Biological Activities and Chemistry of Triterpene Saponins from Medicago Species: An Update Review

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1. Introduction

Plants are known to be a great source of phytochemicals for centuries. Medicago, belonging to the Family Fabaceae, is a large and well spread genus comprising about 83 cosmopolitan species, of which one-third are annuals and span diverse ecological niches. Medicago species are rich in saponins mainly classified into three classes, namely, steroid alkaloid glycosides, triterpene glycosides, and steroid glycosides. These saponins are important compounds having diverse pharmacological and biological activities. As a whole, 95 of saponins are reported to date occurring in Medicago species using various latest extraction/isolation techniques. Considering the multiple biological and pharmacological potential of Medicago species due to saponins along with structural diversity, we compiled this review article to sum up the recent reports for the pharmacological potential of the Medicago’s derived saponins in modern as well as traditional medication systems. The current manuscript produces data of chemical structures and molecular masses of all Medicago species saponins simultaneously. The toxicity of certain pure saponins (aglycones) has been reported in vitro; hederaegenin appeared highly toxic in comparison to medicagenic acid and bayogenin against X. index, while soyasaponin I, containing soyasapogenol B as a glycone, appeared as the least toxic saponin. The diversity in the structural forms shows a close relationship for its biological and pharmacological actions. Moreover, saponins showed antioxidant properties and the mechanism behind antimicrobial potential also elaborated in this review article is mainly because of the side sugar groups on these compounds. The collected data presented herein include chemical structures and molecular masses of all saponins so far. Their biological activity and therapeutic potential are also discussed. This information can be the starting point for future research on this important genus.
Evidence-Based Complementary and Alternative Medicine

Keeping in view the diverse biological and pharmacological activities of saponins along with structural diversity, the most recent available literature about the saponins has been reviewed. Moreover, the nematicidal potential of saponins found in various Medicago species along with antioxidant properties shall also be discussed using latest literature to give an update of this important class of compounds. The collected data presented herein includes chemical structures and molecular masses of all saponins so far. Their biological activity and therapeutic potential are also discussed. This information can be the starting point for future research on this important genus.

2. Extraction, Separation, Identification, and Quantification of Saponins

Saponins are secondary plant metabolites distributed in the plant kingdom in several species, and they encompass triterpenoids, steroids, and steroidal alkaloids glycosylated having single or multiple sugar residues or chains [18]. Contents and composition profile of saponins depend on the cultivar, environmental conditions, physiological stage of growth, and plant organ. The saponin amount varied according to the species, ranging from 0.38 ± 0.04% for M. rugosa Desr. to 1.35 ± 0.08% for M. scutellata (L.) Mill. Medicagenic acid was the dominant aglycone in M. blancheana, M. doliata, M. littoralis, M. rotata, M. rugosa, M. scutellata, M. tornata, and M. truncatula, echinocystic acid in M. polymorpha, hederagenin and bayogenin in M. rigidula and M. arabica, and soyasapogenol B in M. aculeata [19]. The pharmaceutical property discoveries from the Medicago species have driven the emergence of various extraction technologies with the main purpose of maximizing the yield in order to accommodate the recent need. Therefore, Cheok et al. reviewed the extraction and quantification of saponins [20]. In general, the extraction techniques employed in saponin extraction are Soxhlet, maceration, and reflux extraction, microwave-assisted, ultrasound-assisted, and accelerated solvent extraction. The quantification of plant saponins is usually carried out by UV-spectrophotometric and chromatographic (HPLC, UPLC, TLC) methods [19]. Saponins are separated and purified from plant materials using chromatographic methods in many studies to identify a specific saponins compound and investigate its pharmaceutical property [20]. Sapogenins are usually obtained after acid hydrolysis of saponins and evaluated by GC/FID and GC/MS methods [19]. The elucidation and characterization of saponins structure are conducted usually on the basis of EI-MS, 1D, and 2D NMR data [20].

3. Chemical Constituents

For the genus Medicago, saponins make highly complex blend of glycosidic triterpenes originally derived from the isoprenoid pathway via the cyclization of 2,3-oxidosqualene to form β-aminyn nucleus. Oxidative modifications are driven by a series of cytochromes P450 (CYPs) and generate the aglycone moieties (sapogenins) that are subjected to
glycosyl transfer reactions mediated by glycosyltransferases to give the different saponins. On this basis, 2 groups of saponins are reported in the *M. sativa* that can be differentiated: (1) sapogenins with COOH at the C-28 and different oxidation states (zero, OH, CHO, COOH) at C-23 (medicagenic acid, zanthic acid, hedegeranin, bayogenin, \(2\beta\), \(3\beta\)-dihydroxy-23-oxo-olean-12-en-28-oic acid); and (2) sapogenins with an OH group at C-24 with no substituent at C-28 (soyasapogenol A, B, E) [21]. Recently the quericetaric acid and its 2 \(\beta\)-hydroxy derivative, \(2\beta\), \(3\beta\), 30-trihydroxyolean-12-en-28-oic acid have been identified as novel aglycon in *M. arabica* (Figure 1). Quericetaric acid has the olean-12-ene skeleton and, together with glycyrrhetic acid, is one of the few naturally occurring triterpenes which is oxygenated at C-30 [22]. Quericetaric acid is supposed to be synthesized in vivo by a CYP P450 dependent hydroxylation of oleanonic acid [23].

In-depth examinations are conducted to elucidate chemical structures of saponins (compounds 1–95) in *M. arabica*, *M. marina*, *M. polymorpha*, *M. truncatula*, *M. sativa*, and *M. arborea*. Various saponins characterized till now from these species of *Medicago* are described in Tables 1–6. Various aboveground parts of *M. arabica* are well characterized to report the occurrence of saponins comprising of short chain sugar residues such as mono and bidesmosides of hedegeranin, bayogenin, \(2\beta\)-hydroxy oleanolic acid, soyasapogenol B, and oleanolic acid. An exciting quality of saponins derived from *M. arabica* is bidesmosides of \(2\beta\), \(3\beta\), 30-trihydroxyolean-12-en-28-oic acid and \(2\beta\), \(3\beta\), 30-trihydroxyolean-12-en-28-oic acid (compounds 1–5), as new aglycons for saponins of *Medicago* species (Table 1). All the detected saponins in *M. marina* are bidesmosidic compounds with the C-3 position characterized by the presence of the same sugar, glucose or by the disaccharide chain Glc \(1\alpha\)–\(2\alpha\) Glc (Table 2). Compounds 20, 21, 25, and 26 are undescribed in *Medicago* and never reported before in other plant species. Twelve triterpene saponins are recognized as glycosides of echinocystic acid hedegeranin, soyasapogenol B, bayogenin, and caulophylogenin in *M. polymorpha* (Table 3). Compounds 31 and 32 are declared as the novel natural compounds in *Medicago* species. Echinocystic acid is pioneer compounds to be reported in the genus *Medicago*. Saponins in *M. truncatula* seeds consist mainly of mono- and bidesmosides of soyasapogenol B and medicagenic acid (Table 4). Thirty-five pentacyclic triterpenoid saponins in *M. sativa* have been reported to occur as a complex mixture of short and long sugar chains of mono and bidesmosidic compounds having zanthic acid, bayogenin, hedegeranin, medicagenic acid, \(2\beta\), \(3\beta\)-Dihydroxy-23-oxo-olean-12-en-28oic acid and soyasapogenol B (Table 5). Compounds 62, 77, 78, 84–88, and 91 are new triterpenoid saponins, but methyl ester derivative of saponins (compounds 77, 78, and 88) are accepted as artifacts examined through methanolic extraction [24]. *M. arborea* saponins from aerial parts are mainly mono and bidesmosides of medicagenic acid (Table 6).

4. Biological Activity

Being the model plant species, *Medicago* holds a prominent place in Leguminosae family mainly due to its saponins [36].

The presence of diverse class of chemicals holding multiple biological activities is all well reported and utilized for centuries. These saponins are primarily the glycosides having aglycone moiety which is formed involving enzymatic cyclization of 2,3-oxidosoualene catalyzed by the \(\beta\)-amyrin cyclase [22]. Most of the *Medicago* species are being utilized as fodder for the grazing animals, but traditional medication system also clarifies that some of the species such as *M. sativa* herb are also beneficial for the human body. *M. sativa* is well recognized for centuries in traditional medication system in curing loss of memory, kidney issues, asthma, coughing, joint pains, and central nervous system disorders. All these pharmacological activities are detailed in the following text.

4.1. Insecticidal Activity. Due to increasing environmental and public health issues of using synthetic pesticides, the scientists are ever trying their hard to explore safer biological molecules to cure agricultural crops against multiple pathogens, namely, insects, bacterial, and fungal strains. Plant parasitic nematodes are cosmopolitan in distribution and are a major cause of huge economic losses for most of the agricultural crops and often quite hard to control the pathogens [37]. *M. sativa* L. shoot contains large amounts of saponins, which were identified in a recent study for their biological against aphid feeding, and found strong aphid inhibitory effects [38]. In an *in vitro* study, saponin rich mixtures of *M. sativa* showed effective growth inhibition on the viral vector nematodes like *Xiphinema*, the root-knot nematode *Meloïdogyne incognita*, and *Globodera rostochiensis* which are the potato cyst parasites [37]. Three saponins, namely, \(3\)O-[\(\beta\)-D-glucuronopyranosyl]-28-O-[\(\alpha\)-L-arabinopyranosyl\(\alpha\)-L-rhamnopyranosyl]-28-O-[\(\alpha\)-L-arabinopyranosyl] medicagenic acid, Zanthic acid tridesmoside and \(3\)O-[\(\beta\)-D-glucuronopyranosyl]-28-O-[\(\beta\)-D-xylopyranosyl\(\alpha\)-L-rhamnopyranosyl\(\alpha\)-L-rhamnopyranosyl] medicagenic acid were extracted from *M. sativa* L., potentially inhibits feeding of aphid *Acrystosiphon pisum* assessed through electrical penetration graph technique in a dose dependent way [38]. In another recent study, saponins (10, 100 ppm) extracted from *M. sativa* extracts were applied freshly ecysed 3rd larval instar of *Spodoptera littoralis*, and higher dose (100 ppm) caused absolute death while lower dose (10 ppm) caused only 26.7% mortalities [30]. Saponins mainly exert their effects by decreasing viability and rising mortalities, lowering the weights, reducing development and reproductive activities. Moreover, *M. sativa* saponins damaged the hindgut and fat body of *S. littoralis* badly to reduce its populations [30]. Another study examined the nematocidal effects of saponins of three different *Medicago* species (*M. sativa*, *M. arabica*, *M. arborea*) using plant shoots and roots against *Xiphinema index*, which is a plant parasitic nematode. It is said that the presence of prosapogenins and sapogenins in shoots and roots extracts (500 \(\mu g/ml\)) effectively induces absolute (100%) mortality of *X. index*, except the *M. arborea* that is less effective within 48 hours [39]. This nematocidal activity is correlated with the presence of aglycones (medicagenic acid and...
hederagenin) that occur in the roots and shoots saponin extracts [40]. *M. truncatula* saponins mediate caterpillar deterrence as a resistance mechanism in F83005.5 ecotype and associate these saponins as potential antifeedants that could be used in agricultural sustainable pest management strategies.

The seeds flour of *M. truncatula* showed a strong inhibition of the major pest (rice weevil *Sitophilus oryzae*) of cereals including rice [28], which were mainly responsible to the constituent of saponins 3-GlcA-28-AraRhaxylmedicagenate. Furthermore, when the saponin 3-GlcA-28-AraRhaxylmedicagenate was used in less concentration, it showed no effect on *Caenorhabditis elegans* (*C. elegans*) and *E. coli*, but at higher concentrations (100 µg/ml) it may lead to stopping the growth of *Saccharomyces cerevisiae*. Continuing this, the study emphasized the use of this target specific saponin (3-GlcA-28-AraRhaxylmedicagenate) only for mature *S. oryzae* but not others like coleopteran *Tribolium castaneum* and the S9 insect cultured cells [28]. Root knot nematodes *Meloidogyne incognita* is the major cause of huge economic losses and is quiet hard to control. *M. sativa* L. crude extracts are much effective against tomato seedling infection caused by root knot nematode *Meloidogyne incognita*, which is mainly due to less cholesterol levels in root knot nematode eggs controlled by the saponins in plant extracts [40].

Gastrointestinal nematodes are considered as the crucial parasites in ruminants deteriorating the quality dairy products, hence appealing the exploration of natural phytochemicals bearing anthelmintic potential to avoid synthetic chemicals. The extracts of four *Medicago* species (*M. sativa, M. arborea, M. polymorpha, M. polymorpha*) were examined to find in vitro anthelmintic potential of 1% saponins that cause a significant reduction (>80%) in nematode egg hatching of gastrointestinal nematodes of dairy donkeys [41]. In another study, the *Medicago* plant extracts enriched with prosapogenins and saponins were tested for in vitro anthelmintic activity for sheep gastrointestinal strongyles (GISs) by the egg hatch test. The prosapogenins and saponins obtained from extracts of *M. polymorpha* cultivars Anglona showed strong inhibition on GIS eggs following a concentration-dependent manner [42].

4.2. Cytotoxic Effects. The saponins in alfalfa roots extracts (50 µg ml⁻¹) induce over 75% cell death in poplar cells following a dose dependent fashion. This reduction in cell viability was mainly due to saponins-mediated induction of nitric oxide (NO) and reactive oxygen species (ROS) production, where the former found quite responsive to sodium azide and N⁵-monomethyl-L-arginine, which are the specific inhibitors of specific cellular pathways involved in NO biosynthesis in the plant cells isolated from poplar [43]. In another study, brine shrimps (*Artemia Salina*) were treated with extracts of twelve different *Medicago* plant species rich in a range of saponins. But, plant extracts of *M. rigidula* and *M. arabica* showed lethal dose₅₀ of 4.6 and 10.1 µg/mL, which depicts structure-activity relationship [19].

The different saponin extracts from *M. arabica* tops and roots showed best cytotoxic activity at the highest concentrations (200 µg/ml) against MCF-7 and HeLa cells using cisplatin as a positive control, and showed only 14 and 23% of cell survival, respectively. In this study, saponins
Table 1: Saponins identified in *M. arabica* leaves.

| Aglycone | No. | 3-OH substituted | 28-COOH substituted | 30-CH₃ substituted | Formula weight | Ref. |
|----------|-----|------------------|---------------------|-------------------|----------------|-----|
| 2β, 3β, 30-Trihydroxyolean-12-en-28-oic acid | 1* | α-L-Ara(1→2)-β-D-GluA | — | β-D-Glc | C₆₀H₁₀₀O₃₂ 1368 [26] |
| | 2* | β-D-GluA | α-L-Ara(1→2)-β-D-Glc | C₆₀H₁₀₀O₃3 1368 [26] |
| Hederagenin | 6 | α-L-Ara(1→2)-β-D-Glc-(1→2)-α-L-Ara | β-D-Glc | — | C₅₉H₉₈O₂₂ 1060 [26] |
| | 7 | β-D-Glc-(1→2)-α-L-Ara | β-D-Glc | — | C₆₀H₁₀₀O₂₀ 958 [26] |
| | 8 | α-L-Ara | β-D-Glc | — | C₅₉H₉₈O₁₇ 928 [26] |
| | 9 | α-L-Ara(1→2)-β-D-Glc-(1→2)-α-L-Ara | — | — | C₅₉H₉₈O₁₃ 766 [26] |
| 3β, 30-Dihydroxyolean-12-en-28-oic acid | 10 | β-D-Glc-(1→2)-α-L-Ara | — | — | C₅₉H₉₈O₁₃ 766 [26] |
| | 11 | α-L-Ara(1→2)-β-D-GluA | — | — | C₅₉H₉₈O₁₄ 780 [26] |
| | 12 | α-L-Ara | — | — | C₅₉H₉₈O₈ 604 [26] |
| Bayogenin | 13 | α-L-Ara | β-D-Glc | — | C₅₉H₉₈O₁₄ 782 [26] |
| | 14 | α-L-Ara | — | — | C₅₉H₉₈O₈ 620 [26] |
| 2β-Hydroxy oleanolic acid | 15 | α-L-Ara(1→2)-β-D-GluA | β-D-Glc | — | C₅₉H₉₈O₁₉ 942 [26] |
| | 16 | β-D-GluA | — | — | C₅₉H₉₈O₁₀ 648 [26] |
| Soyasapogenol B | 17 | L-Rha(1→2)-β-D-Gal-(1→2)-β-D-GluA | — | — | C₅₉H₉₈O₁₈ 942 [6, 26] |
| Oleanolic acid | 18 | α-L-Ara(1→2)-β-D-GluA | — | — | C₅₉H₉₈O₁₃ 784 [26] |
| | 19 | β-D-GluA | — | — | C₆₃H₁₀₀O₃₂ 632 [26] |

Table 2: Saponins from *M. marina* leaves and roots.

| Aglycone | No. | 3-OH substituted | 28-COOH substituted | Formula weight | Ref. |
|----------|-----|------------------|---------------------|----------------|-----|
| Zanhic acid | 20* | β-D-Glc(1→2)-β-D-Glc | β-D-Xyl(1→4)-β-D-Api(1→3)-α-L-Rha(1→2)-α-L-Ara | C₆₃H₁₀₀O₃₃ 1384 [26] |
| | 21* | β-D-Glc(1→2)-β-D-Glc | β-D-Xyl(1→4)-α-L-Rha(1→2)-α-L-Ara | C₅₉H₉₈O₂₉ 1252 [26] |
| | 22 | β-D-Glc(1→2)-β-D-Glc | β-D-Xyl(1→4)-β-D-Ara(1→3)-α-L-Rha(1→2)-α-L-Ara | C₆₃H₁₀₀O₃₃ 1384 [26] |
| | 23 | β-D-Glc | β-D-Xyl(1→4)-β-D-Ara(1→3)-α-L-Rha(1→2)-α-L-Ara | C₅₉H₉₈O₂₉ 1222 [26] |
| | 24 | β-D-Glc | β-D-Xyl(1→4)-α-L-Rha(1→2)-α-L-Ara | C₅₉H₉₈O₂₄ 1090 [26] |
| Medicagoenic acid | 25* | β-D-Glc(1→2)-β-D-Glc | β-D-Xyl(1→4)-β-D-Ara(1→3)-α-L-Rha(1→2)-α-L-Ara | C₆₃H₁₀₀O₃₂ 1368 [26] |
| | 26* | β-D-Glc | β-D-Xyl(1→4)-β-D-Ara(1→3)-α-L-Rha(1→2)-α-L-Ara | C₅₉H₉₈O₂₇ 1206 [26] |
| | 27 | β-D-Glc(1→2)-β-D-Glc | β-D-Xyl(1→4)-β-D-Api(1→3)-α-L-Rha(1→2)-α-L-Ara | C₆₃H₁₀₀O₃₂ 1368 [26] |
| | 28 | β-D-Glc | β-D-Xyl(1→4)-α-L-Rha(1→2)-α-L-Ara | C₅₉H₉₈O₂₃ 1074 [26] |
| Soyasapogenol B | 29 | α-L-Rha(1→2)-β-D-Gal(1→2)-β-D-GluA | — | — | C₄₈H₇₆O₁₈ 942 [26] |
| Soyasapogenol E | 30 | α-L-Rha(1→2)-β-D-Gal(1→2)-β-D-GluA | — | — | C₄₈H₇₆O₁₈ 940 [26] |
4.3. Antioxidant Potential. The extraction studies reported that Medicago plants extracts bear strong antioxidant potential. For instance, various parts (roots, stem, leaves) of M. sativa plant ethanolic extracts yield various phenolics, flavonoids, and saponins, all of which show higher antioxidant potential [44]. M. lupulina is comparatively less studied species, and its crude methanolic extracts showed antioxidant activity with a Trolox® equivalent antioxidant activity (TEAA) and ferric reducing antioxidant power (FRAP) values of 45.4 μmol Trolox/g dw and 0.2 mmol Fe²⁺/g dw through DPPH and FRAP assay [45]. In vitro free radical scavenging activity using DPPH assay was performed using various extracts of M. sativa seeds, but ethanolic extracts of seeds and seed sprouts showed maximum and ascending radical scavenging activity in a concentration dependent fashion (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 μg) [46].

4.4. Antimicrobial Effects. The extracts of M. sativa have strong inhibitory effect on Proteus vulgaris, Escherichia coli (E. coli), Klebsiella pneumonia, Salmonella typhi, Macor circinelloides, Rhizopus azygosporus, and R. microsporus with less pronounced action on Shigella flexneri, Staphylococcus epidermidis, Candida albicans, and Emericella quadrilineata [10]. Moreover, a reversed influence on Pseudomonas aeruginosa and Streptococcus pyogenes was seen, while Pseudallescheria ellipsoidea, two species of Penicillium, and five of Aspergillus were seen somewhat resistant for these plant extracts [10]. M. sativa plant extracts rich in saponins showed strong antifungal potential to successfully check the growth of Candida albicans along with certain clinical pathogenic fungal strains mainly by inhibiting the germ tube formation, retarded the growth of fungal hyphae, and lessened the adherence of yeast cells and eradication of biofilm development at 24 hours after treatment [47]. It is further stated that saponin extracts of M. sativa in a dosage range harmful to check the growth of fungi are least toxic to the mice fibroblast L929 cells, which showed them being safe to use for human antifungal conditions [47].

### Table 3: Saponins from M. polymorpha leaves and roots.

| Aglycone        | No. | 3-OH substituted | 28-COOH substituted | Formula weight | Ref. |
|-----------------|-----|------------------|---------------------|----------------|------|
| Echinocystic acid | 31* | α-L-Ara          | β-D-Glc             | C₁₂H₁₈O₁₃ 766  | [27] |
|                 | 32* | α-L-Ara          | β-D-Glc             | C₁₂H₁₈O₁₃ 928  | [27] |
|                 | 33  | β-D-Glc(1→2)-α-L-Ara | β-D-Glc(1→6)-β-D-Glc | C₁₃H₁₆O₁₃ 1090 | [27] |
|                 | 34  | α-L-Ara          | —                   | C₁₂H₁₆O₆ 604  | [27] |
|                 | 35  | β-D-Glc          | —                   | C₁₁H₁₆O₆ 634  | [25] |
| Hederagenin     | 36  | α-L-Rha(1→2)-α-L-Ara | β-D-Glc(1→6)-β-D-Glc | C₁₃H₁₆O₁₂ 1074 | [27] |
|                 | 37  | β-D-Glc(1→2)-α-L-Ara | β-D-Glc             | C₁₂H₁₈O₁₃ 928  | [27] |
|                 | 38  | α-L-Ara          | β-D-Glc             | C₁₁H₁₆O₆ 766  | [27] |
|                 | 39  | α-L-Rha(1→2)-α-L-Ara | —                   | C₁₂H₁₆O₁₂ 750  | [25, 27] |
|                 | 40  | α-L-Ara          | —                   | C₁₂H₁₆O₆ 604  | [27] |
| Soyasapogenol B | 41  | α-L-Rha(1→2)-β-D-Gal(1→2)-β-D-GluA | — | C₁₄H₂₀O₁₈ 942 | [27] |
| Cauophylligenin | 42  | α-L-Ara          | —                   | C₁₂H₁₆O₆ 620  | [27] |
| Bayogenin       | 43  | α-L-Ara          | —                   | C₁₂H₁₆O₆ 620  | [27] |

### Table 4: Saponins from M. truncatula seeds.

| Aglycone        | No. | 3-OH substituted | 28-COOH substituted | Formula weight | Ref. |
|-----------------|-----|------------------|---------------------|----------------|------|
| Soyasapogenol B | 44  | β-D-GlcA         | β-D-Xyl(1→4)-α-L-Rha(1→2)-α-L-Ara | C₁₂H₁₈O₁₃ 1044 | [28] |
|                 | 45  | α-L-Rha(1→2)-β-D-Gal(1→2)-β-D-GlcA | — | C₁₄H₂₀O₁₈ 942 | [29] |
| Medicagenic acid| 46  | β-D-GlcA         | α-L-Ara(1→2)-α-L-Rha(1→2)-β-D-Xyl | C₁₃H₁₆O₂₄ 1088 | [29] |
|                 | 47  | β-D-Glc          | α-L-Ara(1→2)-α-L-Rha(1→2)-β-D-Xyl | C₁₃H₁₆O₂₃ 1074 | [29] |
|                 | 48  | β-D-Glc          | β-D-Glc             | C₁₃H₁₆O₁₆ 826  | [29] |

(monodesmosides of hederagenin and bayogenin) rich plant extracts mainly containing 1, 3-O-β-D-glucopyranosyl (1→2)-α-L-arabinopyranosyl hederagenin potentially reduced the proliferation of MCF-7 and HeLa cells at 24 hours.
### Table 5: Saponins from *M. sativa.*

| Aglycone | No. | 3-OH substituted | 28-COOH substituted | Formula weight | Ref. |
|----------|-----|------------------|---------------------|----------------|-----|
| Medicagenic acid | | | | | |
| | 49 | \(\beta\)-D-GlcA | \(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | \(C_{47}H_{72}O_{20}\) 956 | [30] |
| | 50 | \(\alpha\)-L-Rha(1\(\to\)2)-\(\beta\)-D-Gal(1\(\to\)2)-\(\beta\)-D-GluA | — | — | — |
| | 51 | \(\beta\)-D-GlcA | \(\beta\)-D-Xyl(1\(\to\)4)-\(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | \(C_{52}H_{86}O_{24}\) 1088 | [18, 31] |
| | 52 | \(\beta\)-D-GlcA | \(\beta\)-D-GlcA | \(C_{42}H_{66}O_{18}\) 854 | [32] |
| | 53 | \(\beta\)-D-Glc(1\(\to\)3)-\(\beta\)-D-Glc | \(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | \(C_{53}H_{84}O_{24}\) 1104 | [32] |
| | 54 | \(\beta\)-D-GlcA | \(\beta\)-D-Api(1\(\to\)3)-[\(\beta\)-D-Xyl(1\(\to\)4)]-\(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | | |
| | 55 | \(\beta\)-D-Glc | \(\beta\)-D-Glc(1\(\to\)4)-\(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | \(C_{53}H_{84}O_{24}\) 1104 | [33] |
| | 56 | \(\beta\)-D-Glc(1\(\to\)2)-\(\beta\)-D-Glc | \(\beta\)-D-Xyl(1\(\to\)4)-\(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | \(C_{58}H_{96}O_{28}\) 1236 | [18, 33] |
| | 57 | \(\alpha\)-L-Ara(1\(\to\)2)-\(\beta\)-D-Glc(1\(\to\)2)-\(\alpha\)-L-Ara | \(\beta\)-D-Glc | \(C_{52}H_{80}O_{24}\) 1090 | [33] |
| | 58 | \(\beta\)-D-Glc | — | | |
| | 59 | \(\beta\)-D-Glc | \(\beta\)-D-Glc | \(C_{42}H_{66}O_{16}\) 826 | [1, 18, 25] |
| | 60 | \(\beta\)-D-Glc | \(\beta\)-D-Xyl(1\(\to\)4)-\(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | | |
| | 61 | \(\beta\)-D-Glc(1\(\to\)2)-\(\beta\)-D-Glc | \(\beta\)-D-Glc | | |
| | 62* | \(\beta\)-D-Glc(1\(\to\)2)-\(\beta\)-D-Glc | — | — | — |
| | 63 | \(\alpha\)-L-Rha(1\(\to\)2)-\(\beta\)-D-Glc(1\(\to\)2)-\(\beta\)-D-Glc | — | — | — |
| Zanhic acid | | | | | |
| | 64 | \(\beta\)-D-GlcA | \(\beta\)-D-Xyl(1\(\to\)4)-\(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | \(C_{52}H_{80}O_{25}\) 1104 | [30] |
| | 65 | \(\beta\)-D-Xyl(1\(\to\)4)-\(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | — | — | — |
| | 66 | \(\beta\)-D-Glc | \(\beta\)-D-Xyl(1\(\to\)4)-\(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | \(C_{52}H_{80}O_{24}\) 1090 | [32] |
| | 67 | \(\beta\)-D-Glc(1\(\to\)3)-\(\beta\)-D-Glc | \(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | \(C_{53}H_{84}O_{25}\) 1120 | [32] |
| | 68 | \(\beta\)-D-Glc | \(\alpha\)-L-Ara(1\(\to\)3)-[\(\beta\)-D-Xyl(1\(\to\)4)]-\(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | \(C_{57}H_{90}O_{28}\) 1222 | [32] |
| | 69 | \(\beta\)-D-GlcA | \(\alpha\)-L-Ara(1\(\to\)3)-[\(\beta\)-D-Xyl(1\(\to\)4)]-\(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | \(C_{57}H_{88}O_{29}\) 1236 | [32] |
| | 70 | \(\beta\)-D-Glc(1\(\to\)3)-\(\beta\)-D-Glc | \(\alpha\)-L-Ara(1\(\to\)3)-[\(\beta\)-D-Ara(1\(\to\)4)]-\(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | | |
| | 71 | \(\beta\)-D-Glc(1\(\to\)2)-\(\beta\)-D-Glc | \(\beta\)-D-Api(1\(\to\)3)-[\(\beta\)-D-Xyl(1\(\to\)4)]-\(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | \(C_{58}H_{96}O_{33}\) 1384 | [32] |
| | 72 | \(\alpha\)-L-Ara(1\(\to\)2)-\(\beta\)-D-Glc(1\(\to\)2)-\(\beta\)-D-Glc | \(\beta\)-D-Api(1\(\to\)3)-[\(\beta\)-D-Xyl(1\(\to\)4)]-\(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | | |
| | 73 | \(\beta\)-D-Glc(1\(\to\)2)-\(\beta\)-D-Glc(1\(\to\)2)-\(\beta\)-D-Glc | \(\beta\)-D-Api(1\(\to\)3)-[\(\beta\)-D-Xyl(1\(\to\)4)]-\(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | \(C_{56}H_{106}O_{34}\) 1414 | [1, 18, 25] |
| | 74 | \(\beta\)-D-Glc(1\(\to\)2)-\(\beta\)-D-Glc(1\(\to\)2)-\(\beta\)-D-Glc | \(\alpha\)-L-Ara(1\(\to\)3)-[\(\beta\)-D-Xyl(1\(\to\)4)]-\(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | | |
| | 75 | \(\beta\)-D-GluA | \(\alpha\)-L-Ara(1\(\to\)3)-[\(\beta\)-D-Xyl(1\(\to\)4)]-\(\alpha\)-L-Rha(1\(\to\)2)-\(\alpha\)-L-Ara | \(C_{57}H_{86}O_{29}\) 1236 | [3] |
adipocytes [24]. Contemporary studies indicate that extracts rich in saponins are effective in lowering blood cholesterol levels. The potential beneficial effects of alfalfa saponins and flavonoids in agriculture and horticulture with regard to protecting plants against pests seem to be of great interest.

4.6. Saponins in Dietary Supplements. Various studies reported the use of alfalfa saponins in dietary supplements and are said to be linked with blood plasma parameters, nutrients digestibility, and growth performance of the cattle [48]. *Medicago* species mixed as hay and in silage are considered as significant food for herbivorous fauna, and a rich source of proteins and physically effective neutral detergent fiber for grazers [49]. Within natural grazing systems particularly in meadows, the intake of various classes of compounds like alkaloids, tannins, and saponins is being neutralized to give comfort to the grazers [50].

4.7. Bioavailability of the Saponins. The saponins have got permeability barrier across the cellular membranes for their large molecular weights. Hence the bioavailability of saponins should be checked as potential drugs. His major issue with larger molecular structures of saponins rendered them to catch the attention for utilization in drug industry. Recently, huge attempts were made to find the pharmacokinetics potential of these compounds (ginsenosides, astragaloside IV, clematininoside AR, and methylprotodioscin) sourced from different plants. In an attempt to find the reasons for the less permeability and reduced bioavailability of saponins, an in silico comparative study

| Aglycone   | No. | 3-OH substituted | 28-COOH substituted | Formula weight | Ref.  |
|------------|-----|-----------------|---------------------|----------------|-------|
| Bayogenin  |     |                 |                     |                |       |
| 76         | α-L-Ara | β-D-Glc   |                     | C_{41}H_{66}O_{14} 782 | [32]  |
| 77*        | β-D-Gal(1→2)-β-D-GluAME | β-D-Glu |                     | C_{48}H_{76}O_{22} 1016 | [35]  |
| 78*        | β-D-Xyl(1→4)-β-D-GluAME | β-D-Glc |                     | C_{48}H_{76}O_{20} 972 | [35]  |
| Hederagenin|     |                 |                     |                |       |
| 79         | —    | β-D-Glc   |                     | C_{46}H_{68}O_{13} 766 | [1]   |
| 80         | α-L-Ara(1→2)-β-D-Glc | β-D-Glc |                     | C_{47}H_{76}O_{18} 928 | [18]  |
| 81         | α-L-Ara(1→2)-β-D-Glc(1→2)-α-L-Ara | — |                     | C_{46}H_{74}O_{17} 898 | [18]  |
| 82         | β-D-Glc(1→2)-α-L-Ara | — |                     | C_{46}H_{76}O_{13} 766 | [1]   |
| 83         | α-L-Ara(1→2)-β-D-Glc(1→2)-α-L-Ara | β-D-Glc |                     | C_{42}H_{64}O_{16} 844 | [18, 34] |
| 84*        | β-D-Xyl(1→3)-β-D-Glc | β-D-Xyl(1→4)-α-L-Rha(1→2)-α-L-Ara |                     | C_{57}H_{82}O_{23} 1176 | [34]  |
| 85*        | α-L-Ara(1→2)-β-D-Glc(1→2)-β-D-Xyl | β-D-Glc |                     | C_{32}H_{42}O_{12} 1060 | [34]  |
| 86*        | β-D-Xyl(1→2)-β-D-Glc(1→2)-β-D-Glc | β-D-Glc |                     | C_{33}H_{46}O_{23} 1090 | [34]  |
| 87*        | β-D-Glc(1→2)-α-L-Ara | β-D-Glc |                     | C_{24}H_{50}O_{24} 928 | [34]  |
| 88*        | α-L-Ara(1→2)-β-D-Glc(1→2)-β-D-GluAME | β-D-Glc |                     | C_{24}H_{50}O_{24} 928 | [34]  |
| 89         | β-D-Glc(1→2)-α-L-Ara | β-D-Glc |                     | C_{48}H_{76}O_{20} 972 | [35]  |
| 2β, 3β-Dihydroxy-23-oxoolean-12-en-28oic acid |     |                 |                     |                |       |
| 90         | β-D-GlcA | β-D-Glc |                     | C_{42}H_{46}O_{16} 824 | [32]  |
| 91*        | β-D-Xyl(1→2)-β-D-Glc(1→2)-β-D-Glc | β-D-Glc |                     | C_{33}H_{46}O_{23} 1090 | [34]  |
| Soyasapogenol B |     |                 |                     |                |       |
| 92         | α-L-Rha(1→2)-β-D-Gal(1→2)-β-D-GluA | — |                     | C_{48}H_{76}O_{18} 942 | [1, 18, 25, 33, 34] |

| Table 6: Saponins from *M. arborea*. |
|--------------------------------------|
| Aglycone   | No. | 3-OH substituted | 28-COOH substituted | Formula weight | Ref.  |
|------------|-----|-----------------|---------------------|----------------|-------|
| Medicagenic acid |     |                 |                     |                |       |
| 93         | β-D-GlcA | β-D-Xyl(1→4)-α-L-Rha(1→2)-α-L-Ara |                     | C_{32}H_{46}O_{14} 1088 | [1, 25] |
| 94         | β-D-Glc | β-D-Xyl(1→4)-α-L-Rha(1→2)-α-L-Ara |                     | C_{32}H_{46}O_{13} 1074 | [1, 25] |
| 95         | β-D-Glc | —               |                     | C_{36}H_{56}O_{13} 664 | [1]   |
was done with crucial physicochemical parameters of cardiotonic drugs sourced from saponins/natural products to elucidate intestinal absorption and bioavailability [51].

5. Conclusion

The article summarizes the updates and latest advancements in various biological and pharmacological activities of structurally diverse saponins occurring in the genus Medicago. Medicago species (M. sativa L.) are being used in traditional medicine systems due to the presence of unique saponins. The article produces the data of chemical structures and molecular masses of all saponins simultaneously. The biological activity of saponins is dependent on the number of side sugar chains attached to the sapogenins as well as to the nature of the sapogenin itself [52]. Monodesmosidic compounds were generally reported to be more biologically active than the corresponding bidesmosidic saponins [19]. For example, when pure aglycones have been used in in vitro bioassays, hederagenin was shown to be even more toxic than medicagenic acid and bayogenin against X. index, while soyasaponin I, containing soyasapogenol B as a glycone, was the less-active saponin [53]. It is confirmed that structural diversity has a close relationship with its biological and/or pharmacological activities. It is suggested that more sophisticated techniques are needed to isolate more novel saponins for industrial, agricultural, and food manufacturing industries.

Data Availability

All data used to support the findings of this study are included within the paper.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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