Journal of Advanced Pharmaceutical Sciences

Journal homepage: https://japs.journals.ekb.eg/

Review article

Therapeutic potential of *Medicago sativa* (Alfalfa): A comprehensive review of its bioactive compounds and pharmacological properties

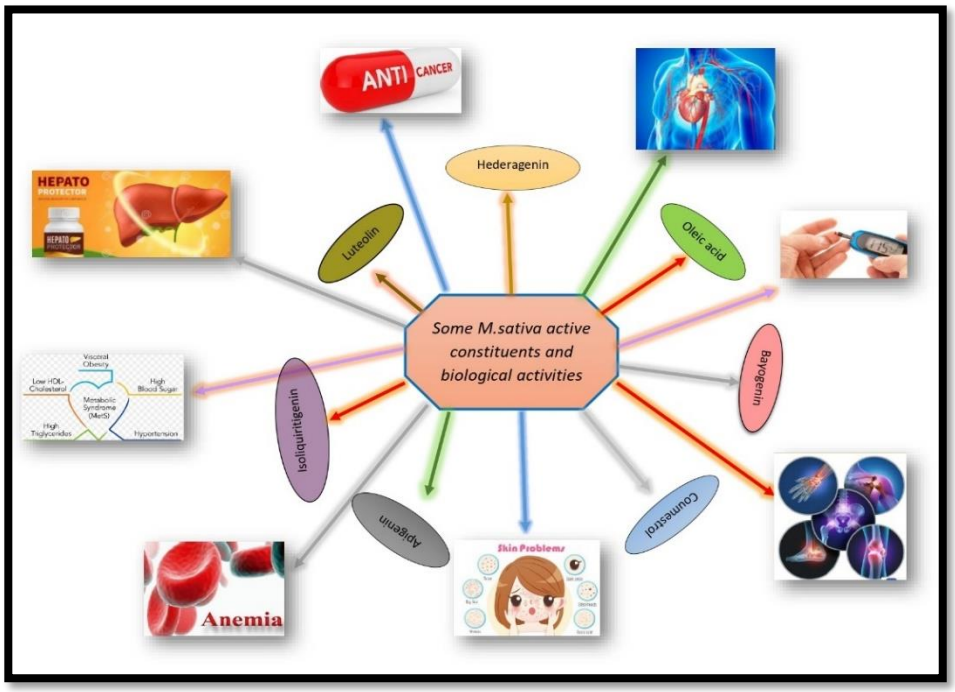
Asmaa Khairy, Hala M. Hammoda, Ali M. Metwally, Hala H. Zaatout & Reham S. Ibrahim*

Faculty of Pharmacy, Department of Pharmacognosy, Alexandria University, Egypt.

***Corresponding author:** Faculty of Pharmacy, Department of Pharmacognosy, University of Alexandria, El-Messalah, Alexandria 21521, Egypt. Tel: 01223821098, Fax: (+203)4868256

E-mail: reham.abdelkader@alexu.edu.eg

Abstract:



Medicago sativa or alfalfa (Fabaceae), commonly known as Berseen in Egypt, has been historically utilized to treat a variety of widely distributed diseases like arthritis, and renal issues, and is also used as a diuretic, antipyretic, antitumor, cardiotonic, anti-rheumatic, emmenagogue, depurative, antiscorbutic, lactagogue, and to treat boils. Sprouts and leaves were also eaten as a vegetable salad. In health food stores, seeds, and leaves were sold in bulk as powdered herb, tablets, and capsules for nutritional supplementation. *M. sativa* phytochemical examination revealed the presence of carbohydrates, proteins, phenolics, lignin, tannins, flavones, saponins, triterpene glycosides, alkaloids, sterols, carotenoids, isoflavonoids, phytoestrogens, and many other bioactive substances. Preceding pharmacological research revealed that the plant experienced a wide range of beneficial effects such as antioxidant, anti-inflammatory, anticancer, phytoestrogens, antimicrobial, antifungal, immunological, hypolipidemic, antidiabetic, neuroprotective, anxiolytic, anti-scorbutic, anti-anemic, dermatological, and hepatoprotective activities. This review investigated the bioactive components together with the pharmacological properties of *M. sativa*.

Received: 25 December 2024 …. Accepted: 29 March 2025 … Published: 6 April 2025

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

Keywords: *Medicago sativa*; Alfalfa; Biological properties; Phytochemical profile.

1. Introduction

The Legumes family is the third biggest angiosperm family behind Asteraceae and Orchidaceae [(1)] with over 770 genera and more than 19,500 species worldwide [(2-4)]. Based on overall output and harvested area, legumes rank as the second most important cereal crop in agriculture [(5)]. Foods, dyes, oils, hardwoods, medicines, fuel, and soil enrichment are among their economically valuable products. By fixing atmospheric nitrogen through root-nodulating symbiotic bacteria, they contribute significantly to the worldwide terrestrial biogeochemistry [(6)]. Fruits of the family Fabacaea are characterized by being typically legumes while inflorescences are usually racemose, of which simple raceme is very common [(7)]. Mimosoideae, Papilionoideae, and Caesalpinioideae are the three subfamilies into which the extremely varied legumes fall [(8)]. Of the three traditionally recognised subfamilies of the Fabacaea, Papilionoideae legumes are the most widely distributed, with approximately 13,800 species distributed among 28 tribes and 478 genera [(2)], one of which is genus *Medicago* [(9)]. The genus *Medicago L.* comprises a total number of 83 different species among annuals and perennials, according to the most recent taxonomic analysis of the genus [(10)]. The natural distribution of *Medicago* genus encompasses broad regions of North Africa, Caucasus, Iran, Turkey, and Eurasia [(11)]. Except for three shrubs, the majority of the species in the genus are herbaceous. The biological cycles may be perennial, biennial, or annual. *M. sativa*, or alfalfa, is an annual flowering shrub that belongs to the Fabaceae family. In several nations worldwide, it is grown as a significant forage crop [(12)]. It is utilized for silage, grazing, and hay. North America uses the term alfalfa, while in South Africa, New Zealand, Australia, and the United Kingdom, the term "lucerne" is more frequently used. It seems like clover and blooms up to approximately two feet tall, with a smooth and upright stem, trifoliate pinnately leaf, racemes of purple to violet flowers that bloom from June to August, and spirally- coiled seeds. It is also called “Father of All Plant” [(13)], “World's Feed Queen” and “King of Forage” [(14)]. Alfalfa is native to warm temperate areas. It has been farmed for cattle feed since at least the time of the ancient Romans and Greeks [(15)].

1.1. The taxonomy:

Kingdom: Plantae, **Subkingdom:** Angiosperms, **Division:** Eudicots, **Class:** Rosids**, Order:** Fabales, **Family:** Fabaceae, **Subfamily:** Papilionoideae, **Genus:** *Medicago,* **Species:** *sativa* [(16)].

1.2. Synonyms

Medica sativa, Medicago asiatica, Medicago afganica, Medicago beipinensis, Medicago ladak, Medicago grandiflora, Medicago orientalis, Medicago mesopotamica, Medicago praesativa, Medicago polia, Medicago praesativa subsp. spontanea, Medicago grandiflora, Medicago sativa f. alba, Medicago tibetana, Medicago tibetana, Medicago sogdiana, Trigonella upendrae [(17)].

1.3. Common names

English: lucerne, alfalfa; Arabic: berseem, jatt; French: alfalfa, luzerne cultivée; German: saatluzerne, blaueluzerne, luzerne; Italian: medica, erbamedica; Hindi: rizka, lasunghas, wilayati-gawuth; Russian: lyutzernasinyaya, lyutzernaposevnaya; Korean: jajukgaejari; Swedish: blalusern; Spanish: mielga, alfalfa rustica [(17)].

1.4. Parts used traditionally

Leaves, roots, seeds and sprouts [(18, 19)].

1.5. Traditional uses of *M. sativa*:

Alfalfa plant has an extended history of use in traditional healthcare in numerous parts around the globe, particularly India, China, Mexico, America, Turkey, and Iraq, for the

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

management of digestive system disorders (stomach ulcers, indigestion, enhancing the peristaltic function of the gastrointestinal tract, and appetite stimulation), vascular disorders (cardiotonic, blood clotting disorders), reproductive system (menopausal symptoms, uterine stimulant, breast cancer, prostate disorders, and cervical cancer) [20, 21], arthritis, kidney disorders (kidney stones, kidney pain, diuretic, fluid retention, gravel, bladder disorders, and dysuria) [22, 23] or respiratory system disorders (cough, asthma, allergy). It was also used for the treatment of fever, diabetes [24], inflammation, wound healing, scurvy, and CNS disorders [25-29].

1.6. Role in sustainable agriculture:

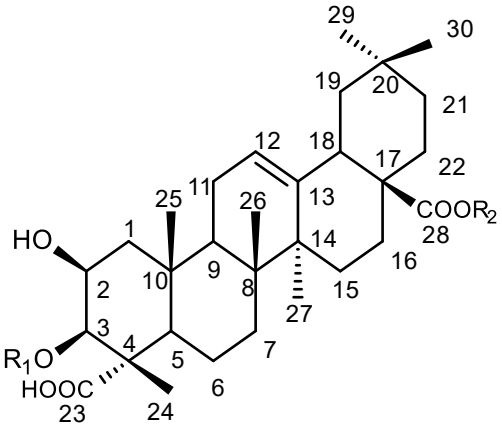
Alfalfa extensive root system is very helpful for retaining soil. The soil is rapidly covered by alfalfa canopy, which prevents soil erosion by water and wind [30]. Alfalfa capacity for capturing atmospheric nitrogen through root-nodulating symbiotic bacteria, so that N is accessible for plant growth is one of its primary advantages [31]. It is also a useful crop for controlling water tables because of its deep roots and substantial capacity to absorb water [32]. Moreover, alfalfa can be used to recycle a wide variety of organic waste materials. Numerous vital vitamins (A, B, E, and D) and minerals are naturally abundant in alfalfa [33].

2. Chemistry of *M. sativa*:

The preliminary phytochemical studies of alfalfa seeds extracts revealed the existence of saponins, proteins, lignin, carbohydrates, alkaloids, phenolic compounds, triterpene glycosides, tannins, phytoestrogens (cumestrol), sterols, carotenoids, isoflavones, flavones and phenolic compounds [34, 35]. Flavonoids and saponins, particularly those of the triterpenic pentacyclic type, are the most prevalent secondary metabolites found in *Medicago* species. Because of their high protein, crucial amino acids, and carbohydrate content, they are very important economically and nutritionally.

Literature review about the previously reported saponins, **Table 1**, flavonoids, **Table 2,** as well as other different classes and miscellaneous compounds in extracts of *M. sativa* are summarized as shown in **Tables 3-8**.

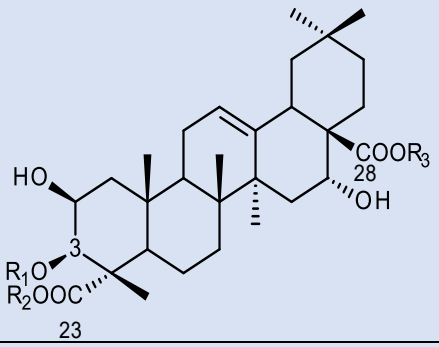
Table 1: Previously reported sapogenins and saponin glycosides in *M. sativa*:

Name	Structure		Ref.
			
	R1	**R2**	
Medicagenic acid (MA)	H	H	(36, 37)
MA-3-*O*-*β*-D-glucopyranoside	Glc	H	(36-41)
MA-3,28-di(*O*-*β*-D-glucopyranoside)	Glc	Glc	(36-41)
MA-3-*O*-[*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl]-28-*O*-[*β*-D-	Glc-Glc	Xyl-Rha-Ara	(37, 39, 41-43)

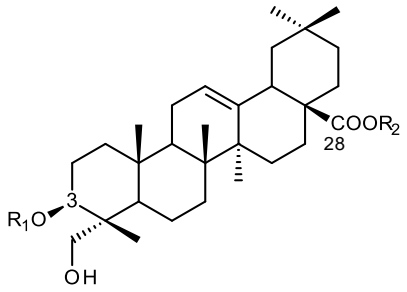
ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

xylopyranosyl(1→4)-*α*-L-rhamnopyranosyl(1→2)-*α*-L-arabinopyranoside]			
MA-3-*O*-*β*–D-glucopyranosyl-28-*O*-[*α*-L-rhamnopyranosyl(1→2)-*α*-L-arabinopyranoside]	Glc	Rha-Ara	(39, 41, 44)
MA-3-*O*-*β*-D-glucopyranosyl-28-*O*-[*β*-D-xylopyranosyl(1→4)-*α*-L-rhamnopyranosyl(1→2)-*α*-L-arabinopyranoside]	Glc	Xyl-Rha-Ara	(36, 39, 41, 43)
MA-3-*O*-*β*-g1ucuronopyranoside	GlcA	H	(36)
MA-3-*O*-*β*-D-glucuronopyranosyl-28- *O*-[*β*-D-xylopyranosyl(1→4)-*α*-L-rhamnopyranosyl(1→2)-*α*-L-arabinopyranoside]	GlcA	Xyl-Rha-Ara	(36, 37, 39, 41)
MA-28-*O*-[*β*-D-xylopyranosyl(1→4)-*α*-L-rhamnopyranosyl(1→2)-*α*-L-arabinoside]	H	Xyl-Rha-Ara	(41, 43)
MA-3-*O*-[*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl(1→2)-*α*-L-rhamnopyranosyl]-28-*O*-*β* -D-glucopyranoside	Glc-Glc-Rha	Glc	(40)
3-*O*-*β*-D-Glucopyranosyl-6″-malonyl MA	Glc-malonyl	H	(40)
MA-3-*O*-*β*-D-glucopyranosyl-6″-malonyl-28-*O*-*β*-D- glucopyranoside	Glc-malonyl	Glc	(40)
3*β*-Medicagenic acid-*β*-maltoside	Glc-Glc	H	(40, 45)
MA-3-*O*-[*α*-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranoside]	Rha-Glc-Glc	H	(39)
MA-3-*O*-[*α*-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl]-28-*O*-*β*-D-glucopyranoside	Rha-Glc-Glc	Glc	(39, 41)
MA-3-*O*-[*α*-L-arabinopyranosyl(1→2)-*β*-D-glucopyranosyl(1→2)-*α*-L-arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside	Ara-Glc-Ara	Glc	(39)
MA-3-*O*-*β*-D-glucuronopyranosyl-28-*O*-[*α*-L-rhamnopyranosyl(1 →2)-*α*-L-arabinopyranoside]	GlcA	Rha- Ara	(44)
MA-3,28-di(*O*-*β*-D-glucuronopyranoside)	GlcA	GlcA	(41)
MA-3-*O*-[*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl]-28-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranoside]	Glc-Glc	Rha-Ara	(41)
MA-3-*O*-[*α*-L-rhamnopyranosyl(1→2)-*β*-D-glucuronopyranosyl(1→2)-*β*-D-glucopyranoside]	Rha-GlcA-Glc	H	(41)
Medicagoside E	Glc-Glc-Glc	H	(46)

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**



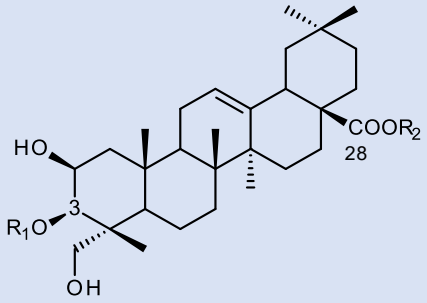
	R1	R2	R3	
Zanhic acid (ZA)	H	H	H	(36, 37)
ZA tridemoside	Glc-Glc-Glc	Ara	Api- Xyl-Rha-Ara	(38, 44)
ZA-3-*O*-*β* -D-glucopyranosyl-28-*O*-[*β*-D-xylopyranosyl(1→4) *α*-L-rhamnopyranosyl(1→2)-*α*-L-arabinoside]	Glc	H	Xyl-Rha-Ara	(41)
ZA-3-*β*-*O*-glucuronopyranosyl-28-*O*-[*β*-D-xylopyranosyl(1→4) *α*-L-rhamnopyranosyl(1→2)-*α*-L-arabinoside]	GlcA	H	Xyl-Rha-Ara	(41)
ZA-3-*O*-[*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranosyl]-28-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranoside]	Glc-Glc	H	Rha-Ara	(41)
ZA-3-*O*- [*β*-D-glucopyranosyl(l→2) -*β*-D-glucopyranosyl(l→2)-*β*-D-glucopyranoside]	Glc-Glc-Glc	H	H	(41)
ZA-3-*O*-[*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl]-28-*O*-[*β*-D-xylopyranosyl(1→4)-*α*-L-rhamnopyranosyl(1→2)-*α*-L-arabinopyranoside]	Glc-Glc	H	Xyl-Rha-Ara	(41)



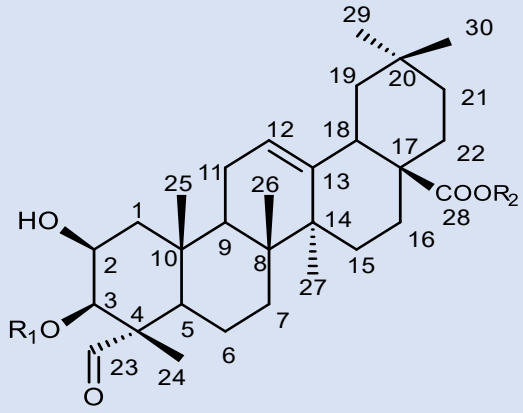
	R1	R2	
Hederagenin	H	H	(36, 37)
Cauloside C	Glc-Ara	H	(36, 39, 41)
Hederagenin-3-*O*-[*α*-L-arabinopyranosyl-(1→2)-*β*-D-glucopyranosyl-(1→2)-*α*-L-arabinopyranoside]	Ara-Glc-Ara	H	(36-41)
Hederagenin-3-*O*-[*β*-D-glucuronopyranosyl methyl ester]-28-*O*-*β*-D-glucopyranoside	GlcA methyl ester	Glc	(41)
Hederagenin-3-*O*-[*α*-L-arabinopyranosyl(1→2)-*β*-D-glucopyranosyl-(1→2)-*α*-L- arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside	Ara-Glc-Ara	Glc	(47)

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

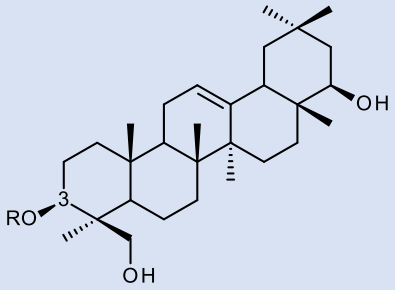
Hederagenin-3-*O*-[*β*-D- galactopyranosyl-(1→2)-*α*-L- arabinopyranosyl]-28-*O*-*β*-D-glucopyranoside	Gal-Ara	Glc	(47)
Medicagoside A	Xyl-Glc	Xyl-Rha-Ara	(46)
Medicagoside B	Ara-Glc-Xyl	Glc	(46)
Medicagoside C	Xyl-Glc-Glc	Glc	(46)
Medicagoside D	Glc-Glc-Ara	Glc	(46)



	R1	**R2**	
Bayogenin	H	H	(36, 37)
Caryocaroside III-9	Gal-GlcA	Glc	(39, 41)
Bayogenin-3-*O*-*α*-L-arabinopyranosyl-28-*O*-*β*-D-glucopyranoside	Ara	Glc	(41)

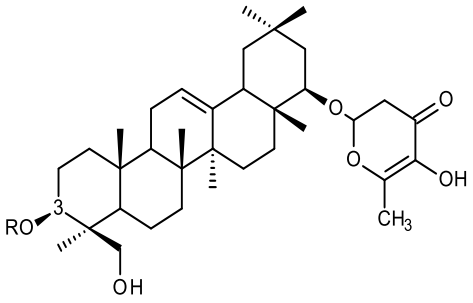
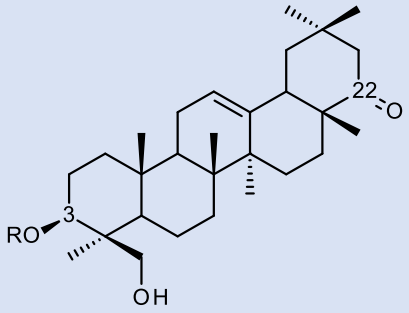
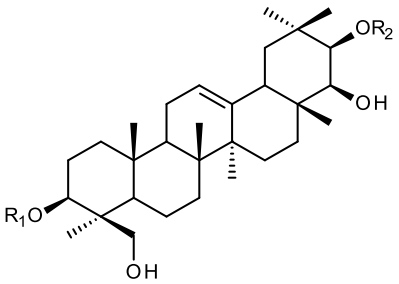


	R1	**R2**	
2β,3β-Dihydroxy-23-oxo-olean-12-en-28-oic acid	H	H	(36, 37)
2β,3β-Dihydroxy-23-oxo-olean-12-en-28-oic acid-3-*O*-*β*-D-glucuronopyranosyl-28-*O*-*β*-D-glucopyranoside	GlcA	Glc	(41)
Medicagoside F	Xyl-Glc-Glc	Glc	(46)

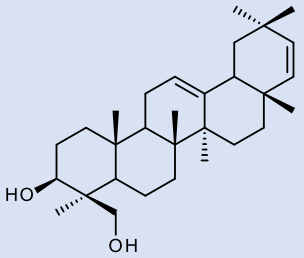
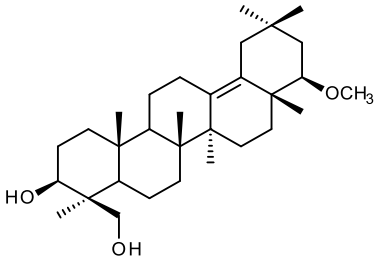
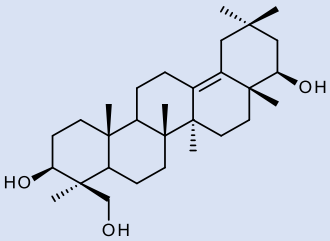
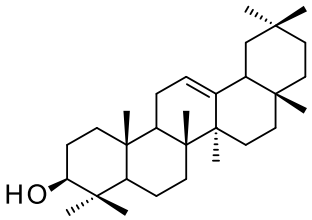


	R	

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

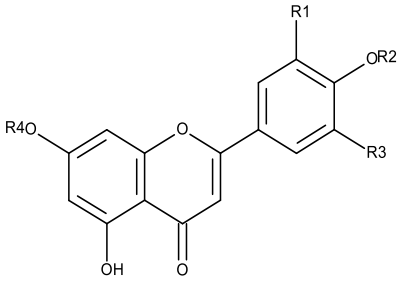
Soyasapogenol B	H	(36, 37)
Soyasapogenol B-3-*O*-[*α*-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranosyl(1→2)-*β*-D-glucuronoside]	Rha-Glc-GlcA	(41, 43)
SoyasaponinI	Rha-Gal-GlcA	(37-41)
Soyasapogenol B-3-*O*-[*β*-D-glucopyranosyl(1→2)-*β*-D-glucuronopyranoside	Glc-GlcA	(41)
Soyasaponin I methyl ester	Rha-Gal-GlcA methyl ester	(39, 41)
Soyasapogenol I	Rha-Gal-Glc	(40)
Soyasaponin VI	 R= Rha-Gal- GlcA	(48)
		
	R	
Soyasapogenol E	H	(49)
Dehydrosoyasaponin I	Rha-Gal-GlcA	(39-41)
		
	R1 **R2**	
Soyasapogenol A	H H	(36, 37)
Soyasapogenol A-3-*O*-[*α*-L-rhamnopyranosyl(1→2)-*β*-D-galactopyranosyl(1→2)-*β*-D-glucuronopyranosyl]-21-*O*-*α*-L-rhamnopyranoside	Rha-Gal-GlcA Rha	(39, 41)

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

Soyasapogenol C		(36, 37)
Soyasapogenol D		(36, 37)
Soyasapogenol F		(36, 37)
β-Amyrin		(36, 37)

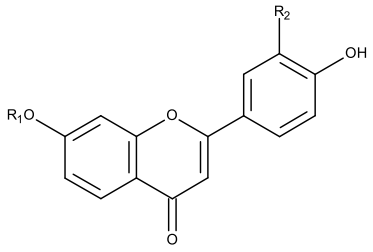
Glc=glucose, GlcA=glucuronide, Gal=galactose, Xyl=xylose, Rha=rhamnose, and Ara=arabinose.

Table 2: Previously isolated flavonoids (flavone (A), flavonol (B), isoflavone (C), flavanone (D), isoflavan (E) and chalcone (F)) from *M. sativa*:

Name	Structure				Ref.
	(A) Flavone				
					
	R1	**R2**	**R3**	**R4**	
Apigenin	H	H	H	H	(50)
Apigenin-4′-*O*-*β*-D-glucuronopyranoside	H	GlcA	H	H	(50)
Clerodendrin	H	H	H	GlcA-GlcA	(50)

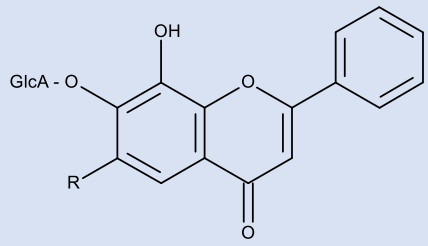
ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

Apigenin-7-*O*-*β*-D-7,5′-Dihydroxy-2′,3′,4′-trimethoxy-isoflavane-5′-*O*-*β*-D-glucopyranoside.	H	H	H	GlcA	(50)
Apigenin-4′-*O*-[2′-*O*-*E*-feruloyl-*O*-*β*-D-glucurono(1→2)-*O*-*β*-D-glucuronopyranoside]	H	GlcA -GlcA - 2-*O*-Feruloyl	H	H	(51)
Apigenin-7-*O*-*β*-D-glucurono-4′-*O*-[2′-*p*-*E*-coumaroyl-*O*-*β*-D-glucurono(1→2)-*O*-*β*-D-glucuronopyranoside]	H	GlcA-GlcA - 2-*O*-Coumaroyl	H	GlcA	(51)
Luteolin	OH	H	H	H	(52)
Luteolin-7-*O*-*β*-D-glucuronopyranoside	OH	H	H	GlcA	(50)
Luteolin-7-*O*-*β*-D-glucopyranoside	OH	H	H	Glc	(52)
Tricin	OCH_3	H	OCH_3	H	(53)
Tricin-7-*O*-*β*-D-glucuronopyranoside	OCH_3	H	OCH_3	GlcA	(53)
Tricin-7-*O*-[*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucuronopyranoside]	OCH_3	H	OCH_3	GlcA-GlcA	(53)
Tricin-7-*O*-[2′-*O*-sinapoyl-*β*-D-glucuronopyranosyl(1→2)-*O*-*β*-D-glucuronopyranoside]	OCH_3	H	OCH_3	GlcA-GlcA-2-*O*-Sinapoyl	(53)
Tricin-7-*O*-[2′-*O*- *p*-coumaroyl-*β*-D-glucuronopyranosyl (1→2)-*O*-*β*-D-glucuronopyranoside]	OCH_3	H	OCH_3	GlcA-GlcA-2-*O*-*p*-Coumaroyl	(53)
3′-*O*-Methyltricetin-7-*O*-*β*-D-glucuronopyranoside	OCH_3	H	OH	GlcA	(53)
Chrysoeriol	OCH_3	H	H	H	(53)
Chrysoeriol-7-glucuronide	OCH_3	H	H	GlcA	(54)
Chrysoeriol-7-diglucuronide	OCH_3	H	H	GlcA-GlcA	(54)
Chrysoeriol-7-triglucuronide	OCH_3	H	H	GlcA- GlcA-GlcA	(54)
Chrysoeriol-7-*O*-*β*-D-glucuronopyranosyl-4′-*O*-*β*-D-glucuronopyranoside	OCH_3	GlcA	H	GlcA	(53)
Chrysoeriol-7-*O*-[2′-*O* feruloyl-*β*-D-glucuronopyranosyl(1→2)-*β*-D-glucuronopyranoside]	OCH_3	H	H	GlcA-GlcA-2-*O*-Feruloyl	(53)



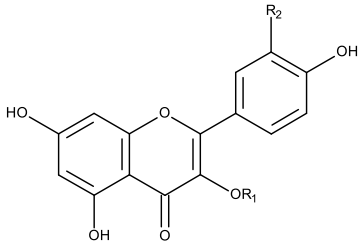
ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

	R1	R2	
4',7-Dihydroxyflavone	H	H	(52)
4',7-Dihydroxyflavone-7-glucopyranoside	Glc	H	(54)
7,3′,4′-Trihydroxyflavone	H	OH	(55)
7,3′,4′-Trihydroxyflavone-7-glucopyranoside	Glc	OH	(56)



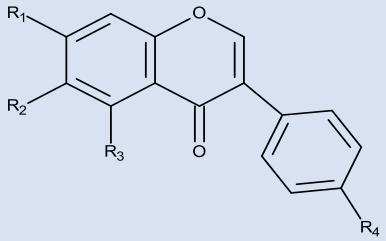
	R	
6,8-Dihydroxyflavone-7-*O*-*β*-D-glucuronopyranoside	OH	(57)
6-Methoxy-8-hydroxyflavone-7-*O*-*β*-D- glucuronopyranoside	OCH_3	(57)

(B) Flavanol



	R1	R2	
Kaempferol-3-*O*-*β*-D-glucopyranoside	Glc	H	(56)
Quercetin	H	OH	(52)
Quercetin-3-*O*-*β*-D-galactopyranoside	Gal	OH	(52)
Quercetin-3-*O*-*β*-D-glucopyranoside	Glc	OH	(56)

(C) Isoflavone

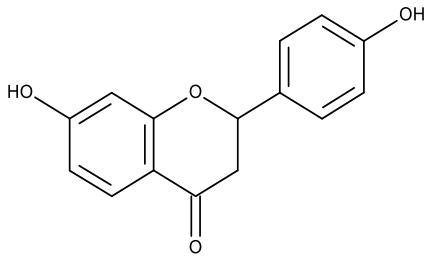


	R1	R2	R3	R4	
Genistein	OH	H	OH	OH	(58)
Genistein-7-glucoside	OGlc	H	OH	OH	(58)
Daidzein	OH	H	H	OH	(58)
Glycitein	OH	OCH_3	H	OH	(58)
Formononetin	OH	H	H	OCH_3	(58)
Biochanin A	OH	H	OH	OCH_3	(59)

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

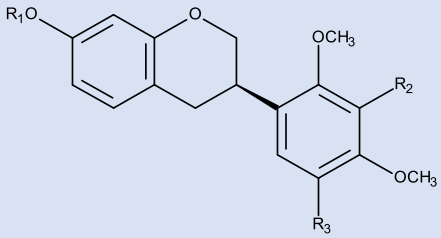
(D) Flavanone

Liquiritigenin



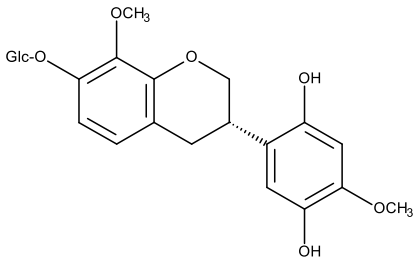
(12)

(E) Isoflavan



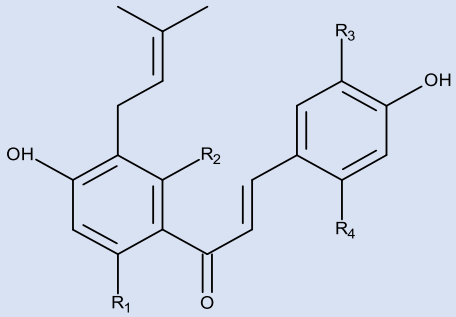
	R1	R2	R3	
7,5′-Dihydroxy-2′,3′,4′-trimethoxy-isoflavane-5′-*O*-*β*-D-glucoside	H	OCH_3	O-Glc	(46)
7-Hydroxy-2′,4′,5′-trimethoxy-isoflavane-7-*O*-*β*-D-glucoside	Glc	H	OCH_3	(46)
7-Hydroxy-2′,3′,4′-trimethoxyisoflavan	H	OCH_3	H	(60)
Sativan	H	H	H	(60)
5′-Methoxysativan	H	H	OCH_3	(60)
Millepurpan	H	OCH_3	OH	(60, 61)

7,2′,5′-Trihydroxy-8,4′-dimethoxy-isoflavane-7-*O*-*β*-D-glucoside



(46)

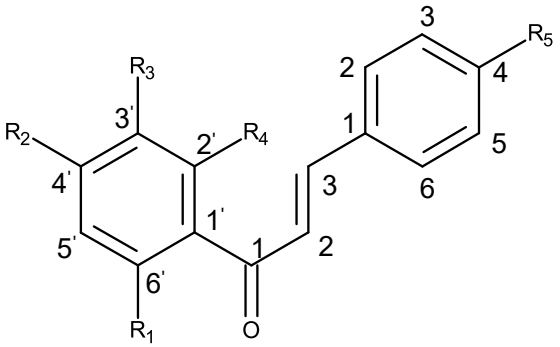
(F) Chalcone



	R1	R2	R3	R4	
Broussochalcone A	OH	H	OH	H	(14)

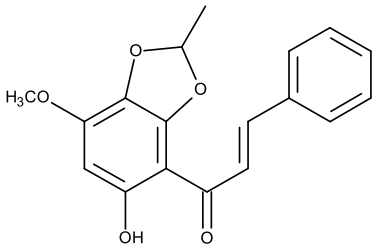
ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

Broussochalcone B	OH	H	H	H	(14)
7,9,2′,4′-Tetrahydroxy-8-isopentenyl-5-methoxychalcone	OCH_3	OH	H	OH	(14)
Xanthohumol	OCH_3	OH	H	H	(14)
Desmethylxanthohumol	OH	OH	H	H	(14)



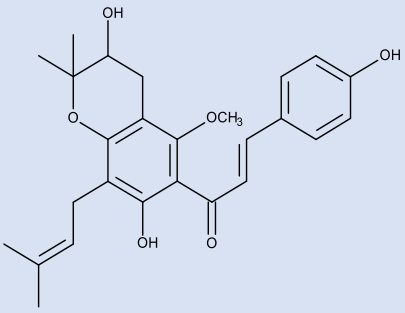
	R1	**R2**	**R3**	**R4**	**R5**	
4,4′-Dihydroxy-2′-methoxychalcone	H	OH	H	OCH_3	OH	(62)
6′-Hydroxy-2′,3′,4′ -trimethoxychalcone	OH	OCH_3	OCH_3	OCH_3	H	(14)
Flavokawin B	OCH_3	OCH_3	H	OH	H	(14)
Isoliquiritigenin	OH	OH	H	H	OH	(14)
2′-Hydroxy-4′,6′-dimethoxychalcone	OH	OCH_3	H	OCH_3	H	(14)

Litseaone B



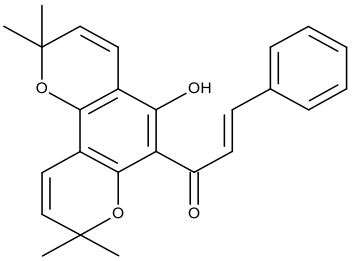
(14)

Xanthohumol M



(14)

Flemiculosin



(14)

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

Table 3: Previously isolated anthocyanidins and anthocyanins from *M. sativa*:

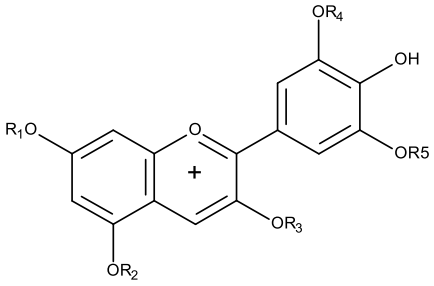
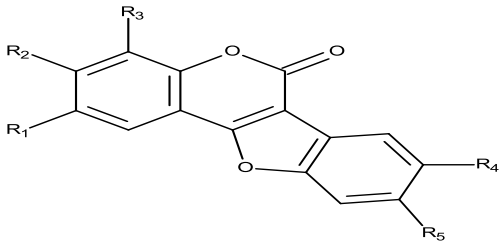
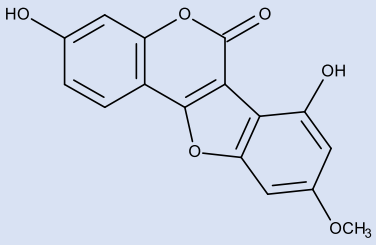
Name	Structure					Ref.
						
	R1	**R2**	**R3**	**R4**	**R5**	
Delphinidin	H	H	H	H	H	(63)
Delphinidin-3,5-diglucoside	H	Glc	Glc	H	H	(63)
Petunidin	H	H	H	CH_3	H	(63)
Petunidin-3,5-diglucoside	H	Glc	Glc	CH_3	H	(63)
Malvidin	H	H	H	CH_3	CH_3	(63)
Malvidin-3,5-diglucoside	H	Glc	Glc	CH_3	CH_3	(63)

Table 4: Previously isolated coumestans from *M. sativa*:

Name	Structure					Ref.
	Coumestans					
						
	R1	**R2**	**R3**	**R4**	**R5**	
Coumestrol	H	OH	H	H	OH	(12)
4′-Methoxycoumestrol	H	OH	H	H	OCH_3	(64)
03′-Methoxycoumestrol	H	OH	H	OCH_3	OH	(64)
Lucernol	OH	OH	H	H	OH	(65)
Sativol	H	OCH_3	OH	H	OH	(65)
3′,4′-Dimethoxycoumesterol	H	OH	H	OCH_3	OCH_3	(64)
Trifoliol						(64)

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

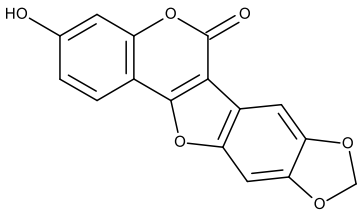
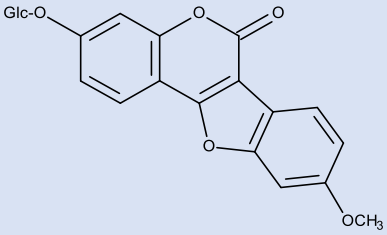
Medicagol		(66)
Licoagroside C		(67)

Table 5: Previously isolated pterocarpans from *M. sativa*:

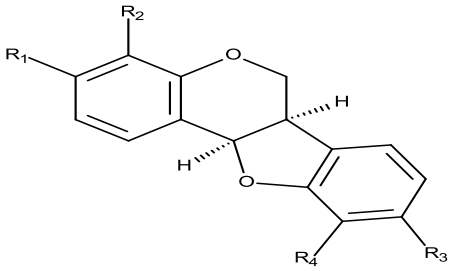
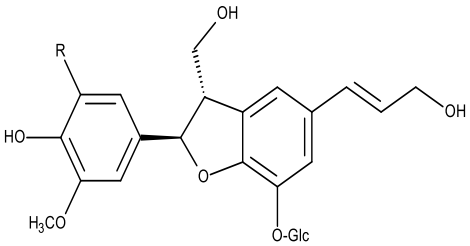
Name	Structure				Ref.
					
	R1	**R2**	**R3**	**R4**	
Medicarpin	OH	H	OCH_3	H	(61)
Medicarpin-3-*O*-*β*-D-glucoside	O-Glc	H	OCH_3	H	(68)
4-Methoxymedicarpin	OH	OCH_3	OCH_3	H	(60)
10-Methoxymedicarpin	OH	H	OCH_3	OCH_3	(60)
Medicarpin-4-*O*-*β*-D-glucoside	OH	O-Glc	OCH_3	H	(46)
Melilotocarpan E	OCH_3	OH	OH	OCH_3	(61)

Table 6: Previously isolated benzofuran neolignans from *M. sativa*:

Name	Structure	Ref.
		
	R	
(7R,8S)-5-Methoxy-3′-demethyl-dehydrodiconiferyl alcohol-3′-*O*-*β*-D-glucopyranoside	OCH_3	(69)
(7R, 8S)-3′-Demethyl-dehydrodiconiferyl alcohol-3′-*O*-*β*-D-glucopyranoside	H	(69)

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

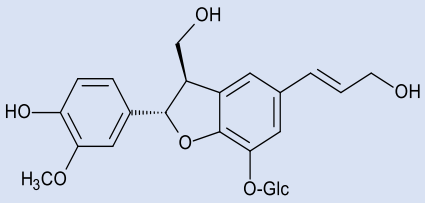
(7S, 8R)-3′-Demethyl-dehydrodiconiferyl alcohol-3′-*O*-*β*-D-glucopyranoside		(69)

Table 7: Previously isolated alkaloids from *M. sativa*:

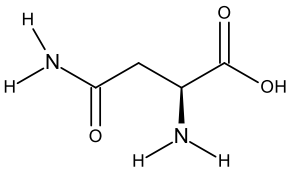
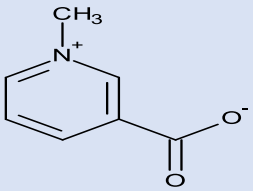
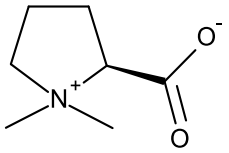
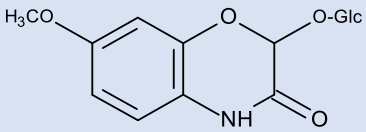
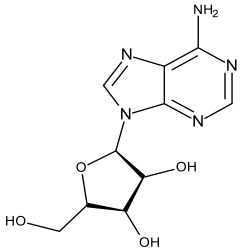
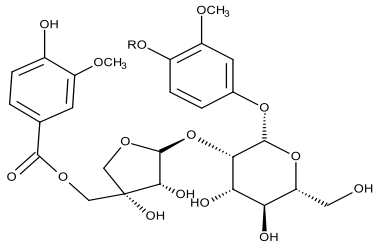
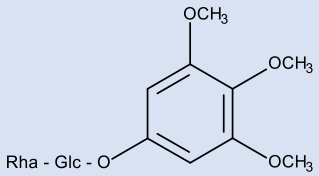
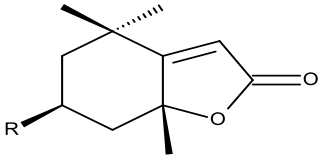
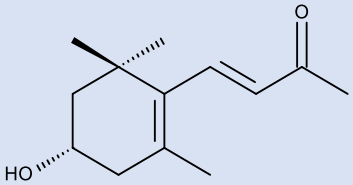
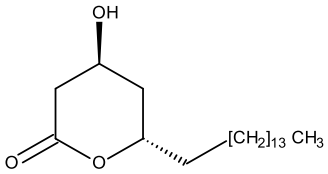
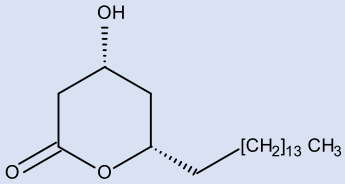
Name	Structure	Ref.
Asparagine		(28)
Trigonelline		(70)
Stachydrine		(70)
2-*O*-*β*-D-Glucopyranosyl-7-methoxy-1,4(2H)-benzoxazin-3-one		(69)
Adenosine		(71)

Table 8: Previously isolated miscellaneous compounds from *M. sativa*:

Name	Structure	Ref.
		
	R	

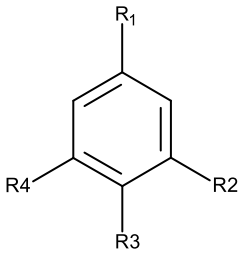
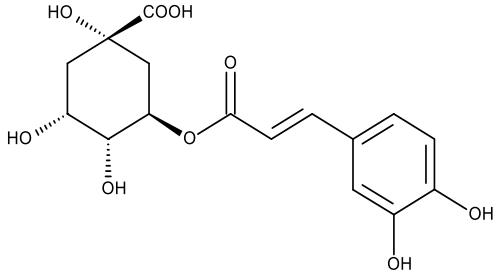
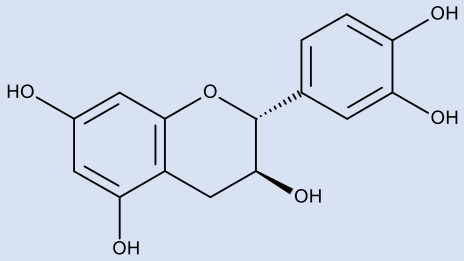
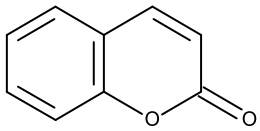
ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

Seguinoside K	R=H	(72)
Seguinoside K 4-methylether	$R=CH_3$	(73)
1-[α-L-Rhamnosyl(1→6)-β-D-glucopyranosyloxy]-3,4,5-trimethoxybenzene		(69)
		
	R	
Loliolide	R=OH	(12)
Dihydroactinidiolide	R=H	(61)
3-Hydroxy-β-ionone		(61)
(4S,6S)-4-Hydroxy-6-pentadecyltetrahydropyr-2-one		(12)
(4R,6S)-4-Hydroxy-6-pentadecyltetrahydropyr-2-one		(12)

2.1. Allelopathic compounds in *M. sativa:* The biological phenomenon called allelopathy takes place when a certain organism releases one or more biochemicals that impair the capacity of other organisms to germinate, grow, survive, and reproduce. Those biochemicals are referred to as allelochemicals, and they can affect the target species and the community in either a beneficial or detrimental way. An essential component of plants' defense against herbivory is the presence of allelochemicals with detrimental allelopathic effects [(74)]. Examples of allelopathic compounds isolated from alfalfa are presented in **Table 9**.

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

Table 9: Allelochemicals previously isolated from *M. sativa*:

Name	Structure				Ref.
					
	R1	**R2**	**R3**	**R4**	
Ferulic acid	$CHCHCO_2H$	OCH_3	OH	H	(75)
p-Coumaric acid	$CHCHCO_2H$	H	OH	H	(75)
Sinapic acid	$CHCHCO_2H$	OCH_3	OH	OCH_3	(76)
Caffeic acid	$CHCHCO_2H$	H	OH	OH	(75)
Trans-cinnamic acid	$CHCHCO_2H$	H	H	H	(75)
Hydrocinnamic acid	$CH_2CH_2CO_2H$	H	H	H	(75)
Vanillic acid	CO_2H	OCH_3	OH	H	(76)
Vanillin	CHO	OCH_3	OH	H	(77)
p-Hydroxy benzoic acid	CO_2H	H	OH	H	(78)
Gallic acid	CO_2H	OH	OH	OH	(77)
Protocatechuic acid	CO_2H	OH	OH	H	(77)
Chlorogenic acid					(78)
Catechin					(77)
Coumarin					(75)

3. Pharmacological activity of *M. sativa*:

3.1. Anti-oxidant activity:

Alfalfa ethyl acetate extract's antioxidant capacity was investigated. Malondialdehyde, an indication of oxidative stress, was found to be substantially lower in animals fed with alfalfa than in the control group that did not receive any supplements. Additionally, there was a noticeably smaller drop in blood antioxidants such as retinol, β-carotene,

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

glutathione (GSH), and vitamin C in the group of animals fed alfalfa extract [79].

Alfalfa and other leguminous plant sprouts are well known for their antioxidant compounds that promote health [80, 81]. Alfalfa sprouts may reduce H2O2-induced DNA destruction, which may reduce the incidence of some malignancies, according to *in-vivo* and *in-vitro* dietary studies [82]. Research carried out utilizing Folin-Ciocalteu and DPPH bioautograhic methods to directly quantify the total polyphenolics and *in-vitro* antioxidant activity, respectively, demonstrated that total polyphenolic content increased in the first day of sprouting, then sharply declined in the second day followed by gradual increase until reaching its highest level in the eleventh day. Analysis of the antioxidant profile throughout the course of several sprouting days revealed a strong correlation with the polyphenolic profile [83].

3.2. Anti-inflammatory activity:

Alfalfa leaves crude extract was studied for anti-inflammatory activity using LPS-stimulated immune responses and it was found to have a moderate activity. It prevents the release of NO from macrophage cells RAW 264.7, activated with IFN-γ and bacterial lipopolysaccharides [19]. Mice administrated LPS alone demonstrated 0% survival rate after 48 hours, whereas, mice given the extract orally showed a 60% survival rate. Sub-fractions of chloroform extract significantly inhibited LPS-mediated induction of the extracellular signal-regulated kinase and nuclear factor Kappa-B [84].

Moreover, *in vivo* and *in vitro* investigations of ethyl acetate extracts from alfalfa sprouts proved that it eliminates acute inflammations and inhibits the synthesis of inflammatory cytokines (IL-1β and IL-6)[85].

Mice supplemented with *M. sativa* sprouts ethyl extracts showed a reduction in acute inflammatory risks and a suppression of pro-inflammatory cytokine production. It dramatically decreased the synthesis of IL-1β, IL-6, TNF-α as well as the trans-activation activity of NF-kappa B. Additionally, the extract demonstrated much greater survival rates than the control group [18].

3.3. Anti-cancer and anti-tumor activity:

Recent studies were carried out on the methyl *tert*-butyl ether and toluene extracts from alfalfa leaves and revealed that they had cytotoxic impacts on two leukemic cell strains from rats. Flavonoids like millepurpan, tricin, chrysoeriol, (-)-melilotocarpan E and (-)-medicarpin were identified in the toluene extract which was found to have the highest cytotoxicity. Despite the structural similarity of medicarpin and melilotocarpan E, melilotocarpan E exhibited more cytotoxic activity, confirming that the quantity of methoxy substitutions in pterocarpans is correlated with their action. Additionally, early studies on recently discovered chalcones showed that some of them have strong antiangiogenic properties and could help prevent tumors from forming new blood vessels [14, 61]. Other investigations indicate that some pure saponins from alfalfa may also function as anticancer agents by enhancing apoptosis [86]. Research on new anticancer drugs is known to concentrate on their possible pro-apoptotic qualities [87]. Although alfalfa saponins have been shown to have pro-apoptotic qualities, there is currently no research on how they work in human cell lines.

3.4. Reproductive activity:

Certain alfalfa flavonoids, such as apigenin, medicarpin, quercetin, coumestrol, luteolin, isoliquiritigenin, and liquiritigenin were discovered to possess phytoestrogen-like properties [12, 88]. Owing to their structural resemblance to 17-*β*-estradiol, these compounds' phytoestrogenic nature allows them to bind to and inhibit the estrogenic receptor sites, hence causing oestrogenic

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

and/or anti-estrogenic actions [89, 90]. This activity is believed to fight against osteoporosis, menopausal symptoms, heart diseases, and malignancies of the breast, lung, colon, stomach, ovary, and uterus [88, 91]. Additionally, it has been demonstrated that coumestrol, liquiritigenin, and isoliquiritigenin bind to both ERβ and ERα estrogen receptors and stimulate ER target genes via ERβ [85]. Recently the ethanolic extracts estrogenic activity of alfalfa plant was investigated in immature female rats. These animals showed a drop in overall cholesterol levels and a spike in total serum proteins. Furthermore, serum progesterone levels were greater than those of the controls, which was thought to be caused by the alfalfa extract's estrogen-like activities through stimulating follicular growth and corpora lutea formation [92]. Alfalfa also, was found to have the ability to change the reproductive hormones testosterone, FSH, and LH in rats [93].

Moreover, alfalfa can change fertility due to its antioxidant activities; because these agents can directly or indirectly inhibit spermatogenesis [94, 95]. Additionally, *in vitro* investigations have convincingly demonstrated that ovarian glutathione shortage speeds up the development of antral follicle atresia, which conveys the great vulnerability of antral follicles to oxidative stress. The development of the embryo and the fertilization process were similarly affected. Research has indicated that women who experience infertility that cannot be explained have greater ROS levels than their fertile counterparts [96]. In females of senior age, the lower quality of oocytes may be explained by the natural build-up of free radicals with aging [97].

3.5. Anti-microbial activity:

Alfalfa saponins were discovered to have a moderate anti-microbial action, mostly against Gram-positive bacteria like *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*, and *Bacillus subtilis*. The antibiotic impact elevated from crude saponins extract to prosapogenins and relevant sapogenins indicating that the existence of sugar moieties may not be necessary for the antibacterial action. Alfalfa antibacterial properties may potentially be attributed to the medicagenic acid [98].

3.6. Anti-fungal activity:

Analysis of the structure-activity relationship of *M.sativa* saponins showed that aglycones possess lower antifungal activity against dermatophytes than their glycosides especially medicagenic acid-3-*O*-β-D-glucopyranoside was the most active component. However, glycosides of zanhic acid and hederagenin demonstrated weak activities [99]. Medicagenic acid-3-*O*-β-D-glucopyranoside is also effective versus *Cryptococcus neoformans* yeast [100]. An extract high in alfalfa saponins also works efficiently against the yeast pathogen *Candida albicans*. It also shows little cytotoxicity to the L929 murine fibroblast cell lines. As a result, it can be employed as a disinfectant or in antifungal treatment [101].

3.7. Ameliorating autoimmune diseases:

Multisystem inflammatory illness and the generation of many autoantibodies are two characteristics of the diverse condition known as systemic lupus erythematosus. SLE is nine times more frequent in females than in males [102, 103] as it is an estrogen-enhanced disease [104, 105]. It is hypothesized that inhibiting the generation of inflammatory cytokines and autoantibodies might prevent the progression of the condition. Alfalfa sprouts ethyl acetate extract significantly suppresses the production of pro-inflammatory cytokines such as IFN-γ and IL-1β and thus has beneficial effects on disease improvement [106]. Additionally, it is a high source of phytoestrogens, which may help SLE by potentially modulating the immune system by reducing inflammatory responses [107].

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

3.8. Cholesterol-lowering activity:

The cholesterol-lowering activity of alfalfa extract has been studied since the 1960s. It has been shown that the triterpene saponins obtained from alfalfa aerial parts are responsible for lowering hypercholesterolemia without affecting the HDL level. The impact of the alfalfa saponins extract was found to surpass that of the widely utilized hypolipidemic medication, Gemfibrozil, which is known to reduce LDL and triglycerides levels which provoke the formation of atherosclerotic plaques and enhance the HDL level [(108)]. Therefore, can be effective in reducing some of the cardiovascular complications [(109)]. Recent studies on rats indicated that the potential mechanisms behind the cholesterol-lowering properties of this extract may involve alterations in the expression of genes correlated to cholesterol esterase and acetyl CoA carboxylase, both of which play crucial roles in cholesterol biosynthesis and the production of classical bile acids, as well as in the encoding of LDL receptors [(110, 111)]. Another mechanism for lowering serum cholesterol is by diminishing intestinal absorption and increasing cholesterol defecation [(112)]. *In vitro* investigations on specific cell lines have demonstrated that chalcones also exhibit notable hypolipidemic effects [(14)]. Moreover, the incorporation of alfalfa sprouts into the diet has been shown to lower plasma and egg cholesterol levels, likely due to the synergistic effects of various compounds present in the sprouts, such as (lignans, PUFA, sterols, and isoflavones) [(113)].

3.9. Blood glucose lowering and antidiabetic effects:

Although there is no definitive cure for diabetes, managing diet and incorporating food supplements can effectively enhance mild forms of the condition. Research indicates that alfalfa sprouts serve as a potent anti-diabetic owing to their capacity to reduce blood glucose levels [(114)]. Maintaining stable blood sugar levels is essential in managing diabetes, and incorporating foods like alfalfa sprouts can help to reduce reliance on insulin. Studies have investigated the anti-hyperglycemic properties of alfalfa aqueous extracts, demonstrating that diabetic mice administered alfalfa extract showed a considerable increase in both the size and quantity of pancreatic islets compared to those receiving no treatment [(115)]. Consequently, it can be inferred that the hypoglycemic effect of alfalfa may be attributed to the repair of islets.

3.10. Neuroprotective & Anxiolytic activities in CNS:

Studies on mice confirmed the traditional use of alfalfa to enhance memory and alleviate CNS disorders because this plant exhibits a neuroprotective effect against reperfusion insult and ischemia in mice. Also, the methanolic extract exerts anxiolytic effect [(28)].

3.11. Supplementary source of vitamin C:

Alfalfa sprouts have been identified as one of the foods highest in vitamin C. Alfalfa seeds sprouting increased ascorbic acid content up to 10 fold. This rise in ascorbic acid in the sprouts represents a significant source of vitamin C in human nutrition, particularly during critical situations such as scurvy, making it vital for numerous healthy bodily functions [(116)].

3.12. In the treatment of anemia:

In place of iron and folic acid supplements, 102 anaemic teenage girls between the ages of 14 and 18 participated in a randomised controlled two-arm experiment using alfalfa leaf extract for three months. None of the remaining 86 girls had severe anaemia at the end of the experiment; nine had moderate anaemia, twenty-six had mild anaemia, and fifty-one had normal haemoglobin levels (12 g/dl). According to the findings, leaf concentrate is a more pleasant and efficient

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

treatment option for anaemia in teenage girls than folic acid and iron supplements [117].

3.13. Dermatological effects

The effectiveness of *M. sativa* extract in treating burn wounds created by NaOH was investigated in rats. In comparison to the conventional medication, the extract dramatically raised the amount of GSH in burned skin tissues at both small and large dosages. Both the low and high-dose treatment groups showed a substantial decline in MDA in skin tissues in comparison to the standard and control groups ($P<0.01$). Rats treated with both low and high doses of the extracts showed lower NO concentrations in their skin homogenate when compared to the control group ($P<0.01$) [118].

3.14. For the treatment of metabolic syndrome:

M. sativa, which is cultivated in high-saline environments, was incorporated into experimental diets and evaluated in a spontaneous hypertensive rat model. The results showed that it improved glucose metabolism, reduced the risk of kidney stones, protected against oxidative insult in fatty liver disorder, and slightly lowered blood pressure. For treating and preventing several metabolic changes related to metabolic syndrome, alfalfa shown to be an effective functional food [119].

3.15. Hepatoprotective effect

In rats, the impact of a lyophilized alfalfa aqueous extract against oxidative stress and liver damage caused by CCl4 was investigated. Alfalfa pre-treatment for three weeks before CCl4 was administered, considerably inhibited the rise in serum hepatic markers, LDL, and VLDL levels. It also decreased oxidative stress, as seen by increasing concentrations of total protein and non-protein sulfhydryl. According to the histological liver analysis, alfalfa extract also decreased the frequency of CCl4-induced liver lesions [120].

3.16. Cardioprotective effect

Rats with isoproterenol-induced myocardial infarction were used to assess the cardioprotective properties of *M. sativa* ethanolic extract. A lipid profile with a lower HDL-C level and elevated blood levels of cardiac and liver markers were seen in the isoproterenol group. The extract pretreatment returned the levels of the liver and heart enzymes as well as the lipid profile to almost normal [17, 121].

4. Toxicity and side effects

The effectiveness of hydroalcoholic *M. sativa* extract on coagulation system parameters, blood biochemical variables, and liver function was investigated in male rats. According to the findings, the experimental groups' serum levels of ALT, ALP, and glucose concentration were considerably lower after using the extract than those of the control group. Additionally, lucerne enhanced fibrinogen and total protein in the experimental groups in a concentration-dependent relation (P 0.001 and P 0.01 respectively) [122].

In general, moderate use of *M. sativa* leaves in capsules and teas is regarded as safe and free of serious adverse effects. Large-scale consumption of *M.sativa* sprouts and seeds has been related to lupus aggravation or promotion of lupus-like symptoms; this effect is ascribed to canavanine [123].

Although there is no research on *M. sativa* in pregnancy or lactation involving humans or animals, herbalists believe that the plant is safe to consume during pregnancy because farmers do not forbid their livestock from eating it during these times. *M. sativa* feed spiked milk production, decreased fat, and boosted milk protein in dairy cows [124].

5. Conclusion

M. sativa emerges as a diverse wild species, esteemed for its functions as both a valuable nutritional resource and a provider of medicinal benefits. Ethnomedicinal research emphasizes the utilization of all the plant

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

parts. *M. sativa* phytochemical makeup was thoroughly examined, and different classes including saponins, glycosides, phenolic acids, anthocyanins, coumestans, pterocarpans, alkaloids, and flavonoids were identified. Various compounds such as luteolin, isoliquiritigenin, and apigenin, derived also from *M. sativa* aerial parts, attributing to its chemical divergence. Furthermore, nutritional examination of *M. sativa* revealed its importance as a vital nutrient source. This detailed characterization of phytoconstituents and their actions improves our understanding of *M. sativa* medicinal potential and gives new intuitions for future research and applications.

Authors' contributions

A.K.: collecting data, analyzing and interpreting it, writing the work. R.S.I., H.H.Z., and H.M.H.: work conceptualization, experimental design, and manuscript revision.

Conflicts of interest

No conflicts need to be declared.

Highlights

- Exact taxonomic classification, synonyms, and different common names of *Medicago sativa* have been illustrated.
- Versatile applications of *Medicago sativa* traditionally, in addition to its pharmacological applications as a pharmaceutical drug both *in vivo* and *in vitro* have been discussed.
- Differential classification of different *Medicago sativa* active constituents was also addressed in this review.

6. References:

(1) Mabberley, D. J. *The plant-book: a portable dictionary of the vascular plants*. Cambridge University Press, 1997.

(2) Lewis, G. P. *Legumes of the World*. Royal Botanic Gardens Kew, 2005.

(3) Lewis, G., Schrire, B., Mackinder, B., Rico, L.& Clark, R. A 2013 linear sequence of legume genera set in a phylogenetic context—a tool for collections management and taxon sampling. South African journal of botany. 2013;89:76-84.

(4) Group, L. P. W. Legume phylogeny and classification in the 21st century: progress, prospects and lessons for other species-rich clades. Taxon. 2013;62(2):217-248.

(5) Sabir, J. *et al.* Evolutionary and biotechnology implications of plastid genome variation in the inverted-repeat-lacking clade of legumes. Plant biotechnology journal. 2014;12(6):743-754.

(6) McKey, D. Legumes and nitrogen: the evolutionary ecology of a nitrogen-demanding lifestyle. Advances in legume systematics. 1994;5:211-228.

(7) Hickey, M.& King, C. *100 families of flowering plants*. Cambridge University Press, 1988.

(8) Doyle, J. J.& Luckow, M. A. The rest of the iceberg. Legume diversity and evolution in a phylogenetic context. Plant Physiology. 2003;131(3):900-910.

(9) Evans, W. Trease and Evans Pharmacognosy 14th edition WB Saunders Company Limited. 1998;

(10) Small, E.& Jomphe, M. A synopsis of the genus Medicago (Leguminosae). Canadian Journal of Botany. 1989;67(11):3260-3294.

(11) Heyn, C. C. The annual species of Medicago. 1963;

(12) Hong, Y.-H. *et al.* Phytoestrogenic compounds in alfalfa sprout (Medicago sativa) beyond coumestrol. Journal of agricultural and food chemistry. 2010;59(1):131-137.

(13) Joy, G. S.& George, P. Antimicrobial screening of Alfalfa (Medicago sativa) in various bacterial strains. Int J Pharm Drug Anal. 2014;2:65-69.

(14) Ma, Q.-G. *et al.* Characterization of chalcones from Medicago sativa L. and their hypolipidemic and antiangiogenic activities. Journal of agricultural and food chemistry. 2016;64(43):8138-8145.

(15) Aganga, A.& Tshwenyane, S. Lucerne, Lablab and Leucaena leucocephala forages: production and utilization for livestock production. Pakistan Journal of Nutrition. 2003;2(2):46-53.

(16) Al-Snafi, A. E. *Encyclopedia of the constituents and pharmacological effects of Iraqi medicinal plants*. Rigi Publication Khanna, 2015.

(17) Al-Snafi, A. E. *et al.* A review on Medicago sativa: A potential medicinal plant. International Journal of Biological and Pharmaceutical Sciences Archive. 2021;1(2):022-033.

(18) Hong, Y.-H., Chao, W.-W., Chen, M.-L.& Lin, B.-F. Ethyl acetate extracts of alfalfa (Medicago sativa L.) sprouts inhibit lipopolysaccharide-induced inflammation in vitro and in vivo. Journal of biomedical science. 2009;16:1-12.

(19) Karimi, E. *et al.* Insight into the functional and medicinal properties of Medicago sativa (Alfalfa) leaves extract. Journal of Medicinal Plants Research. 2013;7(7):290-297.

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

(20) Boué, S. M. *et al.* Evaluation of the estrogenic effects of legume extracts containing phytoestrogens. Journal of agricultural and food chemistry. 2003;51(8):2193-2199.
(21) Huyghe, C., Bertin, E.& Landry, N. Medicinal and nutraceutical uses of alfalfa (Medicago sativa L). A review. Advances in Medicinal Plant Research Trivandrum, India: Research Signpost. 2007:147-172.
(22) Foster, S.& Duke, J. A. *A field guide to medicinal plants: eastern and central North America*. Boston: Houghton Mifflin Company xii, 366p. ISBN, 1990.
(23) Barnes, J. Herbal Medicines, Barnes J, Anderson AL, Phillipson JD. Pharmaceutical Press: London, Chicago; 2002.
(24) Bnouham, M., Ziyyat, A., Mekhfi, H., Tahri, A.& Legssyer, A. Medicinal plants with potential antidiabetic activity-A review of ten years of herbal medicine research (1990-2000). International Journal of Diabetes and Metabolism. 2006;14(1):1.
(25) Adams, M., Gmünder, F.& Hamburger, M. Plants traditionally used in age related brain disorders—A survey of ethnobotanical literature. Journal of Ethnopharmacology. 2007;113(3):363-381.
(26) Haq, I. Safety of medicinal plants. Pak J Med Res. 2004;43(4):203-210.
(27) Cornara, L., Xiao, J.& Burlando, B. Therapeutic potential of temperate forage legumes: a review. Critical reviews in food science and nutrition. 2016;56(sup1):S149-S161.
(28) Bora, K. S.& Sharma, A. Phytochemical and pharmacological potential of Medicago sativa: a review. Pharmaceutical biology. 2011;49(2):211-220.
(29) DerMarderosian, A. *et al.* Alfalfa. Review of Natural Products: Facts and Comparisons. Alphen aan den Rijn, the Netherlands: Wolters Kluwer Health. 2005;
(30) Blann, K. L., Anderson, J. L., Sands, G. R.& Vondracek, B. Effects of agricultural drainage on aquatic ecosystems: a review. Critical reviews in environmental science and technology. 2009;39(11):909-1001.
(31) Fox, J. E., Gulledge, J., Engelhaupt, E., Burow, M. E.& McLachlan, J. A. Pesticides reduce symbiotic efficiency of nitrogen-fixing rhizobia and host plants. Proceedings of the National Academy of Sciences. 2007;104(24):10282-10287.
(32) Bali, K. M., Grismer, M. E.& Snyder, R. L. Alfalfa water use pinpointed in saline, shallow water tables of Imperial Valley. California Agriculture. 2001;55(4)
(33) Radović, J., Sokolović, D.& Marković, J. Alfalfa-most important perennial forage legume in animal husbandry. Biotechnology in Animal Husbandry. 2009;25(5-6-1):465-475.
(34) Joy, G. S.& George, P. Phytochemical analysis of alfalfa (Medicago sativa) seed extract by soxhlet extraction using different solvents. American Journal of Advanced Drug Delivery. 2014;2(2):145-152.
(35) Gaweł, E. Chemical composition of lucerne leaf extract (EFL) and its applications as a phytobiotic in human nutrition. Acta Scientiarum Polonorum Technologia Alimentaria. 2012;11(3):303-309.
(36) Oleszek, W. *et al.* Isolation and identification of alfalfa (Medicago sativa L.) root saponins: their activity in relation to a fungal bioassay. Journal of agricultural and food chemistry. 1990;38(9):1810-1817.
(37) Tava, A., Oleszek, W., Jurzysta, M., Berardo, N.& Odoardi, M. Alfalfa saponins and sapogenins: isolation and quantification in two different cultivars. Phytochemical Analysis. 1993;4(6):269-274.
(38) Nowacka, J.& Oleszek, W. Determination of alfalfa (Medicago sativa) saponins by high-performance liquid chromatography. Journal of Agricultural and Food Chemistry. 1994;42(3):727-730.
(39) Bialy, Z., Jurzysta, M., Oleszek, W., Piacente, S.& Pizza, C. Saponins in alfalfa (Medicago sativa L.) root and their structural elucidation. Journal of agricultural and food chemistry. 1999;47(8):3185-3192.
(40) Huhman, D. V.& Sumner, L. W. Metabolic profiling of saponins in Medicago sativa and Medicago truncatula using HPLC coupled to an electrospray ion-trap mass spectrometer. Phytochemistry. 2002;59(3):347-360.
(41) Witkowska, H. E., Bialy, Z., Jurzysta, M.& Waller, G. R. Analysis of saponin mixtures from alfalfa (Medicago sativa L.) roots using mass spectrometry with MALDI techniques. NATURAL PRODUCT COMMUNICATIONS. 2008;3(9):1395-1410.
(42) Oleszek, W., Nowacka, J., Gee, J., Wortley, G.& Johnson, I. Effects of some purified alfalfa (Medic ago sativa) saponins on transmural potential difference in mammalian small intestine. Journal of the Science of Food and Agriculture. 1994;65(1):35-39.
(43) Massiot, G., Lavaud, C., Besson, V., Le Men-Olivier, L.& Van Binst, G. Saponins from aerial parts of alfalfa (Medicago sativa). Journal of agricultural and food chemistry. 1991;39(1):78-82.

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

(44) Oleszek, W. *et al.* Zahnic acid tridesmoside and other dominant saponins from alfalfa (Medicago sativa L.) aerial parts. Journal of Agricultural and Food Chemistry. 1992;40(2):191-196.
(45) Levy, M., Zehavi, U., Naim, M.& Polacheck, I. Isolation, structure determination, synthesis, and antifungal activity of a new native alfalfa-root saponin. Carbohydrate research. 1989;193:115-123.
(46) Liu, X.-G. *et al.* Bioactive constituents from Medicago sativa L. with antioxidant, neuroprotective and acetylcholinesterase inhibitory activities. Journal of Functional Foods. 2018;45:371-380.
(47) Massiot, G. *et al.* Structural elucidation of alfalfa root saponins by mass spectrometry and nuclear magnetic resonance analysis. Journal of the Chemical Society, Perkin Transactions 1. 1988;(12):3071-3079.
(48) Massiot, G., Lavaud, C., Benkhaled, M.& Le Men-Olivier, L. Soyasaponin VI, a new maltol conjugate from alfalfa and soybean. Journal of natural products. 1992;55(9):1339-1342.
(49) Sen, S., Makkar, H. P.& Becker, K. Alfalfa saponins and their implication in animal nutrition. Journal of Agricultural and Food Chemistry. 1998;46(1):131-140.
(50) Stochmal, A. *et al.* Alfalfa (Medicago sativa L.) flavonoids. 1. Apigenin and luteolin glycosides from aerial parts. Journal of agricultural and food chemistry. 2001;49(2):753-758.
(51) Stochmal, A. *et al.* Acylated apigenin glycosides from alfalfa (Medicago sativa L.) var. Artal. Phytochemistry. 2001;57(8):1223-1226.
(52) Tsai, S. M.& Phillips, D. A. Flavonoids released naturally from alfalfa promote development of symbiotic Glomus spores in vitro. Applied and Environmental Microbiology. 1991;57(5):1485-1488.
(53) Stochmal, A., Simonet, A. M., Macias, F. A.& Oleszek, W. Alfalfa (Medicago sativa L.) flavonoids. 2. Tricin and chrysoeriol glycosides from aerial parts. Journal of agricultural and food chemistry. 2001;49(11):5310-5314.
(54) Saleh, N. A., Boulos, L., El-Negoumy, S. I.& Abdalla, M. F. A comparative study of the flavonoids of Medicago radiata with other Medicago and related Trigonella species. Biochemical Systematics and Ecology. 1982;10(1):33-36.
(55) Bickoff, E., Witt, S.& Livingston, A. 3′, 4′, 7-Trihydroxyflavone in alfalfa. Journal of pharmaceutical sciences. 1965;54(10):1555-1555.
(56) Perez-Garcia, F., Ceresuela, J. L., Gonzalez, A. E.& Aguinagalde, I. Flavonoids in seed coats of Medicago arborea and M. strasseri (Leguminosae): Ecophysiological aspects. Journal of basic microbiology. 1992;32(4):241-248.
(57) Liang, J., Yang, Z., Cao, X., Wu, B.& Wu, S. Preparative isolation of novel antioxidant flavonoids of alfalfa by stop-and-go counter-current chromatography and following on-line liquid chromatography desalination. Journal of Chromatography A. 2011;1218(36):6191-6199.
(58) Rodrigues, F., Almeida, I., Sarmento, B., Amaral, M. H.& Oliveira, M. B. P. Study of the isoflavone content of different extracts of Medicago spp. as potential active ingredient. Industrial Crops and Products. 2014;57:110-115.
(59) Guggolz, J., Livingston, A.& Bickoff, E. Forage estrogens, detection of daizein, formononetin, genistein, and biochanin a in forages. Journal of Agricultural and Food Chemistry. 1961;9(4):330-332.
(60) Spencer, G. F. *et al.* A pterocarpan and two isoflavans from alfalfa. Phytochemistry. 1991;30(12):4147-4149.
(61) Gatouillat, G. *et al.* Cytotoxicity and apoptosis induced by alfalfa (Medicago sativa) leaf extracts in sensitive and multidrug-resistant tumor cells. Nutrition and cancer. 2014;66(3):483-491.
(62) Maxwell, C. A., Hartwig, U. A., Joseph, C. M.& Phillips, D. A. A chalcone and two related flavonoids released from alfalfa roots induce nod genes of Rhizobium meliloti. Plant physiology. 1989;91(3):842-847.
(63) Gupta, S. Thin layer chromatographic separation of anthocyanins and anthocyanidins in Medicago (Papilionaceae). Journal of Chromatography A. 1968;36:115-119.
(64) Bickoff, E., Spencer, R., Knuckles, B.& Lundin, R. 3'-Methoxycoumestrol from alfalfa: isolation and characterization. Journal of Agricultural and Food Chemistry. 1966;14(5):444-446.
(65) Spencer, R., Bickoff, E., Lundin, R.& Knuckles, B. New Alfalfa Compounds, Lucernol and Sativol, Two New Coumestans from Alfalfa (Medicago sativa). Journal of Agricultural and Food Chemistry. 1966;14(2):162-165.
(66) Livingston, A., Witt, S., Lundin, R.& Bickoff, E. Medicagol, a New Coumestan From Alfalfa1. The Journal of Organic Chemistry. 1965;30(7):2353-2355.
(67) Li, W. *et al.* Flavonoids from Glycyrrhiza pallidiflora hairy root cultures. Phytochemistry. 2001;58(4):595-598.
(68) Sakagami, Y., Kumai, S.& Suzuki, A. Isolation and structure of medicarpin-β-D-glucoside in

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

alfalfa. Agricultural and Biological Chemistry. 1974;38(5):1031-1034.
(69) Liu, X. *et al.* Phytochemical and chemotaxonomic study on Medicago sativa L.(Leguminosae). Biochemical systematics and ecology. 2018;80:55-58.
(70) Phillips, D. A., Joseph, C. M.& Maxwell, C. A. Trigonelline and stachydrine released from alfalfa seeds activate NodD2 protein in Rhizobium meliloti. Plant Physiology. 1992;99(4):1526-1531.
(71) Eliasson, B. *et al.* 1 H and 13 C nuclear magnetic resonance reinvestigation of the dibenzo [a, c] cyclononatetraenyl anion and its 5, 9-diphenyl derivative. Planarity vs nonplanarity. Journal of Organic Chemistry. 1989;54(1):171-175.
(72) Yin, T., Tu, G., Zhang, Q., Wang, B.& Zhao, Y. Three new phenolic glycosides from the caulis of Millettia speciosa. Magnetic Resonance in Chemistry. 2008;46(4):387-391.
(73) Tian, J. *et al.* Hepatoprotective phenolic glycosides from Gymnema tingens. Planta medica. 2013;79(09):761-767.
(74) Carroll, L. WHAT IS ALLELOPATHY. The History of Allelopathy. 2007;1
(75) Chon, S. U.& Kim, J. D. Biological activity and quantification of suspected allelochemicals from alfalfa plant parts. Journal of Agronomy and Crop Science. 2002;188(4):281-285.
(76) Hrubcová, M., Cvikrova, M.& Eder, J. Peroxidase activities and contents of phenolic acids in embryogenic and nonembryogenic alfalfa cell suspension cultures. Biologia plantarum. 1994;36(2):175.
(77) Xuan, T. D., Tsuzuki, E., Terao, H., Matsuo, M.& Khanh, T. D. Correlation between growth inhibitory exhibition and suspected allelochemicals (phenolic compounds) in the extract of alfalfa (Medicago sativa L.). Plant production science. 2003;6(3):165-171.
(78) Abdul-Rahman, A. A.& Habib, S. A. Allelopathic effect of alfalfa (Medicago sativa) on bladygrass (Imperata cylindrica). Journal of chemical ecology. 1989;15(9):2289-2300.
(79) Yalinkilic, O.& Enginar, H. Effect of X-Radiation on Lipid Peroxidation and Antioxidant Systems in Rats Treated with Saponin-containing Compounds. Photochemistry and photobiology. 2008;84(1):236-242.
(80) Hesterman, O., Teuber, L.& Livingston, A. Effect of Environment and Genotype on Alfalfa Sprout Production 1. Crop Science. 1981;21(5):720-726.
(81) Oh, M. M.& Rajashekar, C. Antioxidant content of edible sprouts: effects of environmental shocks. Journal of the Science of Food and Agriculture. 2009;89(13):2221-2227.
(82) Ibrahim, R. S., Khairy, A., Zaatout, H. H., Hammoda, H. M.& Metwally, A. M. Digitally-optimized HPTLC coupled with image analysis for pursuing polyphenolic and antioxidant profile during alfalfa sprouting. Journal of Chromatography B. 2018;1099:92-96.
(83) Ibrahim, R. S. *et al.* Chemometric evaluation of alfalfa sprouting impact on its metabolic profile using HPTLC fingerprint-efficacy relationship analysis modelled with partial least squares regression. Journal of Pharmaceutical and Biomedical Analysis. 2020;179:112990.
(84) Choi, K.-C. *et al.* Chloroform extract of alfalfa (Medicago sativa) inhibits lipopolysaccharide-induced inflammation by downregulating ERK/NF-κB signaling and cytokine production. Journal of medicinal food. 2013;16(5):410-420.
(85) Hong, Y.-H., Chao, W.-W., Chen, M.-L.& Lin, B.-F. Ethyl acetate extracts of alfalfa (Medicago sativa L.) sprouts inhibit lipopolysaccharide-induced inflammation in vitro and in vivo. Journal of Biomedical Science. 2009;16(1):64.
(86) Balestrazzi, A., Carbonera, D., Avato, P.& Tava, A. White poplar (Populus alba L.) suspension cultures as a model system to study apoptosis induced by alfalfa saponins. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents). 2014;14(10):1324-1331.
(87) Podolak, I., Galanty, A.& Sobolewska, D. Saponins as cytotoxic agents: a review. Phytochemistry Reviews. 2010;9(3):425-474.
(88) Seguin, P., Zheng, W.& Souleimanov, A. Alfalfa phytoestrogen content: Impact of plant maturity and herbage components. Journal of Agronomy and Crop Science. 2004;190(3):211-217.
(89) Ososki, A. L.& Kennelly, E. J. Phytoestrogens: a review of the present state of research. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2003;17(8):845-869.
(90) Zhao, E.& Mu, Q. Phytoestrogen biological actions on mammalian reproductive system and cancer growth. Scientia pharmaceutica. 2010;79(1):1-20.
(91) Rishi, R. Phytoestrogens in health and illness. Indian journal of pharmacology. 2002;34(5):311-320.
(92) Ahmad, N., Akhtar, N., Ali, S., Ahmad, M.& Ahmad, I. Effects of Medicago sativa on Some Serum Biochemical Metabolites in Rats. International Journal of Agriculture & Biology. 2013;15(2)

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

(93) Mohammadi, G., Fatemi Tabtabaei, S. R.& Zanganeh, S. Effects of alfalfa on reproductive hormones in male rats. Iranian Veterinary Journal. 2021;17(1):76-80.
(94) Aitken, R. J., Baker, M. A.& Sawyer, D. Oxidative stress in the male germ line and its role in the aetiology of male infertility and genetic disease. Reproductive biomedicine online. 2003;7(1):65-70.
(95) Hardy, M. P. *et al.* Stress hormone and male reproductive function. Cell and tissue research. 2005;322:147-153.
(96) Ruder, E. H., Hartman, T. J., Blumberg, J.& Goldman, M. B. Oxidative stress and antioxidants: exposure and impact on female fertility. Human reproduction update. 2008;14(4):345-357.
(97) Agarwal, A., Aponte-Mellado, A., Premkumar, B. J., Shaman, A.& Gupta, S. The effects of oxidative stress on female reproduction: a review. Reproductive biology and endocrinology. 2012;10:1-31.
(98) Avato, P. *et al.* Antimicrobial activity of saponins from Medicago sp.: structure-activity relationship. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2006;20(6):454-457.
(99) Houghton, P., Patel, N., Jurzysta, M., Biely, Z.& Cheung, C. Antidermatophyte activity of medicago extracts and contained saponins and their structure-activity relationships. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives. 2006;20(12):1061-1066.
(100) Polacheck, I., Zehavi, U., Naim, M., Levy, M.& Evron, R. The susceptibility of Cryptococcus neoformans to an antimycotic agent (G2) from alfalfa. Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene Series A: Medical Microbiology, Infectious Diseases, Virology, Parasitology. 1986;261(4):481-486.
(101) Sadowska, B. *et al.* New pharmacological properties of Medicago sativa and Saponaria officinalis saponin-rich fractions addressed to Candida albicans. Journal of medical microbiology. 2014;63(8):1076-1086.
(102) Cooper, G. S. *et al.* Hormonal, environmental, and infectious risk factors for developing systemic lupus erythematosus. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 1998;41(10):1714-1724.
(103) Lahita, R. The importance of estrogens in systemic lupus erythematosus. Clinical immunology and immunopathology. 1992;63(1):17-18.
(104) Carlsten, H., Tarkowski, A., Holmdahl, R.& Nilsson, L. Å. Oestrogen is a potent disease accelerator in SLE-prone MRL lpr/lpr mice. Clinical & Experimental Immunology. 1990;80(3):467-473.
(105) Dhaher, Y. Y., Greenstein, B., de Fougerolles Nunn, E., Khamashta, M.& Hughes, G. R. Strain differences in binding properties of estrogen receptors in immature and adult BALB/c and MRL/MP-lpr/lpr mice, a model of systemic lupus erythematosus. International journal of immunopharmacology. 2000;22(3):247-254.
(106) Hong, Y.-H., Huang, C.-J., Wang, S.-C.& Lin, B.-F. The ethyl acetate extract of alfalfa sprout ameliorates disease severity of autoimmune-prone MRL-lpr/lpr mice. Lupus. 2009;18(3):206-215.
(107) Verdrengh, M., Jonsson, I., Holmdahl, R.& Tarkowski, A. Genistein as an anti-inflammatory agent. Inflammation Research. 2003;52(8):341-346.
(108) Khaleel, A. E., Gad, M. Z., El-Maraghy, S. A., Hifnawy, M. S.& Abdel-Sattar, E. Study of hypocholesterolemic and antiatherosclerotic properties of Medicago sativa L. cultivated in Egypt. Journal of Food and Drug Analysis. 2005;13(3):212.
(109) Fan, W., Du, H., Zhou, L., Shi, P.& Wang, C. Digital gene-expression of alfalfa saponin extract on laying hens. Genomics data. 2015;3:97-99.
(110) Shi, Y. *et al.* The regulation of alfalfa saponin extract on key genes involved in hepatic cholesterol metabolism in hyperlipidemic rats. PloS one. 2014;9(2):e88282.
(111) Liang, X.-p. Effects of alfalfa saponin extract on mRNA expression of Ldlr, LXRÎ±, and FXR in BRL cells. 2015;
(112) JACKSON, I. M. Abundance of immunoreactive thyrotropin-releasing hormone-like material in the alfalfa plant. Endocrinology. 1981;108(1):344-346.
(113) Mattioli, S. *et al.* Alfalfa and flax sprouts supplementation enriches the content of bioactive compounds and lowers the cholesterol in hen egg. Journal of Functional Foods. 2016;22:454-462.
(114) Seida, A., El-Hefnawy, H., Abou-Hussein, D., Mokhtar, F. A.& Abdel-Naim, A. Evaluation of Medicago sativa L. sprouts as antihyperlipidemic and antihyperglycemic

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**

agent. Pakistan journal of pharmaceutical sciences. 2015;28(6)
(115) Farsani, M. K., Amraie, E., Kavian, P.& Keshvari, M. Effects of aqueous extract of alfalfa on hyperglycemia and dyslipidemia in alloxan-induced diabetic Wistar rats. Interventional Medicine and Applied Science. 2016;8(3):103-108.
(116) Plaza, L., de Ancos, B.& Cano, P. M. Nutritional and health-related compounds in sprouts and seeds of soybean (Glycine max), wheat (Triticum aestivum. L) and alfalfa (Medicago sativa) treated by a new drying method. European Food Research and Technology. 2003;216(2):138-144.
(117) Vyas, S., Collin, S. M., Bertin, E., Davys, G. J.& Mathur, B. Leaf concentrate as an alternative to iron and folic acid supplements for anaemic adolescent girls: a randomised controlled trial in India. Public Health Nutrition. 2010;13(3):418-423.
(118) Zadeh, M., Mirzaei, A.& Mohammad, F. Effects of hydro-alcoholic extract of Medicago sativa on the chemical burn injuries in rats. 2014;
(119) Martínez, R. *et al.* Medicago sativa L., a functional food to relieve hypertension and metabolic disorders in a spontaneously hypertensive rat model. Journal of Functional Foods. 2016;26:470-484.
(120) Al-Dosari, M. S. In vitro and in vivo antioxidant activity of alfalfa (Medicago sativa L.) on carbon tetrachloride intoxicated rats. The American journal of Chinese medicine. 2012;40(04):779-793.
(121) Gomathi, R., Vijipriya, M.& Usha, K. Cardioprotective effect of ethanolic extract of Medicago sativa stem on isoproterenol induced myocardial infarction in wistar albino rats. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(2):839-842.
(122) Servatyari, K. *et al.* The effect of hydroalcoholic extract of Medicago sativa on liver function tests, blood biochemical factors and coagulation system in male rats. 2017;
(123) Montanaro, A.& Bardana Jr, E. J. Dietary amino acid-induced systemic lupus erythematosus. Rheumatic diseases clinics of North America. 1991;17(2):323-332.
(124) Beauchemin, K.& Rode, L. Compressed baled alfalfa hay for primiparous and multiparous dairy cows. Journal of Dairy Science. 1994;77(4):1003-1012.

ISSN: 3009-7061 **DOI: 10.21608/japs.2025.347537.1043**