Screening of Protein Tyrosine Phosphatase 1B Inhibitory Activity from Some Vietnamese Medicinal Plants

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Abstract – Protein tyrosine phosphatase 1B (PTP1B), a negative regulator of insulin signaling, has served as a potential drug target for the treatment of type 2 diabetes. The MeOH extracts of twenty-nine medicinal plants, traditionally used in Vietnam as anti-diabetes agents, were investigated for PTP1B inhibitory activity in vitro. The results indicated that, most materials showed moderate to strong inhibitory activity with IC50 values ranging from 3.4 µg/mL to 35.1 µg/mL; meanwhile, eleven extracts (37.9%) could demonstrate PTP1B activity with IC50 values less than 15.5 µg/mL; sixteen extracts (55.2%) could demonstrate PTP1B activity with IC50 values ranging from 15.5 µg/mL to 35.1 µg/mL. The study may provide a proof, at least in a part, for the ethno-medical use in diabetes disease of these plants.

Keywords – Vietnamese medicinal plants, Protein tyrosine phosphatase 1B (PTP1B), diabetes
responsible for the dephosphorylation of tyrosine residues, and are considered negative regulators of insulin signaling. Several PTPs such as PTP-α, leukocyte antigen-related tyrosine phosphatase (LAR), and SH2-domain containing phosphotyrosine phosphatase (SHP2) have been implicated in the regulation of insulin signaling, there is substantial evidence supporting protein tyrosine phosphatase 1B (PTP1B) as the critical PTP-controlling insulin signaling pathway (Johnson et al., 2002). In addition, in cultured cells, overexpression of PTP1B markedly inhibits insulin effect on its receptor phosphorylation (Kenner et al., 1996). A study with PTP1B knockout mice has shown that PTP1B knockout resulted in mice with marked increase in insulin sensitivity and which are resistant to diet induced obesity (Enchebly et al., 1999). Based on these various findings, PTP1B is considered as one of promising targets in the treatment of diabetes but also obesity, and several classes of plant-derived secondary metabolites have been described as PTP1B inhibitors (Tonks, 2003).

Natural products are tremendous sources of lead compounds in the search for new medicaments for the treatment of disease. The largest present underexplored source of such materials lies in tropical and subtropical regions of the world. In these areas, a long tradition of ethno-botanical medicine often exists and offers a rich and relatively untapped source for the discovery of new drugs from natural products. Vietnamese, a Southeast Asian tropical country, has a rich plant biodiversity, with over 12,000 plants and no less than 2,500 species have been used in ethno-medicine (Chi, 1997; Loi, 2004). Thus, as part of a permanent screen program searching for Vietnamese medicinal plants and natural products, the aim of this study was to discover whether there was some scientific basis for the reputed efficacy of selected traditional medicinal plants from Vietnam in treatment of anti-diabetes. Selection was based on literature research of traditional medicinal plant usage in Vietnam (Loi, 2004).

**Preparation of samples** – The air-dried and powdered parts of different amount of plants were extracted with methanol at room temperature (25°C) by maceration process for 72 hr. The crude extracts were obtained after evaporation of solvent under reduced pressure at 40°C. Percentage yields (w/w) were calculated (Table 1). All extracts were stored at ~20 °C prior to screening.

**Protein Tyrosine Phosphatase 1B inhibitory activity assay** – PTP1B (human, recombinant) was purchased from BIOMOL®, International LP (USA) and the enzyme activity was measured using p-nitrophenyl phosphate (p-NPP) as a substrate. To each 96-well (final volume: 200 µL) were added 2 mM p-NPP and PTP1B (0.05 - 0.1 µg) in a buffer containing 50 mM citrate (pH 6.0), 0.1 M NaCl, 1 mM EDTA, and 1 mM dithiothreitol (DTT) with or without test compounds. Following incubation at 37 °C for 30 min, the reaction was terminated with 10 M NaOH. The amount of produced p-nitrophenol was estimated by measuring the absorbance at 405 nm. The nonenzymatic hydrolysis of 2 mM p-NPP was corrected by measuring the increase in absorbance at 405 nm obtained in the absence of PTP1B enzyme.

**Statistical analysis** – The results were expressed as mean± SD of three determinations at each concentration for each sample. The inhibitory concentration 50% (IC50) was calculated using Microsoft Excel program. Statistical significance was calculated by one-way analysis of variance (ANOVA), followed by Dunnett’s test.

**Results and Discussion**

The plants are listed in alphabetical order of their scientific name, family name, local name, part used and also therapeutic application (Table 1). In the present study, twenty-nine plants species which belonging to twenty-two families were selected, and total of twenty-nine extracts were investigated based on their ethno-medical use for the treatment of diabetes and related diseases by the natives of Vietnamese folk medicinal system (Loi, 2001). The inhibitory effect of twenty-nine extracts on PTP1B activity is summarized (Table 2). Of the twenty-nine extracts assayed, twenty-seven extracts (93.1%) could demonstrate PTP1B activity with IC50 values no higher than 36.0 µg/mL (Table 2). The result revealed that the extract of Morus sp. possessed the most potent effect with IC50 as 3.4 µg/mL followed by radix Glycyrrhizae (IC50= 3.7 µg/mL), Phyllanthus reticulatus (IC50= 4.5 µg/mL), Tetrasan scandens (IC50= 5.2 µg/mL), Erythrina variegata (IC50= 7.4 µg/mL), Dioscorea persimilis (IC50= 7.5 µg/mL), Eurya nitida (IC50= 8.7 µg/mL), Plantago asiatica

**Materials and Methods**

**Plant materials** – Vietnamese medicinal plants used in this study were collected in the North of Vietnam in spring, 2010. The botanical samples were identified (Table 1) by Prof. Pham Thanh Ky, Department of Botany, Hanoi University of Pharmacy, Vietnam, where the voucher specimens were deposited. The plants were dried for 7 - 10 days in the shade at environmental temperatures. The dried plants were then ground and transfer to the laboratory for preparation of the plant extracts.