International Journal of Pharmaceutical Science and Research
ISSN: 2455-4685; Impact Factor: RJIF 5.28
Received: 04-01-2021; Accepted: 06-02-2021; Published: 15-03-2021
www.pharmacyjournal.net
Volume 6; Issue 1; 2021; Page No. 28-29

Pharmacognostic studies on *Medicago sativa* L

Vijay Danapur[1*], Sringeswara AN[2]
[1] Vriksha Vijnan Private Limited, Chikkallasandra, Bangalore, Karnataka, India
[2] Mahatma Gandhi Botanical Garden, University of Agricultural Sciences, GKVK Campus, Bangalore, Karnataka, India

Abstract
Alfalfa, also known as Lucerne or 'father of all food', is a vitality augmenter. It is considered as a spring tonic and has no equal. It is one of the best single sources of protein, and is very high in Vitamins A, D, E, B-6, and K, calcium, magnesium, phosphorous, iron, zinc, potassium, trace minerals and several digestive enzymes as well as carotene and chlorophyll. Alfalfa is one of the most studied plants available. It consist of many important substances, including several saponins, many sterols, flavonoids, coumarins, alkaloids, acids, vitamins, amino acids, natural sugars, proteins (*25% by weight*), minerals, trace elements and other essential nutrients. Alfalfa has never really gained widespread acceptance with the general public due to the idea of humans consuming something that is generally grown as a feed for animals. In the present study pharmacognsotic studies are carried out to authenticate and standardize this herb.

Keywords: *Medicago sativa* alfalfa TLC physicochemical anatomical microbial limits

Introduction
Alfalfa, also known as Lucerne or 'father of all food', is a member of the pea family and is native to western Asia and the eastern Mediterranean region. Alfalfa sprouts have become a popular food. For herbal supplements primarily, the dried leaves of the plant are used. As Alfalfa is a fodder crop the idea of humans consuming it has never really gained widespread acceptance. It is a vitality augmenter and considered as a spring tonic being a source of proteins, several vitamins, trace minerals and several digestive enzymes. Other components of Alfalfa greatly enhance the action of the saponins by binding the bile acids that are necessary for cholesterol absorption. Also of interest are the effects of vitamin K, found in high concentrations in Alfalfa. In man, dietary Vitamin K can remedy bleeding disorders, which occur when the delivery of bile to the bowel is hindered, as for example in obstructive jaundice or biliary fistula. It is also important to remember that Alfalfa is a fiber and it has been shown, along with Bran and Pectin, to bind & neutralize various types of agents carcinogenic to the colon. Alfalfa alkalises and detoxifies the body. It is useful for bone and joint disorders as it provides a complete range of vitamins and trace elements needed for muscle and bone repair [1].
A study showed Saponins found in *M. Sativa* has beneficial benefits on Neurodegenerative diseases by regulating the pathway of cell signaling through multiple target combinations [2]. Anti-oxidant property evaluation of herb and seeds *M. sativa* showed efficient free radicals scavenging [3]. hydroalcoholic extract M. sativa seeds have anti-inflammatory and analgesic properties [4].
In view of the medicinal significance of the aforementioned plant, a detailed pharmacognostic analysis was carried out to further authenticate and classify the plant, setting pharmacopoeial standards for the plant.

Materials and Methods
Specimen: The plant materials were collected and Identity was confirmed with the voucher specimen using [5]

Physico-chemical values such as the percentage of total ash, acid-insoluble ash, and water and alcohol-soluble extractives Were calculated as per [6]

TLC fingerprinting profile carried as per [7]
Anatomical studies and powder microscopic studies, transverse sections (TS) were prepared and stained [8, 9].

Microbial limit test was preformed based on the standard provided by WHO Guidelines and also Indian herbal pharmacopoeia [10]

Results and Discussions
Pharmacognosy

Table 1: Pharmacognosy features

Physicochemical Constants		**Organoleptic Characters**	
Parametrs	Values	Parametrs	Values
TA	3.6%	Taste	Characeristic
AIA	0.1%	Color	Pale brown
ASE	4%	Odour	Mild
WSE	20.4%	Texture	Rough

TA - Total Ash; AIA - Acid Insoluble Ash; ASE - Alcohol Soluble Extractive; WSE - Water Soluble Extractive; Physico-chemical parameters assist to find genuine plant material and Inspect for adulteration such as salts, silica or improper handling of raw materials. Organoleptic properties are distinct (table 1)

Table 2: TLC Profile

TLC Finger Printing Profile								
Under Visible Light								
Rf Values	-	-	-	-	-	-	-	-
Sprayed with 10% H_2SO_4								
Rf Values	0.25	0.38	0.56	0.95	-	-	-	-
Sprayed with Anisaldehyde								
Rf Values	0.31	0.4	0.5	0.57	0.61	0.82	0.96	-
Under Short UV (254 nm)								
Rf Values	0.89	-	-	-	-	-	-	-
Under Long UV (366 nm)								
Rf Values	-	-	-	-	-	-	-	-

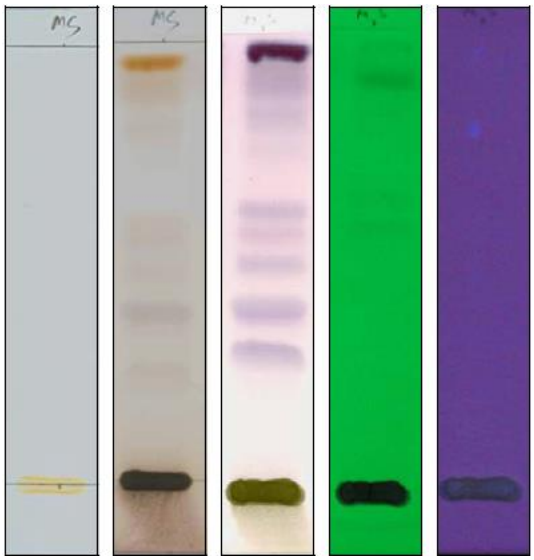

Fig 1: TLC Chromatograms

Medicago sativa showed no band under visible light, 4 bands when sprayed with 10% H_2SO_4 and 7 bands when sprayed with Anisaldehyde. Further, 1, 0 bands were observed under short and long UV light respectively. The results are qualitative TLC finger print profile of plant under study (table 2, fig 1)

Anatomical Characters

Fig 2: Anatomical Characters of *Medicago sativa*

Outer most layer testa single later with thin cuticle, Large embryonic axis present at the center, This embryonic consists of plumule, radical and hypocotyls, Plumule present upper part of the seed portion and radical present lower region of the seed portion, Large vacuole at the center, with full of starch grains (fig 2).

Powder Characters: Powder Colour: Pale orange:

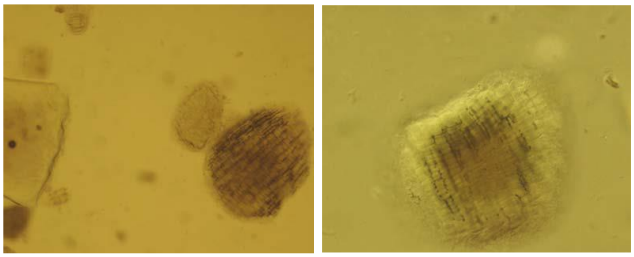

Fig 3: Powder characteristics of *Medicago sativa*

1. Parenchymatous cells are polygonal in shape,
2. Hypodermal and epidermal cells are present,
3. Starch grains are in compound and in groups,
4. Fibers are present.

Powder microscopy permits to acquire knowledge about the various broken bits of the sample that are specific and play a key role in the recognition of the raw sample (fig 3).

Microbial Limit Test

Total Aerobic Bacterial Count (TABC): 1.2 x 10^3

Total Yeast and Mould Count (TYMC): 0.4x10^3

(Microbial contamination limit for raw herbs - TABC: <10^7, TYMC: <10^5)

All criteria were within the limits specified by the WHO Guidelines and Indian Herbal Pharmacopeia.

Conclusion

The macroscopic, microscopic guidelines and physico-chemical parameters are crucial requirements for the determination of the criteria, i.e. identity, quality and purity of medicinal herbs. Keeping this in mind, the current study aimed at standardization on Medicago *sativa* which can serve as a reference tool. Physcio-chemical values will help to authenticate the plant sample. The TLC profile will function as a fingerprint profile for the plant. Organoleptic, anatomical and powder microscopic evaluations are plant-specific. The microbial limit of the raw material was in line with the norms provided

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