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Review Article

A Study on the Wound Healing Properties of *Medicago sativa*

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Abstract

There are lots of herbal plants are using for various treatment, food remedy etc from the prehistoric time. India has taken a great part to garage lots of herbal plants. 3-12 years lifespan containing and cool climate growing *Medicago sativa* is one of them good rank holder herbal plant. After studying the chemical constituents of *M. sativa*, it contains vitamin C, vitamin K, genistein which have great wound healing activity as well as it can cure kidney pain, cough, sore muscle, asthma etc. But it cannot take those people who are suffering breast cancer, ovarian cancer etc. At last, after overall study, *Medicago sativa* has great reflection on human and animals.

Keywords: M*edicago sativa*, wound healing, vitamin C, vitamin K, genistein, food remedy.

INTRODUCTION

Plants are using to cure the humans from various ailments since they first earth. The uses of plants for treatment have been described in Indian literature like the Vedas before modern medical science became available to the masses. Similarly, in antient India, the chaulmoogra fruits were used for antileprotic activity[1].The Brazilians used "Ipecacuanha" for curing the bacterial infections in stomach. Due to the use of these plants by different civilizations around the world as medicine, various traditional forms of medicine like Ayurveda, Siddha and Unani were developed[2].

Medicago sativa Linn. is a perennial herb that has its origins in Asia. Popularly it is called "al-fal-fa" which in Arabic means "Father of all foods". The plant typically has a lifespan of 3-12 years and it generally grows well in a cool climate. Mature plants have a strong taproot which may surpass a length of 6 meters. Many lateral roots may be connected to the crown. The crown of the plant is a complex structure that has perennial meristem activity that produces buds that develop into the stem. The plant grows best in deep, well-drained, moist soils.

Stems range from 5-15 in number and tri or multifoliate leaves form alternatively on the stem and tertiary stems can develop from the leaf axils. Flowers maybe purple, variegated yellow, cream or white in colour. After pollination, flowers mostly produce spiral-shaped seed pods[3].

Binomial name: Medicago sativa Linn, Genus: Medicago, Species: Sativa, Family: Fabaceae, Kingdom: Plantae, Division: Magnoliophyte, Order: Faboideae, Tribe: Trifoliate,[3].

Medicago sativa contains many components like vitamin K, vitamin C, copper, manganese, folate, thiamine, riboflavin, magnesium, iron, etc. It also contains alkaloids, amino acids, coumarins, digestive enzymes, flavonoids, nonprotein amino acids, organic acids, phenolic compounds, etc.

Alfalfa has been used since prehistoric times as fodder and in the treatment of ailments like kidney pain, cough, sore muscles, diabetes, asthma, insect bites, wound healing, etc. In this, we shall focus on the wound healing properties of alfalfa. The wound healing property of the plant can be attributed to the presence of vitamin C or ascorbic acid, vitamin K, and genistein. Seungkuk, et al. in 2019 reported that alfalfa nanofibers showed some wound healing property[4].

The leaves of the plants are very safe for most adults but taking seeds long-term may cause reactions that will lead to autoimmune diseases and may intensify the symptoms of existing autoimmune diseases. The plant may cause the skin of some people to become extra sensitive to sunlight. It is not advisable to give the plant to people with certain conditions like breast cancer, ovarian cancer, endometriosis or uterine fibroids. The plant may have the same effect as the hormone oestrogen on females and may worsen the symptoms of diseases caused by elevated oestrogen levels. It may also cause hypoglycaemia in diabetic patients. Alfalfa has also been reported to boost the immune system, and, in the case

of kidney transplants, it may make the anti-rejection drug cyclosporine less effective. Alfalfa may also interact with other drugs. The major interaction of the plant is with warfarin. The plants are containing huge amounts of vitamin K (helps in synthesis of certain clotting factors) which interacts with the effect of warfarin in slowing down blood clotting. Moderate interactions have been reported with birth control pills, immunosuppressants and photosensitizing drugs [5].

PRODUCTION OF ALFALFA

The major producers of *M. sativa* are the United States of America, Argentina, Australia, South Africa and Middle East. The plant can adapt to various conditions but the optimal growth conditions are given below.

a) Temperature: 25^{0}C in bright sunlight.

b) Rainfall: 600-1200 mm per annum.

c) Soil: deep, well drained, sandy to fertile loamy soil having a pH ranging from 6.5-7.5.

The plant is distributed over a latitude of 36^{0}S to 58^{0}N [3].

PHYTOCHEMICAL CHARACTERS OF ALFALFA

M. sativa is known to contain various phytochemicals some of which are given below.

- Alkaloids: asparaginase, trigonelline, stachydrine, L-homostachydrine.
- Amino acids: medicanine, lysine, arginine, medicagenic acid, histidine, tyrosine, phenylalanine Alkaloids: asparaginase, trigonelline, stachydrine, L-homostachydrine, methionine, aspartic acid, glutamic acid.
- Coumarins: myrsellino;, 4-coumaric acid.
- Digestive enzymes: isoflavone reductase, vestitione reductase, iminopeptidase and two aminopeptidases.
- Flavonoids: quercetin, myricetin, luteolin, aptigenin.
- Minerals: calcium, potassium, magnesium, iron, zinc, copper, aluminium, boron, chromium, manganese, silicon, tin, etc.
- Non protein amino acids: L-canaverin.
- Organic acids: citrate, malate, malonate, succinate, fumarate, lactate, benzoate.
- Phenolic compounds: p-hydroxybenzoic acid, vanillic acid, p-coumaric acid, salicylic acid, sinapic acid, caffeic acid, chlorogenic acid, tannic acid, etc.
- Phytoestrogens: coumesterol, genistein, formometin, biocanine A.
- Phytosterols: ß-sitosterol, stigmasterol.
- Polyamines: norspermidine, norspermine.
- Proteins: ferritin, protein phosphatase 2A holoenzyme, ß-amylase.
- Saponins: soyasaponegols, hederagenin, medicagenic acid and its derivatives.
- Vitamins: A, B_1, B_6, B_{12}, C, D, E, K, niacin, pantothenic acid, biolin, folic acid.
- Volatile components: terpenes, linonene, linalool, trans-ocumene, furanoids, nonadienal, eltyl benzaldehyde, butanol, hexanol, octanol, etc [6-9].

WOUND HEALING PROPERTIES OF ALFALFA

Wound healing is a very complex process that mostly containing three back-to-back processes as reported by Ibrahim, et al. in 2018 [10]. Their study found that the three overlapping phases are inflammatory reaction, proliferation and remodelling and they discussed the following.

- Inflammatory Reaction: In this phase vascular responses like exudation, blood coagulation and haemostatics occur. Immune cells from blood vessels go to the site of the wound and secrete pro-inflammatory cytokines like neutrophils which produce reactive oxygen species (ROS). ROS protect the body against infection but can damage the surrounding tissues when present in excess. Reduction of immune cells and inflammation take place after some day's injury in normal healing process. Simultaneously, the migrating keratinocytes, fibroblasts and endothelial cells start to secrete various growth factors[11].
- Proliferation: In this process, epithelial layer which covers the wound surface is formed and growth of granular tissue occurs which helps fill the dimensions of the wound. The formation of granular tissue involves: proliferation of fibroblasts; deposition of collagens and other extracellular matrices (ECM); development of new blood vessels (angiogenesis). Collagen synthesis is the cause of contraction of wound and reduction of wound size.
- Remodelling: The remodelling phase starts when temporal ECM is slowly converted to a mature scar. During this phase, type III collagen (constituent of granular tissue) is replaced by type I collagen. Remodelling restores the structure and function of the wounded tissue.

The entire process of wound healing is governed by multiple growth factors and cytokines released at the site of the wound. Alfalfa has been found to contain vitamin C (ascorbic acid), genistein (found in the chlorophyll of the leaves) and vitamin K[12].

ROLE OF VITAMIN K IN BLOOD COAGULATION

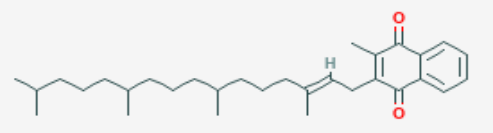

Figure 1: Structure of Vitamin K[13]

Blood coagulation is a complex pathway mediated by a series of proteolytic steps resulting in signal amplification. These steps depend on specific binding to the membranes that contain acidic phospholipids like phosphatidylserine. Stone, et al. in 2004 said that vitamin K aids in blood coagulation reactions by (i) directing the clotting factors to the site of injury and (ii) increasing the concentration of clotting factors through localization to a restricted area. Stone, et al. reported that the vitamin K dependent plasma proteins within the procoagulant cascade contain factors VII, IX, X and prothrombin. These factors are zymogens containing a trypsin like serine peptidase domain. Zymogens are inactive precursors to an enzyme[13]. The corresponding enzymes occur in the Golgi apparatus where a specific part of the zymogen is cleaved and they will be denoted by an "a" after the Roman digit (e.g., VIIa). The exception to the rule is thrombin which is the activated form of thrombin. Two pathways are involved in procoagulation, viz. intrinsic and extrinsic, both of which produce factor Xa. The extrinsic

pathway gets its name because it is dependent on the expression of the cell surface receptor, also called the tissue factor (TF). TF is absent in the blood plasma. Muhammed A Mir in 2019 reported that factor VIIa combines with TF and increases the proteolytic activity by two ways: (i) bringing the binding for both the substrates (factors IX and X) and the enzyme (VIIa) into close proximity and (ii) promoting a conformational change which enhances the enzymatic activity of factor VIIa. The complex between factor VIIa and TF activates factors IX and X. Extrinsic pathway is brought about by vascular injury which exposes the acidic phospholipids like phosphatidylserine. These acidic phospholipids are not exposed on healthy cells. TF is the cofactor of factor VIIa expresses in the subendothelial layers of the cell. Protease activity of factor VIIa is enhanced in complex with TF which catalyses three reactions. The complex activates itself, converting components VII to VIIa, IX to Ixa and X to Xa respectively. Finally, factor Xa finally generates a little amount of prothrombin which in turn activates factor V to Va. Factor Va simulates platelets causing expose an acidic membrane causing platelet aggregation which increases the surface area of the clotting surface. This causes a plug to form at the location of injury[14].

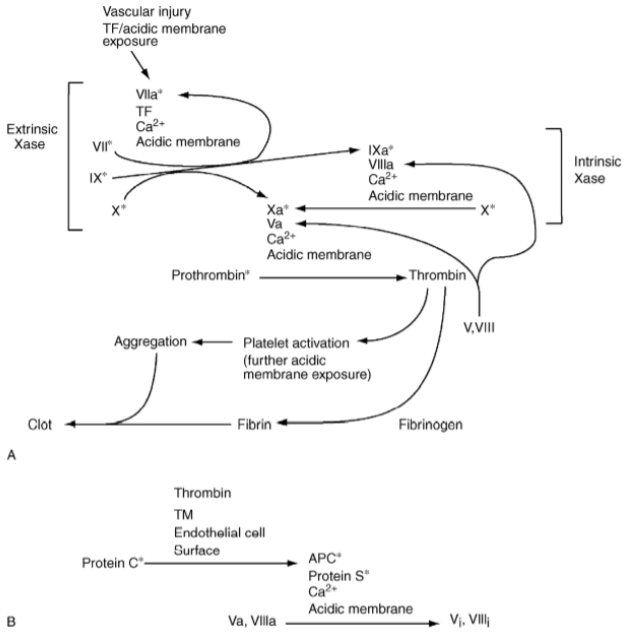

Figure 2: Stone, et al. in 2004 reported that the coagulation cascade is guided by two steps: "A" is the procoagulation cascade which is mediated by a series, i.e., a proteolytic step with feedback activation for signal amplification and "B" is the anticoagulant pathway which inactivates factors Va and VIIIa. Vertical text columns represent enzyme complexes with the proteolytic enzymes, protein cofactors and calicium and membrane components first, second and third respectively. Vitamin K dependent protein is symbolized by an asterisk[16].

The intrinsic pathway follows the extrinsic pathway and its main role is to amplify the clotting stimulus. A complex of membrane, factors IXa and VIIIa catalyses further conversion of factor X into Xa. The large-scale generation of thrombin, which hydrolyses fibrinogen into fibrin is caused by the accumulation of factor Xa and its co factor (Va). This fibrin self-polymerizes into macro molecular protein net, which forms a clot when combined with the platelet plug formed in the extrinsic pathway[15].

ROLE OF VITAMIN C IN WOUND HEALING:

Moores in 2013 reported that Ascorbic acid or 2,3-didehydro-L-threo-hexano-1,4-lactone is a water-soluble antioxidant and cofactor of several enzymes[17]. She stated that according to Linister and van Schaftingen (2006), due to a lack of L- gluconolactone, humans are unable to biosynthesize ascorbic acid[18] .Vitamin C finds application in many biological processes like degradation of tyrosine, synthesis of epinephrine from tyrosine, bile acid formation, absorption of iron and neurotransmitter synthesis. These were reported by various scientists like Jacob in 1999, Murray et al. in 2000 and Villacorta et al. in 2007. Ascorbic acid influences the activity of the immune system via phagocytes, leukocytes and lymphocytes and hence acts as a direct and indirect antioxidant [19].

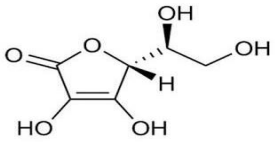

Figure 3: Chemical structure of Ascorbic acid[18]

Moores stated that Collins et al. in 2005 reported that wound healing is negatively influenced by dietary deficiencies. Dietary deficiencies subsequently result in changes in the metabolism profile of macro and micronutrients. Moores states that the role of ascorbic acid in wound healing is disputed by many scientists. However, she concluded that Anderson in 2005 reported that ascorbic acid plays a major role in all stages of wound healing process including cellular apoptosis, antioxidation process and bone formation. Ascorbic acid is specially required in the inflammatory and proliferative phase[20]. According to Vissers and Wilkie (2007) ascorbic acid aids in timely neutrophil death and clearance during the inflammatory phase[21]. In 1996, Ronchetti et al. discovered that ascorbic acid interacts differently in the integral processes of collagen synthesis, maturation, secretion, degradation during the proliferative phase[22].

ROLE OF GENISTEIN IN WOUND HEALING:

In 2012, Behloul and Wu discovered that foods high isoflavones have a high genistein content 4,5,7-trihydroxyisoflavone is the chemical name of genistein[23].

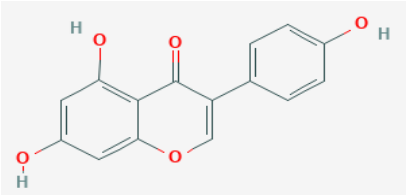

Figure 4: Chemical structure of genistein [25]

Genistein decreased cell proliferation and activity through influencing nuclear translocation of phosphorylated extracellular- signal – regulated kinase (ERK) molecules, according to Irrera et al. in 2017. They also stated that cell proliferation, growth and differentiation is regulated by ERK. Genistein also comprises of p38 pathway which controls stress and inflammatory reactions. The ERK and p38 pathways are linked, genistein may inhibit cell proliferation by interfering with them[24]. Another factor that is important for wound healing and maintenance of skin homeostasis is collagen synthesis (Irrera et al, 2017). Genistein affects collagen biosynthesis by exerting a biphasic effect on it which results in counteraction of collagen biosynthesis inhibition by t- butyl hydroperoxide (t-BHP) in fibroblast

which has an oxidising effect. Nutritionally acquired genistein protects human dermal fibroblasts agents from oxidative stress- induced collagen production inhibition, according to Irrera et al. in 2017. They finally concluded that nutritionally acquired genistein protects human dermal fibroblasts from oxidative stress- induced collagen biosynthesis inhibition[25].

CONCLUSION:

Humans have used *Medicago sativa* for treatment of numerous diseases and as animals' food since prehistoric times. The plant has shown application in various diseases and conditions like management of blood glucose levels, treatment of asthma, as a source of various vitamins like vitamin C and vitamin K. The wound healing properties of alfalfa were investigated in relation to the phytochemical constituents of alfalfa. Based on the phytochemical characters of alfalfa we can conclude that the plant must possess wound healing properties. The plant may use in topical formulations like creams, ointments for wound management. Ibrahim, et al. in 2019 prepared alfalfa nanofibers and tested them on animals as well as human skin for dermal wound healing.

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CONFLICT OF INTEREST:

The authors report no conflict of interest.

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