Salvigenin

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Phytochemical analysis of the Iranian plant Achillea wilhelmsii led to the isolation of 17 pure secondary metabolites belonging to the classes of sesquiterpenoids and phenolics. Two of these compounds, named wilhemsin (7) and wilhelmsolide (9), are new sesquiterpenoids, and the first shows undescribed structural features. Their structures were elucidated through extensive spectroscopic analysis, mainly based on 1D and 2D NMR, and chemical derivatization. Starting from plant traditional use and previous reports on the activity of the plant extracts, all the pure compounds were evaluated on endpoints related to the treatment of metabolic syndrome. The sesquiterpene hanphyllin (8) showed a selective cholesterol-lowering activity (−12.7% at 30 µM), santoflavone (13) stimulated glucose uptake via the GLUT transporter (+16.2% at 30 µM), while the trimethoxylated flavone salvigenin (14) showed a dual activity in decreasing lipid levels (−22.5% palmitic acid biosynthesis at 30 µM) and stimulating mitochondrial functionality (+15.4% at 30 µM).

Keywords: Achillea wilhelmsii ; phytochemical analysis ; sesquiterpenoids ; metabolic syndrome ; mitochondrial stimulatory activity ; yarrow ; salvigenin ; hanphyllin

1. Introduction

Metabolic syndrome is a complex cluster of disorders including dyslipidemia, diabetes mellitus, obesity, osteoporosis, and cardiovascular diseases affecting one-quarter of the world's adult population. Compared to the healthy population, patients present a higher risk to die from heart disease or stroke [1]. Chronic inflammation, insulin resistance, production of abnormal adipocytokines such as tumor necrosis factor α, interleukin-1 (IL-1), IL-6, leptin, and adiponectin are all markers of metabolic syndrome at the cellular level [2].

Lifestyle modification remains the initial intervention of choice for such conditions, but pharmacological treatment is considered for those patients whose risk factors are not adequately reduced with lifestyle changes. Although there is no shortage of pharmacotherapies for the prevention and treatment of metabolic disorders, in several cases the severe adverse effects, their high cost, and insufficient accessibility hamper general use. An increasing number of studies have indicated that some natural products are able to ameliorate metabolic syndrome and its risk factors [3], supporting their eligibility as alternative strategies for treatment. Rigorous research to determine effective plant-derived metabolites, and their cellular mechanisms is needed to design animal and clinical trials.

Achillea wilhelmsii C. Koch is an herbaceous plant belonging to the Asteraceae family (tribe Anthemidae), widely distributed in Asia, especially in Iran (where it is named "Berenjasf"), Pakistan, and the Indian peninsula. This plant holds a remarkable place in traditional Persian medicine, with a particular indication for the treatment of pulmonary affections, and it is used by the local population for a variety of ailments [4]. Recent investigations have substantiated these traditional uses with a scientific approach and activity on irritable bowel syndrome and ulcerative colitis [5][6], and improvement of oxidative stress-related myocardial injury [7] have been demonstrated. Asgary et al. [8] have reported a significant activity of *A. wilhelmsii* extracts in inducing a decrease in triglycerides and LDL-cholesterol levels after 2–4 months of the administration, a result supported by Khazneh et al. [9] in a more recent investigation. However, both these promising studies were carried out mainly on plant extracts and failed to identify an unambiguous correlation between the extract activity and a specific metabolite or a class of compounds.

2. Discussion

The metabolic syndrome refers to the co-occurrence of several known cardiovascular risk factors, including insulin resistance, obesity, atherogenic dyslipidemia, and hypertension. The syndrome is posing substantial concern since it has reached epidemic proportions worldwide. It is widely recognized that botanicals may serve as effective agents for the prevention or treatment of metabolic syndrome since they contain biologically active secondary metabolites that, by exerting multiple mechanisms of action, may potentiate each other's activity, or have a synergistic effect [10]. Although the synergistic effect of structurally related (or sometimes even unrelated) metabolites plays a crucial role in the activity of

plant extracts, a detailed knowledge of the chemical composition and of the biological activity of each single compound is nevertheless required. Indeed, it could allow the optimization of plant extraction, the standardization of extracts or the preparation of extracts enriched in one class of compounds.

We have applied these principles to *A. wilhelmsii*, a plant consumed as a hot beverage in the Middle East region and for its claimed beneficial effects in different conditions, such as treatment of oxidative stress-related myocardial injury [7] or lowering plasma triglycerides and LDL-cholesterol [8][9]. It is known that *A. wilhelmsii* elaborates mainly sesquiterpenoids and phenolic compounds, with special regards to methoxylated flavones and flavone-*C*-glycosides, but a comprehensive characterization of both these classes of compounds was still lacking. In the present investigation, we have obtained in the pure state and chemically characterized 17 compounds from different extractions of the plant (nine sesquiterpenoids and eight phenolic derivatives), including the new sesquiterpene derivatives wilhelmsin (**7**) and wilhelmsolide (**9**). Wilhelmsin is characterized by a *seco*-guaianolide skeleton conjugated to a GABA moiety, a feature not previously known.

We isolated 17 pure compounds and tested them for their cholesterol/fatty acid lowering activity and effects on glucose homeostasis, both hallmarks of beneficial effects against metabolic syndrome. The sesquiterpene hanphyllin (**8**) showed a marked effect in the reduction of cholesterol biosynthesis, with no effect in other bioactivities tested. In our in vitro assay, at the dose of 30 μM compound **8** reduced cholesterogenesis with a potency similar to the statin Simvastatin (12.2% versus 14.8% decrease in cholesterol biosynthesis for **8** and Simvastatin, respectively). Some naturally occurring sesquiterpenoids of the guaianolide and germacranolide classes have already been reported to exert antihyperlipidemic activities, but in most cases their activity is not selective [11]. It has been argued that their activity could be ascribed to electrophilic reactivity toward thiol-bearing enzymes of lipid synthesis, i.e., citrate-lyase, acetyl-CoA synthetase, and beta-hydroxy-beta-methylglutaryl-CoA reductase, with a Michael-type mechanism [11]. In this regard, the α-methylene-γ-lactone moiety should be hypothesized as the pharmacophoric moiety responsible for this activity. The activity of hanphyllin does not disprove this hypothesis, but it must be noted that a similarly (if not more) reactive moiety is also present in compounds **3–7,** thus indicating that the overall skeleton of the compound plays a crucial role in the adaptation to their target enzymes' active site. Indeed, it can be noticed that hanphyllin is the single germacranolide derivative, while **3–7** are all guaianolides.

As mentioned before, hanphyllin did not exert effects on fatty acid homeostasis, while the tetra- and tri-methoxylated flavones santoflavone (**13**) and salvigenin (**14**) showed marked palmitate lowering activity, with a drastic reduction in the number of lipid droplets present intracellularly, via inhibition of de novo lipogenesis. Moreover, in this case, there are reports in the literature about the beneficial effect of flavones in reducing lipid levels in hyperlipidemic rats [12], however, our study updates the available information by showing that: (a) free -OH groups are not essential for activity since polymethoxylated flavones are also very active; (b) the flavone structure is not sufficient for activity since strict structural requirements appear to be operating. Compared to santoflavone (**13**), the most active flavone salvigenin (**14**) possesses an -OH group on ring A which is clearly beneficial for activity. On the contrary, the presence of a methoxy group on ring B of the flavonol artemetin (**12**) plays a dramatically negative effect on bioactivity. Interestingly, santoflavone (**13**) was active in stimulating glucose uptake via GLUT transporter. Further investigation will be necessary to confirm the mechanism of action of this molecule and if it either affects GLUT transporters, insulin receptors, or indirectly promote intracellular catabolism and glucose uptake via GLUT independent transporters.

Mitochondria are master regulators of cellular functions, and their reduced activity is linked to dysmetabolic conditions, while pharmacological stimulation of mitochondria by polyphenols [13] and other plant metabolite can accelerate intracellular catabolic reactions with beneficial effects on metabolic syndrome. Indeed, an accelerated catabolism may promote lipolysis, glucose uptake, and inhibition of lipid and cholesterol biosynthesis in hepatic cells as well as in adipose tissue.

Salvigenin (**14**) was the single *A. wilhelmsii* metabolite to show a significant mitochondrial stimulatory activity, measured as an increase in the activity of the H^+ pumps of the electron transport chain involved in oxidative respiration. The exact mechanism of this activity is not known and could be related either to a direct effect on enzymes of mitochondrial complexes or to a modulation of transcription factors which regulate the expression of mitochondrial proteins. The result of this activity is a complex balance between a pro-oxidant metabolism-stimulating activity (with the consequent increase in ROS production) and the antioxidant effect at molecular level associated to flavones and related flavonoids. Overall, the dual activity detected for salvigenin (**14**) on both lipogenesis and mitochondrial functionality could suggest a link between the two processes. Most likely, the mitochondrial stimulatory activity stimulates fatty acid β-oxidation, with this latter serving as fuel for oxidative phosphorylation and ATP production in mitochondria.

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