. M. at 400 µg/mL concentration for respiratory burst inhibitory activity	Compound 1	330.28 ± 2.46
		Indomethacine *	271.21 ± 5.90
		Aspirin *	50.30 ± 4.42

S. E. M. = Standard error of the mean of 5 assays
* = Positive control used in the assay

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