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A rapid profiling of hypolipidemic agents in dietary supplements by direct injection tandem mass spectrometry

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ABSTRACT

A new, fast-screening method for identification of various pharmacologically active ingredients in cholesterol-lowering dietary supplements using direct injection mass spectrometry was developed. The optimized method was proven to be useful for multitarget screening: 17 compounds of interest were identified, including monacolin K and its acid form, cynarin, green tea catechines, genistein, daidzein, guggulsterones, allicin and L-arginine. The method was successfully applied for the fingerprint analysis of dietary supplements containing only one or several cholesterol-lowering agents including lovastatin registered as a drug. According to the obtained results, it is apparent that cholesterol-lowering dietary supplements are poorly standardized, as two samples did not contain active ingredients (monacolin K, monacolin K acid form and cynarin). Moreover, the application of the proposed direct injection method to quality control of cholesterol-lowering dietary supplements may be proposed for the routine analysis of a large number of samples in the laboratories of regulatory agencies.

1. Introduction

Hyperlipidemia is a heterogeneous group of disorders commonly characterized by an increased flux of free fatty acids, raised levels of triglycerides, low-density lipoprotein (LDL) cholesterol and apolipoprotein B, as well as by reduced plasma high-density lipoprotein (HDL) cholesterol concentration. There are several classes of hypolipidemic drugs (statins, fibrates, nicotinic acid and its derivatives) which may differ in both their impact on the cholesterol profile and adverse effects (Mahamuni et al., 2012). Natural cholesterol-lowering agents are becoming popular as a possible alternative therapy for lowering plasma levels of total cholesterol, especially for patients whose blood cholesterol level is marginally high, but not high enough to warrant the prescription of cholesterol-lowering medication.

Red mold rice is the fermented product of rice on which red mold (*Monascus purpureus*) has been grown. During the fermentation process, 14 monacolins – compounds that inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in hepatic cholesterol synthesis – are produced. The most abundant monacolin is monacolin K, also known as lovastatin, the first commercially available statin drug for treatment of hyperlipidemias (Fig. 1).

Scientists and health practitioners worldwide are in fact questioning whether red mold rice is a dietary supplement or a drug (Gordon and Becker, 2011). In recent years, artichoke (*Cynara scolymus*, *Asteraceae*) has been introduced as a new natural lipid-lowering agent because cynarin (1,5-dicaffeoylquinic acid), the main active ingredient, shows the inhibition effect on HMG-CoA reductase enzyme as well as on phosphatidate phosphohydrolase (PAP) (Heidarian et al., 2011). One of the underlying mechanisms by which green tea (*Camellia sinensis, Theaceae*) catechines ((+)-catechin, (−)-epicatechin, (+)-catechin gallate, (−)-epicatechin gallate, (+)-gallocatechin, (−)-epigallocatechin, (+)-gallocatechin gallate, (−)-epigallocatechin gallate) reduce plasma cholesterol level is up-regulation of LDL receptor activity (Spáčil et al., 2010). Furthermore, it has been discovered that catechins stimulate cholesterol-7α-hydroxylase (CYP7A1) enzyme in human hepatoma cells at mRNA level (Lee et al., 2008). The hypocholesterolemic effect of soybean (*Glycine max, Fabaceae*) has been attributed to the proteins, fiber and phytochemicals including the soy isoflavonoids. Isoflavones, genistein and daidzein, have been shown to inhibit intestinal acyl coenzyme A cholesterol acyltransferaze (ACAT) activity in hepatocyte (Borradaile et al., 2002).

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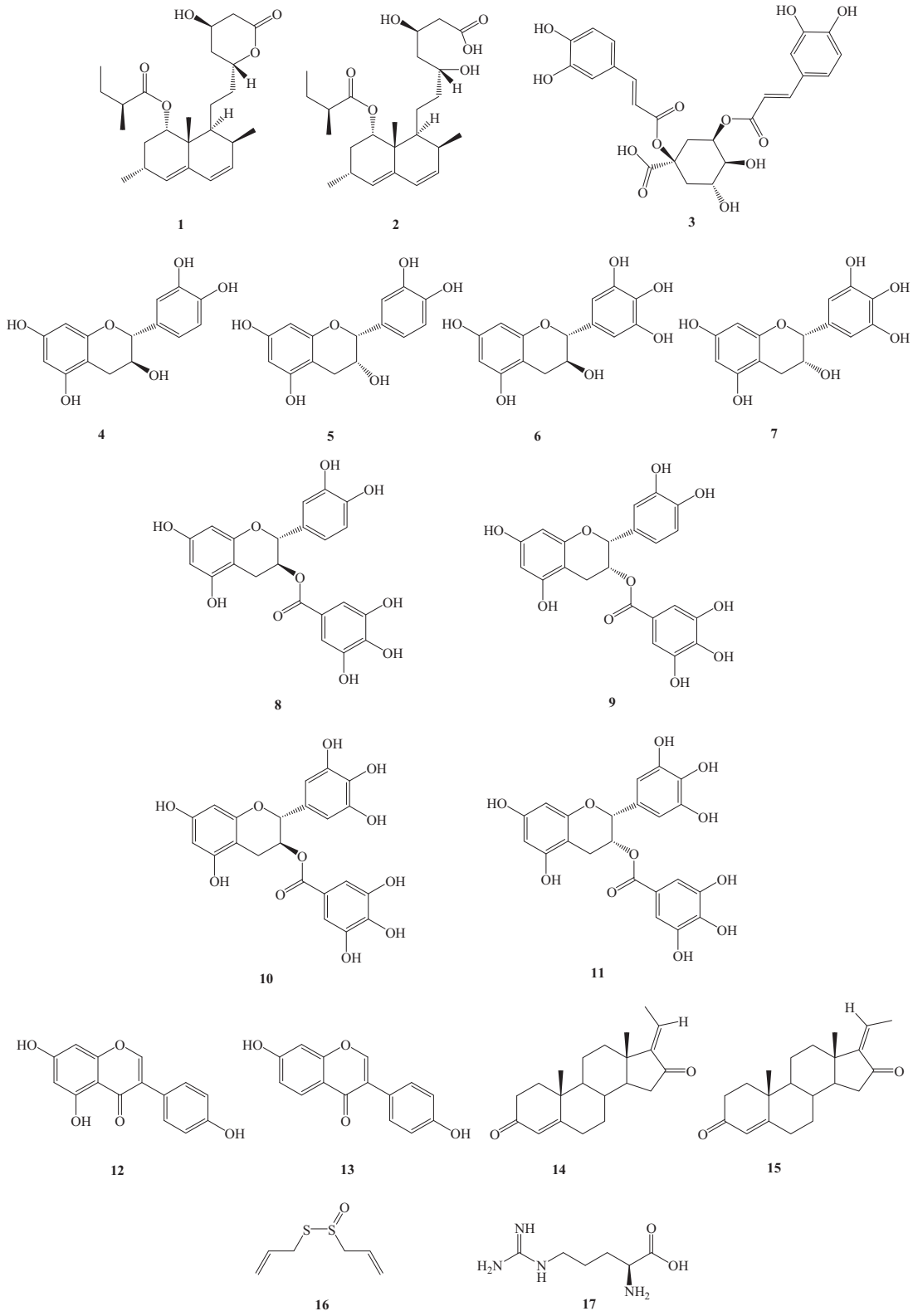

Fig. 1. Chemical structures of investigated natural cholesterol-lowering agents: Monacolin K (1), monacolin K acid form (2), cynarin (3), (+)-catechin (4), (−)-epicatechin (5), (+)-catechin gallate (6), (−)-epicatechin gallate (7), (+)-gallocatechin (8), (−)-epigallocatechin (9), (+)-gallocatechin gallate (10), (−)-epigallocatechin gallate (11), genistein (12), daidzein (13), *E*-guggulsteron (14), *Z*-guggulsteron (15), allicin (16) and L-arginine (17).

A number of studies have shown that two steroidal isomers known as *E*- and *Z*-guggulsterone are active ingredients of guggulipid, responsible for cholesterol-lowering effects of the herbal extract from the resin of the thorny plant *Cammiphora wighitii*, *Burseraceae* (Ulbricht et al., 2005). Both isomers of guggulsterone possess similar hypolipemic activity. Guggulsterones selectively modulate farnesoid X receptor (FXR) gene expression and positively regulate the expression of the cytochrome P450 7A1 and 8B1, thus inducing the cholesterol catabolism into bile acids (Sinal and Gonzalez, 2002). Several