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Medicago sativa as a source of secondary metabolites for agriculture and pharmaceutical industry

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ABSTRACT

Medicago sativa L. (lucerne or alfalfa) is a species which is generally regarded as a cheap source of valuable protein not only for animal fodder but also for nourishment provided in the poorest and developing countries. Moreover, this plant has a long tradition of use in folk medicine. Numerous studies indicate that besides protein, *M. sativa* synthesizes a variety of secondary metabolites. Among secondary metabolite classes produced by alfalfa, the saponins and flavonoids are of most interest and well characterized. In the current review, we have summarized their chemical structure and method of identification. The potential of alfalfa extracts in medicine and pharmacology has been highlighted. Furthermore, new ways of utilizing flavonoids as well as saponins in agriculture and horticulture are discussed. Collected data can be the starting point and inspiration for scientists of various specialties for future research on this plant. This review draws attention to the necessity of developing modern analytical tools for identification and quantification of individual *M. sativa* phytochemicals.

1. Introduction

Plant secondary metabolites can be defined as compounds that are not directly involved in the normal growth, development or reproduction, but they are crucial in the interaction of the plant with its environment. Although it is difficult to determine the precise role of each secondary metabolite, most of them are involved in plant defense against herbivores and pathogens. In recent years the importance of plant secondary metabolites has increased since they are commonly used as drugs, pesticidies, and food additives (Wink, 2010). It is well known that some members of the medicago genus are known to produce a range of bioactive compounds (Barnes et al., 2002). Presently, *Medicago truncatula* is a model species in the Medicago genus. It is an annual legume with rapid generation time and small diploid genome, which is amenable to genetic transformation. Furthermore, the genome of this plant has been sequenced and the knowledge of its DNA sequence helps to understand the pathways of secondary metabolite synthesis (Zhou et al., 2011). The recent years have brought many studies on saponins and flavonoid biosynthesis, their biological functions and their determination in *Medicago truncatula*. These data were analyzed in a comprehensive review by Gholami et al. (2014). However, we focused on the depiction of natural bioactive products in *Medicago sativa* (lucerne or alfalfa) – a plant which is one of the most cultivated forage legumes in the world. It is an economically important crop used as fodder for livestock. This species has been grown since ancient times. Due to its high nutritional value it was referred to by the ancients as al-fac-facah (the father of all food) and nowadays is often called "the queen of forages" (Ehsanpour and Razavizadeh, 2005). Among the legumes lucerne is the plant with the highest yield of crude protein: 2000–3000 kg/ha, *i.e.* 3 times as much as soya and 4 times

Abbreviations: CID, collision induced chromatography; 2D CCC × LC, stop-and-go two-dimensional chromatography coupling of counter-current chromatography and liquid chromatography; ^{1}H, ^{13}C NMR, nuclear magnetic resonance ^{1}H or ^{13}C respectively; DM, dry matter; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EFSA, European Food Safety Authority; ESI MS, electrospray ionisation mass spectrometry; FAB MS, fast atom bombardment in mass spectrometry (ionization technique); GC, gas chromatography; HPLC, high performance liquid chromatography; MS, mass spectrometry; PCD, programmed cell death; RP-HPLC, reversed phase high performance liquid chromatography; TLC, thin layer chromatography; QToF, quadrupole-time of flight; TEM, transmission electron microscopy.

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as much as wheat. According to the data from 2009, global cultivation of alfalfa reached 32 million hectares (Bresson et al., 2009).

Lucerne is easy to grow and normally lives 4–5 years, and each year the crop can be harvested 2–3 times. Due to the atmospheric nitrogen fixation by symbiotic rhizobia it requires only small doses of this component in the mineral form. It is also a perfect forecrop for other plants. Growing alfalfa requires low labor intensity and the whole work can be mechanized. Its well-developed root system enables it to draw up and thoroughly use the soil nutrients (Rashmi et al., 1997).

Alfalfa is rich in essential amino acids such as valine, leucine, threonine and lysine. Moreover, the composition and the ratio of amino acids are considered to be similar to the standard egg white. Its aerial parts are one of the best sources of chlorophyll and vitamins C, E, B_1, B_2, B_6, B_{12}, niacin, folic acid, biotin, inositol, choline, and β-carotene (Stochmal, 2007). It also contains valuable minerals such as Ca, Cu, Fe, Mg, Mn, P, Zn, Si (Zanin, 2009; Bora and Sharma, 2011a, 2011b). Recent studies have demonstrated the presence of lithium (1.12 mg/g) in *M. sativa* growing in the desert of Oman (Hanif et al., 2015). This mineral is a mood stabilizer and has been widely used in treatment of bipolar disorder (Gray and McEwen, 2013). European Food Safety Authority (EFSA) accepted alfalfa leaf extract as a safe dietary supplement rich in proteins and vitamins. Several studies have shown that besides its nutritional properties alfalfa is a rich source of biologically active compounds – secondary metabolites. Karimi et al. (2013) showed that total value of phenolics and flavonoids is 37.0 ± 0.02 mg gallic acid equivalent (GAE)/g dry matter (DM) and 12.6 ± 0.17 mg rutin equivalent/g DM, respectively. Many studies indicate that some cultivars of alfafa are also an especially rich source of bioactive saponins (Oleszek, 1996; Tava et al., 2011). High biomass production together with the high content of phenolic compounds and saponins make *M. sativa* a good source of bioactive compounds. Hence, besides its importance as fodder, this species is considered to be a herb beneficial to the human body. Saponins and flavonoids are valuable components of animal and human diet and have many pharmaceutical applications (Gholami et al., 2014; Bora and Sharma, 2011a, 2011b; Głowniak et al., 2007). This plant has been used for centuries in traditional medicine to improve memory, to cure kidney problems, asthma, cough, arthritis and central nervous system disorders (Finkler, 1985; Inamul, 2004). Contemprorary studies indicate that extracts rich in saponins are effective in lowering blood cholesterol levels. Not without

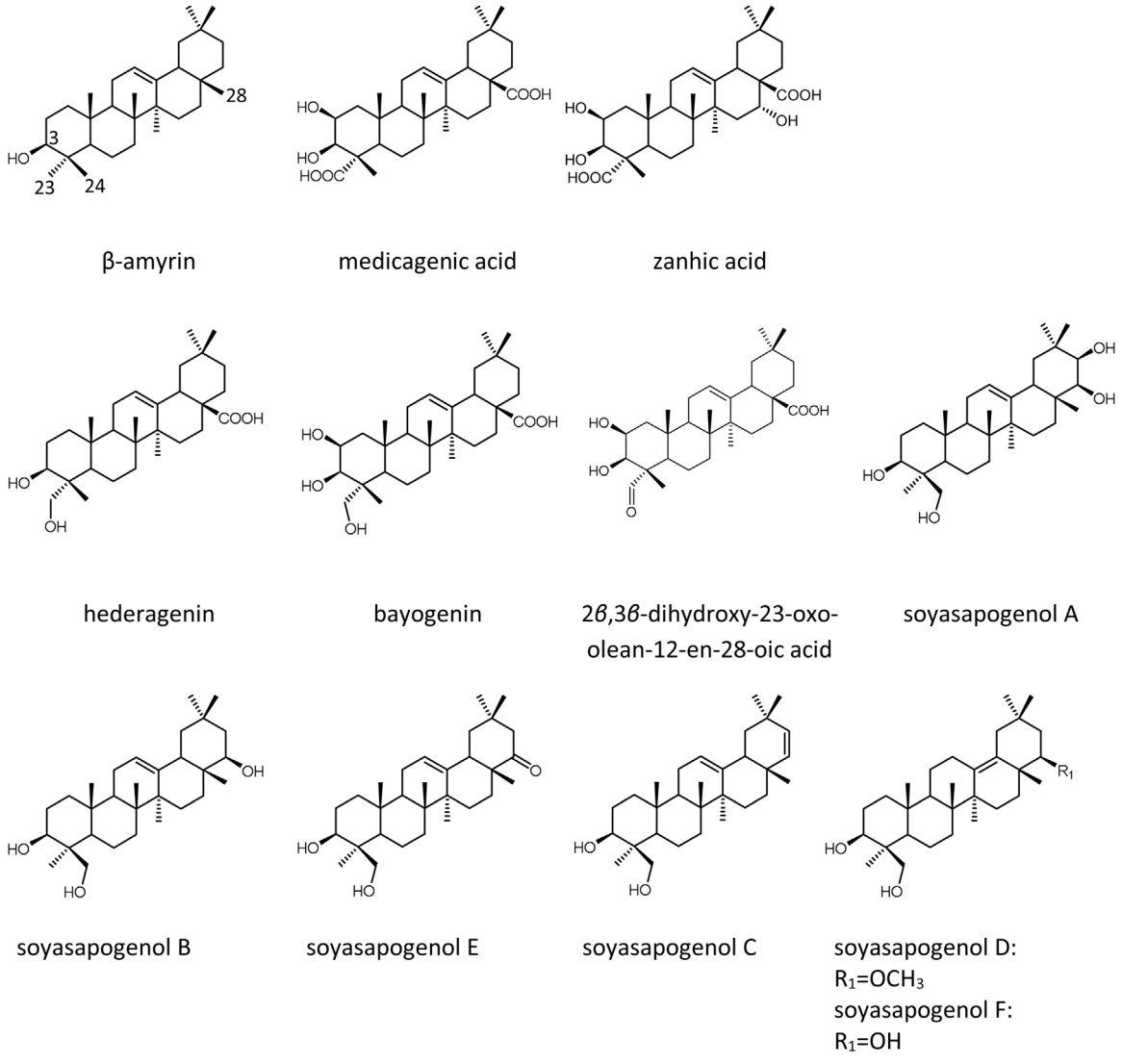

Fig. 1. Sapogenins identified in *Medicago sativa* L. extracts.

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