



Research Article

PHYTOCHEMICAL ANALYSIS AND FREE RADICAL SCAVENGING POTENTIAL OF *MEDICAGO SATIVA* LINN SEEDS

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ABSTRACT

Phytochemical analysis and *in vitro* free radical scavenging activities were carried out in various extracts of *Medicago sativa* raw seeds and germinated seeds. The phytochemical analysis showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, saponins, phytosterols, tannins, anthraquinone, phenols and proteins. Among the various extracts, phytochemicals were extracted best in ethanol in both raw seeds and germinated seeds of *Medicago sativa*. Free radical scavenging activities such as, 2,2-diphenyl-1-picrylhydrazil (DPPH), hydrogen peroxide and hydroxyl radicals, superoxide anion radicals and nitric oxide radicals were analyzed in the ethanolic extract of *Medicago sativa* raw seeds and germinated seeds and were compared with standard antioxidant ascorbic acid. Both the ethanolic extract of *Medicago sativa* raw seeds and germinated seeds scavenged the free radicals in a concentration dependent manner. The antioxidative role was found to be more pronounced in ethanolic extract of *Medicago sativa* germinated seeds. The results observed were comparable to standard antioxidant ascorbic acid.

Keywords: *Medicago sativa*, phytochemicals, free radicals and antioxidant.

INTRODUCTION

All over the world the herbal medicine acts as the representative of the most important fields of traditional medicine. The study on the medicinal plants is essential to promote the proper use of herbal medicine in order to determine their potential as a source for the new drugs ¹.

The World Health Organization (WHO) estimated that 80% population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs. Also, modern pharmacopoeia still contains at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants. Demand for medicinal plant is increasing in both developing & developed countries due to growing recognition of natural products, being non-narcotic, having no side-effects. Medicinal plant sector has traditionally occupied an important position in the socio cultural, spiritual and medicinal arena of rural and tribal lives of India ².

The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases ³.

Reactive oxygen species such as superoxide anions ($O_2^{\cdot-}$), hydroxyl, nitric oxide radicals and hydrogen peroxide (H_2O_2) play an important role in oxidative stress related to the pathogenesis of various important diseases ⁴. Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals. Antioxidant-based drugs/formulations for the prevention and treatment of complex

diseases, like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer have appeared in the last three decades ⁵. Antioxidant supplements or foods containing antioxidants have been reported to protect the human body by reducing oxidative damage. This has attracted a great deal of research interest in natural antioxidants ⁶.

Medicago sativa L., or Alfalfa is a member of Leguminosae or Fabaceae family, which has a long history of dietary and medicinal uses in traditional herbal medicine in China, America, India and many Middle Eastern countries for the treatment of a variety of ailments and is also used as a fodder for animals ⁷. *Medicago sativa* L., sprouts are often consumed as vegetable salad and their leaves or seeds are also sold as bulk powdered herb, capsules and tablets for nutritional supplement in health food stores. The extracts from alfalfa sprouts, leaves and roots have been indicated to be helpful in lowering cholesterol levels in animal and human studies ⁸. In this regard, the present investigation was aimed to analyze the phytochemical constituents and free radical scavenging activity of *M. sativa* raw seeds and germinated seeds.

MATERIALS AND METHODS

Collection of Plant Material

Medicago sativa seeds were collected from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, and authenticated (BSI/SRC/5/23/2014-15/Tech/394) by the authority of the botanical survey of India (BSI), Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Preparation of the Plant Extract

Medicago sativa (L) seeds were taken for this study. The seed samples were collected from TNAU in Coimbatore District, Tamilnadu, India. They were washed with distilled water and allowed to dry using a filter paper, 20g of seeds were germinated for five days and then dried and powdered. 20g of raw seeds were powdered. Powdered samples were extracted separately using different solvents in soxhlet apparatus.

The solvents used were petroleum ether, chloroform, benzene, ethyl acetate, ethanol, methanol and water. Further studies were carried out using the various extracts of *M.sativa* raw seeds and germinated seeds.

Qualitative Analysis of Phytochemicals

The phytochemical constituents of various extracts of *Medicago sativa* seeds were qualitatively analysed using standard procedure⁹⁻¹¹.

Determination of Free Radical Scavenging Activity

Free radical scavenging activities such as DPPH, hydrogen peroxide, hydroxyl, superoxide, and nitric oxide radical scavenging activities were analysed using standard procedures¹²⁻¹⁶.

RESULTS AND DISCUSSION

Phytochemical Analysis of *Medicago sativa* Seeds and Germinated Seeds (extracts)

The phytochemical screening of plant materials to determine the presence of bioactive constituents is thus vital in the knowledge of therapeutic properties of plants. In the present study the extracts of *Medicago sativa* seeds and germinated seeds were analysed for the presence of phytochemical constituents. Ethanol, methanol, chloroform, benzene, ethyl acetate, petroleum ether, and aqueous extracts were prepared and analysed for their bioactive constituents (Table 1 and Table 2).

Table 1 indicates the phytochemical analysis of *Medicago sativa* seeds. The alkaloids and flavanoids were found to be present in all the extracts. The anthraquinones were found to be present in all the extracts except petroleum ether and aqueous extract. Saponins were present in chloroform, ethanol and methanol extracts. Phenols were present in all the extracts except ethyl acetate. Steroids were present in petroleum ether, benzene, ethyl acetate, and aqueous extracts. Tannins were found in chloroform, ethyl acetate, ethanol and methanol extracts. Terpenoids were found in petroleum ether, chloroform, ethanol, methanol, and aqueous extracts.

Table 1: Phytochemical Analysis of *Medicago sativa* Seeds

Phytochemical constituents	Petroleum ether	Benzene	Chloroform	Ethyl acetate	Ethanol	Methanol	Aqueous
Amino acids	-	-	-	+	+	+	-
Anthraquinones	-	+	+	+	+	+	-
Alkaloids	+	+	+	+	+	+	+
Carbohydrates	+	+	-	+	+	-	-
Flavonoids	+	+	+	+	+	+	+
Glycosides	-	-	-	-	-	-	-
Saponins	-	-	+	-	+	+	-
Steroids	+	+	-	+	-	-	+
Tannins	-	-	+	+	+	+	-
Terpenoids	+	-	+	-	+	+	+
Phenols	-	-	+	-	+	+	-
Fixed oils and fats	+	-	-	-	-	-	-

(+) – indicates the presence of phytocomponents ; (-) – indicates the absence of phytocomponen

Table 2: Phytochemical Analysis of *Medicago sativa* Germinated Seeds

Phytochemical constituents	Petroleum ether	Benzene	Chloroform	Ethyl acetate	Ethanol	Methanol	Aqueous
Amino acids	-	+	+	-	+	+	-
Anthraquinones	-	+	+	+	+	-	-
Alkaloids	+	+	+	+	+	+	-
Carbohydrates	+	-	-	+	+	+	+
Flavonoids	+	+	+	+	+	+	+
Glycosides	+	-	-	-	-	-	-
Saponins	-	-	+	-	+	+	-
Steroids	+	+	-	+	+	-	+
Tannins	-	-	+	-	+	+	-
Terpenoids	+	-	-	-	+	+	+
Phenols	+	-	+	-	+	+	-
Fixed oils and fats	+	-	+	-	-	-	-

(+) – indicates the presence of phytocomponents; (-) – indicates the absence of phytocomponents

Table 2 indicates the phytochemical analysis of *Medicago sativa* germinated seeds. Phenols were found to be present in all the extracts. Alkaloids were present in all the extracts except

aqueous extract. Steroids were present in petroleum ether, benzene, ethyl acetate, ethanol and aqueous extracts. Flavonoids and tannins were present in petroleum ether, chloroform, ethanol

and methanol extracts. Among the various extracts, the ethanolic extract of *Medicago sativa* seeds and germinated seed extract showed maximum phytochemical constituents. So, the ethanolic extract of *Medicago sativa* raw seeds and germinated seeds were taken for further study.

The phytochemical screening of *C. lanatus* seeds showed the presence of phytochemical constituents such as alkaloids, flavonoids, tannins, aminoacids, carbohydrates, cardioglycosides, terpenoids, oils and fats in the ethanolic extract of plant material when compared with other solvents¹⁷.

Free Radical Scavenging Activity

The scavenging of stable DPPH radical model is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. DPPH radical is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule¹⁸.

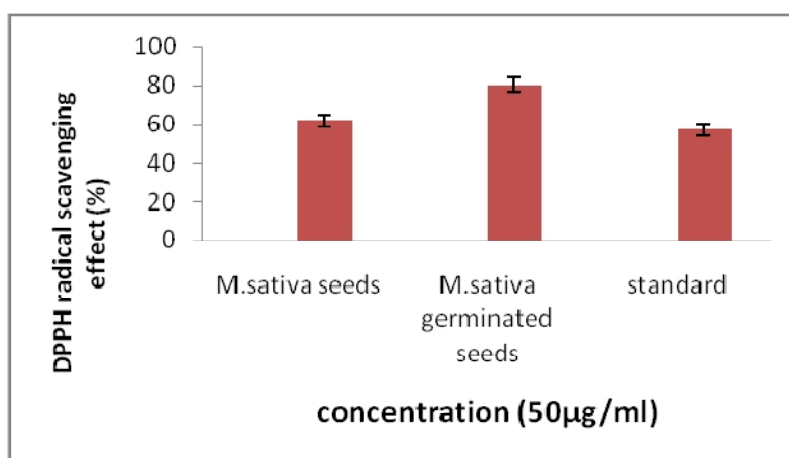


Figure 1: Free radical scavenging activity of ethanolic extract of *M. sativa* seeds(EMsS), *M.sativa* germinated seeds (EMsgS) and standard ascorbic acid at the same concentration (50µg/ml) by 2,2-diphenyl-1-picryl hydrazyl radicals (DPPH).

Figure 1 illustrates a significant decrease in the concentration of DPPH radical due to the scavenging ability of the ethanolic extract of *Medicago sativa* seeds (EMsS), ethanolic extract of *Medicago sativa* germinated seeds (EMsS) and standard ascorbic acid. The scavenging effect of EMsS extracts and standard on the DPPH radical decreased in the order of EMsgS > EMsS > ascorbic acid and were found to be 80.2%, 61.9% and 57.1% at the same concentration (50 µg/ml) respectively. These results indicated that EMsgS have a noticeable effect on

scavenging free radicals. Free radical scavenging activity was also increased with an increasing concentration.

Scavenging of Hydrogen Peroxides (H₂O₂)

Hydrogen peroxide has a strong oxidizing property. Scavenging of hydrogen peroxide by the plant extracts may be attributed to their phenolics which donate electron to hydrogen peroxide thus reducing it to water¹⁹.

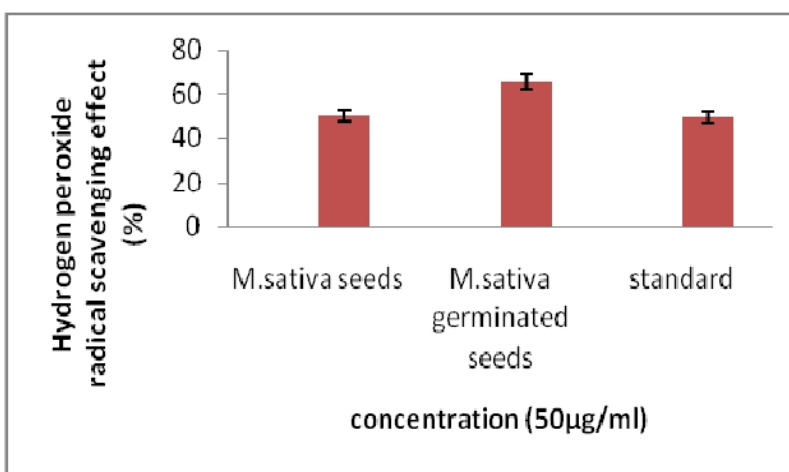


Figure 2: Hydrogen peroxide (H₂O₂) scavenging effects of of ethanolic extract of *M. sativa* seeds(EMsS), *M.sativa* germinated seeds (EMsgS) and standard ascorbic acid at the same concentration (50µg/ml)

Figure 2 shows the percentage H₂O₂ scavenging effect by 50µg/ml of EMsS, EMsgS and compared with the same dose of standard antioxidant ascorbic acid. EMsgS had strong H₂O₂

scavenging activity when compared to standard and EMsS. The percentage H₂O₂ scavenging effect at the same concentration (50µg/ml) of EMsS, EMsgS and ascorbic acid was found as

50.32%, 65.36% and 49.56%, respectively. H_2O_2 scavenging activity of those samples followed the order: EMsS > EMsS > Ascorbic acid. H_2O_2 is highly important because of its ability of penetrate biological membranes. H_2O_2 itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells. Thus, removing H_2O_2 is very important for the protection of living systems.

Hydroxyl Radical Scavenging Activity

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology capable of damaging the biomolecules of living cells²⁰.

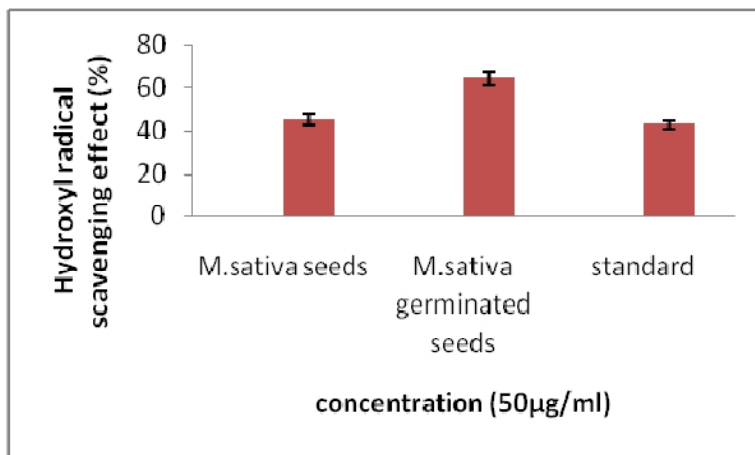


Figure 3: Hydroxyl radical scavenging effects of of ethanolic extract of *M. sativa* seeds(EMsS), *M.sativa* germinated seeds (EMsGS) and standard ascorbic acid at the same concentration (50µg/ml)

The hydroxyl radical is very reactive and can be generated in biological cells through the Fenton reaction. EMsS and EMsGS extracts exhibited concentration dependent scavenging activities against hydroxyl radicals generated in a Fenton reaction system (Fig 3). EMsGS showed significant hydroxyl radical scavenging activity, exhibited higher hydroxyl radical scavenging activity than ethanolic extract of *Medicago sativa* seed extract and standard ascorbic acid.

Superoxide Radical Scavenging Activity

Superoxide can cause oxidation of solutes depending on their reduction potential. Both aerobic and anaerobic organisms possess superoxide dismutase enzymes, which catalyse the breakdown of superoxide radical²¹.

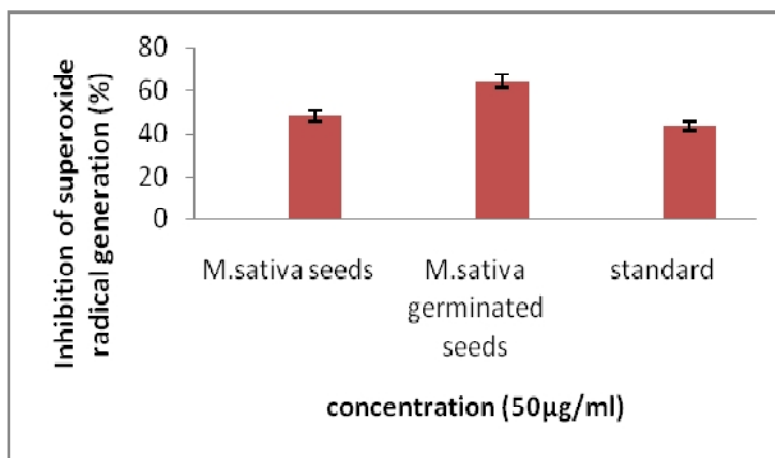


Figure 4: Comparison of superoxide anion radical scavenging activity of ethanolic extract of *M. sativa* seeds(EMsS), *M.sativa* germinated seeds (EMsGS)and standard ascorbic acid at the same concentration (50µg/ml) by the PMS-NADH-NBT method at the same concentration (50 µg/ml).

Figure 4 shows the percentage inhibition of superoxide radical generation by 50 µg/ml EMsS, EMsGS and comparison with the same concentration of ascorbic acid. At concentrations of 50µg/ml, EMsGS exhibited concentration-dependent scavenging activities against $O_2^{\bullet-}$ generated in PMS-NADH systems.

EMsGS showed significant superoxide radical scavenging activity, exhibited higher superoxide radical scavenging activity than EMsS and standard ascorbic acid. Superoxide radical scavenging activity of those samples followed the order: EMsGS > EMsS > ascorbic acid.

Nitric Oxide Scavenging Activity

Nitric oxide is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuron signaling,

inhibition of platelet aggregation and regulation of cell mediated toxicity²².

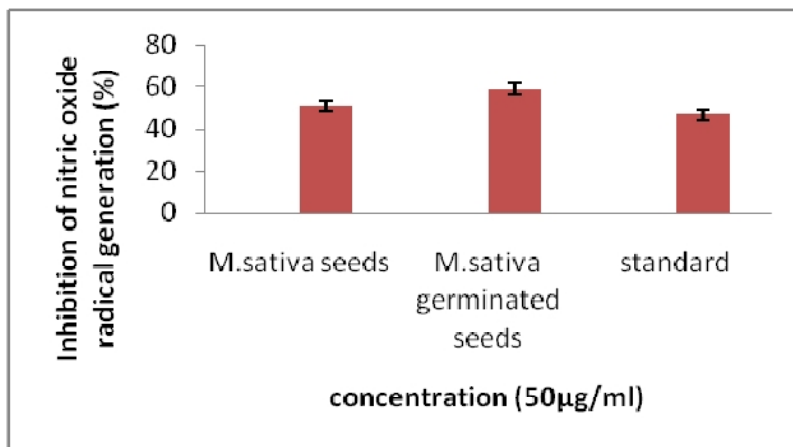


Figure 5: Comparison of nitric oxide radical scavenging activity of ethanolic extract of *M. sativa* seeds (EMsS), *M. sativa* germinated seeds (EMgS) and standard ascorbic acid at the same concentration (50 µg/ml) by the PMS-NADH-NBT method at the same concentration (50 µg/ml).

Figure 5 illustrates the percentage inhibition of nitric oxide generation by EMsS, EMgS and standard ascorbic acid. EMgS showed significant nitric oxide radical scavenging activity, exhibited higher nitric oxide radical scavenging activity than EMsS and standard ascorbic acid. Nitric oxide radical scavenging activity of those samples followed the order: EMgS > EMsS > ascorbic acid.

The aqueous and ethanolic extracts of *Mentha pulegium* leaves showed higher scavenging activity towards nitric oxide and superoxide radicals compared to various extracts and standard²³. The free radical scavenging activity of ethanolic extract of *Actinopteris radiata* showed good capacity to scavenge hydroxyl and superoxide ions²⁴. The free radical scavenging activity of ethanolic extract of *Ficus religiosa* leaves which showed effective scavenging of H₂O₂ radicals²⁵.

CONCLUSION

The results of the present study indicated that the various extract of *M. sativa* seeds and germinated seeds have a rich source of phytochemicals and exhibits the greatest antioxidant activity through the scavenging of free radicals which participate in various pathophysiology of diseases including ageing. Thus, the ethanolic extract of *Medicago sativa* germinated seeds could be recommended as a potent antioxidant to the patients suffering from various oxidative degenerative diseases such as diabetes, cardiovascular disease, arthritis, and cancer.

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