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Review Article

Medicago Sativa: A Potential Health Plant An Overview

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ABSTRACT

To put it simply, phytoestrogens are plant-based substances that mimic oestrogen (17β-estradiol) in both structure and action. The plants Medicago sativa L., Marsilea crenata Presl., Chrysophyllum cainito L., Elaeis guineensis Jacq., and Lannea acida Rich. all contain phytoestrogens. The purpose of this study was to provide evidence that these five plants contain phytoestrogens, which were detected using various equipment. Additionally, the review attempted to evaluate the effects of these plants on bone formation in female rats and mice. The publications included in this systematic review were located using a combination of Google Scholar, PubMed, and Science Direct. A flowchart outlining research inclusion and exclusion criteria was generated using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standards as a basis for the article selection procedure. The frequency of hybrids created has been increased by pedigree selection among derivatives of the two original seed parents of M. sativa (MB and M8). Different hybrid individuals have shown a variety of M. arborea-specific features, even if Alborea individuals are more closely related to M. sativa. Features such as indeterminate growth, a small crown, lodging, resistance to cold and anthracnose, big seeds, yellow blooms, and single-coil flat pods are all part of this plant. These characteristics of M. arborea might reshape alfalfa, making it more adaptable and useful. A growing body of research is indicating that some Alborea varieties may enhance the productivity of adapted alfalfa varieties in North America, South America, and Australia. Trait introgression from M. arborea into alfalfa is an ongoing effort.

INTRODUCTION

The soil and plant quality may be greatly affected by the tractor traffic caused by harvest equipment used for alfalfa cultivation. Different pieces of equipment are needed for alfalfa harvesting at different points in the production cycle. Swather, rake, bailer, and bail waggon movement is anticipated to cover an estimated 60–70% of the field. Changes in soil and plant characteristics

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might occur as a consequence of this flow. Soil bulk density and water penetration rates were assessed in two alfalfa trials in California from 1982 to 1988, with traffic serving as the variable under investigation. Soil bulk density in frequented regions rose by 17% at 0.05 m, 12% at 0.15 m, and 7% at 0.25 m depths, with no change continuing down to 0.65 m, in comparison to non-trafficked areas [01]. In the third year, the field that did not see traffic had greater infiltration rates and soil hydraulic conductivity at the surface. The same trials showed that traffic stress altered plant functions including yield and the dispersion of seasonal fine roots, according to many published research. Data on changes in alfalfa relative growth rates, unit leaf rates, and leaf area ratios as a function of traffic stress was reported by Rechel and Novotny (1996). Furthermore, information on the leaf/stem (L/S) ratios was provided from four separate harvest cycles, two of which occurred in 1984 and two in 1985 [02]. The results demonstrated that trafficked alfalfa had noticeably higher values compared to non-trafficked alfalfa. Rechel and Novotny (1996) proposed that harvest traffic might enhance alfalfa quality, since L/S is known to be associated with improved quality. It is necessary to measure the impacts of harvest traffic on alfalfa quality since it is a universal element of alfalfa production and involves the swather, rake, bailer, and bail waggon. If you want to know how good an alfalfa crop is, you may measure its quality by looking at its acid detergent fibre (ADF), neutral detergent fibre (NDF) (lower values mean better quality), and crude protein (CP) (higher values mean better quality). It is usual practice to measure the impact of various environmental and administrative strategies on alfalfa using the aforementioned three plant quality metrics. If farmers want to know how much their feed is worth, they may use the relative feed value (RFV) formula, which takes ADF and NDF into account [03].

DESCRIPTION: GENERAL MORPHOLOGY

Perennial legume Melodiflora sativa may live anywhere from three to twelve years, depending on the kind and the weather. Both Teuber and Brick (1988) and Barnes and Sheaffer (1995) discussed the overall shape of the M. sativa plant. A robust taproot characterises the mature plant of Melilotus sativa. In deep, well-drained, damp soils, M. sativa may have a taproot that is 6 metres long or longer and a plethora of lateral roots that branch out from the crown. There is perpetual meristem activity in the crown, a complex structure close to the soil surface, which causes buds to grow into stems [04]. Secondary and tertiary stems may emerge from the axils of tri-or multi-foliolate leaves, which grow alternately on the stem. In a normal field fodder production, a plant may grow to be around 1 metre tall and have 5–15 stems. The most frequent flower colours are white, cream, yellow, variegated purple, and yellow with white spots. The most typical outcome of pollination is the development of spiral-shaped seed pods by these blooms [05].

CHEMICAL CONSTITUENTS-

Proteins, carbs, saponins, lignin, phenolic compounds, tannins, alkaloids, triterpene glycosides, carotenoids, sterols, phytoestrogens (cumestrol), isoflavonoids, flavones, and phenolic compounds were identified in the first phytochemical study of alfalfa seed extracts. Medicago sativa contains a variety of substances that have pharmacological activity. These substances include alkaloids (such as stachydrine and homostachydrine), aminoacids (such as arginine, asparginine, cystine, histidine, isoleucine, leucine, methionine, tryptophan, and valine), coumarins (such as medicagol, sativol, trifoliol, lucernol, 4-o-methyl coumesterol, 3-methoxycoumesterol, 11,12 - dimethoxy -7-hydroxyl coumesterol), and flavonoids (such as quercetin, myricetin, luteolin, apigenin, chrysoeriol, tricin, coumest [06]

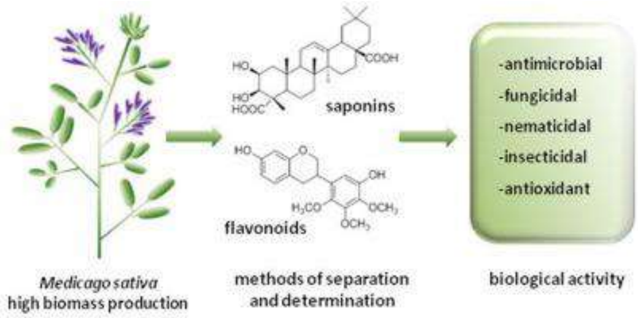

Fig no 1 - M. sativa Chemical constituent

(A, genistein), saponins, steroids (stigmasterol, campestrol, cycloartenol, β-sitosterol), volatile components (nonadienal, benzaldehyde, 2-methyl 4-pentenai, terpenes, limonene, linalool, transocimene, furanoids, ethyl benzaldehyde, butanol, hexanol, octanol, alcohols), substances such as 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, methyl phenyl ketone, esters, pentan-3-one, octan-3-one, pent-1-en-3-one, aldehydes, hexanal, trans-3-hexenylbutanoate, trans-3-hexenylacetate [07], trans-2- pentenal, trans-2-hexenal, trans-2-nonenal, trans-2,4-hexadienal, furane-2-ethyl), acids (lauric, maleic, malic, malonic, myristic, oxalic, palmitic, quinic), purines (adenine, guanine, xanthine, hypoxanthine), canavanine, amino acids (medicanine, lysine, arginine, histidine, tyrosine, phenylalanine, methionine, aspartic acid, glutamic acid, asparagine, serine, alanine, threonine), vitamins (A, B1, B6, B12, C, D, E, K), ketones (myristone, alfalfone) and other constituents such as fructose, pectin, chlorophyll, minerals and trace elements [08]

Table no 1 - Medicago Charactorization with its observation [09-13]

Trait	Observations
Flower colour	Yellow flowers per se and variegated flowers
Indeterminate growth	Plants grow up to 4 m in height
Minimal crown	Observed in ca. 20% of plants
Large leaves	Leaves larger than in both parents in some plants
M. arborea pod shape and size	1- to 1.5-coil flat pods ca. 1 cm in diameter observed in ca. 20% of plants
Large seeds	Seeds twice the size of alfalfa and half the size of M. arborea
Short racemes	5–6 florets versus 15–25 in alfalfa
Fewer seeds per pod	0–50% of alfalfa, although 8–9 per pod observed in one plant
Pollen quantity	0–25% of alfalfa
Autogamy	Full seed set not observed; 10–25% of cross-fertility level
Late flowering	May take some plants 2 years to flower, as for M. arborea

GENOMIC CHARACTERIZATION-

It was discovered that Alborea has a root-tip chromosomal number that is close to tetraploid (2n = 4x = 30-32). The five hybrids and their parents were analysed using an AFLP technique in Australia. The seed parent was an MB derivative called WA2071. While the study did find that certain hybrids did include up to 4% of the M. arborea-specific AFLP bands, 27% of the bands were monomorphic in both the parents and the hybrid, suggesting that more than 4% of the M. arborea genome was likely transmitted. There may have been chromosomal rearrangements as a result of the introgression of M. arborea chromosome(s) or chromosome(s) contributing novel alleles at the introgression locations, as the hybrid accounted for 7% of the total bands [14]. Since each M. arborea chromosome theoretically accounts for an average of 1/32, or 3.1%, of the genome, the presence of around 4% of M. arborea-specific alleles in the hybrid might suggest the transfer of a whole chromosome or the introgression of many smaller pieces. By using marker profiles produced by 46 SSR primers of known M. sativa genomic sites encompassing all 8 linkage groups, we evaluated M. arborea introgression into 7 hybrids produced in Wisconsin. A total of two hybrids exhibited introgression from each of the three linkage groups, indicating that M. arborea-specific alleles had entered the population via each of those links. The offspring of test crossings between the hybrids and M. sativa showed evidence of the transfer of alleles peculiar to M. arborea. It is still unclear whether the introgressed M. arborea genome represents a single chromosome, an arm, or many fragments of chromosome(s), however the latter is the more probable option [15].

ETHNOPHARMACOLOGICAL USES-

Memory enhancement, cough relief, aching muscles, antidiabetic, antioxidant, anti-inflammatory, antifungal, anti-asthmatic, antibacterial, diuretic, galactagogue, and diseases of the central nervous system (CNS) are all traditional uses of melissa sativa. For many years, Ayurvedic and homoeopathic practitioners have relied on M. sativa to treat central nervous system (CNS) diseases. Since the sixth century, the Chinese have utilised M. sativa for a variety of medical purposes, including the treatment of kidney stones, fever, gravel, dysuria, and edoema and fluid retention [16]. Ayurvedic doctors in ancient India utilised Melissoma sativa to alleviate the symptoms of arthritis, fluid retention, and ulcers. Mexicans traditionally use M. sativa for memory enhancement, muscular pain, and inflammation. M. sativa was a common remedy for a variety of ailments among the indigenous Americans, including gout, cancer, boils, scurvy, and arthritis. The Iraqis utilise M. sativa for arthritic pain. As a cardiotonic, it also alleviates scurvy and arthritis in Turkey [17]. In addition, it is believed to be helpful in a variety of medical conditions, including but not limited to: disorders involving the bladder or blood clotting, boils, coughing, diuresis, gastrointestinal tract disorders, cancers of the breast or cervical region, kidney disorders, disorders involving the prostate, inflammation, stimulation of the appetite, asthma, indigestion, insect bites, jaundice, menopausal symptoms, allergies, increased excretion of neutral steroids and bile acids in faecal matter, nutritional support, stomach ulcers, skin damage from radiation exposure, galactagogic, increased peristaltic action of the stomach and bowels, thrombocytopenic purpura, uterine stimulant, rheumatoid arthritis, scurvy, vitamin supplementation [18].

PHARMACOLOGICAL EFFECTS-

Antioxidant effect-

We used the DPPH radical to test the antioxidant properties of Medicago sativa floral extracts. Findings showed that bioactive chemicals, especially total phenolic compounds, were best extracted from alfalfa flowers using water rather than acetic acid or methanol. On average, there were 263.5±1.02 mg GAE/100g of dry methanol extract weight of active compounds. The radical decomposition activity was shown to be associated with the phenolic concentration. All three extracts demonstrated antioxidant activity, while the water extract outperformed the acetic and methanol ones. Water extract (0.924 mg/ml), acetic acid extract (0.154 mg/ml), and methanol (0.079 mg/ml) were ranked according to their inhibitory potencies (IC50) [19]. Medicago sativa raw seed and germinated seed extracts were tested for their ability to scavenge free radicals in vitro utilising DPPH, hydroxyl, superoxide anion, and nitric oxide assays. Ethanolic extracts from both ungerminated and germinated Medicago sativa seeds scavenged free radicals in a concentration-dependent manner. The ethanolic extract from germinated seeds of Medicago sativa has a stronger antioxidant activity [20]. At a concentration of 250 µg/ml of leaves crude extract, the experiment with Alfalfa's antioxidant activity using the DPPH and ferric reducing antioxidant power (FRAP) techniques revealed a 54.42 and 56.71% suppression of free radicals, respectively. The NO inhibition experiment showed that at a dose of 250 µg/ml, NO had an inhibitory activity of 50.99% [21].

Antidiabetic effect

Researchers examined the effects of a water-based ethanol extract from Medicago sativa on the histopathological and blood sugar alterations in rats that had been driven to diabetes by streptozotocin. The findings showed that the diabetic rats' blood sugar levels were considerably lowered by the extract. In contrast to the diabetic + extract group, the diabetic control and extract-treated rats' ultimate body weights were much higher than their initial weights. A statistically significant increase in kidney weight was seen in the diabetic + extract group of rats when contrasted with the control + extract group. All groups had comparable total kidney and cortical volumes, but the diabetes + extract group had a much larger medulla volume than the control + extract group. Also, when comparing diabetic rats to controls, the overall glomerular volume was much higher in the former [22-24]. For four weeks, rats with type 2 diabetes that had been generated by streptozotocin were studied for the hypoglycemic impact of an aqueous extract of Medicago sativa. In both type 2 diabetic and non-diabetic rats, medicago sativa extract reduced postprandial glycaemia. The impact was mediated by the extract's ability to enhance insulin secretion [25]. Researchers studied the effects of an aqueous alfalfa extract on blood glucose and serum lipids in rats that had been driven to diabetes by alloxan. The doses used were 250 and 500 mg/kg for 21 days. The diabetic rats' blood glucose, cholesterol, triglyceride, and LDL levels were markedly reduced while their HDL levels were markedly boosted by the aqueous extract. The levels of ALT and AST were also found to be lower. Based on histological analysis, it was shown that the water-based extract helped repair liver damage and increased the diameter of the pancreatic Langerhans islets [26, 27].

Reproductive effects

Female rats were given alfalfa ethanolic extracts at doses of 9, 18, and 36 mg/kg for 15 days, and their serum oestradiol levels, ovarian weights, and uterine weights were all considerably enhanced [28]. The concentration of plasma luteinizing hormone (LH) was measured in sheep that were given alfalfa. The sheep given phyto-estrogenic alfalfa had a higher peak LH level (66.0 ± 16.8 ng/ml), compared to the control group whose peak level was 40.1 ± 5.5 ng/ml, which was lower ($P<0.05$). In addition, the oestrous phase of ewes that were given phyto-estrogenic alfalfa had a later LH peak (15.4 ±4.5 h) than the control group ($P<0.05$). A transactivation test for ERα and ERβ was used to monitor the activity of phytoestrogenic substances (coumestrol, liquiritigenin, isoliquiritigenin, loliolide, and (4S,6S)- and (4R,6S)-4-hydroxy-6-pentadecyl tetrahydropyr-2-one) that were extracted from Medicago sativa. The substances that were evaluated showed a greater level of transactivation via ERβ as compared to ERα. In comparison to ERβ activation, 1 nM E2-induced ERα inhibition was more pronounced in loliolide, isoliquiritigenin, and (4S,6S)- and (4R,6S)-4-hydroxy-6-pentadecyltetrahydropyr-2-one, but not in coumestrol. Researchers in this study examined the impact on the reproductive system of adult female mice of an aqueous extract of the aerial portions of a combination of Medicago sativa and Salvia officinalis. Oral administration of the plant mixture's aqueous extract with a water supplement was done for two and four weeks, respectively, at dosages of 100 and 200 mg/kg/day. Weight gain was seen across the board in the treated groups, with the exception of the reproductive organs, which showed a significant rise in the groups given the highest dosages. During the oestrous phase, levels of LH and estradiol were considerably higher and levels of FSH were significantly lower in all treatment groups. A striking rise in the quantity of corpora lutea and ovarian follicles was shown by the histological testing. The endometrial glands' width expanded, particularly in the groups who took the extract for an extended period of time, and the height of the uterine epithelial cells rose dramatically across the board [29].

Antiinflammatory effect

Lipopolysaccharide (LPS)-stimulated immune responses were used to investigate alfalfa's anti-inflammatory potential. Compared to ether, butanol, or water-soluble extracts, the aerial parts chloroform extract had a greater inhibitory effect on immunological responses triggered by LPS. In macrophages, nitrite concentrations reached 44.3 μM when exposed to 1 μg/ml of LPS; however, after adding 100 μg/ml of chloroform extract, these concentrations dropped to 10.6 μM. Pre-treating with 100 μg/ml of the extract reduced the levels of TNF-α, IL-6, and IL-1β in the cell culture supernatants from 41.3, 11.6, and 0.78 ng/ml, respectively, after LPS treatment. After 48 hours of injection, mice given 30 mg/kg bw of LPS alone had no chance of survival, while mice given the extract orally had a 60% chance of survival. The activation of nuclear factor kappa-B and extracellular signal-regulated kinase was significantly reduced by chloroform extract subfractions in response to LPS [30]. Medicago sativa sprout ethyl extract supplementation reduced acute inflammatory risks and decreased pro-inflammatory cytokine production in mice. The extract had a notable impact on mitogen-stimulated RAW264.7 cells, decreasing their IL-6 and IL-1β production as well as their NFkappa B trans-activation activity. In addition, compared to the control group, the extract exhibited considerably lower levels of serum TNF-α, IL-6, and IL-1beta at 9 hours after LPS exposure, as well as significantly greater survival rates [31].

Hypolipidemic effect

Alfalfa seed extract lowered blood cholesterol and low-density lipoprotein (LDL) levels in rabbits by 38–41.7% and 48–53.3%, respectively, in both the initial and established hyperlipidaemic models of cholesterol feeding. In a mouse that was given cholesterol-free alfalfa meals, the efficacy of decreasing LDL was 64.4% [32]. Researchers looked at the hypolipidemic effects of Medicago sativa sprouts in diabetic rats that had been given streptozotocin. When compared to rouvastatin, the levels of triglycerides, total cholesterol, LDL, and VLDL were markedly reduced after four weeks of treatment with methanol extract (500 mg/kg), petroleum ether (32.5 mg), and butanol fractions (60 mg). The fraction of petroleum ether showed the most effective efficacy against hyperlipidemia (12.23%). However, when compared to metformin, the ethyl acetate fraction had superior hypoglycemic efficacy. The biochemical findings were corroborated by the histological improvement of the liver, pancreas, and spleen [33]. Research was conducted on the effects of various alfalfa products on diet-induced changes in liver cholesterol buildup, bile acid excretion, jejunal and colonic morphology, and the effects of saponin-free alfalfa plant and sprout. In both the ethanol solution and the micellar suspension, the alfalfa plant saponins bind large amounts of cholesterol. Cholesterol and alfalfa sprout saponins had a small but notable interaction. The removal of saponins from the plant material did not diminish the alfalfa plant's maximum bile acid adsorption. Although alfalfa sprouts did not inhibit cholesterol buildup in the livers of rats given cholesterol, the elimination of saponins improved alfalfa's capacity to do so. The modifications in intestinal morphology that had been previously documented were mitigated when alfalfa saponins were removed; however, it did not seem that this saponin impact was caused by contact with membrane cholesterol [34].

Antimicrobial effect

Antibacterial activity against Staphylococcus aureus, Streptococcus pyogens MTCC 1928, Proteus mirabilis MTCC 425, Escherichia coli MTCC 2961, Pseudomonas aeruginosa MTCC 4676, Klebsiella pneumoniae MTCC 432, and Salmonella typhi MTCC 733 was assessed in the petroleum ether, chloroform, benzene, methanol, ethanol, and water extracts of Medicago sativa. Among the examined microorganisms, methanol extract showed the most activity, followed by chloroform and ethanol extracts. No discernible action was seen in the petroleum ether and benzene extracts. When comparing solvent extracts to aqueous extracts, the antibacterial activity of the former is clearly superior. Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus aureus, and Listeria monocytogenes were tested for their antibacterial effects by use of an aqueous alfalfa seed extract. Gramme positive bacteria were moderately affected, however Gramme negative bacteria were unaffected [35].

Antibacterial efficacy against Streptococcus pneumoniae, Haemophilus influenza, and Moraxella catarrhalis was investigated in Medicago sativa root extract. To combat Streptococcus pneumoniae, Haemophilus influenza, and Moraxella catarrhalis, the minimum inhibitory concentration (MIC) of the root extract of Medicago sativa was 125 mg/ml. While Staphylococcus aureus was unaffected by the extract, the inhibition zones for Moraxella catarrhalis, Streptococcus pneumoniae, and Haemophilus influenza were 16 mm, 13 mm, and 10 mm, respectively [36].

Toxicity/side-effects

Research has shown that female monkeys may develop a disease similar to systemic lupus erythematosus (SLE) when exposed to either the seed or the herb of the M. sativa plant. A nonprotein amino acid component known to affect human immunoregulatory cells in vitro, canavanine is thought to be responsible for this action. It has been discovered that using M. sativa pills, which contain canavanine, can trigger the reactivation of dormant SLE in people. Whether the pills were made of herbs or seeds was not specified. Because it is chemically similar to arginine and may prevent this amino acid from attaching to enzymes and being incorporated into proteins, canavanine is poisonous to all animals. Canavanine concentrations in M. sativa seeds range from 8.33 to 13.6 mg/kg, but in the plant the reported values are much lower[37]. In humans, taking 80–160 g of pulverised M. sativa seeds daily to reduce plasma cholesterol levels has been linked to pantopenia. Studies on rats and monkeys that included alfalfa top saponins (ATS) in their diets found that it decreased blood lipid contents and did not cause any harm. Also, blood lipid concentrations were shown to decrease in rats that were fed cholesterol and then administered ATS. It has been reported that ATS do not contain the SLE-inducing chemical found in the seeds. When M. sativa was evaluated for mutagenicity using Salmonella strains TA98 and TA100, the findings were negative. Any patient on blood-thinning medicine, hormone replacement treatment, or taking birth control pills should consult with their healthcare provider before utilising M. sativa [38-42].

CONCLUSIONS-

It was shown experimentally that the production of partial hybrids between M. sativa and M. arborea, with a prevalence of the M. sativa genome, is influenced by reproductive defects, such as unreduced eggs, in the M. sativa seed parents. It was feasible to transmit a variety of M. arborea-specific features to the hybrids, which may have significance in improving alfalfa, even though each hybrid only had around 5% of the M. arborea-specific genes. These characteristics were seen in alfalfa crosses and included a bigger seed size, indeterminate development, resistance to cold, and heterosis for persistence and production. It is reasonable to assume that the evaluated studies over the last two decades have shown that interspecific partial hybrids have the ability to improve alfalfa via continuous breeding. Microwave extraction, active compound isolation and identification, pharmacological screening of extracts and isolated compound(s), and other extraction methods can be explored in future studies to improve the reliability of results and explore their potential use in herbal formulations. Although medicinal uses of M. sativa date back centuries, there has been very little systematic pharmacological research to back up these claims. The plant has also found therapeutic use in a variety of herbal and homoeopathic remedies for a wide range of illnesses. Extensive research into the many biological functions of M. sativa seems to be very promising. Consequently, its full potential must be realised in the realm of pharmaceutical and medical sciences for the sake of innovative and beneficial applications. In order to isolate bioactive phytoconstituent(s), the authors are now interested in assessing the central nervous system effects of historically used therapeutic plants, such as M. sativa.

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