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Chemical and Biological Activity of Triterpene Saponins from *Medicago* Species

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Dedicated to the memory of Professor Ivano Morelli.

Naturally occurring saponins are a large group of triterpene and steroid glycosides characterized by several biological and pharmacological properties. The *Medicago* genus represents a valuable source of saponins which have been extensively investigated. This review summarizes the chemical features of saponins from *Medicago* species and their biological activity, with particular attention to their antimicrobial, insecticidal, allelopathic and cytotoxic effects. Influence of saponins on animal metabolism is also reported.

Keywords: *Medicago*, *M. sativa*, saponins, chemical structure, biological activity.

Saponins are a large group of plant metabolites including triterpenoids, steroids and steroidal alkaloids glycosylated with one or more sugar chains [1, 2]. They are commonly distinguished by their surfactant and hemolytic activities. Naturally occurring saponins display a broad spectrum of biological and pharmacological properties such as fungicidal, molluscicidal, antibacterial, antiviral and antitumor activities [2-6]. Due to their chemical, physical and physiological characteristics, commercial products containing plant saponins are available and used in the pharmaceutical, cosmetic and food industries [7-8]. Some saponins are the starting material for the semisynthesis of drugs and some are used as emulsifiers and foaming agents in food. Plant extracts rich in saponins have been used as folk detergents and are ingredients of cosmetic preparations such as lipsticks, shampoos and toothpaste.

Saponins are produced by many plant species and their distribution in the plant kingdom seems to be correlated with the structural type. That is, steroidal saponins have been found almost exclusively in the Monocotyledons, while triterpenoid saponins mainly occur in the Dicotyledons and are practically absent in the Gymnosperms [6, 9]. The *Leguminosae* have been extensively investigated for their saponin content and within this family of plants, the *Medicago* genus represents a particularly rich source of bioactive saponins [10-15].

The genus *Medicago* includes 83 different species, the most known represented by *M. sativa* L. (syn. *M. media* Pers.), or alfalfa, a highly valued forage crop [16, 17]. The chemical structure of saponins from several species within the genus has been determined [18-46]. Generally they are complex mixtures of high-molecular weight triterpene glycosides with medicagenic acid, hederagenin, zanhic acid, bayogenin and soyasapogenols A and B as the dominant aglycones. Recently the 2β,3β-dihydroxy-23-oxo-olean-12-en-28-oic acid has been identified as a new aglycone moiety in the two species *M. arborea* [45] and *M. hybrida* [46]. A summary of the structural types of sapogenins isolated from the various species of *Medicago* is reported in Figure 1.

Sugars or sugar chains are generally linked at the C-3 position of the aglycone (monodesmosides) and

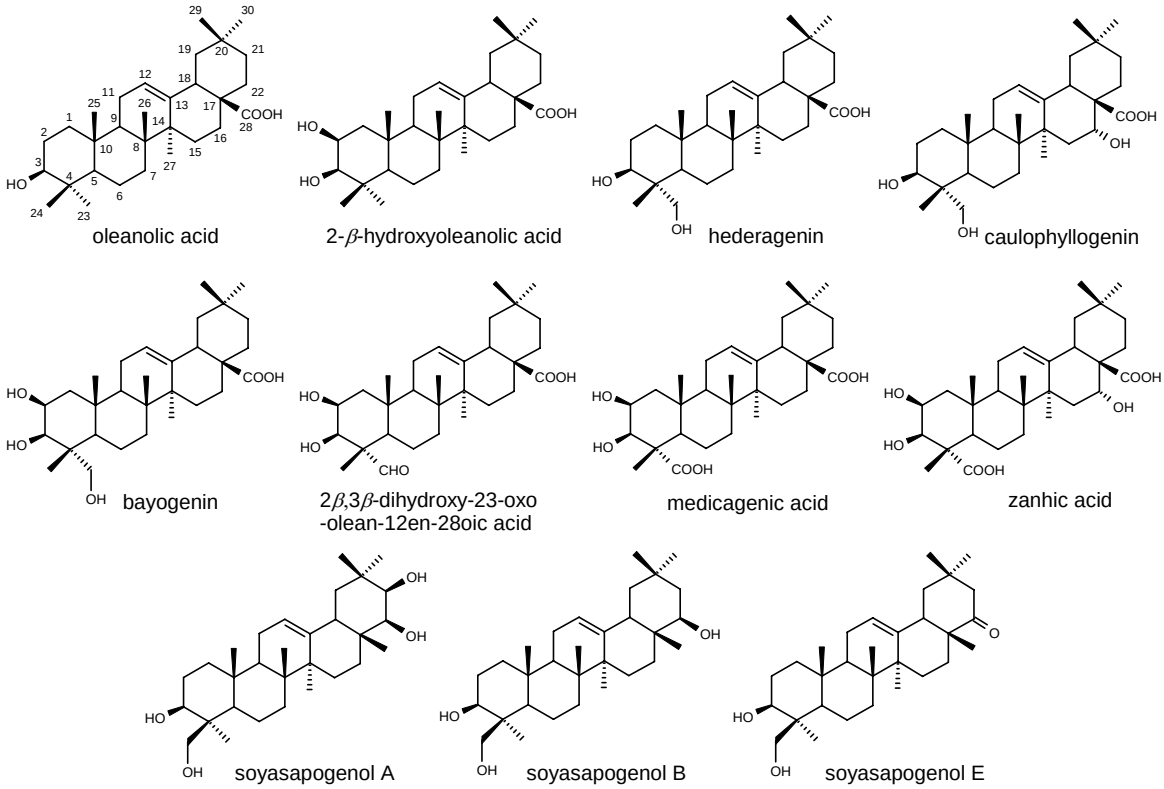

Figure 1: Chemical structure of sapogenins detected in *Medicago* species.

additionally at the C-28 position, giving the corresponding bidesmosides. A tridesmoside saponin (extra sugar at the C-23 position) has only been identified in *M. sativa* [36] and *M. truncatula* [42].

The most abundant monosaccharide units found in the *Medicago* saponins are: arabinose, rhamnose, xylose, glucose, and glucuronic acid. Saponins in the *Medicago* species are produced in all the plant organs: leaves, flowers, roots, seeds and sprouts [10-49]. Their content in the plant material changes as a function of several factors, such as plant organs, genotype, cutting, year and stage of growth, and environmental effects, as reported for *M. sativa*, the most studied species of the genus [50-55]. The chemotaxonomic significance of saponins has also been investigated as their composition can discriminate among *Medicago* species [56-59]. The occurrence of saponins in the *Medicago* genus is long known [60], and their composition has been studied in several species. Structure elucidation of complex saponin mixtures differentiates their aglycone composition [61, 62]. In particular, investigation of several annual and perennial wild and cultivated *Medicago* species, showed variability in the aglycone composition of the saponins from each species. Medicagenic acid was detected in some of them and soyasapogenol B was often present in the form of soyasaponin I, a common saponin of the *Leguminosae* family [10, 63].

Chemical analysis of saponins is not simple due to their 'soapy' properties due to sugars in the molecules. Their presence can be evaluated by biological tests involving their toxic haemolytic [64], fungicidal [65] and insecticidal [66] properties. Chemical methods also have been used, such as TLC [10], HPLC [67], GC and GC/MS [52, 68, 69], the last technique being used to analyze and quantify only the aglycone moieties. Capillary electrophoresis [70] and LC/MS methods [40, 42, 43] have also been employed for the identification and quantification of saponins in the plant extracts.

Structure investigation of *Medicago* saponins is usually performed by preliminary identification of the sapogenins and sugars released after acid hydrolysis from pure saponins obtained by direct and reverse-phase chromatographic separation of the raw saponin mixtures. Detailed information on the saponin structure, however, could be obtained only by a combination of analytical methods, including

MS [71] and NMR analyses [72, 73], performed on pure compounds. The MS spectra allowed subsequent fragmentation of the sugar chains to give the corresponding aglycones, NMR analyses (^{1}H, ^{13}C and 2D experiments) allowed the determination of all the carbon atoms and the sugar linkage in the molecules, while the absolute configuration of monosaccharides was generally obtained by GC analyses with a chiral capillary column. Detailed investigations on saponin chemical structures have until now been reported for *M. arabica, M. arborea, M. hybrida, M. lupulina, M. polymorpha, M. sativa* and *M. truncatula* [19-46]. Characterized saponins from these species of *Medicago* are listed in Tables 1-7.

Table 1: Saponins identified in *M. arabica* leaves [41].

Aglycone	3 OH substituted	28 COOH substituted
2*β*-Hydroxy oleanolic acid	*α*-L-Ara(1→2)-*β*-D-Glc	*β*-D-Glc
Hederagenin	*α*-L-Ara	-
Hederagenin	*α*-L-Ara	*β*-D-Glc
Hederagenin	*β*-D-Glc(1→2)-*α*-L-Ara	-
Hederagenin	*β*-D-Glc(1→2)-*α*-L-Ara	*β*-D-Glc
Hederagenin	*α*-L-Ara(1→2)-*β*-D-Glc(1→2)-*α*-L-Ara	*β*-D-Glc
Bayogenin	*α*-L-Ara	-
Bayogenin	*α*-L-Ara	*β*-D-Glc

M. arabica leaves are characterized by the presence of short sugar chain saponins, including mono and bidesmosides of 2-*β*-hydroxyoleanolic acid, hederagenin and bayogenin (Table 1). *M. arborea* leaves produce saponins containing up to seven sugars, identified as mono and bidesmosides of medicagenic and zanhic acid (Table 2). Saponins from *M. hybrida* roots are characterized by the presence of short sugar chain bidesmosides of hederagenin and medicagenic acid (Table 3). *M. lupulina* leaves contain mono and disaccharide saponins of hederagenin and medicagenic acid (Table 4), while saponins from the leaves of *M. polymorpha* predominantly consist of short sugar chain bidesmosides of hederagenin and caulophyllogenin (Table 5). Saponins from the roots and the aerial parts of *M. sativa* are a complex mixture of both short and long sugar chains of mono and bidesmosidic compounds with hederagenin, medicagenic acid, zanhic acid and soyasapogenols as the most representative aglycones. In this species a tridesmoside saponin containing eight monosaccharide units and a *β*-maltoside derivative, 3-*O*-[*α*-D-glucopyranosyl(1→4)-*β*-D-glucopyranosyl] medicagenic acid, were also identified in the aerial parts (Table 6). *M. truncatula* saponins from both roots and aerial parts (Table 7) are long sugar chain bidesmosides of medicagenic and zanhic acid.

Table 2: Saponins identified in *M. arborea* leaves [45].

Aglycone	3 OH substituted	28 COOH substituted
2*β*-Hydroxy oleanolic acid	*α*-L-Rha(1→2)-*α*-L-Ara(1→2)-*β*-D-Glc	-
Bayogenin	*β*-D-GlcA	*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara
2*β*,3*β*-Dihydroxy-23-oxo-olean-28-oic acid	*β*-D-GlcA	*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara
Medicagenic acid	*β*-D-Glc	*α*-L-Rha(1→2)-*α*-L-Ara
Medicagenic acid	*β*-D-GlcA	*α*-L-Rha(1→2)-*α*-L-Ara
Medicagenic acid	*β*-D-Glc	*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara
Medicagenic acid	*β*-D-GlcA	*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara
Medicagenic acid	*β*-D-Glc(1→2)-*β*-D-Glc	*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara
Medicagenic acid	*β*-D-GlcA	*β*-D-Api(1→3)-[*β*-D-Xyl(1→4)]-*α*-L-Rha(1→2)-*α*-L-Ara
Zanhic acid	*β*-D-Glc	*α*-L-Ara(1→3)-*α*-L-Rha(1→2)-*α*-L-Ara
Zanhic acid	*β*-D-GlcA	*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara
Zanhic acid	*β*-D-Glc	*α*-L-Ara(1→3)-[*β*-D-Xyl(1→4)]-*α*-L-Rha(1→2)-*α*-L-Ara
Zanhic acid	*β*-D-GlcA	*β*-D-Api(1→3)-[*β*-D-Xyl(1→4)]-*α*-L-Rha(1→2)-*α*-L-Ara
Zanhic acid	*β*-D-GlcA	*α*-L-Ara(1→3)-[*β*-D-Xyl(1→4)]-*α*-L-Rha(1→2)-*α*-L-Ara
Zanhic acid	*β*-D-Glc(1→2)-*β*-D-Glc	*α*-L-Ara(1→3)-[*β*-D-Xyl(1→4)]-*α*-L-Rha(1→2)-*α*-L-Ara
Zanhic acid	*α*-L-Ara(1→2)-*β*-D-Glc(1→2)-*β*-D-Glc	*β*-D-Api(1→3)-[*β*-D-Xyl(1→4)]-*α*-L-Rha(1→2)-*α*-L-Ara
Soyasapogenol A	*α*-L-Rha(1→2)-*β*-D-Gal(1→2)-*β*-D-GlcA	*α*-L-Rha
Soyasapogenol B	*α*-L-Rha(1→2)-*β*-D-Gal(1→2)-*β*-D-GlcA	-

Table 3: Saponins identified in *M. hybrida* roots [46].

Aglycone	3 OH substituted	28 COOH substituted
Oleanolic acid	*β*-D-Gal(1→2)-*β*-D-GlcA	*β*-D-Glc
Oleanolic acid	*β*-D-Gal(1→2)-*β*-D-GlcA	*α*-L-Rha(1→4)-*β*-D-Glc
Hederagenin	*β*-D-Glc	-
Hederagenin	*β*-D-GlcAMe	-
Hederagenin	*β*-D-Glc(1→2)-*α*-L-Ara	-
Hederagenin	*β*-D-GlcA	*β*-D-Glc
Hederagenin	*β*-D-GlcAMe	*β*-D-Glc
Hederagenin	*α*-L-Rha(1→2)-*β*-D-Glc(1→2)-*β*-D-Glc	*β*-D-Glc
Bayogenin	*β*-D-Glc	*β*-D-Glc
2*β*,3*β*-Dihydroxy-23-oxo-olean-12-en-28-oic acid	*β*-D-GlcA	*β*-D-Glc
Medicagenic acid	*β*-D-Glc	-
Medicagenic acid	*β*-D-Glc	*β*-D-Glc
Medicagenic acid	*β*-D-GlcA	*β*-D-Glc
Medicagenic acid	*β*-D-Glc(1→2)-*β*-D-Glc	*β*-D-Glc

Table 4: Saponins identified in *M. lupulina* [28].

Aglycone	3 OH substituted	28 COOH substituted
Hederagenin	*β*-D-Glc	-
Medicagenic acid	*β*-D-Glc	-
Medicagenic acid	*β*-D-Glc	*β*-D-Glc
Soyasapogenol B	*α*-L-Rha(1→2)-*β*-D-Gal(1→2)-*β*-D-GlcA	-

Table 5: Saponins identified in *M. polymorpha.*

Aglycone	3 OH substituted	28 COOH substituted	Ref.
Oleanolic acid	*α*-L-Rha(1→2)-*α*-L-Ara	*β*-D-Glc-(1→6)-*β*-D-Glc	[38]
Hederagenin	*α*-L-Rha(1→2)-*α*-L-Ara	-	[38]
Hederagenin	*α*-L-Ara	*β*-D-Glc-(1→6)-*β*-D-Glc	[38]
Hederagenin	*α*-L-Rha(1→2)-*α*-L-Ara	*β*-D-Glc	[38]
Hederagenin	*α*-L-Rha(1→2)-*α*-L-Ara	*β*-D-Glc-(1→6)-*β*-D-Glc	[38]
Caulophyllogenin	*α*-L-Rha(1→2)-*α*-L-Ara	*β*-D-Glc	[38]
Caulophyllogenin	*α*-L-Rha(1→2)-*α*-L-Ara	*β*-D-Glc-(1→6)-*β*-D-Glc	[38]
Soyasapogenol B	*α*-L-Rha(1→2)-*β*-D-Glc(1→2)-*β*-D-GlcA	-	[33]

Branched sugar chain saponins were identified in this species, as in *M. arborea* and *M. sativa.* Methyl ester derivative of saponins were also found in *M. hybrida* and *M. sativa*, but these were recognized as artifacts obtained during the extraction with methanol [74].

The nature of the saccharide units, their position on the molecule and the similarity of the sugar chains on saponins from the different species, have suggested high enzymatic selectivity for the sugar position. Hederagenin often contains an *α*-L-arabinopyranose unit as the first sugar in its 3-*O* position. Alternatively a *β*-D-glucopyranose or the corresponding uronic derivative are present as in *M. hybrida* root saponins. The second monosaccharide unit linked at the C-2 position of *α*-L-arabinopyranose can be *α*-L-rhamnopyranose, as in *M. polymorpha*, or *β*-D-glucopyranose as in *M. arabica* and *M. sativa*.

By contrast, in all the studied species of *Medicago*, saponins of medicagenic and zanhic acids are always characterized by the presence of *β*-D-glucopyranose or *β*-D-glucuronopyranose units as the first sugar in the 3-*O* position. The second monosaccharide *β*-D-glucopyranose, linked predominantly at the C-2 position, as in *M. arborea*, *M. hybrida* and *M. sativa*, or at the C-3 position, as in *M. truncatula* suggesting the presence of a specific glucosyltransferase in this species. Different sugar linkage positions were also detected in the 3-*O* disaccharide chain of *M. sativa*, in which the 1→3 and 1→4 linkage between the first and the second monosaccharide were found. Trisaccharides are predominantly 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl derivatives.

The C-28 glycosylated saponins showed the presence of the *β*-D-glucopyranose unit esterified at the carboxylic group, the disaccharide chain 28-*O*-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside (only found in *M. polymorpha*), and chains with more than two sugars, always characterized by *α*-L-arabinopyranose, directly linked at the C-28, and an *α*-L-rhamnopyranose in the central position, linked (1→4) with a *β*-D-xylopyranose. Branching points are formed by *α*-L-arabinopyranose or *β*-D-apiofuranose linked (1→3) at the *β*-D-xylopyranose unit. These features are typical of saponins extracted from *M. arborea*, *M. sativa* and *M. truncatula* and suggest high enzymatic selectivity for the sugar position independent of the involved genin.

Table 6. Saponins identified in *M. sativa* leaves and roots.

Aglycone	3 OH substituted	28 COOH substituted	Ref.
Hederagenin	*β*-D-Glc(1→2)-*α*-L-Ara	-	[13]
Hederagenin	*β*-D-Glc(1→2)-*α*-L-Ara	*β*-D-Glc	[37]
Hederagenin	*β*-D-Glc(1→2)-*α*-L-Ara	*β*-D-Glc	[27]
Hederagenin	*β*-D-Glc(1→3)-*β*-D-Xyl	*β*-D-Glc	[37]
Hederagenin	*α*-L-Ara(1→2)-*β*-D-Glc(1→2)-*α*-L-Ara	-	[23]
Hederagenin	*α*-L-Ara(1→2)-*β*-D-Glc(1→2)-*α*-L-Ara	*β*-D-Glc	[24]
Bayogenin	*β*-D-Gal(1→2)-*β*-D-GlcA	*β*-D-Glc	[39]
Medicagenic acid	-	*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara	[34]
Medicagenic acid	*β*-D-Glc	-	[19]
Medicagenic acid	*β*-D-GlcA	-	[31]
Medicagenic acid	*β*-D-Glc	*β*-D-Glc	[22]
Medicagenic acid	*β*-D-Glc	*α*-L-Rha(1→2)-*α*-L-Ara	[30]
Medicagenic acid	*β*-D-GlcA	*α*-L-Rha(1→2)-*α*-L-Ara	[36]
Medicagenic acid	*β*-D-Glc	*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara	[25]
Medicagenic acid	*β*-D-GlcA	*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara	[31]
Medicagenic acid	*β*-D-GlcA Me ester	*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara	[39]
Medicagenic acid	*α*-D-Glc(1→4)-*β*-D-Glc	-	[29]
Medicagenic acid	*β*-D-Glc(1→3)-*β*-D-Glc	*β*-D-Glc	[39]
Medicagenic acid	*β*-D-Gal(1→2)-*β*-D-Glc	*β*-D-Glc	[27]
Medicagenic acid	*β*-D-Glc(1→2)-*β*-D-Glc	*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara	[27]
Medicagenic acid	*α*-L-Rha(1→6)-*β*-D-GlcA(1→2)-*β*-D-Glc	-	[20]
Medicagenic acid	*β*-D-Glc(1→6)-*β*-D-Glc(1→3)-*β*-D-Glc	-	[21]
Medicagenic acid	*α*-L-Rha(1→2)-*β*-D-Glc(1→2)-*β*-D-Glc	-	[39]
Medicagenic acid	*α*-L-Rha(1→2)-*β*-D-Glc(1→2)-*β*-D-Glc	*β*-D-Glc	[27]
Medicagenic acid	*β*-D-Glc(1→2)-*β*-D-Glc(1→2)-*β*-D-Glc	*β*-D-Glc	[39]
Medicagenic acid	*β*-D-Glc(1→2)-*β*-D-Glc(1→2)-*β*-D-Glc	*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara	[39]
Medicagenic acid	*β*-D-Glc(1→2)-*β*-D-Glc(1→2)-*β*-D-Glc	*β*-D-Api(1→3)-[*β*-D-Xyl(1→4)]-*α*-L-Rha(1→2)-*α*-L-Ara	[39]
Medicagenic acid	*β*-D-Glc(1→2)-[*α*-L-Rha(1→3)]-*β*-D-Glc (1→2)-*β*-D-Glc	*β*-D-Glc	[32]
Medicagenic acid	Glc-malonyl	-	[40]
Medicagenic acid	Glc-malonyl	Glc	[40]
Zanhic acid	*β*-D-Glc(1→2)-*β*-D-Glc(1→2)-*β*-D-Glc	*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara	[39]
Zanhic acid	*β*-D-Glc(1→2)-*β*-D-Glc(1→2)-*β*-D-Glc	*β*-D-Api(1→3)-[*β*-D-Xyl(1→4)]-*α*-L-Rha(1→2)-*α*-L-Ara	[39]
Zanhic acid	*β*-D-Glc(1→2)-*β*-D-Glc(1→2)-*β*-D-Glc	*β*-D-Api(1→3)-*β*-D-Xyl(1→4)-*α*-L-Rha 23 COOH substituted: *α*-L-Ara	[36]
Zanhic acid	*β*-D-Glc(1→2)-*β*-D-Glc(1→2)-*β*-D-Glc	*β*-D-Api(1→3)-*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara 23 COOH substituted: *α*-L-Ara	[36]
Soyasapogenol A	*α*-L-Rha(1→2)-*β*-D-Gal(1→2)-*β*-D-GlcA	*α*-L-Rha	[39]
Soyasapogenol B	*β*-D-Glc(1→2)-*β*-D-GlcA	-	[26]
Soyasapogenol B	*α*-L-Rha(1→2)-*β*-D-Glu(1→2)-*β*-D-GlcA	-	[26]
Soyasapogenol B	*α*-L-Rha(1→2)-*β*-D-Gal(1→2)-*β*-D-GlcA	-	[26]
Soyasapogenol E	*α*-L-Rha(1→2)-*β*-D-Gal(1→2)-*β*-D-GlcA	-	[26]
Soyasapogenol E	*α*-L-Rha(1→2)-*β*-D-Gal(1→2)-*β*-D-GlcA	22-*O*-maltol	[35]

A very interesting structural feature of these substances, is the presence of an aldehyde group at the C-23 position in 2*β*,3*β*-dihydroxy-23-oxo-olean-12-en-28-oic acid (Figure 1), a new aglycone of saponins from *M. arborea* and *M. hybrida*. This metabolite might in fact represent an interesting biosynthetic intermediate in the oxidative steps that lead from a methyl group to the corresponding carboxylic acid [1, 75]. That is, if we consider the following genins found in the genus *Medicago*: 2*β*-hydroxyoleanolic acid, bayogenin, 2*β*,3*β*-dihydroxy-23-oxo-olean-12-en-28-oic acid and medicagenic

Table 7: Saponins identified in *M. truncatula* leaves and roots.

Aglycone	3 OH substituted	28 COOH substituted	Ref.
Hederagenin	GlcA	-	[40]
Hederagenin	Glc-Ara	Glc	[40]
Medicagenic acid	Glc	-	[40]
Medicagenic acid	Glc-malonyl	-	[40]
Medicagenic acid	Glc-Glc	-	[40]
Medicagenic acid	Glc	Glc	[40]
Medicagenic acid	Glc-malonyl	Glc	[40]
Medicagenic acid	*β*-GlcA	*β*-Glc	[44]
Medicagenic acid	*β*-Glc	*β*-Xyl(1→4)-*α*-Rha(1→2)-*α*-Ara	[42, 44]
Medicagenic acid	*β*-GlcA	*β*-Xyl(1→4)-*α*-Rha(1→2)-*α*-Ara	[42, 44]
Medicagenic acid	*β*-Glc(1→3)-*β*-Glc	*α*-Rha(1→2)-*α*-Ara	[44]
Medicagenic acid	*β*-Glc(1→3)-*β*-Glc	*β*-Xyl(1→4)-*α*-Rha(1→2)-*α*-Ara	[44]
Medicagenic acid	*β*-Glc(1→3)-*β*-Glc	*α*-Ara(1→3)-[*β*-Xyl(1→4)]-*α*-Rha(1→2)-*α*-Ara	[44]
Medicagenic acid	*β*-Glc(1→3)-*β*-Glc	*β*-Api-(1→3)-[*β*-Xyl(1→4)]-*α*-Rha(1→2)-*α*-Ara	[44]
Zanhic acid	*β*-Glc	*β*-Xyl(1→4)-*α*-Rha(1→2)-*α*-Ara	[44]
Zanhic acid	*β*-Glc(1→3)-*β*-Glc	*α*-Rha(1→2)-*α*-Ara	[44]
Zanhic acid	*β*-Glc(1→3)-*β*-Glc	*α*-Rha[4-Ac](1→2)-*α*-Ara	[44]
Zanhic acid	*β*-Glc(1→3)-*β*-Glc	*β*-Xyl(1→4)-*α*-Rha(1→2)-*α*-Ara	[44]
Zanhic acid	*β*-Glc(1→3)-*β*-Glc	*α*-Ara(1→3)-*α*-Rha(1→2)-*α*-Ara	[44]
Zanhic acid	*β*-Glc(1→3)-*β*-Glc	*β*-Api(1→3)-*α*-Rha(1→2)-*α*-Ara	[44]
Zanhic acid	*β*-Glc(1→3)-*β*-Glc	*α*-Ara(1→3)-[*β*-Xyl(1→4)]-*α*-Rha(1→2)-*α*-Ara	[44]
Zanhic acid	*β*-Glc(1→3)-*β*-Glc	*β*-Api-(1→3)-[*β*-Xyl(1→4)]-*α*-Rha(1→2)-*α*-Ara	[44]
Zanhic acid	Glc-Glc-Glc	Xyl-Rha-Ara, 23 COOH substituted: Ara	[42]
Zanhic acid	Glc-Glc-Glc	Api-Xyl-Rha-Ara, 23 COOH substituted: Ara	[42]
Soyasapogenol B	*α*-Rha(1→2)-*β*-Gal(1→2)-*β*-GlcA	-	[40, 43]
Soyasapogenol B	*α*-Rha(1→2)-*β*-Xyl(1→2)-*β*-GlcA	-	[43]
Soyasapogenol E	*α*-Rha(1→2)-*β*-Gal(1→2)-*β*-GlcA	-	[40, 43]

acid, all the oxidative products at C-23 can be observed. The above genins all possess the same stereochemistry (2β,3β*)* in the hydroxylated triterpene carbons with the different functional groups at the C-23 position. The presence of an aldehyde group in 2*β*,3*β*-dihydroxy-23-oxo-olean-12-en-28-oic acid, identified for the first time in *Medicago* spp, indicates a possible biosynthetic pathway for the sapogenins of this genus. Accordingly, medicagenic acid may originate from bayogenin by subsequent oxidative enzymatic steps involving the formation of 2*β*,3*β*-dihydroxy-23-oxo-olean-12-en-28-oic acid while bayogenin may originate by a selective oxidative demethylation at C-23 from 2*β*-hydroxyoleanolic acid. In a similar way, the two 16*α*-hydroxy triterpenes found in this genus, caulophyllogenin and zanhic acid (Figure 1) probably originate by enzymatic oxidation of hederagenin and medicagenic acid, respectively. The biosynthesis of these compounds in the genus *Medicago* has never been extensively investigated, and only a few papers have been published [76-78].

Saponin extracts as well as purified saponins from selected species of *Medicago* have different biological properties [10-14]. Their antimicrobial, insecticidal, allelopathic and cytotoxic effects are described below. The influence of saponins on animal metabolism is also reviewed.

Antimicrobial activity

Saponins are likely to be implicated in plant defense mechanisms against microbial or fungal infections. In some plants wounding of tissues in response to a pathogenic attack causes the hydrolysis of saponins to derivatives with strong antibiotic activity [79, 80]. On the other hand, resistance to infestation by certain fungi in plants such as oat is associated with the specific presence of saponins (e.g. avenacins). Nevertheless, antifungal efficacy of saponins has been demonstrated *in vitro* for a number of plant species [2, 7, 79, 81] but little data is available on their antibacterial activity [2].

A compilation of microorganisms used to assess antifungal and antibacterial activity of saponins from *Medicago* spp. is reported in Table 8. Data derive from incubation of *Medicago* dry meals, saponin extracts and purified saponins from different species and plant organs.

Antifungal efficacy of *Medicago* has been primarily studied with the model fungus *Trichoderma viride* [10, 29, 31, 63, 65, 82-85] which appeared particularly sensitive to the presence of saponins in the growth medium. A bioassay to determine the content of saponins in the plant was developed [58] based on saponin toxicity towards this fungus. Growth of *T. viride* was in fact found inversely correlated with the amount of *Medicago* saponins in the incubation medium thus representing a useful index to evaluate the total percentage of these metabolites.

As described (Table 8), saponins from *Medicago* have been assayed *in vitro* against phytopathogenic species and their activity well established not only against specific pathogens of *Medicago*, but also against some fungi generally pathogenic to cereals [10, 14, 28, 31, 65, 85-95]. A higher antifungal activity was found for the saponins from the roots than from the aerial parts of *M. sativa* [92, 93, 95]. Furthermore, assays with purified saponins from the same species [93] indicated that the growth of the two pathogens *Botrytis tulipae* and *Phloma narcissi* was mostly affected by the following compounds: medicagenic acid; 3-*O*-*β*-D-glucopyranosyl-medicagenic acid; 3-*O*-*β*-D-glucopyranosyl-28-*O*-[*β*-D-xylopyranosyl(1→4)-*α*-L-rhamopyranosyl(1→2)-*α*-L-arabinopyranoside] medicagenic acid and 3-*O*-*β*-D-glucuronopyranosyl- 28-*O*-[*β*-D-xylopyranosyl (1→4)-*α*-L-rhamopyranosyl (1→2)-*α*-L-arabino-pyranoside]medicagenic acid. The screening of saponin extracts from several *Medicago* spp. has shown that *M. arabica* possesses antifungal efficacy several times higher than that of *M. sativa* [95] and the most sensitive pathogens were *Rhizoctonia solani*, *B. tulipae*, *P. narcissi*, *Fusarium oxysporium* ssp. *tulipae* and *Pestalotia* ssp.

Besides their phytopathogenic potential the antimicrobial activity of saponins from *Medicago* against human pathogens has also been investigated [14, 96-104]. Preliminary studies have concerned the effect against some yeasts and dermatophytes of a gluco derivative of medicagenic acid named G2 and later identified as 3-*O*-*β*-D-glucopyranosyl-medicagenic acid. The compound was found to be particularly effective against *Cryptococcus neoformans* with an MFC of 4 μg/mL [2, 7, 79, 80]. More recent investigations [104] on dermathophytes have shown that *Trichophyton interdigitale* and *Microsporium gypseum* were susceptible to *Medicago* saponins especially to glycosides of medicagenic acid, such as 3-*O*-*β*-glucopyranoside (MIC < 62.5 μg/mL) the most bioactive phytochemical.

The study of the antifungal activity of saponins from *M. sativa*, *M. arborea* and *M. arabica* against a selection of medically important yeasts (*Candida albicans*, *C. tropicalis*, *Saccharomyces cerevisiae*, *Cryptococcus laurentii* and *Blastomyces capitatus*) [103] has shown that *S. cerevisiae* was the most susceptible, being highly inhibited when treated with the sapogenin mixtures from the aerial parts of the three different species of *Medicago* (MICs of 125, 62.5 and 175 μg/mL for *M. sativa*, *M. arabica* and *M. arborea*, respectively). A very low MIC value (42.5 μg/mL) was observed when the same strain was treated with medicagenic acid, which represents the dominant aglycone found in *M. sativa* (50%) and *M. arborea* (30%) aerial organs. Medicagenic acid also inhibited the two mycetes *C. tropicalis* and *B. capitatus*, with an MIC of 125 μg/mL.

Although strongly antifungal, saponins are reported to have only weak or no growth inhibitorial effects against bacteria [80]. To the best of our knowledge only one investigation has been carried out to evaluate the antibacterial activity of saponins from *Medicago* species and they were found not very active (MICs > 500 μg/mL) against Gram negative bacteria [103]. Nevertheless, they displayed some efficacy against selected Gram positive bacteria [103]. In particular, sapogenins obtained on acid hydrolysis of saponins from *M. arabica* aerial parts and roots were the most effective, showing good growth inhibitorial activity towards three different strains of *S. aureus*, two strains of *E. faecalis*, and against *B. subtilis* and *B. cereus* (Table 8). *In vitro* antibacterial assays with purified aglycones from *Medicago* saponins showed that medicagenic acid had significant activity against *S. aureus* (MIC 52.5 μg/mL) and two strains of *E. faecalis* (MICs 50 and 32.5 μg/mL)

The *in vitro* effects of some saponins from *M. sativa* on rhizosphere bacteria suspension, showed that 3-*O*-*β*-D-glucopyranosylmedicagenic acid sodium salt

Table 8: Overview of antimicrobial studies with saponins from *Medicago* spp.

Saponin source	Microorganisms	Ref.
	a) Phytopathogenic fungi	
M. sativa leaves Total saponins	*Fusarium oxysporum, F. solani, Phytophtora drechsleri* *Phoma sp,. Rhizoctonia solani, Verticillium albo-atrum*	[65]
M. sativa tops *M. sativa* roots Total saponins	*Alternaria solani* *Pytium myriotylum* *P. butleri, P. sp. PRL2142, Sclerotium rolfsii*	[86, 87]
M. sativa roots Total extract Total saponins Saponin sugars Sapogenins	*Rhizoctonia solani*	[88]
M. sativa roots 3-*O*-*β*-D-Glc Medicagenic acid	*Aspergillus niger,* *Fusarium oxysporum* sp. *Lycopersici, Phytophtora cinnamommi, Rhizopus mucco,Sclerotium rolfsii*	[82]
M. sativa roots 3-*O*-[*α*-D-Glc(1→4)-*β*-D-Glc] Medicagenic acid (Medicagenic acid *β*-maltoside)	*Aspergillus niger* *Fusarium oxysporum* sp. *Lycopersici, Phytium aphanidermatum, Rhizoctonia solani, Sclerotium rolfsii*	[29]
M. sativa roots Compound G2 (3-*O*-*β*-D-Glc Medicagenic acid)	*Aspergillus niger* *Fusarium oxysporum, Geotrichum candidum* *Phytium aphanidermatum,Phytophtora cinnamommi* *Rhizoctonia solani, Rhizopus mucco, Sclerotium rolfsii*	[14]
Meal from aerial parts of: *M. arabica, M. doliata, M. heyniana, M. murex, M. sativa* Total saponins from aerial parts of: *M. arabica, M. heyniana, M. murex, M. polymorpha, M. sativa* *M. sativa* roots Total saponins Total prosapogenins 3-*O*-*β*-D-Glc Medicagenic acid Medicagenic acid	*Cephalosporium gramineum*	[89, 91, 94]
Meal from aerial parts of: *M. arabica, M. doliata, M. heyniana, M. murex, M. sativa* *M. sativa* roots Total saponins Total prosapogenins 3-*O*-*β*-D-Glc Medicagenic acid Medicagenic acid	*Gaeumannomyces graminis* v. *tritici*	[90, 91]
M. sativa aerial parts *M. sativa* roots Total saponins	*Alternaria zinniae,* *Botrytis cinerea, B. tulipae,* *Phoma narcissi, P. poolensis, Rhizoctonia solani*	[92]
M. sativa leaves and roots 3-*O*-[*α*-L-Ara(1→2)-*β*-D-Glc(1→2)-*α*-L-Ara]-28-*O*-*β*-D-Glc Hederagenin 3-*O*-*β*-D-Glc Medicagenic acid 3-*O*-*β*-D-Glc-28-*O*-*β*-D-Glc Medicagenic acid 3-*O*-*β*-D-Glc-28-*O*-[*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara] Medicagenic acid 3-*O*-*β*-D-GlcAc-28-*O*-[*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara] Medicagenic acid 3-*O*-[*β*-D-Glc(1→2)-*β*-D-Glc(1→2)-*β*-D-Glc]-23-*α*-L-Ara-28-*O*-[*β*-D-Api(1→3)-*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara] Zanhic acid Soyasaponin I Hederagenin, Medicagenic acid, Soyasapogenol B	*Botrytis tulipae* *Phoma narcissi*	[93]

Table 8 (*Contd.*)

M. arabica shoots Total saponins	*Alternaria tenui, Botrytis cinerea, B. tulipae, Fusarium oxysporium* sp. *Callistephi, F. oxysporium* sp. *Narcissi, F. oxysporium* sp. *Tulipae, Pestalotia ssp., Phoma narcissi P. poolensis, Pythium ultimum, Rhizoctonia solani, Stangospora curtisii*	[95]
	b) Human pathogenic fungi	
M. sativa Compound G2 (3-*O*-*β*-D-Glc Medicagenic acid)	*Candida albicans,C. guilliermondii, C. krusei, C. parapsilopsis, C. pseudotropicalis, C. tropicalis Cryptococcus neoformans, Epidermophyton floccosum Geotrichum candidum, Microsporium canis Rhodotorula glutinis, Torulopsis candida, T. glabrata Trycophyton mentagrophytes, T. mentagrophytes* var. *granulare, Trichopyton rubrum,T. tonsurans*	[14, 96-100]
3-*O*-*β*-D-Glc Medicagenic acid	*Scopulariopsis brevicaulis, Trycophyton mentagrophytes*	[101, 102]
M. arabica tops Total saponins, Sapogenins, Bayogenin	*Blastomyces capitatus, Candida albicans, C. tropicalis Cryptococcus laurentii, Saccharomyces cerevisiae*	[103]
M. arabica roots Total saponins, Sapogenins		
M. arborea tops Total saponins, Prosapogenins, Sapogenins		
M. sativa tops Total saponins, Prosapogenins, Sapogenins Medicagenic acid, Hederagenin		
M. sativa roots Total saponins, Sapogenins		
Medicago sp. 3-*O*-*α*-L-Ara-Hederagenin 3-*O*-[*α*-L-Ara(1→2)-*β*-D-Glc(1→2)-*α*-L-Ara]-Hederagenin 3-*O*-*β*-D-Glc-Medicagenic acid 3-*O*-*β*-D-Glc-28-*O*-*β*-D-Glc-Medicagenic acid 3-*O*-*β*-D-GlcAc-28-*O*-*β*-D-Glc-Medicagenic acid 3-*O*-[*β*-D-Glc(1→2)-*β*-D-Glc]-28-*O*-*β*-D-Glu-Medicagenic acid 3-*O*-*β*-D-Glc-28-*O*-[*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara]-Medicagenic acid 3-*O*-*β*-D-GlcAc-28-*O*-[*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara]-Medicagenic acid 3-*O*-[*β*-D-Glc(1→2)-*β*-D-Glc]-28-*O*-[*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara]- Medicagenic acid 3-*O*-[*β*-D-Glc(1→2)-*β*-D-Glc(1→2)-*β*-D-Glc]-28-*O*-[*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara]-Zanhic acid Soyasaponin I Hederagenin, Medicagenic acid	*Micropsorum gypseum, Trichophyton interdigitale*	[104]
	c) Bacteria	
M. arabica tops Total saponins, Sapogenins, Bayogenin	*Acinebacter baumanii, Bacillus subtilis, B. cereus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*	[103]
M. arabica roots Total saponins, Sapogenins, Hederagenin		
M. sativa roots Total saponins, Sapogenins		
M. arborea tops Total saponins, Prosapogenins, Sapogenins		
M. sativa tops Total saponins, Prosapogenins, Sapogenins, Medicagenic acid		
	d) Soil Bacteria	
M. sativa 3-*O*-*β*-D-Glc-28-*O*-*β*-D-Glc Medicagenic acid Soyasaponin I 3-*O*-*β*-D-Glc Medicagenic acid Na^+ salt Medicagenic acid Na^+ salt	*Agrobacterium tumefaciens, Bacillus thuringensis Curtobacterium flacumafaciens, Pseudomonas fluorescens*	[105]

Table 8 (*Contd.*)

	e) Others	
Total saponins from aerial parts of: *M. aculeata, M. arabica,M. blancheana, M. carstiensis* ***M. ciliaris, M. coerulea, M. coronata , M. disciformis*** *M. doliata, M. falcata,M. glutinosa, M. granadensis* *M. hemicycla, M. heyniana, M. hybrida, M. intertexta* *M. laciniata, M. lupulina, M. minima, M. murex* *M. muricoleptis, M. noeana,M. orbicularis, M. polyceratia* *M. polymorpha, M. praecox, M. radiata, M. rigidula* *M. rotata , M. rugosa, M. sativa , M. sauvagei , M. scutellata* *M. soleirolii, M. tornata, M. turbinata, M. truncatula*	*Trichoderma viride*	[10, 29, 31, 51, 63, 65, 82-85]
M. sativa roots Total saponins 3-*O*-[*α*-L-Ara(1→2)-*β*-D-Glc(1→2)-*α*-L-Ara] Hederagenin 3-*O*-*β*-D-Glc Medicagenic acid 3-*O*-*β*-D-GlcAc Medicagenic acid 3-*O*-[*α*-D-Glc-(1→4)-*β*-D-Glc] Medicagenic acid (Medicagenic acid *β*-maltoside) 3-*O*-*β*-D-Glc-28-*O*-*β*-D-Glc-Medicagenic acid 3-*O*-*β*-D-Glc-28-*O*-[*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara] Medicagenic acid 3-*O*-*β*-D-GlcAc-28-*O*-[*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara] Medicagenic acid 3-*O*-[*β*-D-Glc(1→2)-*β*-D-Glc]-28-*O*-[*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara] Medicagenic acid		
M. lupulina roots 3-*O*-*β*-D-Glc Medicagenic acid 3-*O*-*β*-D-Glc-28-*O*-*β*-D-Glc Medicagenic acid Medicagenic acid		

could negatively affect them and, could negatively affect them and, in most cases, its activity corresponded to that of the corresponding aglycone, medicagenic acid disodium salt [105].

Investigations on the structure-activity relationships of *Medicago* saponins have led to contrasting results [11, 86, 106]. The number, kind and sequence of the sugar residues in the molecules have been differently correlated with their antimicrobial effects. A detailed study on the activity of different saponins from alfalfa roots against *T. viride* indicated that the monodesmoside derivatives of medicagenic acid were more active than the related bidesmosides, even though no straight correlation between the number of sugars in the molecule and its bioactivity could be established [31]. Moreover, the antifungal activity of medicagenic acid and its derivatives were reported as dependent on the presence of functional groups, such as carboxy and hydroxy in the molecule. In some studies, reduction of bioactivity was related to the presence of a sugar moiety at the 28-*O*-position of the saponin [11, 86, 106].

Bioassays with saponins from *Medicago* sp. against human pathogenic fungi and bacteria indicated that the sugar moieties are not required for antimicrobial activity [103]. This study proved that sapogenins were more active than the related prosapogenins and saponins.

Insecticidal activity

Toxicity of saponins to insects is known, and it has been suggested that they might also provide plant protection from insect predation [107]. To support this hypothesis, the herbivore-induced response of alfalfa was recently examined through assays with *Spodoptera littoralis* larvae, and it was observed that the levels of total saponins increases in the young foliage of damaged plants [108].

Several works on the insecticidal and antifeedant properties of saponins against several classes of insects have been published [109, 110]. A list of saponin source and related insects and pests on which they have been tested, is reported in Table 9. Saponins from alfalfa roots and shoots were reported to be active against the peach aphid (*Myzus persicae*) [109], and found to be toxic to the larvae of the grass grub (*Costelytra zealandica*) [111]. Several species of locusts have shown increasing mortality when fed

on alfalfa; their larvae developed more slowly and the emerging adults were smaller than when they were fed saponin-free herbage [109]. Alfalfa root saponins, rich in medicagenic acid, are toxic to the flour beetle (*Tribolium castraneum*) and their toxicity increased when some of the sugars were removed by hydrolysis [109]. On the contrary, it has been described that several alfalfa pests, such as alfalfa weevil (*Hypera postica*), spotted aphid (*Tereoaphid maculata*), clover root curculio (*Stona hispidulus*), and seed chalcid (*Bruchophagus roddi*) are hardly affected by a saponin-rich diet, suggesting that they have evolved strategies to overcome the toxicity of the saponins of the plant on which they prey [109].

Crude mixtures and purified saponins from alfalfa leaves were tested against potato leafhopper (*Empoasca fabae* Harris) and pea aphid (*Acyrthosiphon pisum* Harris). Larvae were fed with a diet containing 0.01-5.0% saponins for a few days. An increase of mortality was observed for all the tested organisms, in particular for those fed on saponins containing medicagenic acid [112].

Saponins extracted from the leaves of 41 alfalfa varieties, with a different content of saponins and sapogenins, were assayed *in vitro* against larvae of the yellow mealworm (*Tenebrio molitor* L.). Results showed a good correlation between larvae mortality and saponin concentration so this biological assay was proposed to detect alfalfa saponins in plant material [66, 113].

Alfalfa saponin mixtures also have been tested against the summer fruit tortrix moth (*Adoxophyes orana* F.v.R.), the European grape moth (*Lobesia botrana* Den. & Schiff.) and the European corner borer (*Ostrinia nubilalis* Hb.). The increasing amount of saponins added to the diet (from 1 to 1000 ppm) increased larval mortality from 11.3% at 1 ppm to 46.1% at 1000 ppm. The contact effect accounted for a maximum of 22.7% mortality. No appreciable differences were detected in the insecticidal activity exerted by crude saponins derived from alfalfa leaves and roots [12].

Saponins isolated from the aerial parts of alfalfa were tested against the Colorado potato beetle (*Leptinotarsa decemlineata* Say). Larvae were fed on potato leaves sprayed with 0.5 and 1% saponin solutions; no repellent effects were observed for any of the tested compounds, but insect feeding proved to be less intense on saponin-treated leaves. The larvae fed on saponin treated leaves had the lowest body weight gain, suggesting the antifeedant activity of the compounds. The insect mortality from eating saponin-treated leaves was 100% at both tested concentrations [114]. Other experiments showed that the larvae of Colorado potato beetles reared on potato leaves treated with a 0.5% solution of total saponins from *M. sativa* roots and tops, died after 4-6 days because of fasting. Lower saponin doses (from 0.1 to 0.001%) reduced the insect feeding less causing an inhibition in growth and an extension of the larval stage. Mortality was reached at a level of 76.7-100%. No evident differences have been found in saponin activity from the tops or the roots of alfalfa [115].

The Colorado potato beetle was also used to differentiate insecticidal activity of saponins from *M. arabica*, *M. hybrida* and *M. murex* roots and tops. Total saponins were included in the insect diet as a solution applied on potato leaves on which larvae were reared. All saponins reduced larval feeding, growth rate and mortality in a dose dependant manner. All the saponins showed a high insecticidal activity at the concentration of 0.5%. Saponins from *M. murex* roots and from *M. arabica* and *M. hybrida* aerial parts were found to be the most active, probably due to the differences in their saponin composition [116].

Crude alfalfa root saponins, their prosapogenins produced by alkaline hydrolysis, and medicagenic acid sodium salt, were tested in field trials against spider mite (*Tetranychus urticae* Koch.) and hop aphid (*Phoron humuli* Schrank). Plants were sprayed with a 0.1 and 0.2% solution of saponin products. Prosapogenins were the most active against both phytophages, while crude saponins and medicagenic acid sodium salt were less active [117].

Dried alfalfa leaf and root tissues incorporated in an artificial diet to give the final saponin concentration of 0.1, 0.5 or 1.6% mg/g fresh weight, a cholesterol-precipitable saponin fraction from the plant leaves and a total saponin mixture from the roots were used to evaluate their toxic potential against the polyphagus insect european corn borer. The growth and development of larvae were significantly inhibited after feeding. Root saponins were somewhat more harmful than saponins from the tops [118].

Total saponins from *M. sativa* roots and leaves and individual saponins and sapogenins were tested on a polyphagous pest, the army-worm *Spodoptera*

Table 9: List of pests and insects used to evaluate the insecticidal activity of saponins from the *Medicago* spp.

Saponin source	Insect	Ref.
M. sativa tops Total saponins	Grass grub (*Costelytra zealandica*)	[111]
	Pea aphid (*Acyrthosiphon pisum* Harris) Potato leafhopper (*Empoasca fabae* Harris)	[112]
	Yellow mealworm (*Tenebrio molitor*)	[66, 113]
	Colorado potato beetle (*Leptinotarsa decemlineata* Say)	[114]
M. sativa tops *M. sativa* roots Total saponins	Alfalfa weevie (*Hypera postica*) Clover root curculio (*Stona hispidulus*) Flour beetle (*Tribolium castraneum*) Peach aphid (*Myrus persicae*) Seed chalacid (*Bruchophagus rodoli*) Spotted aphid (*Thereoaphid maculata*) Several species of locusts	[109]
	European corner borer (*Ostrinia nubilalis* Hb.) European grape moth (*Lobesia botrana* Den. & Schiff.) Summer fruit tortrix moth (*Adoxophyes orana* F.v.R.)	[12]
	Colorado potato beetle (*Leptinotarsa decemlineata* Say)	[115]
M. arabica *tops* M. arabica *roots* *Total saponins*	Colorado potato beetle (*Leptinotarsa decemlineata* Say)	[116]
M. hybrida *tops* M. hybrida *roots* Total saponins		
M. murex tops *M. murex* roots Total saponins		
M. sativa roots Total saponins Prosapogenins Medicagenic acid Na^+ salt	Hop aphid (*Phoron humuli* Schrank) Spider mite (*Tetranychus urticae* Koch.)	[117]
M. sativa tops Total saponins Cholesterol-precipitable saponins	European corner borer (*Ostrinia nubilalis* Hb.)	[118]
M. sativa roots Total saponins		
M. sativa 3-*O*-*β*-D-Glc-28-*O*-[*α*-L-Ara(1→2)-*β*-D-Glc(1→2)-*α*-L-Ara] Hederagenin 3-*O*-*β*-D-Glc Medicagenic acid 3-*O*-*β*-D-Glc-28-*O*-*β*-D-Glc Medicagenic acid 3-*O*-*β*-D-Glc-28-*O*-[*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara] Medicagenic acid 3-*O*-*β*-D-GlcAc-28-*O*-[*β*-D-Xyl(1→4)-*α*-L-Rha(1→2)-*α*-L-Ara] Medicagenic acid Soyasaponin I, Hederagenin, Medicagenic acid, Soyasapogenol A, Soyasapogenol B, Soyasapogenol E, 3-*O*-*β*-D-Glc Medicagenic acid Na^+ salt 3-*O*-*β*-D-Glc-28-*O*-*β*-D-Glc Medicagenic acid Na^+ salt Soyasaponin I Na^+ salt, Medicagenic acid Na^+ salt	Army-warm (*Spodoptera littoralis* Boisd.)	[119]

littoralis. Total saponins (1, 10 and 100 ppm) and a series of pure saponins (10 ppm) and sapogenins (20 ppm) were given in the food and their effects examined during larval development as well as in the resulting pupae and adults. At 1 ppm, root saponins caused a nearly 70% mortality and the emerged females exhibited about 60% fertility reduction. Total saponins from the aerial parts were less active, although the increase of mortality and the reduction of fecundity were significant. All the pure saponins lowered the food consumption and reduced the larval growth rate although to a different extent. Aglycones

influenced the larval development in a similar way, medicagenic acid was found to be the most active, hederagenin and soyasapogenols A and B exhibited only moderate activity, while soyasapogenol E was inactive. Medicagenic acid sodium salt and its 3-*O*-*β*-D-glucopyranosyl and medicagenic acid 3-*O*-*β*-D-glucopyranosyl-28-*O*-*β*-D-glucopyranoside derivatives were the most active substances. Additionally, all the tested *α*-L-arabinopyranosyl glycosides were inactive, while the corresponding aglycones or glycosides were active. Based on those results it has been suggested that glycosylated saponins are bioactive only when they are hydrolyzed by insect gut glycosidases and release an active aglycone; complex glycosides containing arabinopyranosyl units apparently resist the action of the enzymes [119].

The spraying of winter wheat with different saponin concentrations at various phenological phases had no negative effects on growth parameters, grain yield and quality of wheat flour. Results indicated that saponins (0.01-0.1%) can be applied on a wheat crop as fungicides or insecticides [120].

Allelopathic effects

Alfalfa, as other forage legumes, has the reputation as an important rotation crop to improve nitrogen availability in the soil. In some cases the increase of nitrogen by *Medicago* does not correspond to an increase of grain yield in the succeeding rotated crop, suggesting that some factors might interfere with the utilization of nitrogen. This effect has been experimentally correlated with the presence, in alfalfa plant material, of saponins which display allelopathic activity [11, 13, 121-124].

The role of alfalfa saponins as allelopathic agents was first reported in 1955 by Mishutin and Naumova [125] who observed that growth of cotton was influenced by the use of alfalfa as a rotation crop. Detrimental effects on cotton-seed germination was also shown in *in vitro* assays with alfalfa saponins. Later investigations have shown that saponins from various species of *Medicago* act as allelochemicals, some with a defined specificity towards different plants [126-130]. The allelopathic potential of medicagenic acid glycosides has been noted. Depending on their concentration they may function as plant growth inhibitors (high concentrations) or stimulators (low concentrations) [121, 129, 130]. They also inhibit the growth of several weeds and cereals [11, 13, 121, 128-130]: elongation of roots and shoots of *Bromus secalimus* and *Echinochloa crus-galli* was inhibited by 10 ppm saponin (19-11 and 28-17%, respectively), while growth of wheat roots was 50% reduced at the concentration of 100 ppm compared to the control [129, 130]. Other saponins such as soyasapogenol B and hederagenin glycosides were in general found less active as growth inhibitors than medicagenic acid derivatives [121, 129].

The different allelopathic potential of *Medicago* species was related to their different content of saponins. Thus, for example, saponins (medicagenic and soyasapogenol glycosides) isolated from the seeds of *M. lupulina* were able to inhibit the growth of the cereals oat, barley, wheat and rye, whereas saponins from the seeds of *M. sativa* (containing only soyasapogenol glycosides) had no effects on wheat and rye development, but only on that of barley and oat [121, 129]. The use of plant material of various physiological ages indicated that alfalfa at immature stages is more phytotoxic since it likely contains higher amounts of allelochemicals [126].

Soil texture also was found to influence the inhibitory activity of alfalfa saponins [121, 127]. In a detailed study it has been in fact shown that finely powdered alfalfa roots in sandy soil causes a more pronounced detrimental effect on wheat growth than incorporation in heavy clay soils.

An autotoxic effect of alfalfa also has been reported [131].Despite their allelopathic activity, however, saponins produced by the plant species seem not to be involved in the autoxic effects which instead have been attributed to water-soluble phenolic components [132-135]. In particular the isoflavonoids medicarpin and its methoxy analogue, 4-methoxymedicarpin, and chlorogenic acid purified from alfalfa leaves were assayed in *in vitro* experiments and found to contribute to the plant autoallelopathy which results in a yield decrease, low seed germination and poor growth when alfalfa is sown in soils where the same species was previously cropped.

The physiological mechanism of action of saponins as allelopathic agents is not clear. Inhibition of seed germination has been correlated with a decrease in

oxygen diffusion through the seed coat [121], while seedling growth retardation has not been well studied. Comparison of the allelopathic effects of structurally different saponins from *Medicago* species revealed some structure-activity relationships. As found for antimicrobial activity, monodesmosides were in general more active than the related bi- and tridesmosides while medicagenic acid glycosides having glucose at the C-3 position were more active than similar compounds substituted with glucuronic acid, and zanhic acid glycosides were more effective than the 3-*O*-glucuronides of medicagenic acid [11, 121, 129].

Effects on animals

The significance of natural saponins in animal nutrition has been widely investigated [136, 137]. Studies of the effects of the saponins from *Medicago* species have been carried out only for *M. sativa*, due to the importance of this species as forage and as an industrial source of leaf protein concentrate used in animal diets. An excellent review on this topic is available [138]. Saponins may have significant effects on all the phases of animal metabolism from ingestion to excretion. Alfalfa saponins influence rumen fermentation and affect microbial protein synthesis in the rumen, the site of nutrient digestion. Moreover they suppress fermentation in rumen cultures [139], and *in vivo* investigations [140] have confirmed a general decrease of fermentation associated with a symptomatic decrease of volatile fatty acids and cellulose digestion. A significant reduction of protozoa in rumen of sheep receiving alfalfa saponins was also reported [140]. Moreover, endogenous bacteria appeared morphologically modified when treated with alfalfa saponins [139]. All these effects on animal nutrition have been related to the ability of saponins, or their aglycones, to interact with cell membrane sterols and other metabolites [138, 141]. Saponins are in fact able to complex cholesterol, and their anti-nutritional effects were lowered by addition of cholesterol to the diet. Retardation of growth by alfalfa dietary saponins has been observed in livestock and laboratory animals, probably due to the bitter and astringent sensory characteristics of the processed grain products. One mechanism that might account for the growth depressing effects of saponins is the lowering of feed intake because of unpalatability.

No clear information is available on the lethal dose or minimum inhibition concentration of alfalfa saponins towards livestock. Animal species differ in their susceptibility to saponins, however. Poultry are more sensitive than other farm animals. A variety of alfalfa with 1.47% of saponins caused an average reduction of 11% in weight gain of chicks compared to a low-saponin variety containing 0.59% of the active compounds [142, 143]. No effects were reported when calves were fed with alfalfa hay containing up to 2.62% saponin [144]. Though accurate estimates of detrimental saponin levels are lacking, high- and low-saponin germplasm has been defined in the literature (and set as a goal in breeding programs), mostly based on responses of monogastric animals, or biological assays (e.g. *Trichoderma viride* test and the hemolytic test). Conventionally an average concentration of about 2.0% and 0.8% were considered to be high and low, respectively [143, 145].

Determination of saponins by semi-quantitative methods based on biological assays may give erroneous results. For instance, glycosides of zanhic acid are weakly detectable by biological tests, although they are classified as toxic/moderately toxic compounds, with an LD_{50} value of 562 mg/kg body weight calculated for hamsters [146]. Sensory test trials on human volunteers, using saponins isolated from alfalfa aerial parts, showed that zanhic acid tridesmoside is the most bitter, astringent and throat-irritating compound of all the tested saponins [36]. This compound is also reported to have the highest intestinal membrane depolarizing activities compared to other alfalfa saponins [147]. It also has been described as causing breathing problems and nervous system perturbations to hamsters, followed by death after 24h. Bloat syndromes were observed at necropsy [146].

Rats fed alfalfa saponins at levels of 1% in the diet for up to 26 weeks showed no toxic effects; a potentially beneficial reduction of serum cholesterol and triglycerides was observed instead [148]. No adverse reactions have been detected in the non-human primate, *Macaca fascicularis*, following consumption of a mixture of alfalfa top saponins for up to 78 weeks. The metabolites decreased cholesterolemia without changing the level of high density lipoprotein-cholesterol; hence, they reduced the total cholesterol/high density lipoprotein-

cholesterol ratio. Furthermore, saponins decreased intestinal adsorption of cholesterol, and increased excretion of neutral steroids and bile acids [149]. As these compounds interact with cholesterol and directly interfere with its absorption, a possible application in some human pathologies can be hypothesized, although toxicity of alfalfa saponins for human consumption needs detailed investigation.

Furthermore, *in vitro* studies indicated that saponins from *M. sativa* roots and aerial parts have some effects on pancreatic lipase activity. Results showed that they stimulated lipolytic activity and did not influence the proteolytic and amylolytic activities of Neopancreatium, a mixture of porcine pancreatic enzymes such as trypsin, chimotrypsin, lipase and amylase. An increase of the stimulatory effects of saponins was observed when sodium cholate was added to the medium [150].

Cytotoxic and tumor-promoter inhibitory activities

Although the cytotoxicity of triterpenoid saponins is known [151], saponins from *Medicago* species have never been extensively investigated, although saponins from *M. sativa* leaves showed dose-dependant growth inhibition *in vitro* of human leukemic cell line K562 [12]. No significant effects on clonogenic survival were observed when purified saponins from *M. sativa* roots, leaves and seeds were tested *in vitro* against MCF7 human breast carcinoma cells and HeLa human cervical carcinoma cells, although MCF7 was more sensitive to the treatment. Inhibition of tumoral cell growth was instead observed when saponins were used in association with cis-platin. The growth of MCF7 cells was 18-33% (saponin concentration 25 μg/mL; cis-platin 4 μg/mL), compared to 40% survival when only cis-platin was used. Root and seed saponins were found to be more active than saponins from leaves. All the tested saponins enhanced the cis-platin induced toxicity, although HeLa cells were significantly less affected [152]. As reported [153], saponins seem to act as promotors probably affecting cell membrane permeability cis-platin diffusion in the cells.

The lack of information on the cytotoxicity of saponins from *Medicago* spp. does not allow additional indications of their activity, but their antitumoral, chemopreventive and antimutagenic properties can be extrapolated from those of bioactive saponins from other plants but found in *Medicago* spp. For example, saponins from soybean, including soyasaponin I (soyasapogenol B 3-*O*-α-L-rhamopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranosyl) found in almost all the studied *Medicago* species, are known for their chemopreventive properties [154-156]. Orally consumed soybean saponins are not adsorbed in the small intestine and appear to reach the colon [157] where they exert their beneficial effects. They are able to suppress the growth of human colon carcinoma cells *in vitro* [158, 159], and to inhibit the chemically induced colonic aberrant crypt formation in CF1 mice [160]. Soyasaponin I from *W. brachybotrys* has also been shown to strongly inhibit mouse skin tumor promotion [161].

Antimutagenic and antiproliferative [162-164] activity has also been observed for some hederagenin monodesmosides from *Hedera helix*, including 3-*O*-α-L-arabinopyranosyl hederagenin and 3-*O*-α-L-rhamopyranosyl(1→2)-α-L-arabinopyranosyl hederagenin in *M. arabica* and *M. polymorpha*, respectively.

The saponin 3-*O*-α-L-rhamopyranosyl(1→2)-β-D-glucopyranosyl (1→2) -β-D- glucuronopyranosyl soyasapogenol E from *Wistaria brachybotrys* (Leguminosae), named wistaria saponin D, showed antitumor promoting activity [165]. The same sapogenin has also been found in *M. sativa* and *M. truncatula*.

In this context, the mutagenic activity of some saponins and sapogenins from *M. sativa* have been evaluated. Soyasaponin I, in a concentration up to 500 μg, medicagenic acid (up to 200 μg) and its 3-*O*-glucopyranosyl derivative (up to 200 μg), were tested according to the Ames assay against *Salmonella typhimorium* strains TA97, TA98, TA100 and TA102. Results showed that saponins did not increase the number of $\underline{his}^+$ revertants in any of the strains, neither in the absence nor in the presence of metabolic activation (S9 fraction from rat liver) [166].

Conclusion

Saponins from the *Medicago* genus are a complex group of pentacyclic triterpene glycosides which

display antimicrobial, insecticidal, allelopathic and cytotoxic properties, together with antinutritional effects. Particularly studied *M. sativa*, the most important species within the genus from an agronomic point of view.

The biological activities of *Medicago* saponins are related to their chemical structure in that monodesmosides are more active than the corresponding bidesmosides, and the aglycone and the nature and position of the sugar in the molecule might be important factors in determining their efficacy.

Based on their bioactivity, plant saponins are already used commercially. Data summarized here might suggest further applications of saponins from *Medicago*.

References

[1] Dewick PM. (**2001**) *Medicinal Natural Products – A Biosynthetic approach.* Wiley, New-York.

[2] Hostettmann K, Marston A. (**1995**) *Chemistry and Pharmacology of Natural Products*. *Saponins*. Phillipson JD, Baxter H (Eds). Cambridge University Press, UK.

[3] Milgate J, Roberts DCK. (**1995**) The nutritional and biological significance of saponins. *Nutritional Research*, ***8***, 1223-1249.

[4] Lacaille-Dubois MA, Wagner H. (**2000**) Bioactive saponins from plants: an update. In *Studies in Natural Products Chemistry*. Vol. ***21***, Atta-ur-Rahman (Ed). Elsevier, London, UK. 633-687.

[5] Francis G, Keem Z, Makkar HPS, Becker K. (**2002**) The biological action of saponins in animal systems. A review. *British Journal of Nutrition*, ***88***, 587-605.

[6] Sprag SG, Light ME, van Staden J. (**2004**) Biological activities and distribution of plant saponins. *Journal of Ethnopharmacology*, ***94***, 219-243.

[7] Tanaka O, Tamura Y, Masuda H, Mizutani K. (**1996**) Application of saponins in food and cosmetics: saponins of *Mohave yucca* and *Sapindus mukurossi*. In *Advances in Experimental Medicine and Biology*. *Saponins Used in Food and Agriculture*. Vol. ***405***. Waller GR, Yamasaki K. (Eds). Plenum Press, New-York. 1-11.

[8] San Martin R, Briones R. (**2000**) Quality control of commercial quillaja (*Quillaja saponaria* Molina) extracts by reverse phase HPLC. *Journal of the Science of Food and Agriculture*, ***88***, 587-605.

[9] Bruneton J. (**1999**) *Pharmacognosy – Phytochemistry, Medicinal Plants.* Lavoisier Publishing, Paris, 671-719.

[10] Jurzysta M, Waller GR. (**1996**) Antifungal and haemolytic activity of aerial parts of alfalfa (*Medicago*) species in relation to saponin composition. In *Advances in Experimental Medicine and Biology*. *Saponins Used in Traditional and Modern Medicine*. Vol. ***404***. Waller GR, Yamasaki K. (Eds). Plenum Press, New-York. 565-574.

[11] Oleszek W. (**1996**) Alfalfa saponins: structure, biological activity and chemotaxonomy. In *Advances in Experimental Medicine and Biology*. *Saponins used in Food and Agriculture*. Vol. ***405***. Waller GR, Yamasaki K. (Eds). Plenum Press, New-York. 155-170.

[12] Tava A, Odoardi M. (**1996**) Saponins from *Medicago* spp.: chemical characterization and biological activity against insects. In *Advances in Experimental Medicine and Biology*. *Saponins Used in Food and Agriculture*. Vol. ***405***. Waller GR, Yamasaki K. (Eds). Plenum Press, New-York. 97-109.

[13] Timbekova AE, Isaev MI, Abubakirov NK. (**1996**) Chemistry and biological activity of triterpenoid glycosides from *Medicago sativa*. In *Advances in experimental medicine and biology. Saponins Used in Food and Agriculture.* Vol. ***405***, Waller GR, Yamasaki K (Eds). Plenum Press, New York USA. 171-182.

[14] Zehavi U, Polacheck I. (**1996**) Saponins as antimycotic agents: glycosides of medicagenic acid. In *Advances in Experimental Medicine and Biology. Saponins Used in Traditional and Modern Medicine*. Vol. ***404***. Waller GR, Yamasaki K. (Eds). Plenum Press, New-York. 535-546.

[15] Oleszek W. (**2000**) Alfalfa saponins: Chemistry and Application. In: *Phytochemicals as Bioactive Agents.* Technomic Publ. Comp., Inc., USA, 167-188.

[16] Lesins KA, Lesins I. (**1979**) *Genus Medicago (Leguminosae). A taxogenetic Study*. Junk W. (Ed), Publisher, London.

[17] Heyn CC. (**1963**) *The Annual Species* of *Medicago*. Magnes Press, Hebrew University, Jerusalem.

[18] Potter GC, Kummerow FA. (**1954**) Chemical similarity and biological activity of the saponins isolated from alfalfa and soybeans. *Science*, ***120***, 224-225.

[19] Morris RJ, Dye WB, Gisler DS. (**1961**) Isolation, purification and structural activity of an alfalfa root saponins. *Journal of Organic Chemistry*, ***26***, 1241-1243.

[20] Morris RJ, Hussey EW. (**1965**) A natural glycoside of medicagenic acid. An alfalfa blossom saponin. *Journal of Organic Chemistry*, ***30***, 166-168.

[21] Gestetner B. (**1971)** Structure of a saponin from lucerne (*Medicago sativa*). *Phytochemistry*, ***10***, 2221-2223.

[22] Timbekova AE, Abubakirov NK. (**1984**) Triterpene glycosides from alfalfa. I. Medicoside G. A novel bidesmoside from *Medicago sativa*. *Khimiya Prirodnykh Soedinenii*, 451-458.

[23] Timbekova AE, Abubakirov NK. (**1985**) Triterpene glycosides from alfalfa. II. Medicoside C. *Khimiya Prirodnykh Soedinenii*, 805-808.

[24] Timbekova AE, Abubakirov NK. (**1986**) Triterpene glycosides from alfalfa. VII. Medicoside I. *Khimiya Prirodnykh Soedinenii*, 607-610.

[25] Timbekova AE, Abubakirov NK. (**1986**) Triterpene glycosides of alfalfa. IV. Medicoside J. *Khimiya Prirodnykh Soedinenii*, 610-613.

[26] Kitagawa I, Taniyama T, Murakami T, Yoshihara M, Yoshikawa M. (**1988**) Saponin and sapogenol. XLVI. On the constituents of aerial part of american alfalfa, *Medicago sativa* L. The structure of dehydrosoyasaponin I. *Yakugaku Zasshi*, ***108***, 547-551.

[27] Massiot G, Lavaud C, Le Men-Olivier L, van Binst G, Miller SPF, Fales HM. (**1988**) Structural elucidation of alfalfa root saponins by mass spectrometry and nuclear magnetic resonance analysis. *Journal of Chemical Society Perkin Transaction* ***I***, 3071-3079.

[28] Oleszek W, Price KR, Fenwick GR. (**1988**) Triterpene saponins from the roots of *Medicago lupulina* L. (black medic trefoil). *Journal of Science of Food and Agriculture*, ***43***, 289-297.

[29] Levy M, Zehavi U, Naim M, Polacheck I. (**1989**) Isolation, structure determination and antifungal activity of a new alfalfa root saponin. *Carbohydrate Research*, ***193***, 115-123.

[30] Timbekova AE, Larin MF, Yagudaev MR, Abubakirov NK. (**1989**) Triterpene glycosides of alfalfa. V. Medicoside H. *Khimiya Prirodnykh Soedinenii*, 673-677.

[31] Oleszek W, Price KR, Colquhoun IJ, Jurzysta M, Ploszynski M, Fenwick GR. (**1990**) Isolation and identification of alfalfa (*Medicago sativa* L.) root saponins: their activity in relation to a fungal bioassay. *Journal of Agricultural and Food Chemistry*, ***38***, 1810-1817.

[32] Timbekova AE, Verechagin AL, Semenov AA, Abubakirov NK. (**1990**) Triterpene glycosides of alfalfa. VI. Medicoside L *Khimiya Prirodnykh Soedinenii*, 221-227.

[33] Mahato SB. (**1991**) Triterpenoid saponins from *Medicago hyspida*. *Phytochemistry*, ***30***, 3389-3393.

[34] Massiot G, Lavaud C, Besson V, Le Men-Olivier L, van Binst G. (**1991**) Saponins from aerial parts of alfalfa (*Medicago sativa*). *Journal of Agricultural and Food Chemistry*, ***39***, 78-82.

[35] Massiot G, Lavaud C, Benkhaled M, Le Men-Olivier L. (**1992**) Soyasaponin VI, a new maltol conjugate from alfalfa and soyabean. *Journal of Natural Products*, ***55***, 1339-1341.

[36] Oleszek W, Jurzysta M, Ploszynski M, Coloquhoun IJ, Price KR, Fenwick GR. (**1992**) Zahnic acid tridesmoside and other dominant saponins from alfalfa (*Medicago sativa* L.) aerial parts. *Journal of Agricultural and Food Chemistry*, ***40***, 191-196.

[37] Timbekova AE, Shashkov AS, Abubakirov NK. (**1993**) Triterpene glycosides from alfalfa. VII. Medicosides E and F. *Khimiya Prirodnykh Soedinenii*, 701-705.

[38] Kinjo J, Uemura H, Nakamura M, Nohara T. (**1994**) Two new triterpenoidal glycosides from *Medicago polymorpha* L. *Chemical and Pharmacological Bulletin*, ***42***, 1339-1341.

[39] Bialy Z, Jurzysta M, Oleszek W, Piacente S, Pizza C. (**1999**) Saponins in alfalfa (*Medicago sativa* L.) root and their structural elucidation. *Journal of Agricultural and Food Chemistry*, ***47***, 3185-3192.

[40] Huhman DV, Sumner LW. (**2002**) Metabolic profiling of saponins in *Medicago sativa* and *Medicago truncatula* using HPLC coupled to an electrospray ion-trap mass spectrometer. *Phytochemistry*, ***59***, 347-360.

[41] Zbigniew B, Jurzysta M, Mella M, Tava A. (**2004**) Triterpene saponins from aerial parts of *Medicago arabica* L. *Journal of Agricultural and Food Chemistry*, ***52***, 1095-1099.

[42] Huhman DV, Berhow MA, Sumner LW. (**2005**) Quantification of saponins in aerial and subterranean tissues of *Medicago truncatula*. *Journal of Agricultural and Food Chemistry*, ***53***, 1914-1920.

[43] Kapusta I, Bogdan J, Stochmal A, Oleszek W. (**2005**) Determination of saponins in aerial parts of barrel medic (*Medicago truncatula*) by liquid chromatography-electrospray ionization/mass spectrometry. *Journal of Agricultural and Food Chemistry*, ***53***, 7654-7660.

[44] Kapusta I, Stochmal A, Perrone A, Piacente S, Pizza C, Oleszek W. (**2005**) Triterpene saponins from barrel medic (*Medicago truncatula*) aerial parts. *Journal of Agricultural and Food Chemistry*, ***53***, 2164-2170.

[45] Tava A, Mella M, Avato P, Argentieri MP, Bialy Z, Jurzysta M. (**2005**) Triterpene saponins from leaves of *Medicago arborea* L. *Journal of Agricultural and Food Chemistry*, ***53***, 9954-9965.

[46] Bialy Z, Jurzysta M, Mella M, Tava A. (**2006**) Triterpene Saponins from the Roots of *Medicago hybrida*. *Journal of Agricultural and Food Chemistry*, ***54***, 2520-2526.

[47] Pedersen MW. (**1975**) Relative quantity and biological activity of saponins in germinated seeds, roots, and foliage of alfalfa. *Crop Science*, ***15***, 541-543.

[48] Gorski PM, Miersch J, Ploszynski M. (**1991**) Production and biological activity of saponins and canaverine in alfalfa seedlings. *Journal of Chemical Ecology*, ***17***, 1135-1143.

[49] Oleszek W. (**1998**) Composition and quantitation of saponins in alfalfa (*Medicago sativa* L.) seedlings. *Journal of Agricultural and Food Chemistry*, ***46***, 960-962.

[50] Berrang B, Davis KH Jr, Wall ME, Hanson CH, Pedersen ME. (**1974**) Saponins of two alfalfa cultivars. *Phytochemistry*, ***13***, 2253-2260.

[51] Quazi HM (**1975**) Effect of cultivar and season on the concentration of saponins in lucerne (*Medicago sativa* L.). *New Zealand Journal of Agricultural Research*, ***18***, 227-232.

[52] Tava A, Oleszek W, Jurzysta M, Berardo N, Odoardi M. (**1993**) Alfalfa saponins and sapogenins: isolation and quantification in two different cultivars. *Phytochemical Analysis*, ***4***, 269-274.

[53] Tava A, Pecetti L. (**1998**) Hemolytic activity and saponin content in lucerne (*Medicago sativa* complex) genotypes. *Journal of Genetics and Breeding*, ***52***, 33-37.

[54] Tava A, Odoardi M, Oleszek W. (**1999**) Seasonal changes of saponin content in five alfalfa (*Medicago sativa*) cultivars. *Agricoltura Mediterranea*, ***129***, 111-116.

[55] Pecetti L. Tava A, Romani M, De Benedetto MG, Corsi P. (**2006**) Variety and environment effects on the dynamics of saponins in lucerne (*Medicago sativa* L.). *European Journal of Agronomy*, ***25***, 187-192

[56] Jurzysta M, Burda S, Oleszek W, Ploszynski M. (**1988**) The chemotaxonomic significance of laricytrin and medicagenic acid in the tribe Trigonellae. *Canadian Journal of Botany*, ***66***, 363-367.

[57] Jurzysta M, Small E, Nozzolillo C. (**1988**) The evolution of hemolytic saponin content in wild and cultivated alfalfa (*Medicago sativa*, Fabaceae). *Economic Botany*, ***44***, 226-235.

[58] Small E, Jurzysta M, Nozzolillo C. (**1990**) Hemolysis, a sinapomorphic discriminator for an expanded genus *Medicago* (Leguminosae). *Taxon*, ***37***, 354-363.

[59] Jurzysta M, Burda S, Oleszek W, Ploszynski M. (**1992**) Chemical composition of seed saponins as a guide to the classification of *Medicago* species. *Canadian Journal of Botany*, ***70***, 1384-1387.

[60] Walter ED, Van Atta GR, Thompson CR, Maclay WD. (**1954**) Alfalfa saponins. *Journal of the American Chemical Society*, ***76***, 2271-2273.

[61] Jurzysta M. (**1973**) Isolation and chemical characterization of saponins from lucerne seeds (*Medicago media* Pers.) *Acta Societatis Botanicorum Poloniae*, ***42***, 201-207.

[62] Gorsky PM, Jurzysta M, Burda S, Oleszek W, Ploszynnsky M. (**1984**) Studies on *Medicago lupulina* saponins. 2. Isolation, chemical characterization and biological activity of saponins from *Medicago lupulina* tops. *Acta Societatis Botanicorum Poloniae*, ***53***, 527-533.

[63] Jurzysta M, Nowacki E. (**1979**) Saponins in the genus *Medicago*. *Acta Agrobotanica*, ***32***, 13-17.

[64] Jurzysta M. (**1979**) Haemolytic micromethod for rapid estimation of toxic alfalfa saponins. *Acta Agrobotanica*, ***32***, 5-11.

[65] Zimmer DE, Pederson MW, McGuire CF. (**1967**) A bioassay for alfalfa saponins using the fungus *Trichoderma viride* Pers. *Crop Science*, ***7***, 223-224.

[66] Pracros P. (**1988**) Mesure de l'activité des saponins de la lucerne par les larves du ver de la farine: *Tenebrio molitor* L. (Colèoptére, *Tenebrionidae*). I. Comparaison avec les résultats de divers tests biologiques. *Agronomie*, ***8***, 257-263.

[67] Nowacka J, Oleszek W. (**1994**) Determination of alfalfa (*Medicago sativa* L.) saponins by high performance liquid chromatography. *Journal of Agricultural and Food Chemistry*, ***42***, 727-729.

[68] Jurzysta M. Jurzysta A. (**1978**) Gas-liquid chromatography of trimethylsilyl ethers of soyasapogenols and medicagenic acid. *Journal of Chromatography*, ***148***, 517-520.

[69] Rao D, Boris G. (**1987**) Simple gas chromatographic method for the determination of medicagenic acid in alfalfa (*Medicago sativa*). *Journal of Chromatography*, ***410***, 169-175.

[70] Tava A, Chiari M, Oleszek W. (**2000**) Separation of alfalfa (*Medicago sativa* L.) saponins as their borate complexes by capillary electrophoresis. In *Saponins in Food, Feedstuffs and Medicinal Plants*. Chapter ***5***, Oleszek W, Marston A (Eds). Kluwer Academic Publishers, Dordrecht The Netherlands. 43-56.

[71] Lee MK, Ling YC, Jurzysta M, Waller GR. (**1996**) Saponins from alfalfa, clover, and mungbeans analysed by electrospray ionization-mass spectrometry as compared with positive and negative FAB-mass spectrometry. In *Advances in Experimental Medicine and Biology. Saponins Used in Food and Agriculture.* Vol ***405***, Waller GR, Yamasaki K (Eds). Plenum Press, New York USA. 353-364.

[72] Agrawal PK. (**1996**) A systematic NMR approach for the determination of the molecular structure of steroidal saponins. In *Advances in Experimental Medicine and Biology. Saponins Used in Food and Agriculture.* Vol ***405***, Waller GR, Yamasaki K (Eds). Plenum Press, New York USA. 299-315.

[73] Mella M, Jurzysta M, Ricci M, Tava A. (**2002**) NMR investigation of saponins and sapogenins from *Medicago* species. *Proceedings of XXXII National Congress on Magnetic Resonance*. Pavia, Italy P-3.

[74] Tava A, Mella M, Bialy Z, Jurzysta M. (**2003**) Stability of saponins in alcoholic solutions: ester formation as artifacts. *Journal of Agricultural and Food Chemistry*, ***51***, 1797-1800.

[75] Torsell, KBG. In *Natural Product Chemistry - A Mechanistic, Biosynthetic and Ecological Approach*. Swedish Pharmaceutica Society, **1997**.

[76] Morris RJ, Tankersley DL. (**1963**) The synthesis of the β-D-glucoside of medicagenic acid, an alfalfa root saponin. *The Journal of Organic Chemistry*, ***28***, 240-242.

[77] Nowacki E, Jurzysta M, Dietrych-Szostak D. (**1976**) Zur biosynthese der medicagensaure in keimenden luzernesamen. *Biochemie und Physiologie der Pflanzen*, ***156***, 183-186.

[78] Suzuki H, Achnine L, Xu R, Matsuda SPT, Dixon RA. (**2002**) A genomic approach to the early stages of triterpene saponin biosynthesis in *Medicago truncatula*. *The Plant Journal*, ***32***, 1033-1048.

[79] Osbourn A. (**1996**) Saponins and plant defence - a soap story. *Trends in Plant Science*, ***1***, 4-9.

[80] Osbourn A. (**2003**) Saponins in cereals. *Phytochemistry*, ***62***, 1-4.

[81] Wina E, Muetzel S, Becker K. (**2005**) The impact of saponin-containing plant materials on ruminant production-A review. *Journal of Agricultural and Food Chemistry*, ***53***, 8093-8105.

[82] Levy M, Zehavi U, Naim M, Polacheck I. (**1986**) An improved procedure for the isolation of medicagenic acid 3-O-β-D-Glucopyranoside from alfalfa roots and its antifungal activity on plant pathogens. *Journal of Agricultural and Food Chemistry*, ***34***, 960-963

[83] Nonaka M. (**1986**) Variable sensitivity of *Trichoderma viride* to *Medicago sativa* saponins. *Phytochemistry*, ***25***, 73-75.

[84] Gruiz K. (**1996**) Fungitoxic activity of saponins: practical use and fundamental principles. In *Advances in Experimental Medicine and biology*. *Saponins Used in traditional and modern medicine*. Vol. ***404***. Waller GR, Yamasaki K. (Eds). Plenum Press, New-York. 527-534.

[85] Jurzysta M, Bialy Z. (**1999**) Antifungal and haemolytic activity of roots of alfalfa (*Medicago* spp.) in relation to saponin composition. In *Modern fungicides and antifungal compounds II*. Lyr H, Russel PE, Sisler HD (Eds). Intercept: Andover, UK. 445-451.

[86] Gestetner B, Assa Y, Henis Y, Birk Y, Bondi A. (**1971**) Lucerne saponins IV.- Relationship between their chemical constitution, and haemolytic and antifungal activities. *Journal of Agricultural and Food Chemistry*, ***22***, 168-172.

[87] Assa Y, Gestetner B, Chet I, Henis Y. (**1972**) Fungistatic activity of Lucerne saponins and digitonin as related to sterols. *Life Sciences*, ***11***, Part II, 637-647.

[88] Levanon D, Henis Y, Okon Y, Dovrat A. (**1982**) Alfalfa saponins and microbial transformations of nitrogen in peat. *Soil Biology & Biochemistry*, ***14***, 501-504.

[89] Martyniuk S, Jurzysta M, Bialy Z, Wróblewska B. (**1995**) Alfalfa root saponins affect the growth and sporulation of *Cephalosporium gramineum*. In *Environmental Biotic Factors in Integrated Plant Disease Control*. Manka M (Ed). The Polish Phytopathological Society, Poznan. 395-398.

[90] Martyniuk S, Wróblewska B, Jurzysta M, Bialy Z. (**1995**) Saponins as inhibitors of cereal pathogens: *Gaeumannomyces graminis* v. *tritici* and *Cephalosporium gramineum*. In *Modern Fungicides and Antifungal Compounds*. Lyr H, Russel PE, Sisler HD (Eds.) Intercept Ltd., UK. 193-197.

[91] Martyniuk S, Jurzysta M, Wróblewska B. (**1999**) Influence of powdered aerial parts of various *Medicago* species on the growth of *Gaeumannomyces graminis* and *Cephalosporium gramineum*. *Bulletin of the Polish Academy of Sciences, Biological Sciences*, ***47***, 2-4.

[92] Saniewska A, Jurzysta M, Bialy Z. (**2001**) Differential antifungal activity of alfalfa (*Medicago sativa* L.) saponins originated from roots and aerial parts of some ornamental plant pathogens. *Acta Agrobotanica*, ***54***, 31-43.

[93] Saniewska A, Jurzysta M, Bialy Z. (**2003**) The effect of alfalfa (*Medicago sativa*) saponins on *Botrytis tulipae* and *Phoma narcissi* growth. *Phytopathologica Polonica*, ***27***, 15-27.

[94] Martyniuk S, Bialy Z, Jurzysta M. (**2004**) Antifungal activity of aerial parts and saponins of *Medicago* ssp. against *Cephalosporium gramineum*. *Allelopathy Journal*, ***13***, 75-82.

[95] Saniewska A, Jarecka Z, Bialy Z, Jurzysta M. (**2005**) Antifungal activity of saponins from *Medicago arabica* L. shoots against some pathogens. *Allelopathy Journal*, ***16***, 105-112.

[96] Polacheck I, Zehavi U, Naim M, Levy M, Evron R. (**1986**) Activity of compound G2 isolated from alfalfa roots against medically important yeasts. *Antimicrobial Agents and Chemotherapy*, ***30***, 290-294.

[97] Polacheck I, Zehavi U, Naim M, Levy M, Evron R. (**1986**) The susceptibility of Cryptococcus neoformans to an antimycotic agent (G2) from alfalfa. *Zentralblatt fur Bakteriologie, Mikrobiologie und Hygiene*. Series A, ***261***, 481-486.

[98] Evron R, Polacheck I, Guizie M, Levy M, Zehavi U. (**1988**) Activities of compound G2 isolated from alfalfa roots against dermatophytes. *Antimicrobial Agents and Chemotherapy*, ***32***, 1586-1587.

[99] Evron R, Guizie M, Zehavi U, Polacheck I. (**1990**) Activity of compound G2 isolated from alfalfa roots in experimental dermatophyte infection. *Antimicrobial Agents and Chemotherapy*, ***34***, 1600-1601.

[100] Polacheck I, Zehavi U, Naim M, Levy M, Evron R. (**1986**) The susceptibility of *Cryptococcus neoformans* to an antimycotic agent (G2) from alfalfa. *Zentralblatt fur Bakteriologie, Mikrobiologie und Hygiene. Series A*, ***261***, 481-486.

[101] Spiewak R, Szostak W, Jurzysta M, Bialy Z. (**2000**) Inhibiting action of medicagon acid 3-glucoside on the growth of *Scropulariopsis brevicaulis in vitro*. *Abstract Book*, 10th International Mycological Symposium of PDS – Mycology 2000, Poland, pg 113.

[102] Spiewak R, Szostak W, Jurzysta M, Bialy Z. (**2000**) Inhibiting action of medicagon acid 3-glucoside on the growth of *Trichophyton mentagrophytes in vitro*. *Abstract Book*, 10th International Mycological Symposium of PDS – Mycology 2000, Poland, pg 114.

[103] Avato P, Bucci R, Tava A, Vitali C, Rosato A, Bialy Z, Jurzysta M. (**2006**) Antimicrobial activity of saponins from *Medicago* sp.: structure –activity relationship. *Phytotherapy Research*, ***20***, 454-457.

[104] Houghton PJ, Patel N, Cheung CY, Jurzysta M, Bialy Z. (**2005**) Antifungal activity of saponins from *Medicago* species. *Abstract Book*, 53rd GA Annual Congress, Florence, p. 192.

[105] Hoagland R. (**2001**) Effects of alfalfa saponins on in vitro physiological activity of soil and rhizosphere bacteria. *Journal of Crop Protection*, ***4***, 349-361.

[106] Oleszek W. (**2000**) Saponins. In *Natural Food Antimicrobial Systems*. Naidu AS (Ed). CRC Press, London, 1-30.

[107] Applebaum SW, Marco S, Birk Y. (**1969**) Saponins as a possible factor of resistance of legume seed to attack of insects. *Journal of Agricultural and Food Chemistry*, ***17***, 618-621.

[108] Agrelli J, Oleszek W, Stochmal A, Olsen M, Anderson P. (**2003**) Herbivore-induced responses in alfalfa (Medicago sativa). *Journal of Chemical Ecology*, ***29***, 303-320.

[109] Applebaum SW, Birk Y. (**1979**) In *Herbivores. Their Interaction with Secondary Plant Metabolites.* Rosenthal GA, Janzen DH (Eds). Academic Press, New York. 539-566.

[110] Oakenfull D, Sidhu GS. (**1989**) Saponins. In *Toxicant of Plant Origin. Volume II. Glycosides.* Cheeke PR (Ed), CRC Press, Boca Raton, Florida (USA), 97-141.

[111] Sutherland ORW, Hutchins RFN, Greenfield WJ. (**1982**) Effect of lucerne saponins and lotus condensed tannins of survival of grass grub, *Costelytra zealandica. New Zealand Journal of Zoology*, ***9***, 511-516.

[112] Horber E, Leath KT, Berrang B, Macarian V, Hanson CH. (**1974**) Biological activities of saponin components from DuPuits and Lahontan alfalfa. *Entomology Experimental & Application*, ***17***, 410-424.

[113] Pracros P. (**1988**) Mesure de l'activitè des saponines de la luzerne par les larves du ver de farine: *Tenebrio molitor* L. (Coléoptère, *Tenebriolidae*). II – Recherche des fractions de saponines responsables des effets antinutritionnelles observès. *Agronomie*, ***8***, 793-799.

[114] Waligora D, Krzymanska J. (**1994**) The influence of secondary plant substances: glucosinolates, alkaloids and saponins on the feeding of Colorado potato beetle (*Leptinotarsa decemlineata* Say). *Proc. XXXIV Session IOR, vol. **II***, Poznan, pp. 9-12.

[115] Szczepanik M, Krystkowiak K, Jurzysta M, Bialy Z. (**2001**) Biological activity of saponins from alfalfa tops and roots against colorado potato beele larvae. *Acta Agrobotanica*, ***54***, 35-45.

[116] Szczepanik M, Bialy Z, Jurzysta M. (**2004**) The insecticidal activity of saponins from varius *Medicago* spp. against Colorado potato beetle, *Leptinotarsa decemlineata* Say. *Allelopathy Journal*, ***14***, 177-186.

[117] Puszkar L, Jastrzebski A, Jurzysta M, Bialy Z. (**1994**) Alfalfa saponins as a chance in the integrated hop protection. *Proc. XXXIV Session IOR, vol. II*, Poznan, pp. 255-259.

[118] Nozzolillo C, Arnason JT, Campos C, Donskov N, Jurzysta M. (**1997**) Alfalfa leaf saponins and insect resistance. *Journal of Chemical Ecology*, ***21***, 995-1002.

[119] Adel MM, Sehnal f, Jurzysta M. (**2000**) Effects of alfalfa saponins on the moth *Spodoptera littoralis. Journal of Chemical Ecology*, ***26***, 1065-1078.

[120] Podolska G, Bialy Z, Jurzysta M, Waller GR. (**2003**) Effect of application of alfalfa root saponins aqueous solution on the plant structure, yield and quality of winter wheat. *Allelopathy Journal*, ***11***, 171-184.

[121] Waller GR. (**1989**) Biochemical frontiers of allelopathy. *Biologia Plantarum*, ***31***, 418-447.

[122] Oleszek W, Jurzysta M, Gorski PM. (**1992**) Alfalfa saponins the allelopathic agents. In *Allelopathy: Basic and Applied Aspects.* Rizvi SJH, Rizvi V. (Eds). Chapman & Hall, London. 151-167.

[123] Oleszek W. (**1993**) Allelopathic potential of alfalfa (*Medicago sativa*) saponins: their relation to antifungal and hemolytic activites. *Journal of Chemical Ecology*, ***19***, 1063-1074.

[124] Oleszek W, Hoagland RE, Zablotowicz RM. (**1999**) Ecological significance of plant saponins. In: *Principles and Practices in Plant Ecology. Allelochemical Interactions.* Inderjit Dakshini KMM, Foy CL (Eds.). CRC Press LLC, Washington DC, 451-465.

[125] Mishustin BN, Naumova AN. (**1955**) Secretion of toxic substances by alfalfa and their effect on cotton and soil microflora. *Akademia Nauk USSR Izvestija*, Seriya Biologicheskaya, ***6***, 3-9.

[126] Guenzi WD, Kehr WR, McCalla TM. (**1964**) Water-soluble phytotoxic substances in alfalfa forage:variation with variety, cutting, year, and stage of growth. *Agronomy Journal*, ***56***, 499-500.

[127] Oleszek W, Jurzysta M. (**1987**) The allelopathic potential of alfafa root medicagenic acid glycosides and their fate in soil environments. *Plant and Soil*, ***98***, 67-80.

[128] Wyman-Simpson CL, Waller GR, Jurzysta M, McPherson JK, Young CC. (**1991**) Biological activity and chemical isolation of root saponins of six cultivars of alfalfa (*Medicago sativa* L.). *Plant and Soil*, ***135***, 83-94.

[129] Waller GR, Jurzysta M, Thorne RLZ. (**1993**) Allelopathic activity of root saponins from alfalfa (*Medicago sativa* L.) on weeds and wheat. *Botany Bulletin Academia Sinica*, ***34***, 1-11.

[130] Waller GR, Jurzysta M, Thorne RLZ. (**1995**) Root saponins from alfalfa (*Medicago sativa* L.) and their allelopathic activity on weeds and wheat. *Allelopathy Journal*, ***2***, 21-30.

[131] Hall MH, Hederlong PR. (**1989**) Alfalfa autotoxic fraction characterization and initial separation. *Crop Science*, ***29***, 425-428.

[132] Miller RW, Kleiman R, Powell RG. (**1988**) Germination and growth inhibitors of alfalfa. *Journal of Natural Products*, ***51***, 328-330.

[133] Dornbos DL Jr, Spencer GF, Miller RW. (**1990**) Medicarpin delays alfalfa seed germination and seedlings growth. *Crop Science*, ***30***, 162-166.

[134] Chung I-M, Seigler D, Miller DA, Kyung S-H. (**2000**) Autotoxic compounds from fresh alfalfa leaf extracts: identification and biological activity. *Journal of Chemical Ecology*, ***26***, 315-327.

[135] Xuan TD, Tsuzuki E, Terao H, Matsuo M, Khanh TD. (**2003**) Correlation between growth inhibitory exhibition and suspected allelochemicals (phenolic compounds) in the extract of alfalfa (*Medicago sativa* L.). *Plant Production Science*, ***6***, 165-171.

[136] Price KR, Johnson IT, FenwickGR. (**1987**) The chemistry and biological significance of saponins in food and feedstuffs. *CRC Critical Reviews in Food Science and Nutrition*, ***26***, 27-135.

[137] Cheeke PR. (**1996**) Biological effects of feed and forage saponins and their impacts on animal production. In *Advances in experimental medicine and biology. Saponins Used in Food and Agriculture.* Vol ***405***, Waller GR, Yamasaki K (Eds). Plenum Press, New York USA. 377-385.

[138] Sen S, Makkar HPS, Becker K. (**1998**) Alfalfa saponins and their implication in animal nutrition. *Journal of Agricultural and Food Chemistry*, ***46***, 131-140.

[139] Lu CD, Jorgensen NA. (**1987**) Alfalfa saponins affect site and extent of nutrient digestion in ruminants. *Journal of Nutrition*, ***117***, 919-927.

[140] Lu CD,Tsai LS, Schaefer DM, Jorgensen NA. (**1987**) Alteration of fermentation in continuous culture of mixed rumen bacteria. *Journal of Dairy Science*, ***70***, 799-805.

[141] Wallace RJ, McEwan NR, McIntosh FM, Teferedegne B, Newbold CJ. (**2002**) Natural products as manipulators of rumen fermentation. *Asian-Australian Journal of Animal Science*, ***15***, 1458-1468.

[142] Pedersen MW, Zimmer DE, McAllister DR, Anderson JO, Wilding MD, Taylor GA, McGuire CF. (**1967**) Comparative studies of several alfalfa varieties using chemical and biochemical assay. *Crop Science*, ***7***, 349-352.

[143] Pedersen MW, Wang L. (**1971**) Modification of saponin content of alfalfa through selection. *Crop Science*, ***11***, 833-835.

[144] Howard RE. (**1988**) Antiquality factors and nonnutritive chemical components. In *Alfalfa and alfalfa Improvements.* Hanson AA, Barnes DK, Hill RR Jr. (Eds). ASA Inc. CSSA Inc. SSSA Inc. Publishers, Madison, USA. 493-514.

[145] Majak W, Fesser AC, Goplen BP, Pedersen MW. (**1980**) Relationship between ruminant bloat and composition of alfalfa herbage. II. Saponins. *Canadian Journal of Animal Science*, ***60***, 699-708.

[146] Oleszek W, Nowacka-Zaborska JM, Minta M, Zmudzki J. (**1995**) Effect of alfalfa saponin-zanhic acid tridesmoside on hamsters (*Mesocricetus auratus*). In *Current trends in Fruit and Vegetable Phytochemistry*. Garcia-Viguera C, Castaner M, Gil MI, Ferreras F, Tomas-Barberan FA (Eds). Consejo Superior Investigaciones Cientificas, Madrid, Spain. 293-297.

[147] Oleszek W, Nowacka J, Gee JM, Wortley GM, Johnson IT. (**1994**) Effects of some purified alfalfa (*Medicago sativa*) saponins on transmural potential difference in mammalian small intestine. *Journal of Science of Food and Agriculture*, ***65***, 35-39.

[148] Malinow MR, McLaughlin P, Papworth L, Stafford C, Kohler GO, Livingston AL, Cheeke PR. (**1977**) Effects of alfalfa saponins on intestinal cholesterol adsorption in rats. *The American Journal of Clinical Nutrition*, ***30***, 2061-2067.

[149] Malinow MR, Connor WE, McLaughlin P, Stafford C, Lin DS, Livingston AL, Kohler GO, McNulty WP. (**1981**) Cholesterol and bile acid balance in *Macaca fascicularis*: effects of alfalfa saponins. *The Journal of Clinical Investigation*, ***67***, 156-162.

[150] Sroka Z, Jurzysta M, Tylcz J, Rzadkowska-Bodalska H. (**1997**) Stimulation of pancreatic lipase activity by saponins isolated from *M. sativa* L. *Zeitschrift fur Naturforschung*, ***52c***, 235-239.

[151] Bader G, Plohmann B, Hiller K, Franz F. (**1996**) Cytotoxicity of triterpenoid saponins. Part 1: activities against tumor cells *in vitro* and haemolytic index. *Pharmazie*, ***51***, 414-417.

[152] Singuaroli I. (**2002**) Attività citotossica di saponine da *M. sativa* in linee tumorali umane. Doctoral Thesis. Università degli Studi di Pavia, Facoltà di Farmacia, Dipartimento di Farmacologia Sperimentale ed Applicata.

[153] Gaidi G, Correia M, Chauffert B, Beltramo JL, Wagner H, Lacaille-Dubois MA. (**2002**) Saponin-mediated potentiation of cisplatin accumulation and cytotoxicity in human colon cancer cells. *Planta Medica*, ***68***, 70-72.

[154] Messina MJ, Persky V, Setchell KDR, Barnes S. (**1994**) Soy intake and cancer risk: a review of the *in vitro* and *in vivo* data. *Nutrition and Cancer*, ***21***, 113-131.

[155] Rao AV, Sung MK. (**1995**) Saponins as anticarcinogens. *Journal of Nutrition*, ***125***, 117s-124s.

[156] Fournier DB, Erdman JW Jr, Gordon GB. (**1998**) Soy, its components, and cancer prevention: a review of the *in vitro*, animal, and human data. *Cancer Epidemiology Biomarkers Prevention*, ***7***, 1055-1065.

[157] Gestneter B, Birk Y, Tencer Y. (**1968**) Soybean saponins. Fate of ingested soybean saponins and the physiological aspect of their hemolytic activity. *Journal of Agricultural and Food Chemistry*, ***16***, 1031-1035.

[158] Sung MK, Kendall CWC, Koo MM, Rao AV. (**1995**) Effect of soybean saponins and gypsophylla saponin on growth and viability of colon carcinoma cells in culture. *Nutrition and Cancer*, ***23***, 259-270.

[159] Oh YJ, Sung MK. (**2001**) Soybean saponins inhibit cell proliferation by suppressing PKC activation and induce differentiation of HT-21 human colon adenocarcinoma cells. *Nutrition and Cancer*, ***39***, 132-138.

[160] Koratkar R, Rao AV. (**1997**) Effect of soya bean saponins on azoxymethane-induced preneoplastic lesions in the colon of mice. *Nutrition and Cancer*, ***27***, 206-209.

[161] Konoshima T, Kokumai M, Kozuka M, Tokuda H, Nishino H, Iwashima A. (**1992**) Anti-tumor-promoting activities of afromosin and soyasaponin I isolated from *Wisteria brachybotrys. Journal of Natural Products*, ***55***, 1776-1778.

[162] Elias R, De Meo M, Vidal-Ollivier E, Laget M, Balansard G, Dumenil G. (**1990**) Antimutagenic activity of some saponins isolated from *Calendula officinalis* L., *C. arvensis* L. and *Hedera helix* L. *Mutagenesis*, ***5***, 327-331.

[163] Danloy S, Quetin-Leclercq J, Coucke P, De Pauw-Gillet MC, Elias R, Balansard G, Angenot L, Bassleer R. (**1994**) Effects of α-hederin, a saponin extracted from *Hedera helix*, on a cell cultured *in vitro*. *Planta Medica*, ***60***, 45-49.

[164] Park HJ, Kwon SH, Lee JH, Lee KH, Miyamoto KI, Lee KT. (**2001**) Kalopanaxsaponin A is a basic saponin structure for the anti-tumor activity of hederagenin monodesmosides. *Planta Medica*, ***67***, 118-121.

[165] Konoshima T, Kozuka M, Haruma M, Ito K. (**1991**) Constituents of leguminous plants, XIII. New triterpenoid saponins from *Wisteria brachybotrys. Journal of Natural Products*, ***54***, 830-836.

[166] Czeczot H, Rahden-Staron I, Oleszek W, Jurzysta M. (**1994**) Isolation and studies of the mutagenic activity of saponins in the Aimes test. *Acta Poloniae Pharmaceutica – Drug Research*, ***51***, 133-136.