IJAYUSH
International Journal of AYUSH
AYURVEDA, YOGA, UNANI, SIDDHA AND HOMEOPATHY
http://internationaljournal.org.in/journal/index.php/ijayush/

International Journal
Panacea
Research library
ISSN: 2349 7025

Review Article | Volume 13 Issue 12 | December2024

REVIEW ARTICLE ON ANTIDIABETIC ACTIVITY OF SILVER NANOPARTICLES FROM USING MEDICAGO SATIVA EXTRACT

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Abstract

India has a great affluence of different herbal drugs that occur naturally and have great pharmacological activities.Medicago sativa L. is one of the widely well-known folklore medicinal herb. The Medicago sativa is a medicinal plant and has been shown to have substantial pharmacological potential with great usefulness and use in folklore medicine. The plant has provided to different Pharmacological activities in the scientific field of Indian medicinalsystem, such as Anti-diabetic, anticancer, cholesterol and anti-inflammatory activities. The leave and sees were sold as bulk powdered herb, capsule and tablets for nutritional supplement in health food stores. The silver Nanoparticles were characterized by UV-vis, XRD and HR-TEM analysis, UV-vis, spectroscopic studies provided evidence for the formation of nanoparticles. The plant phytochemicals act as stabilizing agent around the Ag NPS, XRD and HR-TEM Analysis clearly proved the crystalline nature of the nanoparticles. This paper provides an split review on the plants phytoconstituents, pharmacological and silver nanoparticles.

Keywords: Anti-diabetic, Medicago sativa L, folklore medicinal

INTRODUCTION

Medicinal plants are the Nature's gift to human beings to help them pursue a disease-free healthy life. Plants have been used as drugs by humans since thousands of years ago. As a result of accumulated experience from the past generations, today, all the world's cultures have an extensive knowledge of herbal medicine. The phytochemical analysis of Medicago sativa showed the presence of proteins, carbohydrates, saponins, lignin, phenolic compounds, tannins, alkaloids triterpene glycosides, carotenoids, sterols, phytoestrogens, flavones, isoflavonoids and phenolic compounds. The previous pharmacological investigation showed that the plant possessed antioxidant, antidiabetic, reproductive, anti-inflammatory, antimicrobial, dermatological, anxiolytic, hepatoprotective, neuroprotective, immunological, cardioprotective, cytotoxic, anti-scorbutic, anti-anemic, xanthine oxidase inhibition and many other pharmacological effects. The current review was designed to discuss the bioactive constituents and pharmacological activities of Medicago sativa.

Phenolic compounds in plants include flavonoids. Flavonoids are usually found in plants as glycosides, i.e. Provided with sugar substituents such as galactose, Rhamnose or glucose, or glycoside malonates. Apigenin Glycosides play a very important role in plant development And physiology, especially during their interactions with Other living organisms. Flavonoid glycosides and free Aglycones are involved in pathogenic and symbiotic Interactions with microorganisms. In addition to the Nutritional components (proteins and carbohydrates) that are important in the use of alfalfa and other plants as Animal feed or food supplements, the plants produce a Variety of secondary metabolites. Many of these secondary Metabolites help to protect the plant against herbivores.It is one of the commonest endocrine disorders and its Prevalence is expected to intensify almost five times in Another 10 years. Chronic hyperglycaemia leads to the Production of free radicals as a result of glucose oxidation, Non-enzymatic glycation of proteins and subsequent Degradation of glycated proteins which in turn can lead to Damage of cellular organelles and enzymes, all Contributing to the promotion and development of Complications of diabetes mellitus.

M. sativa has a long tradition of use as Ayurvedic and Homoeopathic medicine in central nervous and digestive system disorders, and for the treatment of various other M. sativa has a long traditional of use as Ayurvedic and homoeopathic medicine in central nervous and digestive system disorders, and for the treatment of various other ailments. However, only limited research has been conducted on this plant species. The present review emphasizes the

traditional uses and phytopharmacological potential of M. sativa. Additionally, sporadic pharmacological work has so far been carried out to prove its traditional claims. Through this review,the authors hope to attract the attention of natural product researchers throughout the world to focus on the unexplored potential of M. sativa. No review so far has been compiled on this species, and therefore it was considered important to take stock of what has been done in the past, in order to provide a solid foundation and direction to any future research that may be conducted on this planAilments. However, only limited research has been conducted on this plant species. The present review emphasizes the traditional uses and phytopharmacological Potential of M. sativa. Additionally, sporadic pharmacological work has so far been carried out to prove its traditional claims. Through this review, the authors hope to attract the attention of natural product researchers throughout the world to focus on the unexplored potential of M. sativa. No review so far has been compiled on this species, and therefore it was considered important to take stock of what has been done in the past, in order to provide a solid foundation and direction to any future Research that may be conducted on this plant.

Nanomaterials have brought immense interest these days because of their promising applications in numerous areas of Science and technology. Among these, metal nanoparticles are most versatile owing to their properties that depend on their size and Morphology which makes them a proper candidate in various applications.[19-22] Several methods are available for nanoparticle Synthesis such as chemical,[23] photochemical,[24]electrochemical[25] and biological methods.[26] Many of these production routes Involve the use of toxic chemicals and require harsh reaction conditions. Chemical method of nanoparticle synthesis is still widely used because of its short reaction time. However, in this method, the chemical reagents used as reducing and capping agent are usually Toxic and lead to environmental pollution.

With increasing focus on green chemistry, bio mediated synthesis of metal nanoparticles is getting more attention currently. Because of its simplicity, environment benign nature and cost effectiveness. Plant extracts and several microorganisms such as Bacteria, fungi and yeast have been used for the synthesis of nanomaterials.[27-29] Several reports are obtainable on the biological Synthesis of nanoparticles using plant extract as both reducing and capping agent.[30-34]Even though, plant mediated biosynthesis Can be carried out at ambient conditions, the time required for nanosynthesis is much longer than the chemical methods. Microwave Assisted biosynthetic strategy provides a remedy to this problem. The reaction

time can be significantly reduced by using microwave Irradiation. Microwave-assisted synthesis using plant extracts as both reducing and stabilizing agent is a feasible way for the rapid And simple green synthesis of silver nanoparticles. It has several attractive features such as shorter reaction time, lower energy Consumption and better product yield.[34]Microwave irradiation offers rapid and uniform heating of the reaction medium and thus provides uniform nucleation and growth conditions for nanoparticles.[35]

METHODS AND MATERIAL

Plant Profile(37)

Fig: 1*medicago sativa*

Taxonomical

- **Tamil name:**குதிரைமசால்
- **Syno:***Alfalfa,* lucerne, buffalo herb. Purple medic[1]
- **Kingdom**: Plantae
- **Clade**: Tracheophytes
- **Clade**: Angiosperms
- **Clade**: Eudicots
- **Clade**: Rosids
- **Order**: Fabales
- **Family**: Fabaceae
- **Subfamily**: Faboideae
- **Tribe**: Trifolieae
- **Genus**: Medicago
- **Species**: Medicagosativa[2]

Habit: It is perennial tetraploid species, forage crop[4,5]

REVIEW LITERATURE ON PHYTOCHEMISTY AND PHARMACOLOGY ACTIVITY

M. sativa has been reported to contain a variety of Phytochemicals. It has following different classes ofPhytoconstituents.

Alkaloids: Asparagines, trigoneline, stachydrine, l-homostachydrine (Duke, 1985; Mills, 1994; Tamsynet al., 2009).

Amino acids: Medicanine, lysine, arginine, histidine, tyrosine, phenylalanine, methionine, aspartic Acid, glutamic acid, asparagine, serine, alanine, threo-Nine (Worthington & Breskin, 1983; Fushiya et al., 1984; Lihu&Pedak, 1979; Mego&Erdelsky, 1977). Carotene (Gupta et al., 1981). Coumarins: myrsellinol, scopole-Tin, esculetin, 4-coumaric acid (Duke, 1985; El-Khrisy Et al., 1994; Orr et al., 1993). Digestive enzymes (Duke, 1985).[6]

Enzymes: Isoflavone reductase, vestitone Reductase, iminopeptidase, and two aminopeptidases (Xiaoqiang et al., 2006; Shao et al., 2007; Biagioni et al., 1990).

Flavonoids: Quercetin, myricetin, luteolin, api-Genin, chrysoeriol, tricin, glucuronopyranosyl(1→3)]-O-β-lucuronopyranosyl(1→2)-O-β-glucurono-pyranoside} apigenin, O-glycosyl-Transferases, medicarpin, coumestrol, sativan, vestitol, Formononetin (Bickhoff et al., 1964; Stochmal et al., 2001; Kowalska et al., 2007; Stochmal&Oleszek, 2007; Parry & Edwards, 1994; Dutu et al., 2002; Dixon & Steele, 1999; Sumner et al., 1996; Piretti et al., 1982).[8,9]

Minerals: Ca, K, P, Mg, Fe, Zn, Cu, Al, B, Cr, Co, Mn, Mo, Se, Si, Na, Sn (Worthington & Breskin, 1983; Potgieter, 2005).[10]

Non-protein amino acids: l-canaverin (Albourine et al., 1991; Tamsyn et al., 2009). Organic acids: citrate, malate, Malonate, succinate, fumarate, lactate, benzoate (Francoise Et al., 1991; Tamsyn et al., 2009).

Phenolic compounds: Ip-hydroxybenzoic acid, vanillic acid, p-coumaric acid, ferulic acids, salicylic acid, sinapic acids, caffeic acid, hesperetin, naringenin, chlorogenic acid, tannic acid, heterosides (Newby et al., 1980; Volynets et al., 1979; Dutu et al., 2002).

Phytoestrogens: coumestrol, genistein, Formometin, diadzein, biocanine A (Adler, 1962; Bickhoffet al., 1960).

Phytosterols: β-sitosterol, stigmasterol (Timbekova et al., 1996).

Polyamines: norspermidine, Norspermine (Rodriguez-Garay et al., 1989).

Protein: Ferritin, protein phosphatase 2A holoenzyme, β-amylase (Doehlert et al., 1982; Toth et al., 2000; Barcelo et al., 1997).

Vitamins: A, B1, B6, B12, C, D, E, K, niacin, pantothenic acid, biotin, folic Acid (Worthington & Breskin, 1983; Horst et al., 1984).[10]

Volatile components: terpenes, limonene, linalool, trans-Ocimene, furanoids, nonadienal, 2-methyl 4-pentenai, Benzaldehyde, ethyl benzaldehyde, alcohols, butanol, Hexanol, octanol, pentan-3-ol, 3-methylbutanol, trans2-pentenol, trans-2-hexenol, trans-3-hexenol, pent-1-En-3-ol, ott-I-en-3-ol, octa-1,5-dien-3-ol, benzyl alcohol, 2-phenylethanol, ketones, pent-1-en-3-one, pentan-3-One, octan-3-one, methyl phenyl ketone, esters, trans-3-Hexenylacetate, trans-3-hexenylbutanoate, aldehydes, Hexanal, trans-2-pentenal, trans-2-hexenal, trans-2-Nonenal, trans-2,4-hexadienal, furane-2-ethyl.

PHARMACOLOGICAL ACTIVITY

Using the same animal model at the tested Dose level. Moreover, it was also observed that all M. Sativa preparations produced significant antioxidant Properties.

Anti-diabetic Effect

The level of hyperglycemia was reduced when M. sativaWas supplied in the diet (6.25% by weight) and infusion (1 g/400 mL) in streptozotocin-induced diabetes. Aqueous Extract of the plant (1 mg/mL) stimulated 2-deoxy-glucose Transport (1.8-fold), glucose oxidation (1.7-fold) and Incorporation of glucose into glycogen (1.6-fold) in mouse Abdominal muscle. In acute, 20 min tests, 0.25-1 mg/mL Aqueous extract of M. sativa evoked a stepwise 2.5-6.3-Fold stimulation of insulin secretion from the BRIN-BD11 Pancreatic beta cell line. This effect was abolished by 0.5 mM diazoxide, and prior exposure to the extract did not affect subsequent stimulation of insulin secretion by10mM l-alanine, thereby negating a detrimental effect on cell viability. The effect of the extract was potentiated by 16.7 mM glucose and by 1 mM 3-isobutyl-1-methylxanthine.l-Alanine (10 mM) and a depolarizing concentration of KCl (25 mM) did not increase the insulin-releasing activity of M. sativa. Sequential extraction with solvents revealed Insulin-releasing activity in both the methanol and water Fractions, indicating a cumulative effect of more than one Constituent.[11] The manganese content of M. sativa (45.5 Mg/kg) is reported to be the active principle responsible For a hypoglycemic effect documented for M. sativa. A Diabetic patient, treated with soluble insulin but poorly Controlled, found that an M. sativa extract adequately Controlled his diabetes. When

administered separately, Only small doses of manganese chloride (5-10 mg) were Required to have a hypoglycemic effect. However, no effect Was seen on the blood sugar concentrations of non-Diabetic controls or of other diabetic patients, who were Also administered manganese. It was concluded that Manganese lowered the blood sugar concentration in thisParticular diabetic patient because he was unable to utilize Manganese stored in his body.[12]

Effect on cholesterol

In a study, the ability of M. sativa plant to reduce liver Cholesterol accumulation in cholesterol-fed rats was Enhanced by the removal of saponins. Therefore, M. sativaSaponins appear to play an important role in neutral Steroid excretion but are not essential for increasing bile Acid excretion. In an experiment with prairie dogs, the Lowest incidence of cholesterol gallstones was served with The diet of the higher fiber content (85% alfalfa).[13-14]An evaluation was carried out on alfalfa seeds lower low-Density lipoprotein cholesterol and apolipoprotein B Concentration in patients with type II Hyperlipoproteinemia. In a short-term study involving Three normolipidemic individuals given M. sativa seeds (80-60 g daily), serum cholesterol concentrations were Reported to be reduced. In another small study in which Heat-treated M. sativa seeds (40 g three times daily for Eight weeks) were taken by eight type-IIA Hyperlipoproteinemic patients and three type IIB patients,a significant decrease was noted in total serum cholesterol Concentrations, low-density lipoprotein (LDL) cholesterol and apolipoprotein B. The LDL cholesterol concentration Fell by less than 5% in two of the 11 patients Cholestaid™, A product available in the USA containing 900 mg of M. Sativa extract with 100 mg citric acid, is said to neutralize the cholesterol in the stomach before it reaches the liver, Thus facilitating the excretion of cholesterol from the body with no side effects or toxicity.[15-16]

M. sativa top saponins have been shown to decrease Cholesterolemia without changing the levels of high-Density lipoprotein-cholesterol; hence, they reduced the Total cholesterol/high-density lipoprotein-cholesterol ratio In Macacafascicularis. Furthermore, they decreased Intestinal absorption of cholesterol, increased fecal Excretion of endogenous and exogenous neutral steroids and bile acids, and decreased the percentage distribution Offecal deoxycholic and lithocholic acids.[17]

Health beverage manufactured from M. sativa buds was Found beneficial in maintaining normal digestive function And nutrition balance in the human body, reducing Cholesterol, and preventing osteoporosis, arteriosclerosis And aging. Xiong (2003) evaluated medical–use

extracts of Medicago sativa root and demonstrated that extracts Prepared from M. sativa roots may be used to prepare Medical preparations like powder, pill, or decoction for Lowering the levels of cholesterol and lipid in blood, Improving the liver function and the control and Transmission of nerve tissue, and treating calculus.[18]

SLIVER NANO PARTICLE OF MEDICAGO SATIVA

Procedure:

Materials:

Silver nitrate (AgNO3), methyl orange and sodium borohydride (NaBH4) of analytical grade were purchased from Merck (India) and used as such without further purification. All aqueous solutions were prepared using double distilled water.

Preparation of Medicago sativa leaf extract:

Fresh leaves of Medicago sativa were collected and identified taxonomically. It is washed thoroughly with distilled water and leaves are separated. The leaves are then air dried and 30gm of leaf is weighed using a chemical balance. The sample was taken In a round bottom flask fitted with condenser and boiled for 10 min with 100 mL of double distilled water. It was cooled and filtered through Whatman No. 1 filter paper. The extract thus obtained was stored in a refrigerator for further use.

Synthesis of silver nanoparticles (AgNP- Medicago)[38]

In a typical microwave synthesis, 90 mL of 1 mM silver nitrate solution was taken in a 250 mL beaker. To this, 10 mL Medicago sativa leaf extract was added and stirred well. It was then placed in a domestic microwave oven (Sharp R-219T (W)) operating at a power of 800 W and frequency 2450MHz. The solution was then subjected to microwave irradiation for 90 sec. The Formation of AgNPs was monitored using UV-vis. Spectrophotometer by analyzing the reaction mixture after 30, 60 and 90 sec ofMicrowave action. The silver nanoparticle solution was then centrifuged at 10000 rpm for 10 min. The supernatant was decanted and Thenanoparticles were redispersed in distilled water. The above process was repeated three times. The purified sample thus obtained Was freeze dried to get dry sample.

In order to synthesize AgNP- Medicagoat room temperature, 10 mL of Medicago sativa extract was added to 90 mL of 1 mM aqueous solution of silver nitrate and allowed to react at room

temperature for 4 hours. The reaction mixture was subjected toIntermittent UV-vis. Analysis at an interval of 60 minutes to examine the formation of AgNP- Medicago

IDENTIFICATION TEST[38]

Reduction of methyl orange:

The reduction of methyl orange by NaBH4 was used to look into the catalytic efficiency of AgNP- Medicago. To track this Reaction, 0.5 mL freshly prepared NaBH4 solution (0.06 M) was added to 2 mL of methyl orange solution (0.1 × 10-3 M) taken in a Quartz cell. Then 0.5 mL of AgNP- Medicagosolution was added to start the reaction. The change in the concentration of methyl Orange with time was monitored by UV-vis. spectrophotometer. The absorption spectra were recorded at 30 second intervals in the Range of 200-600 nm at ambient temperature (26^{o}C).

Result

Previous studies showed the adding Alfalfa seed in human diet reduced triglycerides and LDL, improved HDL levels and decreased blood glucose. Alfalfa in Formulation is used as livestock fees, while Alfalfa nanoparticles have a variety of applications, including antioxidant properties and potential benefits for certain diseases. In future, the article is proved the antidiabetics diseases.

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