Bioassay-guided Isolation of Constituents of Piper sarmentosum Using a Mitochondrial Transmembrane Potential Assay

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INTRODUCTION

Piper sarmentosum Roxb. (Piperaceae), known as "wild betel", is a terrestrial herb with long creeping stems and a characteristic purgant odor. The plant is cultivated mainly in tropical and subtropical regions of Asia, from northeast India to southern mainland China and Malaysia, and the Andaman Islands. 1-3 P. sarmentosum is used as a folk medicine in certain countries of Southeast Asia for the treatment of various ailments including asthma, cough, dyspepsia, fever, fungal dermatitis of the feet, headache, pleurisy, and toothache. 1-4 Besides its medicinal uses, the leaves of P. sarmentosum are also consumed as a popular spice.5

Several studies have been carried out to evaluate the biological activities of extracts of different parts of P. sarmentosum. An aqueous extract of the whole plant was reported to show hypoglycemic effects.6 An aqueous extract of the leaves was found to possess antinociceptive and anti-inflammatory activities.7 Methanol extracts of the leaves exhibited neuromuscular blocking action8 and antibacterial properties,9 and a crude methanol extract of the roots exhibited antimarial activity.10 A ethanol extract of the whole plant showed insecticidal activities against mosquitoes,11,12 while a chloroform extract of the leaves was reported to have antimalarial activity.12 In addition, both the aqueous and organic phase extracts of the fruits and leaves were found to exhibit antioxidant activity.13 Previous phytochemical investigations on P. sarmentosum have resulted in the isolation of aromatic alkenes, amide, lignan, phenylpropanoid, and sterol chemical constituents.13,16 Among these compounds, certain amides from P. sarmentosum exhibit antiplasmodial, antimycobacterial, antifungal, and antituberculosis properties,14,16 and thus may be considered as a major class of active principles from this plant.

ISOLATION AND STRUCTURE ELUCIDATION OF COMPOUNDS

As part of an ongoing investigation on the discovery of natural anticancer agents from tropical plants, a CHCl₃-soluble extract of the aerial parts of P. sarmentosum, including the leaves, twigs, stems, and inflorescence, collected in Vietnam, exhibited mitochondrial transmembrane potential (MTP) inhibitory activity in HT-29 human colon cancer cells, using an initial screening procedure. This extract was not appreciably cytotoxic for HT-29 cells, when evaluated using a standard protocol.17 In the present investigation, activity-guided fractionation of this extract using this MTP assay led to the isolation of the four new C-benzylated dihydrafloavones (1-4), together with 13 known compounds. The structures of the four new compounds 1-4 were established by spectroscopic data interpretation. The known compounds were identified as isocamphanetin (5), 7-methoxychamanetin (6), dichamalin (7), 7-methoxychamalexin (8), 8E-2',6'-dihydroxybenzyluravilin (2E)- hydroxy-(2S,6R)-2',6'-benzylisouvarilin (B) (8), prodigiosin (10), pipercallosidine (11), 5,4'-dodecadienamide, pipercallosidine, 7-methoxychamanetin, sesamin benzoic acid, and trans-cinnamic acid, by spectroscopic analysis and comparison of the data obtained with literature values.

Figure 1. Active compounds isolated from P. sarmentosum.

BIOLOGICAL ACTIVITY EVALUATION

Induction of apoptosis, or programmed cell death, has been considered as an important mechanism of action for many antineoplastic agents, so targeting the apoptotic signaling pathway is a promising approach for anticancer drug discovery. The mitochondria play an important role in regulation of the intrinsic apoptotic pathway, and the loss of mitochondrial transmembrane potential (MTP) is one of the key events that may occur during the apoptotic process. Thus, measurement of mitochondrial membrane potential (ΔΨm) is a suitable method to study signaling mechanisms involved in the initiation of the apoptotic cascade in a similar fashion, inhibition of the proteasome results in accumulation of many ubiquitinated proteins that are involved with multiple cellular functions and eventually triggers apoptosis. The proteasome is the catalytic core of the complex ubiquitin-dependent protein degradation pathway, and plays an important role in cell cycle regulation. Since tumor cells are more susceptible to proteasome inhibition than normal cells, targeting the proteasome pathway has been demonstrated as a strategy to investigate potential new cancer chemotherapeutic agents. Accordingly, the isolates obtained in this study were evaluated for their effects in the MTP and proteasome assays.

Table 4. Bioactivity Evaluation of Compounds Isolated from P. sarmentosum.1,2,3

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CONCLUSIONS

This study was supported by grant P01 CA125666 (awarded to A.D. Kinghorn) from NCI, NIH. We thank Mr. John Fowle, College of Pharmacy, The Ohio State University, and Dr. Chun-Hua Yuan, OIST Campus Chemical Instrument Center, for facilitating the acquisition of the 48.00 MHz NMR spectra. We acknowledge Ms. Nan Kleinholz, Mr. Mark Apsoga and Dr. Kari Green-Church, Campus Chemical Instrument Center, The Ohio State University, for the mass spectrometric measurement.

ACKNOWLEDGMENTS

REFERENCES