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TRADITIONAL, ETHNOBOTANICAL, PHYTOPHARMACOLOGICAL AND NUTRACEUTICAL POTENTIAL OF *MEDICAGO SATIVA* LINN.

Vaibhavi Savalia[1*], Devang Pandya[2]

[1*,2]School of Pharmacy, RK University, Kasturbadham, Bhavnagar Highway, Rajkot. 360020. Gujarat, India.

Corresponding Email: [1*]vaibhavi.savalia@rku.ac.in

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ABSTRACT:

Medicago sativa Linn. (Leguminosae), known as the (Al-fal-fa) "father of all foods". It is an erect, green perennial herb and one of the finest fodder plants. Alfalfa has been traditionally used in digestive system disorders, central nervous system disorders, fluid retention, ulcer, and for the treatment of various other ailments in Ayurveda and Homeopathy. Pharmacological and clinical trial reports suggest beneficial role of Alfalfa as hypocholestremic, hypoglycemic, anti-anxiety, antioxidant, immune potentiating, antimicrobial and estrogenic activity in menopausal symptoms in women. Ethnomedicinal reports suggest use of Alfalfa in arthritis, scurvy, improve the memory, inflammation and jaundice. Moreover, Alfalfa also improve health of moderately polluted soil by phytoremediation. Alfalfa contains alkaloids, phytosterols, saponins, phytoestrogens, flavonoids, coumarins, terpenes and digestive enzymes, as major classes of phytoconstituents. Sprout, leaves and seeds are important source of nutraceutical due to presence of proteins, amino acids, minerals, and vitamins. Important phytoconstituents found were lutein, soyasapogenol, medicagenic acid, kaempferol, quercetin, myricetin, beta-sitosterol and stigmasterol. A detailed survey of literature was performed to compile scientific literature published on Medicago sativa. Present review focus on important aspect of Medicago sativa like pharmacological, traditional, ethnomedicinal uses, phytochemical analysis and phytoremediation.

Keywords: Medicago sativa, Alfalfa, Nutraceutical, Hypocholestremic, Saponin, Phytoremediation.

1. INTRODUCTION

Medicago sativa Linn. (Leguminosae) known as the (*Al-fal-fa*) "father of all foods", is a herbaceous perennial plant that originated in Asia (Fig.1). It is also known as Lucerne, Vilaayati gawuth, Lasun ghaas, and Lusan. *Medicago sativa* is the most ancient fodder plant, cultivated throughout the world. Habitat of *Medicago sativa* in India are Punjab, Uttar Pradesh, Gujarat, Maharashtra, Tamil Nadu, West Bengal, as a farm crop (Khare, 2007). *Medicago sativa* have verities of utilization such as soil improvement, medicinal uses, and animal feed (Steppler and Nair, 1987). *Medicago sativa* have long been used Ayurvedic and Homoeopathic medicine in Gastrointestinal disorders, Central nervous system disorders, and for the treatment of various other health problems (Inamul, 2004; Bora and Sharma, 2011a). A literature survey revealed that *Medicago sativa* possess saponins, flavonoids, phytoestrogens, coumarins, alkaloids, phytosterols, and terpenes as major classes of phytoconstituents. However, not detailed literature review was available on its traditional, pharmacological, phytochemical, nutraceutical and phytoremediation aspect (Khare, 2007; Bora and Sharma, 2011a). The present review emphasizes on pharmacological, ethnomedicinal, traditional, phytochemical, nutraceutical and phytoremediation potential of *Medicago sativa*.

Taxonomy

Domain: Eukaryote
Kingdom: Plantae
Phylum: Angiosperms
Class: Magnoliopsida
Order: Fabales
Family: Fabaceae
Genus: *Medicago*
Species: *sativa* (Bora and Sharma, 2011a)

Morphology

Medicago sativa is perennial legume herb. Leaves are compound ovate to obovate, trifoliate, 2-3cm X 1-1.5cm, glabrous, symmetrical base, with acute-mucronate apex, short petiole, entire margin except near apex serrate and both surface dark green in color. Venation reticulate and Phyllotaxy alternate. Leaves like two stipules were observed at base of leaflet. The flowers are yellow to violet-blue and clover-like. Flowers are 9 - 10mm long, appears in oblong, and blossomed racemes. The fruit is a pod with 2 or 3 twists (spiral); the centre is hollow. Stems are erect, smooth and sharply angled. *Medicago sativa* plant when mature have strong taproot. This taproot may be upto 6 m or more in length with several lateral roots connected at the crown in deep, well drained, and moist soils (Bora and Sharma, 2011a; Savalia and Desai, 2013).

Phytochemical Analysis

Qualitative phytochemical analysis

Qualitative phytochemical analysis of leaves revealed presence of terpenoids, sterol, flavonoid, phenol and tannin in *Medicago sativa* extracts (Savalia and Desai, 2013). Seed of *Medicago sativa* revealed presence of carbohydrates, flavonoids, saponins, amino acids, anthraquinones, alkaloids, steroids, tannins, terpenoids, phenols, fixed oils and fats (Gomathi *et al.*, 2016). The herb contains carotenoids (including lutein), triterpene saponins, isoflavonoids coumarins, triterpenes (including sitgmasterol, spinasterol); and, cyanogenic glycosides (corresponding to less than 80 mg HCN/ 100g). Seed contain saponins with the aglycones, soyasapogenol B and E and polymines, diamino propane and norspermine. Two storage globulins, alfin and medicagin are found in the seeds. The flowers contain flavonoids, kaempferol, quercetin, myricetin and laricytrin. The fruits contain beta amyrin, alpha- and

beta-spinasterol, beta-sitosterol, stigmasterol, myrsellinol, scopoletin and esculetin. Alfalfa is a good source of isoflavones. The coumestan derivative coumestrol is the major estrogenic phytochemical. The saponins reduce intestinal absorption of cholesterol in rabbits (Khare, 2007). Table 1 shows phytoconstituents present in different parts of *Medicago sativa*. While, Table 2 shows phytoconstituents present in *Medicago sativa* as per class of phytoconstituents.

Table 1: Phytoconstituents Present in Different parts of *Medicago sativa* (Bora and Sharma, 2011a; Duggal *et al.*, 2011; Duke, 1992)

Sr. no.	Name of Phytoconstituents	Plant part
1	(-)-5'-Methoxysativan, 10-Methoxy-Medicarpin, 11,12-Dimethoxy-7- Hydroxycoumestin, 4',7-Dihydroxyflavone, 7,5'-Dihydroxy-2',3',4'- Trimethoxy isoflavan, 7-Hydroxy-2',3',4- Trimethoxy isoflavan, Adenine, Adenosine, Alfalfone	Plant
2	(S)-N-(3- Hydroxypropyl)- Azetidine- Carboxylic- Acid	Sprout Seedling
3	2-(4-Hydroxy-2- Methoxy-Phenyl)-6- Methoxy-Benzofuran- 3- Carboxylic-Acid, 4-Amino-Butyric-Acid, 8-Methoxycoumestrol	Root
4	2-Carboxyarabinitol, 4',7-Dihydroxyflavone	Leaf
5	2-Methyl-Propanol, 3-Methyl-Butanol, Acetone	Essential Oil
6	3',4',5-Trihydroxy-7- Methoxy-Flavone, 3',4',7-Trihydroxyflavone, 3'-Methoxycoumestrol, 4',7-Dihydroxyflavone, 4'-Methoxycoumestrol	Shoot
7	3-Beta-Galactosidase, 4',7-Dihydroxyflavone, Alfalone	Tissue Culture
8	9-O-Methyl coumestrol	Wood
9	Aesculetin	Fruit

Table 2: Classification of Phytoconstituents Present in *Medicago sativa* (Bora and sharma, 2011a; Duke, 1985; Duke, 2002)

Phytoconstituents class	Name of phytoconstituents
Amino acids	Lysine, Arginine, Tyrosine, Phenylalanine, Methionine, Alanine, & Cysteine etc.
Vitamins	A**,** B1, B6, B12, C, D, E**,** K, Biotin, Folic Acid, Pantothenic Acid, Niacin
Flavonoids	Quercetin, Myricetin, Luteolin, Apigenin etc.
Phenolic compounds	*P*-Hydroxybenzoic Acid, Salicylic Acid, Vanillic Acid, Caffeic Acid, Chlorogenic Acid Ferulic Acids, Sinapic Acids, Hesperidin**,** Naringenin, & Tannic acid Etc.
Non protein amino acids	L-Canavanine
Phytosterols, & Sterols	β-Sitosterol, Stigmasterol, α-Spinasterol, β-Sitosterol, Stigmasterol, Myrsellinol, Scopoletin, Esculetin etc.
Phytoestrogens	Coumestrol, Genistein, Diadzein, Liquiritigenin, Biocanine A, Isoliquiritigenin, Loliolide etc.
Proteins	Ferritin, Protein Phosphatase 2A Holoenzyme, β-Amylase
Saponins	Soyasapogenols, Hederagenin, Medicagenic Acid etc.

Coumarins	Myrsellinol, Scopoletin, Esculetin, 4-Coumaric Acid etc.
Alkaloids	Asparagines, Trigoneline etc.
Other phytoconstituents found in *M. Sativa* are Carotene, Organic acids, Enzymes, Minerals, Polyamines, Volatile components	

Quantitative Phytochemical Analysis

Estimation of Total Phenolic Content: Folin-Ciocalteu reagent method was used to determine total phenolic content of leaves. Results obtained were expressed in Gallic acid equivalence at µg/mg of dry extract. *Medicago sativa* methanolic extract (1 mg), 46.69 ± 3.855µg Gallic acid equivalent of phenols was detected (Savalia and Desai, 2013).

Another Folin-Ciocalteu experiment as illustrated by Karimi et al of methanol extract of leaves of *Medicago sativa* was measured at absorbance 765nm and the result expressed as 37.0 ± 0.02mg Gallic acid equivalent per gm of dried methanol extract of *Medicago sativa* leaves (Ehsan *et al.,* 2013).

Different parts, leaves, flowers, stems, seeds, third cut roots and first cut roots of *Medicago sativa* were extracted by different methods like maceration (M), Accelerated solvent extraction (ASE), Ultrasound-assisted extraction (UAE) and Supercritical fluid extraction (SFE). Total phenolic content was measured by Folin-Ciocalteu method. Total phenolic content was found in the range of 14.6–30.2 mg gallic acid equivalent /g dry material. Highest total phenolic Content values were found in flowers and seeds, whereas the lowest were found in roots, ranging from 6.9 mg gallic acid equivalent /g dry material in root extracts obtained by UAE with 96% EtOH to 48.4 mg gallic acid equivalent /g dry material in flower extracts obtained by UAE with 70% EtOH. In the case of third-cut leaves and roots, the highest results obtained with SFE were 30.2 and 28.9mg gallic acid equivalent/g dry material, respectively (Aneta *et al.*, 2017).

Estimation of Total Flavonoid Content: Total flavonoid content was determined using rutin as standard as described by Karimi et al. (2010). The extract was measured using absorbance at 510nm and the result was expressed as milligrams of rutin equivalents per gram of dry matter. Result showed that the total flavonoids was 12.62 ± 0.17mg rutin equivalent/g dry matter (Ehsan *et al.*, 2013).

Different parts, leaves, flowers, stems, seeds, third cut roots and first cut roots of *Medicago sativa* were extracted by different methods like maceration (M), Accelerated solvent extraction (ASE), Ultrasound-assisted extraction (UAE) and Supercritical fluid extraction (SFE). Total flavonoid contents of EtOH extracts were measured by was evaluated by aluminum colorimetric assay using rutin as the standard. Total flavonoid content was in between the range of 7.4–139.0mg Rutin equivalent/g dry material in *Medicago sativa* extracts obtained from leaves. The highest Total flavonoid content was 139.0mg Rutin equivalent /g dry material in leaf extract obtained using scCO2 and EtOH; the level was 19 times higher than maceration extracts and 3.34 times higher than ASE extracts. A high TFC value was also present in third-cut root SFE extract (50.9mg Rutin equivalent/g dry material). The lowest TFC was found in first-cut root extracts, ranging from 0.0 to 7.3mg Rutin equivalent/g dry material for the different extraction methods. The TFC in extracts obtained using scCO2 and EtOH was as follows: leaves > third-cut roots > flowers > stems > seeds > first-cut roots (Aneta *et al.*, 2017).

Estimation of Total Saponin Content: Different parts, leaves, flowers, stems, seeds, third cut roots and first cut roots of *Medicago sativa* were extracted by different methods like maceration (M), Accelerated solvent extraction (ASE), Ultrasound-assisted extraction (UAE) and Supercritical fluid extraction (SFE). Total saponin content was determined by

spectrophotometric method. Total saponin content in the individual parts of the *Medicago sativa* extracts was in range of 54.6 to 622.2mg Oleanoic acid equivalents/g dry material. The highest saponin content, 622.2mg Oleanoic acid equivalents/g dry material, was revealed in the SFE extracts of leaves, whereas the lowest content was observed in the maceration extract from stems (54.6mg Oleanoic acid equivalents/g DM) (Aneta *et al.*, 2017).
The saponin, medicagenic acid, is found in leaves and roots (leaves 1.49%, roots 2.43% of dry matter) (Khare, 2007).

Traditional, Pharmacological and Ethnomedicinal Study:
Pharmacological reports revealed that *Medicago sativa* is used as hypocholestremic, antimicrobial, estrogenic, neuroprotective, anti-wrinkle, antiulcer, hypolipidemic, and in the treatment of atherosclerosis, stroke, heart disease, diabetes, cancer, and menopausal symptoms in women (Table 3) (Bora and Sharma, 2011a; Bora and Sharma, 2011b; Rana *et al.*, 2010; Bonte *et al.*, 2002; Bora and Sharma, 2011c; Mikaili and Shayegh, 2011; Aggarwal *et al.*, 2006).
Traditionally, *Medicago sativa* is used to improve the memory, to cure kidney pain, cough, in sore muscles, as a rejuvenator, diuretic, antidiabetic, anti-inflammatory, antifungal, antioxidant, anti-asthmatic, antimicrobial, galactagogue and in central nervous system (CNS) disorders (Bora and Sharma, 2011a). Moreover, it is also considered beneficial in bladder disorders, boils, blood clotting disorders, diuresis, cough, gastrointestinal tract disorders, and breast cancer, inflammation, prostate disorders, cervical cancer, kidney disorders, appetite stimulation, allergies, increasing breast milk, asthma, indigestion, insect bites, jaundice, menopausal symptoms, increasing excretion of neutral steroids and bile acids in fecal matter, stomach ulcers, nutritional support, skin damage from radiation, increasing peristaltic action of the stomach and bowels, uterine stimulant, rheumatoid arthritis, scurvy, vitamin supplementation (vitamins A, C, E, & K) and wound healing(Bora and Sharma, 2011a; BHMA; 1996; Der *et al.*, 2005). Worldwide ethnopharmacological reports of *Medicago sativa* are tabulated in Table 4. According to literature survey, *Medicago sativa* is a preventive of high blood pressure (Khare, 2007). *Medicago sativa* seed possess 69% Angiotensin- converting enzyme inhibition at 0.33mg/ml (Kouchmeshky *et al.*, 2012; Wenno and Suprayitno, 2016). Furthermore, root and aerial parts of *Medicago sativa* have been evaluated for antioxidant activity and found to possess significant antioxidant properties (Colin and Volhard, 1992; Sorensen *et al.*, 1998; Eruygur *et al.*, 2018). *Medicago sativa* is widely grown in Asia and United States as fodder plant, but availability of seed is scarce (Savalia and Desai, 2013). Stem, leaf and root are easily available than seed. Because, to produce *Medicago sativa* seeds, usually, the crop is left for fodder production in the first three years after planting, then only in the fourth year it is left for seed production (Abu and Abusuwar, 2015).

Table 3: Pharmacological study on *Medicago sativa* Linn. (Khare, 2007; Bora and Sharma, 2011a; Duggal *et al*., 2011; Smith *et al.*, 1981)

Sr. No.	Pharmacological study and clinical trials	Part used	Dose
1	Hypocholesterolemic activity, hypolipidemic and anti-atherosclerotic activity in animal study & clinical trials	Stem, & leaves, Seed extract, seed	900mg extract, 80-60 g daily
2	Hypoglycemic effect in animal and clinical trials	Whole plant	1 mg/mL, 1 g/400 mL
3	Stimulation of insulin secretion	Whole plant	0.25-1 mg/mL

4	Cerebro-protective effect	Aerial parts	100mg/kg
5	Anti-anxiety activity	Aerial parts	100mg/kg
6	Antioxidant properties and iron chelation activity	Aerial parts, leaves	250µg/ml, 50µg/ml
7	Immuno-potentiating activity	Polysaccharide from plant	250-500 micrograms.ml-1
8	Antimitotic activity, Colon cancer, pancreatic adenocarcinoma	L-Canavanine	3g/kg, IC50 value of 10 µM
9	Estrogenic activity and Anti-dopaminergic& in menopausal symptoms in women	Sprout	100µg/ml
10	Inhibit the activities of reverse transcriptase and protease of HIV	Polysaccharides refined components	-
11	Antimicrobial activity & Nematicidal	Leaves	1mg/ml, 500 mg/ml
12	Anti-inflammatory	Leaves	147.2 µg/ml

Table 4: Ethnopharmacological Reports of *Medicago sativa* (Bora and Sharma, 2011a; Duggal *et al*., 2011)

Sr. No.	**System/Country**	**Use**
1	Ayurvedic system of medicine	Ulcers, arthritis pain and fluid retention, CNS disorders
2	Chinese system of medicine	Digestive system problem, fever, kidney stones, gravel, dysuria, in fluid retention and in swelling.
3	Mexico	To improve the memory, in sore muscles and inflammation
4	Native American Indians	To promote the clotting of blood to avoid excessive blood loss and to treat jaundice
5	Spanish	Treatment of cancer, arthritis and painful boils, scurvy and urinary tract problems
6	Turkey	Cardiotonic, scurvy, arthritis
7	Iraq	Arthritis

***Medicago sativa* as Nutraceutical**

Medicago sativa is utilized as nutritional with many health benefits for humans as well as livestock. Research on *Medicago sativa* suggest that it is nutrition rich food, containing vitamins A, B6, B12, C, D, E, and K. It is also rich in minerals such as iron, calcium, magnesium, potassium, and phosphorus, all in easily absorbable forms. It is a good natural source of fiber and antioxidants, and bioflavonoids. It contains over 300 nutrients and 8 of the essential amino acids. It also contains highest concentration of chlorophyll of any plant. *Medicago sativa* supplements today are easily available in various forms, such as capsules, teas, powdered, and nutritional drinks. They are used as supplement and nutraceutical for a wide range of conditions, including allergies, morning sickness, anemia, arthritis, digestion, rheumatism, gout, blood purifier, blood clotting agent, bone strengthener, tooth decay, and urinary problems (Mikaili and Shayegh, 2011).

Freeze dried material and residual material of *Medicago sativa* have been evaluated for its macronutrient analysis, mineral content, amino acid quantification and chlorophyll content. Total amino acid content ranged from 80.4 to 124.4mg/g in RM and from 108.9 to 215.8

mg/g in Freeze dried material. All essential amino acids (EAA), namely, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine, were identified. Higher EAA (essential amino acid) content of *Medicago sativa* makes it utmost important source of vegetable protein. High content of Ca, (1485.83mg/100g) in residual material and (1939.55 mg/100g) in FDJ and Zn (5.90 mg Fe/100 g and 2.83 mg Zn/100 g) makes them good natural sources of minerals for consumers with deficiency in these micronutrients or even lactose intolerant people. Chlorophyll a content ranged between 12.41–505.58 and 8.19–66.32 lg/g in residual material and Freeze dried material, respectively. In contrast, RM contained higher amounts of chlorophyll b (55.44–269.8 lg/g) than that found in Freeze dried material (36.67–80.53 g/g). Study demonstrates *Medicago sativa* used as nutraceuticals with low caloric content and considerable amounts of protein (Soto *et al.*, 2017).
Herb contains pro-vitamins A, B_6, B_{12}, D, K, E and P; calcium, phosphorus, iron, potassium, magnesium, choline, sodium, silicon and essential enzymes. The seeds contain 33.2% protein and 4.4% mineral matter (Khare, 2007).

***Medicago sativa* in Phytoremediation:**
Phytoremediation comprises of emerging biological remediation technologies that use plants to remove pollutants from the environment or to make them harmless. It has emerged as one of the most acceptable cleanup technologies for many organic pollutants from contaminated soil and improve soil health (Salt *et al.*, 1998).
Medicago sativa could be applied for phytoremediation of soil polluted moderately by Cd, Zn, and Pb. During one of the experiments, the *Medicago sativa* grew for 10, 40 and 80 days in different soils which were polluted by Cd, Zn, and Pd. *Medicago sativa* had better ability of bioaccumulate Cd than Pb and Zn (Fu-giang *et al.*, 2015).
Medicago sativa can tolerate a heavy metal and petroleum hydrocarbon co-contaminated soil, which is an essential characteristic of any plant species useful in phytoremediation. A bioremediation study was performed for organic contaminant like and Trinitrotoluene (2,4,6-TNT, Polychlorinated biphenyls (PCBs), and Polycyclic aromatic hydrocarbons (PAHs). Results showed that *Medicago sativa* played a significant role in Trinitrotoluene and Polychlorinated biphenyls transformation in the low organic matter soil conditions, but was not a significant factor for the transformation of pyrene (Tesema and Lester, 2011).
Another study of phytoremediation was mainly adopted as an ultimate remediation solution to rehabilitate degraded soils and alleviate weathered Polycyclic aromatic hydrocarbons (PAHs) dissipation to completely clean up contaminated soils. After six months, there was a significant improvement of the physicochemical properties of degraded soils owing to *M. staiva* cultivation. These changes impacted positively on microbial activities and soil toxicity reduction. Dissipation of benzopyrene was also observed under vegetated conditions, but this hydrocarbon was still persistent after six months, although at very low concentrations as compared to the initial spiked level up to 64% for treatments initially spiked at the highest level of 100mg Benzopyrene/ kg of soil (Helmi *et al.*, 2012).
One more study evaluated the phytoremediation potential of *Medicago sativa* in co-contaminated soil and the effects of citric acid and Tween 80, applied individually and combined together, in the phytoremediation process. The results showed that *Medicago sativa* plants could tolerate and grow in a co-contaminated soil. Heavy metals were uptake by *Medicago sativa* to a limited extent, by plant roots. Heavy metal concentration in plant tissues were found in the following order: Zn > Cu > Pb. Soil amendments did not significantly enhance plant metal concentration or total uptake. In contrast, the combination of Tween 80 and Citric acid significantly improved alkane-degrading microorganisms (2.4-fold increase) and lipase activity (5.3-fold increase) in the rhizosphere of amended plants, after 30 days of experiment. This evidence supports a favourable response of *Medicago sativa* in terms of

tolerance to a co-contaminated soil and improvement of rhizosphere microbial number and activity, additionally enhanced by the joint application of citric acid and Tween 80, which could be promising for future phytoremediation applications (Agnello *et al.*, 2016).

2. CONCLUSION

Medicago sativa is used as a therapeutic agent for various diseases. The main phytochemical constituents like flavonoids, saponin, alkaloids and phytoestrogens extracted from the plant might be solely responsible for its pharmacological actions. *Medicago sativa* possesses hypocholestremic activity, estrogenic, hypolipidemic, hypoglycemic and antimicrobial activity, and other pharmacological activities. *Medicago sativa* is also an important nutraceutical due to presence of appreciable amount of Iron, Zinc, Calcium and essential amino acid. *Medicago sativa* is also important herb for phytoremediation of contaminated soil from hydrocarbon and heavy metals. Present review provides an insight into traditional, ethnobotanical, phytochemical, pharmacological and nutraceutical information. Ethnobotanical and traditional claims are supported by modern pharmacological study and presence of certain valuable phytochemical. Presence of important proteins, vitamins, amino acids, and minerals proves important position of *Medicago sativa* as nutraceutical. Thus, *Medicago sativa* can be potential candidate for treatment for various diseases and not only an important source of nutraceutical and but also as an important plant to improve health of soil.

Conflict Of Interest
The authors declare no conflict of interest.

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