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In vitro effect of important herbal active constituents on human cytochrome P450 1A2 (CYP1A2) activity

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ABSTRACT

This study was designed to investigate eight herbal active constituents (andrographolide, asiaticoside, asiatic acid, madecassic acid, eupatorin, sinensetin, caffeic acid, and rosmarinic acid) on their potential inhibitory effects on human cytochrome P450 1A2 (CYP1A2) activity. A fluorescence-based enzyme assay was performed by co-incubating human cDNA-expressed CYP1A2 with its selective probe substrate, 3-cyano-7-ethoxycoumarin (CEC), in the absence or presence of various concentrations of herbal active constituents. The metabolite (cyano-hydroxycoumarin) formed was subsequently measured in order to obtain IC_{50} values. The results indicated that only eupatorin and sinensetin moderately inhibited CYP1A2 with IC_{50} values of 50.8 and 40.2 μM, while the other active compounds did not significantly affect CYP1A2 activity with IC_{50} values more than 100 μM. K_i values further determined for eupatorin and sinensetin were 46.4 and 35.2 μM, respectively. Our data indicated that most of the investigated herbal constituents have negligible CYP1A2 inhibitory effect. *In vivo* studies however may be warranted to ascertain the inhibitory effect of eupatorin and sinensetin on CYP1A2 activity in clinical situations

Introduction

Andrographolide (Fig. 1A), a diterpenoid lactone, is the most medicinally active phytochemical substance in the plant *Andrographis paniculata*, which has been traditionally used as folklore herbal remedy in India, China, and European countries for dysentery, cholera, diabetes, consumption, influenza, bronchitis, and gonorrhea (Lim et al. 2012). Many reports revealed that andrographolide exhibited anti-cancer, anti-inflammation and anti-diabetic activities (Lim et al. 2012). Several *in vitro* and *in vivo* studies indicated the ability of this compound to modulate the expression and activity of cytochrome P450 (CYP) enzymes including that of CYP1A (Chatuphonprasert et al. 2009; Pekthong et al. 2009; Jaruchotikamol et al. 2007).

Asiaticoside, asiatic acid, and madecassic acid (Fig. 1B, C, and D) are three major active substances derived from herb *Centella asiatica*, which has been worldwide cultivated as a vegetable or spice in many Asian, African, and Oceanic countries for centuries. Traditionally this herb is used for boosting memory, wound healing, and nowadays it is often used as an active ingredient in tonics, oral slimming formulas, body-beautiful preparations, and anti-aging skin care products (Brinkhaus et al. 2000). *Centella asiatica* contains a good number of active constituents accounting for its beneficial effects such as preventing age-related cognitive deficits, improving wound healing, anti-inflammation, and anti-cancer action (James and Dubery, 2009). Only limited data are available on the effect of its active ingredients on CYP activities (Pan et al, 2010; Winitthana et al. 2011; Chatchanee, 2003).

Sinensetin (Fig. 1E) is one of the major polymethoxyflavones contained in citrus fruits and O*rthosiphon stamineus*. Its bioactivities include anti-diabetes, anti-cancer, and anti-inflammation (Wei et al. 2013). As a methoxylated flavone, sinensetin is likely to be mainly metabolized by CYP1A subfamily (Walle and Walle,

Abbreviations: CEC, 3-cyano-7-ethoxycoumarin; CYP, cytochrome P450; DMSO, dimethylsulfoxide; K_m, Michaelis–Menten constant; V_{max}, maximum velocity; IC_{50}, the half maximal (50%) inhibitory concentration; K_i, the inhibitor concentration required for a half-maximal inhibition; NADPH, nicotinamide adenine dinucleotide phosphate.

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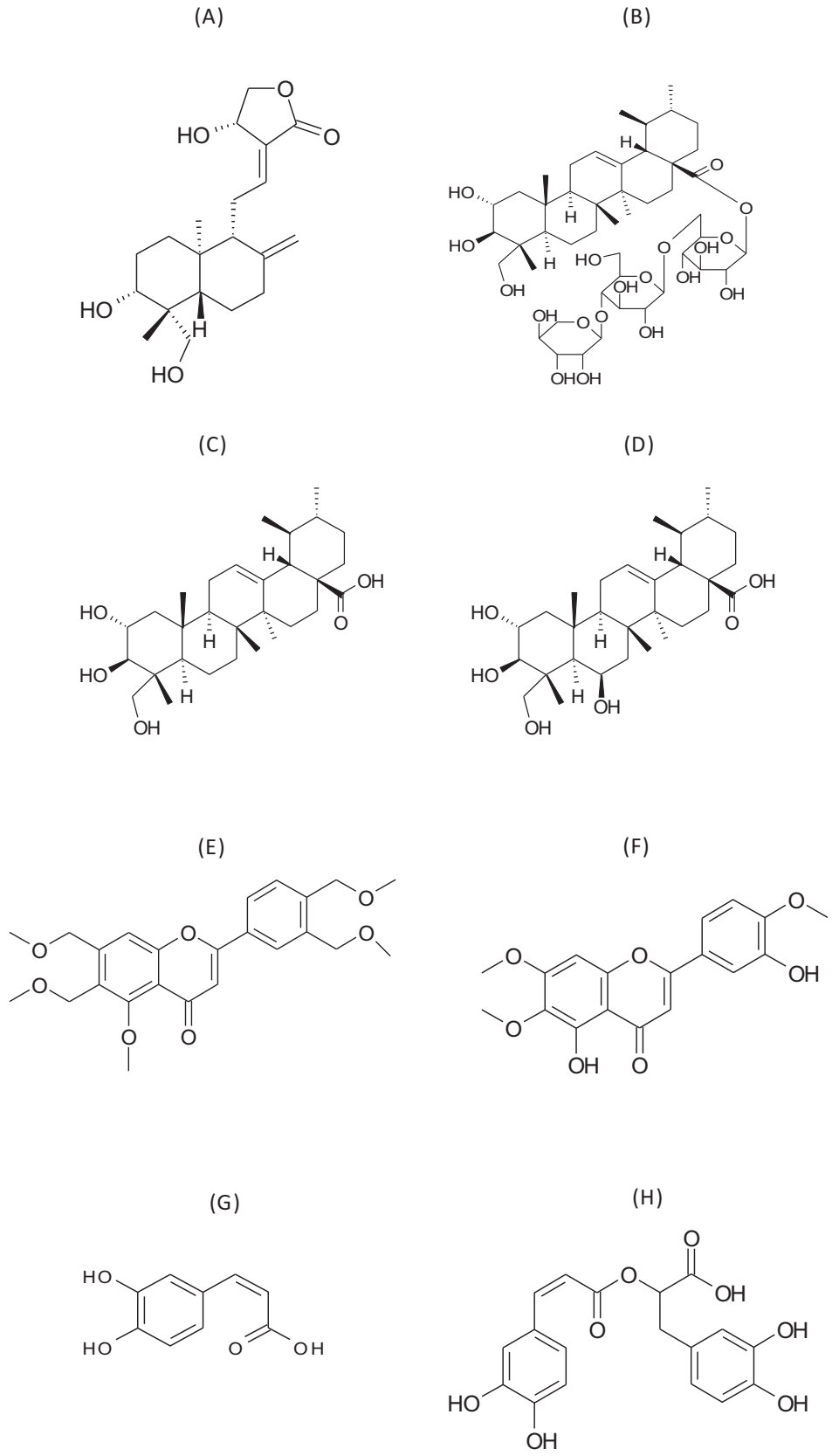

Fig. 1. Chemical structures of herbal active constituents, (A) andrographolide, (B) asiaticoside, (C) asiatic acid, (D) madecassic acid, (E) sinensetin, (F) eupatorin, (G) caffeic acid, and (H) rosmarinic acid.