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PHYTOCHEMICAL SCREENING AND FREE RADICAL SCAVENGING ACTIVITY OF *MEDICAGO SATIVA* LEAVES

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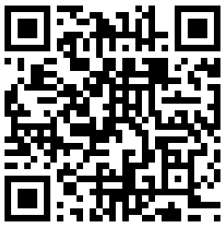
Abstract: *Medicago sativa* L. commonly known as "Alfalfa" belongs to the family leguminosae, found throughout the temperate zone. *Medicago sativa*, the most reputed medicinal plant is traditionally used to improve memory, to cure kidney disorder, cough and sore muscles. In the present study, phytochemical analysis, *in vitro* free radical scavenging and total antioxidant activity were carried out in various extracts of *Medicago sativa* leaves. The phytochemical analysis showed the presence of amino acids, alkaloids, carbohydrates, flavonoids, glycosides, saponins, steroids, tannins, terpenoids and phenols. Free radical scavenging activity was determined using DPPH (2, 2-diphenyl-1-picrylhydarzyl) assay. The DPPH radical scavenging activity was compared with standard antioxidant Butylated hydroxyl toluene (BHT). The highest free radical scavenging was observed in ethanolic extract of *Medicago sativa* leaves when compared with other extracts. High Performance Thin Layer Chromatography analysis (HPTLC) was also carried to find out for the the presence of glycosides.

Keywords: *Medicago sativa*, phytochemical, DPPH, glycosides, BHT, HPTLC



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INTRODUCTION

Nature is the lifeline of our health as it provides all necessary things for survival. Medicinal plants are nature's gift to human beings to make disease free healthy life and play a vital role to preserve our health. In ancient days people did not have any advanced science and technology to protect their life, but they were well aware of values of medicinal plants to preserve their health[1].

Medicinal plants have been traditionally used in the treatment of several human diseases and their pharmacological and therapeutic properties have been attributed to different chemical constituents isolated with antioxidant activities. These antioxidants may be responsible for their preventive effects in various degenerative diseases including cancer, neurological and cardiovascular disease[2].

Phytoconstituents are the natural bioactive compounds found in plants. These phytoconstituents work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions. Phytochemicals are basically divided into two groups, i.e. primary and secondary constituents according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acid, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids, saponins, phenolic compounds, flavonoids, tannins and so on[3].

Phytochemicals are naturally occurring biochemicals in plants that help to give plants their characteristic colour, flavour, smell and texture. Apart from that, phytochemicals could prevent diseases (including cancer and cardiovascular diseases) and inhibit pathogenic microorganisms[4].

Living cells may generate free radicals and other reactive oxygen species by- products as a result of physiological and biochemical processes. Free radicals can cause oxidative damage to lipids, proteins and DNA, eventually leading to many chronic diseases such as cancer, diabetes, aging, and other degenerative diseases in humans[5].

The involvement of free radicals in the pathogenesis of large number of diseases was well documented. A potent scavenger of free radicals may serve as a possible preventative intervention for diseases[6].

Antioxidants are our first line of defense against free radical damage and are critical for maintaining optimum health and wellbeing. Living cells possess a protective system of antioxidants which prevents excessive formation and enables the inactivation of reactive oxygen species. The antioxidants protect the body from potentially damaging oxidative stress which arises as a result of an imbalance between the formation of reactive oxygen species and the body's antioxidant defense[7]. In this view, the present study was carried out to

analyze the phytochemical constituents of various extracts of *Medicago sativa* leaves and to compare the free radical scavenging activity among various extracts.

MATERIALS AND METHODS

(i) Collection of plant material:

The fresh leaves of the *Medicago Sativa* were collected from Department of Forage Crops, Tamilnadu Agricultural University, Coimbatore District, Tamilnadu. The plant was identified by its vernacular name and later taxonomical identification was made by the taxonomist of the Botonical survey of India, Southern circle, Tamilnadu Agricultural University, Coimbatore.

(ii) Preparation of the plant sample:

After that, the plant material was washed with water to remove contamination and shade dried at room temperature. The dried samples were cut into small pieces and then ground into coarse powder with the help of mechanical grinder and stored in airtight containers for futher studies. 20 grams of powdered samples were weighed and wrapped separately with whatmann No.1 filter paper and extracted using various solvents with the help of soxhlet extractor. Then, the various plant extracts were concentrated using rotary evaporator and then preserved for future analysis.

(iii) Qualitative analysis of phytochemicals

The phytochemical constituents of various extracts of *Medicago Sativa* leaves were qualitatively analysed using standard procedure[8, 9, 10].

(iv) Determination of Free Radical Scavenging Activity

A number of *in vitro* methods have been developed to measure the efficiency of dietary antioxidants either as pure compounds or in food mixtures. These methods can be classified into two major types based on the mechanisms involved: The assays by electron- transfer reaction, including ferric ion reducing antioxidant parameter (FRAP) and assay based on 2, 2'-diphenyl-1-picryl hydrazyl (DPPH) [11].

The stable 2, 2'-diphenyl-1-picryl hydrazyl radical (DPPH) was used for the determination of free radical scavenging activity of the plant extracts. Different concentrations (10-100 µg) of various extracts (benzene, chloroform, ethanol, ethyl acetate, methanol and petroleum ether) of *Medicago sativa* leaves were added with an equal volume of methanolic DPPH solution (0.5 mM). DPPH solution with methanol was used as positive control and methanol acted as negative control. When DPPH reacts with antioxidant, DPPH was reduced and the colour changed from deep violet to light yellow. After 30 minutes incubation in dark at room temperature, the absorbance of the mixture was measured at 517 nm[12].

The percentage antioxidant activity was calculated using the following formula,

$$\% \text{ scavenging activity} = \frac{\text{Absorbance of the sample} - \text{Absorbance of the control}}{\text{Absorbance of the control}} \times 100$$

(v) HPTLC analysis for Glycosides:

A densitometric HPTLC analysis was performed for the development of characteristic finger printing profile. The methanolic extract of *M.sativa* leaves was centrifuged at 3000 rpm for 5 min. Supernatant was used as test solution for HPTLC analysis. 3µl of test solution and 4µl of standard solution was loaded as 5mm band length in the 3 x 10 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase (Glycoside) and the plate was developed in the respective mobile phase up to 90mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at visible light, UV 254nm and UV366nm. The developed plate was sprayed with respective spray reagent (Glycoside) and dried at 100°C in Hot air oven. The plate was photo-documented in Visible light and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber.After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at visible light 500nm. The Peak table, Peak display and Peak densitogram were noted. The software used was winCATS 1.3.4 version[13].

RESULTS

1. Qualitative analysis for the presence of phytochemicals:

Medicago sativa leaves were subjected to qualitative analysis of phytochemicals. The phytochemical screening of the various extract of *Medicago sativa* leaves indicated the presence of saponins, steroids, alkaloids, flavonoids, tannins, terpenoids and glycosides.

Among the various extracts, ethanolic extract was found to contain highest amount of phytochemical constituents followed by methanolic extract for the presence of phytochemical constituents. Alkaloids, flavonoids, terpenoids and glycosides were present in all the extracts. Phytochemical constituents of various extracts of *Medicago sativa* leaves were shown in table 1.

Table 1

Phytochemical analysis of various extracts of *Medicago sativa* leaves

	EXTRACTS					
Phytochemical Constituents	**Ethyl Acetate**	**Chloroform**	**Petroleum ether**	**Ethanol**	**Methanol**	**Benzene**
Alkaloids	+	+	+	+	+	+
Anthraquinones	+	-	-	-	-	-
Flavonoids	+	+	+	+	+	+
Saponins	-	+	-	+	+	-
Phenol	+	-	+	+	-	-
Reducing Sugar	+	+	-	+	-	+
Tannins	+	+	-	+	-	+
Terpenoids	-	+	+	+	+	-
Glycosides	+	+	+	+	+	+
Phytosterol	-	-	+	+	+	-
Protein	-	-	+	+	+	-
Fixed oils and fats	-	-	-	-	-	+

Phytochemical analysis of various extracts of *Medicago sativa* leaves

2. Free radical scavenging activity of *Medicago sativa* leaf extracts:

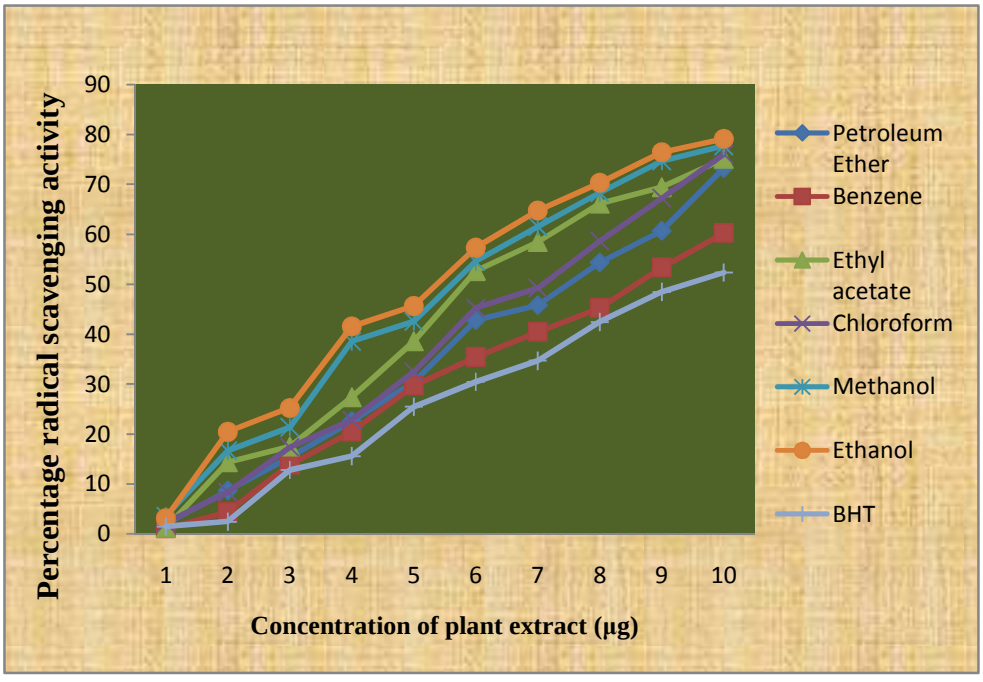
DPPH is a stable nitrogen-centered free radical, the colour of which changes from violet to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers [14].

The various extracts of *Medicago sativa* leaves showed a dose dependent DPPH scavenging activity. Figure 1 show the dose dependent DPPH radical scavenging activity of various extracts and it was compared with the standard antioxidant BHT. The result indicated the antioxidative role of all the extracts and was found to be more pronounced than that of the standard antioxidant BHT. The antioxidative role was found to be more pronounced in ethanolic extract.

Figure 1 indicates the DPPH radical scavenging activity of *Medicago sativa* leaf extracts in different solvent extracts with various concentrations.

Figure 1

DPPH radical scavenging activity



In this study percentage inhibition of free radicals was carried out with different extractions of *Medicago sativa* leaves. The result showed that the ethanolic extract of *Medicago sativa* gave highest percentage of DPPH radical scavenging activity when compared with other extract. Very less percentage of scavenging activity was noted

in ethyl acetate followed by petroleum ether and benzene. The DPPH radical scavenging activity increases with increase in concentration in all the extracts.

3. HPTLC analysis

A densitometric HPTLC analysis was performed for the development of characteristic fingerprint profile, which may be used as marker for quality evaluation and standardization of the drug. R*f* values and the relative percentage area of the separated compounds are recorded in Table 2. HPTLC densitogram of standard and ethanolic extract of *Medicago sativa* leaves are given in Plate 1 and 2 respectively.

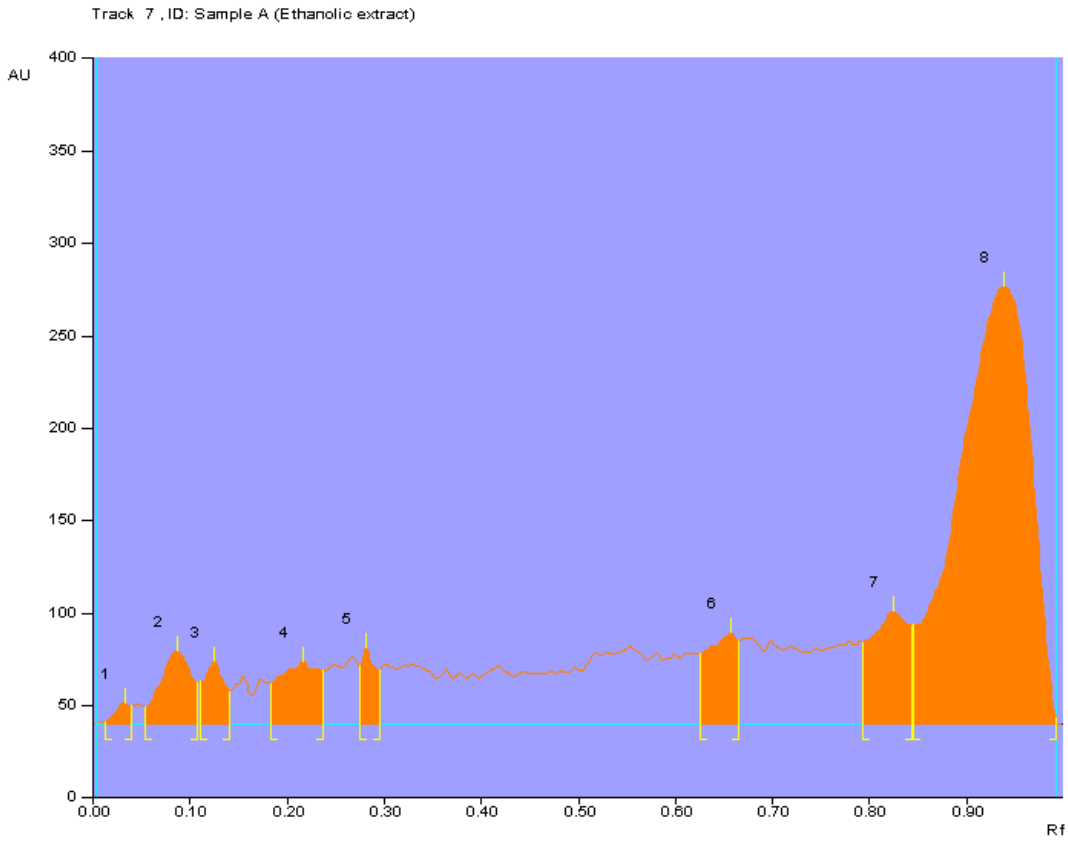
R_f value, height and peak area was shown in table 2

Table 2

HPTLC glycoside profile of ethanolic extract of *Medicago sativa* leaves

Track	Peak	Rf	Height	Area	Assigned substance
STD	1	0.22	185.5	5680.4	Glycoside standard1
STD	2	0.34	270.6	12566.4	Glycoside standard2
Sample A	1	0.03	11.1	144.7	Unknown
Sample A	2	0.09	39.0	1124.2	Glycoside 1
Sample A	3	0.13	33.5	635.5	Unknown
Sample A	4	0.22	33.0	1198.3	Unknown
Sample A	5	0.28	40.2	559.9	Glycoside 2
Sample A	6	0.66	47.3	1379.1	Unknown
Sample A	7	0.82	58.4	2129.3	Glycoside 3
Sample A	8	0.94	233.9	15940.8	Unknown

Figure 2



Peak densitogram of ethanolic extract of *Medicago sativa* leaves

DISCUSSION:

Phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids were present in seven medicinal plants including *Bryophyllum pinnatum, Ipomea aquatica, Oldenlandia corymbosa, Ricinus communis, Terminalia bellerica, Tinospora cordifolia, and Xanthium strumarium*[15].

The qualitative phytochemical analysis in of fruit extract of *Ziziphus mauritiana* Lam showed the presence of secondary metabolites such as flavonoids, glycosides, phenol, lignin, saponins, sterols and tannins except alkaloids[16].

The *Rosmarinus officinalis* extract showed highest antioxidant activities in DPPH assay with 94.3% inhibition. The significant decrease in the concentration of DPPH radical is due to the scavenging ability of this plant extracts[17].

Conclusion:

The results indicated that *Medicago sativa* leaves have a rich source of phytochemicals and antioxidants which may be responsible

for its medicinal property. Phytochemicals were extracted best in ethanol among the various solvents and the ethanolic extract showed the maximum DPPH radical scavenging activity. The antioxidant activity of *Medicago sativa* leaves may be due to the presenece of polyphenols. HPTLC analysis would be definitely useful in deciding the purity and quality of the herbal drug, particularly for market acceptability and competency of commercial formulation. Further studies are under progress in our laboratory for the isolation of the active compounds.

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