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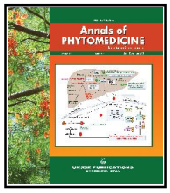

Review Article : Open Access

A comprehensive review on potential benefits of *Annona squamosa* L. leaves for the treatment of diabetic wounds

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Abstract

Wound care in diabetic patients is a major clinical and societal concern. Diabetes is a major obstacle to wound healing, which can have devastating repercussions for patients. The delayed and compromised healing increases its importance for research. The leaves of *Annona squamosa* L. (Custard apple) are widely utilised as a popular dietary supplement in diabetes in many places. The leaves of *A. squamosa* encompass significant bioactive chemicals that hinder the growth of numerous bacteria, virus and cells and shows early regeneration of dermis and epidermis. The phytochemical studies on *A. squamosa* leaves showed numerous flavonoids, polyphenols, glycosides, *etc*. The leaves demonstrated antioxidant, antidiabetic, anti-inflammatory, antitumour, antimicrobial properties proving their usefulness to treat diabetic wounds. Novel investigations on *A. squamosa* leaf in the healing process of impaired diabetic wounds can be conducted in order to find a better alternative source to produce newer drugs for diabetic wounds.

1. Introduction

Diabetes is a serious metabolic ailment affecting more than 340 million population of the whole world. Out of this, nearly 20% diabetic patients suffer the diabetic wound complicacy. People suffering from diabetes possess a reduced capability of metabolising glucose leading to hyperglycemic condition ultimately hindering their process of wound healing. The world-wide incidence of late wound healing in diabetic people is rising because of dearth of proper prevention and managemental strategies. Diabetes mellitus consumes around 2.5%-15% of global health budgets each year, with diabetic wounds playing a significant role. As per the prediction of WHO, diabetes will become the seventh major cause of death by 2030 (Patel *et al*., 2019). Wound repair is a multi-step dynamic task that finally helps for re-establishment of structural and functional integrity of skin. The most important prerequisite for wound management is speedy and complete healing with no spread of infection or sepsis. The main problem in wound healing is changes in body physiological functions with ageing such as compromised blood circulation, obesity, ailments like diabetes, and stressful environmental factors (Banerjee *et al*., 2021).

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Wounds are classified into two types depending on their capability of healing or recovering, *viz*., acute and chronic. The wounds which heal quickly without any complications are termed as acute wounds. The tissue injuries which not follow a rational sequence of repair and take more than three months to get healed completely are classed as chronic wounds. Normally, the process of healing begins with haemostasis, which stops the blood loss from the body along with microbial invasion to injury site (Palai and Patra, 2021). Thereafter, up-regulation of pro-inflammatory cells like neutrophils and macrophages occurs which help to wipe out debris and microbes from the wound area. This is called inflammatory phase of wound healing (Vin *et al*., 2002). The inflammatory phase gets overlapped by proliferative phase. In the proliferative phase, there is synthesis of new tissue, angiogenesis (budding of new blood vessels), and formation of ground substance. All these help to pervade the injured area. Lastly, the remodelling phase ensues where all the newly deposited tissues arrange themselves in appropriate pattern with enhanced tensile strength of extracellular matrix (Gonzalez *et al*., 2016).

The wound healing process is slow in case of diabetes because of hindrance in each phase of wound repair involving haemostatic, inflammatory, proliferative, and remodelling phases. In long run, it has a deleterious effect on life quality, morbidity, and mortality (Mohanty and Sahoo, 2017). Diabetic wounds are differentiated based on prolonged duration of healing due to slow, defective, or unorganized healing. This occurs because of hurdle in mature granulation tissue formation and acquiring sufficient tensile strength of wound. The vascular disintegration due to ischemia may attribute to this. Basing on the origin, wounds can be external and internal.

Bruises, cuts, burns and injuries are categorized under external wounds. These exterior sores frequently get undiagnosed by patients suffering from diabetes due to peripheral neuropathy (Mahmoudvand *et al*., 2023). There are skin and nearby tissues damages with probable risk of microbial infection in case of diabetic wounds. The current conventional technique involves a chain of clinical intervention to clean and take off the affected tissue at the same time preserving moisture and vascular supply. These may also have an impact on the healing process that promote normal tissue repair and reduce bad wound healing (Boateng and Catanzano, 2015). The current study aims to uncover the molecular basis of consequences causing retarded wound healing, enlist the molecular targets for wound healing and encompass pharmacological effects of *A. squamosa* leaves that can synergistically aid in healing of diabetic wounds.

2. Complications of diabetic wound at molecular level

Diabetes slows down the healing process, causing complications and a variety of repercussions such as psychological stress and despair. These complications also include functional limits, walking difficulties, and infections such as abscess, gangrene, osteomyelitis, cellulitis, and septicaemia. In diabetic patients, the non-healing wounds are related to diabetes and its pathophysiology. The basic mechanism of wound healing is proper co-ordinated action between the inflammatory cells and the biochemical mediators activated by various stimuli. Any disturbance in this balanced action among the cellular entities and metabolic components may be the root to failure or delay in healing of diabetic wound (El-Ashram *et al*., 2021).

The cellular components involved in the wound healing process include macrophages, monocytes, neutrophils, T and B lymphocytes, fibroblasts, keratinocytes, endothelial cells and mast cells. These cells activate the process of formation and control of growth factors and cytokines (Palai *et al*., 2021). Both in case of diabetic and non-diabetic wounds, monocytes which eventually differentiate to macrophages are the main source of pro-inflammatory cytokines IL-12, IL-64, TNF-3, IGF-16, VEGF-5, and TGF-7 production. Mononuclear cells like T-lymphocyte, B-lymphocyte and neutrophils are the prime producers of IL-10 and tissue necrosis factor. Rest of the cells, *viz*., endothelial cells, mast cells, fibroblasts and keratinocytes also produce IGF-1, TGF-α and VEGF (Barrientos *et al*., 2008).

A complicated mechanism at the molecular level is involved in diabetes and is responsible for delayed healing of diabetic wound. Oxidative stress and hyperglycemia disturb the epigenetic coding which causes a shift in polarization and regulation of macrophage. This imbalanced macrophage polarization primarily leads to delay in the process of wound healing. The reports on diabetic animal models suggest that parameters like disturbed angiogenic response along with blood vascular complications, impaired pathophysiology of macrophages and neutrophils, sustained release of pro-inflammatory cytokine, disturbance in the migration of fibroblasts and keratinocytes, and irregularity in the formation of healing-associated factors like growth factors are the key causes of non-healing or delay in healing of diabetic wounds (Surai *et al*., 2021).

Other than these factors, metabolic inadequacies, diminished physiological responses, *viz*., hypoxia due to haemoglobin glycation, alteration in the cell membrane of RBCs, and tightening of vascular channels hinder the healing method in diabetic wounds. Hypoxic condition occurs due to reduction in supply of oxygen to the site of wound because of vascular constriction (Palai *et al*., 2017). Haemoglobin glycation decreases the availability of oxygen and nutrients to wound site aggravating the delayed healing. Hypoxia, glucose deprivations as well as malformed proteins induce stress in the cell by accumulating non-folded proteins inside the endoplasmic reticulum (Han *et al*., 2023).

Localized ischemia due to narrowing of microvasculature significantly slows the repair of wound in diabetes. Alteration in the level of miRNAs has been observed in diabetic wound. A miRNA called miR-210 is elevated during hypoxia which inhibits the proliferation of keratinocytes delaying wound healing. Angiogenesis gets inhibited by miR-200b by regulating VEGFR2 and globin transcription factor 2. Similarly, miRNAs like miR-21, miR-130 a, miR-146a and miR-198 play an important role in healing of diabetic wound by influencing epithelium reconstruction, causing delay in inflammation, affecting migration of fibroblasts and keratinocytes and new blood vessel formation (Wei *et al*., 2022).

3. Factors responsible for delayed wound healing in diabetes

3.1 Free radicals and oxidative stress

Excessive oxidative stress is a chief contributor to diabetes complications and poor healing. NRF212, a transcription factor that manages the adaptive reciprocation to oxidative stress, also reduces apoptosis, cell proliferation, migration and differentiation. NRF2 is activated by oxidative stress and hyperglycemia to combat the impairment (Bisht *et al*., 2023). Numerous pro-inflammatory cytokines, such as MIP114, MIP-215, and KC16, play a vital mode in accumulating mononuclear cell such as macrophage/neutrophil/monocyte/immature dendritic cells and contribute to the antimicrobial action during the steps of wound healing. CX3CL117, a membrane bound soluble chemokine formed on the surface of cells stimulates the macrophage and fibroblast accumulation (Matough *et al*., 2012).

In diabetic wound, there is an abnormal expression of MIP1, MIP-2, and CX3CL1, asymmetric activation of STAT318 and supressed activation of NF-B19 and AKT/PKB. All these factors lead to the interruption of healing in diabetic wounds. Hyperglycemia and oxidative stress also lead to ischemia and nerve dysfunction by unusual glycation of the proteins present on nerve cells and the unfavourable triggering of protein kinase C (Sikora *et al*., 2017).

3.2 Immune system

Proper coordination of the innate immune system is critical in wound repair. TLRs22 are crucial initiators of the inflammatory response and the innate immunity. In case of diabetes, there is down-regulation of TLRs-2 in wound tissue which ultimately impedes the immunity of body and inflammatory response. This causes sluggish chemotactic stimulation leading to delay in the recruitment of various defence cells. Bacterial infection causing biofilm formation on the wound area plays a contributory role in delaying healing process. These biofilms form a protective layer for the bacteria so that the antimicrobial drugs and body defence system cannot affect them. This causes hindrance in healing (Hopkins and Sriskandan, 2005). Diabetic host immunity may be subdued due to dysregulation of these cells. The inflammatory cascade gets disturbed by diabetes. Increased level of pro-inflammatory cytokines, *viz*., TNF and IL-6 facilitate insulin resistance and hyperinflammation. Elevation of TNF level may occur due to effector T cell accumulation. Wound healing gets promoted by heat shock proteins through recruitment of

fibroblasts, activation of cellular proliferation, differentiation of keratinocytes, reduction of oxidative stress, alleviation of actin filaments, migration of endothelial cells, synthesis of pro-collagen, and promotion of protein homeostasis. HSP levels (HSP90, HSP70, HSP47, and HSP27) decrease in diabetic condition, due to downstream regulation of its p38-MAPK and TLR4 molecules. These molecules are believed to be responsible for the poor wound repair (Hodgson *et al*., 2015).

3.3 Growth factors in impaired wound healing

Growth factors, various metalloproteinase present in the matrix, inflammatory cells, cytokines, keratinocytes, endothelial cells, and fibroblasts together function to synchronize and harmonize the cascade of wound healing (Palai and Patra, 2021). Thus, these growth factors are highly needed for commencement and maintenance of different stages of wound repair. The growth factors are biologically dynamic polypeptides help in tissue regeneration during the granulation phase of wound healing. Compromise wounds usually reveal inadequacy in growth factor due to its variation in expression, declined production, diminished release. For better healing there is need of extracellular matrix (ECM) synthesis which is accomplished by appropriate equilibrium between matrix synthesis and matrix degradation. Various factors like IGF-I and II, KGF-24, TGF-23, PDGF-25, EGF-26, FGF-27, *etc*., influence ECM synthesis (Zubair and Ahmad, 2019).

In case of diabetes, there may be changes like down-regulation of receptors for growth factors and quick degradation of growth factors which is a cause of delayed wound healing. In the late phase of inflammation of wound healing, the macrophages continuously synthesise PDGF which acts as a pivotal mediator in fibroblast multiplication and migration, production of tissue proteins required for granulation, and new blood vessel formation. In diabetic wounds, the expression of PDGF along with its receptors gets reduced resulting hamper in healing process. A reputed angiogenic cytokine, VEGF helps in the process of angiogenesis. The irregular pattern of VEGF receptor, diminished levels of VEGF mRNA, enhanced level of VEGFR-1 and reduced level of VEGFR-2 result in delayed healing (Xiao *et al*., 2019). The quantity of TGF and IGF-1 are less in diabetic animals and humans, resulting in a slower wound healing process. TGF fascinates and activates inflammatory cells like fibroblasts, neutrophils, lymphocytes, macrophages, and keratinocytes. It also helps in synthesis of growth factors. All these factors speed up vasculogenesis and matrix synthesis and prevent ECM degradation. In the diabetic wound, the TGF concentration has been found to be lower. In the region of MMP-encoding genes promoter, there is presence of a TGF-1-dependent inhibitory element which inhibits the TGF gene expression. This condition of diabetes leads to enhanced glucose level causing prompt macrophage activity. This ultimately results in formation of reactive oxygen species to a great extent and prolonged inflammatory phase (Portou *et al*., 2015).

3.4 Matrix metalloproteinase

Metalloproteinase of the matrix (MMP) that belongs to a family of 26 zinc-dependent endopeptidases plays vital role in the debridement of wound, vasculogenesis, epithelialization and remodelling of extracellular matrix. Its activity includes degradation of all matrix proteins like dermal collagens, collagens present in the basal lamina, elastin, proteoglycans and fibronectin. MMP-2 and 9 are responsible for breakdown of type-IV collagen that forms the basic matrix. The proteinases are present in an inactive form which needs to get activated by unlocking the pro domain. TIMPs29 tend to control the activity of MMP for forming complex with it. This complex structure competitively prevents the interaction of MMP with its receptor. The healthy balance of MMPs and TIMPs necessary for proper wound healing is disturbed in diabetic wound (Kandhwal *et al*., 2022).

4. Molecular targets for promoting wound healing in diabetes

Diabetes have impaired wound healing because of unusual expression of cells involved in healing process including imbalance in the expression of growth factors, cytokines, and other molecular entities required for synchronizing the customary healing steps. Over recent years, several molecular components have been discovered for the treatment of diabetic wound. These handling techniques are categorized basing on molecular factors or targets which either directly or indirectly control their function (Pradhan *et al*., 2023). The factors that directly interact include stem cells autologous keratinocytes or autologous fibroblasts, and numerous growth factors (VEGF, EGF, KGF, FGF, PDGF, TGF). Different entities also affect indirectly the molecular targets by amplifying or declining the expression of pro- and anti-inflammatory cytokines, growth factors, MMPs, level of nitrous oxide, synthesis and breakdown of collagen and factors contributing to angiogenesis (Pop *et al*., 2017).

5. Beneficial effects of *A. squamosa* leaves in diabetic wounds

Traditionally, natural materials collected from many sources, such as ginseng, castor leaves, rosemary, turmeric, neem bark, and many others, have been of primary importance in wound healing (Egbuna *et al*., 2019). As per reports over 13,000 natural treatments have been developed specifically to accelerate the process of wound healing, 75% of commercialised treatments comprise plant-based active components, 22% are mineral-based, and 12% are animal-based. Flavonoids, glycosides, mucilage, saponins, steroids, resins are plant bioactive compounds involved in the healing progression (Patel *et al*., 2019).

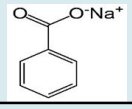
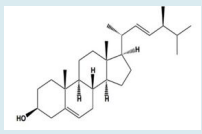
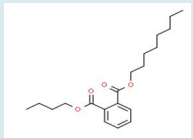
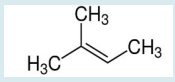
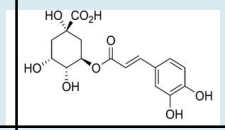
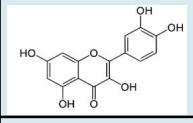
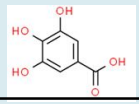
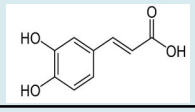
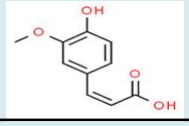
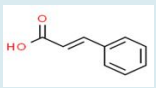
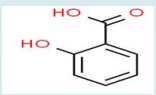
For the treatment of diabetes, villagers in the Aligarh area consume a mixture of 4-5 freshly emerging leaves of *A. squamosa* and five *Piper nigrum* in the early morning as a sustained therapy with 80% success rate. The approach is particularly popular in the Lodha community. This equation has been used by both Unani and Allopathic practitioners (Chowdhury *et al*., 2021).

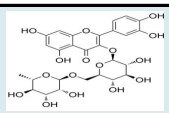
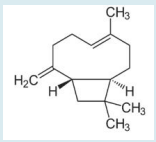
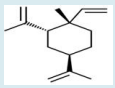
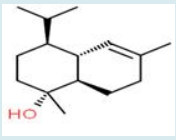
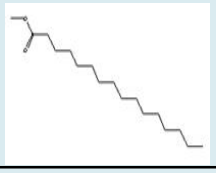
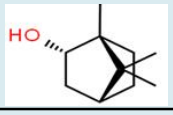
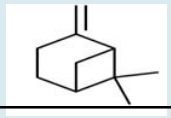
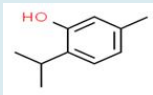
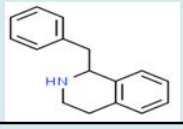
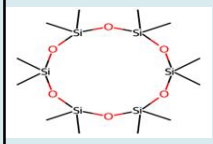
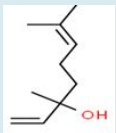
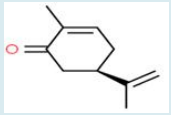
A. squamosa is a tiny tree with fragrant pendulous blooms and tasty fruit. The roundish, sweet-tasting fruit has a knobby surface. *A. squamosa* leaves are typically thrown or burned as a waste agricultural product. *A. squamosa* leaves, on the other hand, have been popularly used as an old-style medicine in subtropical and tropical countries like India, Vietnam, Laos, Malaysia, *etc*., for the treatment of fever, constipation, cardiac difficulties, diarrhoea, fainting, bleeding, worm infections and dysuria (Ponrasu and Suguna, 2012).

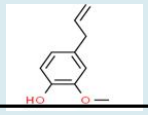
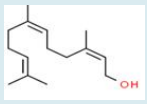
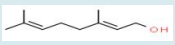
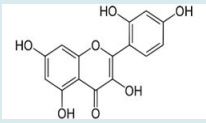
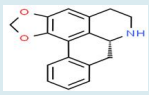
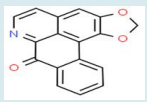
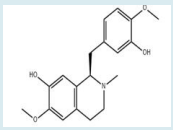
Researches have revealed that application of *A. squamosa* leaf extract in diabetic wound enhances DNA content and granulation substance of the wound tissue indicating multiplication of cells followed by consequent enhancement in content of collagen, uronic acid and hexosamine. This indicates synthesis and settlement of matrix protein.

Collagen, a vital component of extracellular matrix is essential for wound healing. *A. squamosa* leaf extract revealed positive correlation with new collagen proliferation and maturation thus attributing to better wound healing. Uronic acid and hexosamine are the molecules of the ground substance which helps to form a bed for deposition of newly formed collagen in proper pattern thus aids in remodelling phase of wound healing. It has been observed that wounds applied with leaf extract of *A. squamosa* shows early regeneration of dermis and epidermis (Ponrasu and Suguna, 2012). Most of the phytochemicals found in *A. squamosa* leaves have pharmacological properties that can aid in diabetic wound healing (Figure 1, Table 1, 2).

Table 1: Phytochemical composition and pharmacological effects of *A. squamosa* leaves

Phytochemicals found in *A. squamosa* leaves	Method of extraction	Structure of the phytochemical	Structural formula	Pharmacological effects that aid in wound healing effect	References
Sodium benzoate	GC-MS		$C_7H_5NaO_2$	Antimicrobial effect	Kulkari *et al.*, 2011
Stigmasterol acetate	GC-MS		$C_{31}H_{50}O_2$	Anti-inflammatory, antidiabetic, immunomodulatory, antifungal, antibacterial, antioxidant effect	Kulkari *et al.*, 2011; Bakrim *et al.*, 2022
Butyloctylpthalate	GC-MS		$C_{20}H_{30}O_4$	Antioxidant, antibacterial, antiviral impacts	Almarri *et al.*, 2023
Isoamylacetyate	GC-MS		$C_7H_{14}O_2$	Antidiabetic, antioxidant, antimicrobial, antiviral, hepato-protective effect	Kumar *et al.*, 2021
Chlorogenic acid	-		$C_{16}H_{18}O_9$	Antioxidant, anti-inflammatory effects	Kumar *et al.*, 2019
Quercetin	-		$C_{15}H_{10}O_7$	Antioxidant, antimicrobial effect	Guillermo Gormaz *et al.*, 2015
Gallic acid	-		$C_7H_6O_5$	Antitumour, antibacterial, antidiabetic, antiobesity, antimicrobial effect	Bai *et al.*, 2021
Caffeic acid	-		$C_9H_8O_4$	Anticancer, antioxidant, antibacterial, anti-inflammatory, antifungal effect	Lv *et al.*, 2021
Ferullic acid	-		$C_{10}H_{10}O$	Antiapoptotic, antiplatelet, anti-inflammatory effects	Li *et al.*, 2021
Cinnamic acid	-		$C_9H_8O_2$	Antidiabetic, anticholesterolemic, antioxidant, hepatoprotective, anxiolytic effect	Prateek, 2011
Salicylic acid	-		$C_7H_6O_3$	Anti-inflammatory, antimicrobialeffects	Klessig *et al.*, 2016

Rutin (quercetin-3-rhamnosyl glucoside)	NMR - MS		$C_{27}H_{30}O_{16}$	Antioxidant, antimicrobial effect	Malik *et al*., 2018; Ganeshpurkar and Saluja, 2017
β-Caryophyllene	GC-FID GC-MS		$C_{15}H_{24}$	Hepatoprotective, antioxidant, anti-inflammatory, antimicrobial, immunemodulator activities	Garg and Gupta, 2005; Sharma *et al*., 2016
β-elemene	GC-FID GC-MS		$C_{15}H_{24}$	Anticancer effect	Jiang *et al*., 2017
Epi-β-cadinol	GC-FID GC-MS		$C_{15}H_{26}O$	Anti-inflammatory, wound healing agent	Grafakou *et al*., 2021
Methyl hexadecanoate	GC-MS		$C_{17}H_{34}O_2$	Strong antibacterial, antifungal activities	Marahatta *et al*., 2019
Borneol	GC-MS		$C_{10}H_{18}O$	Antioxidant, anticoagulant, anti-inflammatory activities	Liu *et al*., 2021
B-Pinene	GC-MS		$C_{10}H_{16}$	Antibiotic resistance modulation, anticoagulant, antitumor, antimicrobial, antioxidant effect	Salehi *et al*., 2019
Thymol	GC-MS		$C_{10}H_{14}O$	Antioxidant, anti-inflammatory, analgesic, antibacterial, anti-fungal, antiseptic effect	Nagoor Meeran *et al*., 2019
Benzyltetrahydro-isoquinoline	GC-MS		$C_{16}H_{13}N$	Antioxidant and acetylcholine-sterase inhibitory properties	da Silva Mendes *et al*., 2021
Cyclohexasiloxane, dodecamethyl-	GC-MS		$C_{12}H_{36}O_6Si_6$	Antioxidant, antibacterial effects	Mustanir *et al*., 2021; Abdelsattar *et al*., 2022
Linalool	TLC		$C_{10}H_{18}O$	Analgesic, anti-inflammatory, antitumour antibacterial effects	Gowdhami *et al*., 2014
Carvone	TLC		$C_{10}H_{14}O$	Anti-inflammatory, antidiabetic, and anticancer effects	Bouyahya *et al*., 2021

Eugenol	TLC		$C_{10}H_{12}O_2$	Antioxidant, anti-inflammatory and cardiovascular properties	Pramod *et al*., 2010
Farnesol	TLC		$C_{15}H_{26}O$	Antimicrobial, antitumor effects	de Araújo Delmondes *et al*., 2019
Geraniol	TLC		$C_{10}H_{18}O$	Antifungal, antibacterial effect	Lira *et al*., 2020
Morin	-		$C_{15}H_{10}O_7$	Nephroprotective, antioxidant, immunostimulant effect	Mishra *et al*., 2021
Anonaine	UPLC-ESI-MS-MS		$C_{17}H_{15}NO_2$	Antimicrobial, anticancer effect	Pinto *et al*., 2017; Nugraha *et al*., 2019
Asimilobine	UPLC-ESI-MS/MS		$C_6H_5NH_2$	Antibacterial, antioxidant, anticancerous effect	Al Kazman *et al*., 2022
Liriodenine	UPLC-ESI-MS/MS		$C_{17}H_9NO_3$	Antifungal, antimicrobial, antiplatelet, antiviral effect	Chen *et al*., 2013
Reticuline	UPLC-ESI-MS/MS		$C_{19}H_{23}NO_4$	Antioxidant, anti-inflammatory, hypotensive effect	Dias *et al*., 2004

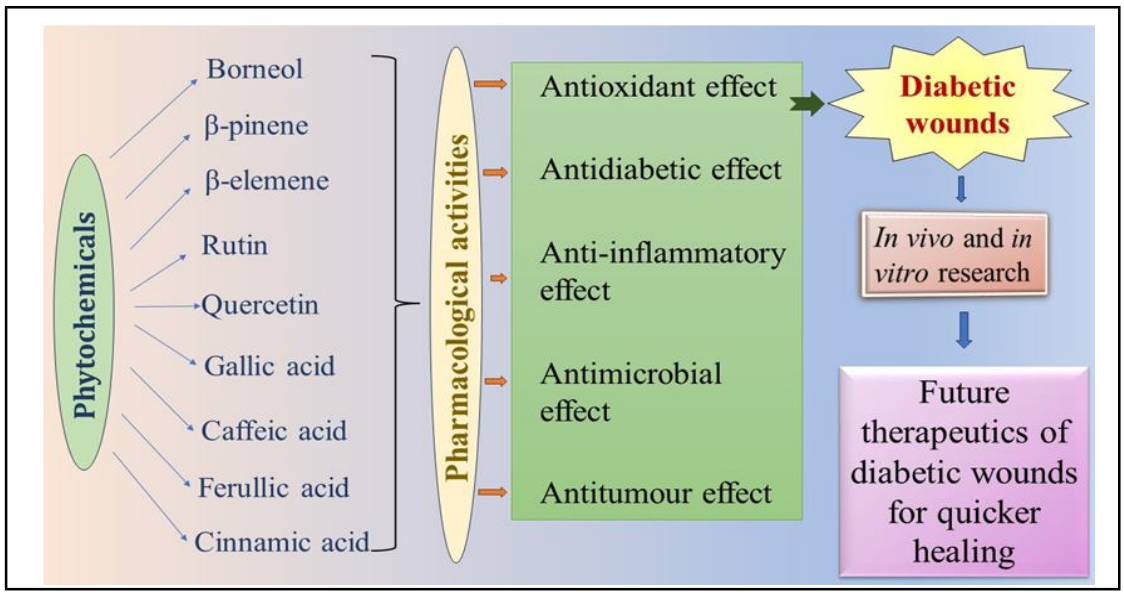


Figure 1: Pharmacological effects of phytochemicals of *A. squamosa* leaves can act synergistically to heal diabetic wounds.

6. Other pharmacological activities of *A. squamosa* leaves

A. squamosa leaves possesses many other beneficial effects like antioxidant, antidiabetic, anti-inflammatory, antibacterial, antiviral, antifungal, antitumour effects. These effects can act synergistically to aid in wound healing in diabetic patients.

6.1 Antioxidant effect

The phenolic chemicals found in *A. squamosa* could be used as a natural antioxidant. The antioxidant effect of phenolic compounds from *A. squamosa* leaves was tested through widely recognised *in vitro* systems. The absorbance capacity of oxygen radical, DPPH, and MTT assays were used to measure antioxidant activity, and the polyphenols profile was determined using HPLC analysis. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay showed a significant flavonoid concentration in *A. squamosa* leaf extracts. The antioxidant action of the various extracts was measured through oxygen radical absorbance capacity and MTT test methods (Mariod *et al*., 2012).

Methanolic extract and its fractionated extracts with n-hexane, ethyl acetate were used to test the antioxidant activity of *A. squamosa* leaf. Using the 1-diphenyl-2-pycrilhydrazil technique. The antioxidant activity of methanolic extract and fractions of n-hexane, ethyl acetate, and residue was tested, and the IC_{50} values were 6.87, 169.99, 31.55, and 44.75 ppm, respectively. The third subfraction contained 19 phytochemical compounds, with highest concentrations of dodecanoic acid, methyl ester, and 4,4'-((p-phenylene) diisopropylidene) diphenol, phthalic acid, isobutyl 2-methylpent-3-yl ester isobutyl 2-methylpent-3-yl ester, *etc*. (Mustanir and Ginting, 2021).

A. squamosa phytochemical studies revealed of numerous flavonoids with antioxidant, anti-inflammatory, cytotoxic, and antibacterial properties. The phenolic content was discovered to be 242.886.13 mg GAE/g extract. The flavonoid content of *A. squamosa* leaves was 82,610.82 mg QE/g dry weight ethanol extract. The antioxidant effect of *A. squamosa* ethanol extract is associated with total phenolic and flavonoid amount having IC_{50} values of 132.961.33 g/ml for DPPH and 64.740.52 g/ml for ABTS scavenging activities (Nguyen *et al*., 2020).

The antioxidant capability of each extract was measured by the scavenging activity of free radicals (hydrogen peroxide, nitric oxide, and 1,1-diphenyl-2-picrylhydrazyl) and the reducing effect of *A. squamosa* leaves. The antioxidant action and reducing effect of all extracts were dependent on their concentration where methanolic extract of *A. squamosa* leaves outperformed the chloroform extract, which outperformed the aqueous extract (Kalidindi *et al*., 2015).

6.2 Antidiabetic effect

A. squamosa leaves are a common dietary supplement for diabetic management. To test the antioxidants, the crude extract was first separated using HPLC-DAD along with DPPH technique. The antioxidant capabilities of five major flavonoids isolated as pure components were researched. Furthermore, crude flavonoid extract outperformed crude ethanol extract in terms of glucose absorption. Thus, the effectiveness of *A. squamosa* leaves in decreasing glucose levels, as well as the antioxidant capabilities of isolated pure components can be primarily responsible for the hypoglycemic characteristics in HepG2 cells (Zhu *et al*., 2020).

Quercetin-3-O-glucoside was extracted from *A. squamosa* leaves and allowed to modulate alloxan-induced hyperglycemia and lipid peroxidation in rats using UV, IR, MS, and NMR studies. In alloxan-treated rats, an increased serum glucose and decreased insulin level were seen. It also decreased hepatic and renal LPO while augmenting the effect of antioxidative enzymes including glutathione, superoxide dismutase and catalase showing antiperoxidative actions. Quercetin-3-O-glucoside is established as a bioactive ingredient in *A. squamosa* leaf extract. Owing to its insulin boosting and/or free radical scavenging properties, it can be used in the treatment of diabetes (Panda and Kar, 2007).

Hot water extract of *A. squamosa* leaves have antidiabetic properties. *In vivo* investigations were conducted on glucose intolerant high-fat fed rats. HPLC, LCMS, and NMR were used to isolate and characterise active molecules. It increased the secretion of insulin from clonal cells and mice islets. The extract increased cellular glucose absorption and insulin effect while decreased glucose diffusion, protein glycation, DPP-IV enzyme activity and starch digestion. It improved beta cell mass, calorie intake, islet size, pancreatic insulin content, and body weight. Isolated insulinotropic substances, including rutin ($C_{27}H_{30}O_{16}$) replicated its beneficial effects on beta cells as well as plasma insulin responses and *in vivo* glucose tolerance. It can be used as therapeutics of T2DM as dietary supplement since it contains possible anti-diabetic substances such as rutin (Ansari *et al*., 2020).

Diabetes was developed using streptozotocin-nicotinamide, and normal and experimental diabetic rats were given *A. squamosa* aqueous leaf extracts in drinking water in graded doses for 12 days. Serum insulin and lipid profiles, fasting plasma glucose, and differences in body weight were measured in normal rats, pancreatic TBARS and glycogen were measured in diabetic rats and compared to conventional glibenclamide. In streptozotocin-nicotinamide-brought type 2 diabetic rats, the aqueous extract lowered plasma glucose levels, demonstrating that *A. squamosa* aqueous extract had a robust hypoglycemic activity (Shirwaikar *et al*., 2004).

6.3 Anti-inflammatory effect

Pain and inflammation ensue as first-line injury defence. Various medicinal plants can aid with pain and inflammation treatment. The effects of *A. squamosa* L. leaf extract on pain and inflammation were examined in Wistar rats and mice. The ethanol extract of *A. squamosa* was tested for centrally acting analgesic activity using the hot plate method, while peripherally acting analgesic effect was tried using the acetic acid-induced writhing method. Plethysmometer was used to assess the anti-inflammatory action. In comparison to pentazocine, the extract showed a significantly increased reaction time in acetic acid produced writhing and hot plate generated nociception (Singh *et al*., 2012).

The antioxidant action of Lebanese *A. squamosa* leaf extracts were explored. The total phenolic and flavonoid content ranged from 27.3 to 179.5 mg GAE/g and 8.3 to 150.8 mg RE/g of plant extract respectively. The DPPH investigation revealed that the methanolic extracts produced in natural dry leaves had strong antioxidant activity. Significant anti-inflammatory action was reported in lipopolysaccharide (LPS)-stimulated THP-1 cells due to a reduction in IL-6 production (Awada *et al*., 2023).

6.4 Antibacterial effect

The leaves of *A. squamosa* includes significant bioactive chemicals that hinder the bacterial and colon cancer cell lines growth making them an effective alternative antibiotic for colon cancer therapy. *A. squamosa* leaf extracts were discovered comprising of bioactive chemicals, the most abundant of which were sesquiterpenes ($C_{15}H_{24}$). These extracts demonstrated significant antioxidant actions as well as antibacterial actions against both gram-positive and gram-negative bacteria. In colon cancer cells, various *A. squamosa* leaf extracts have shown outstanding antiproliferative, cytotoxic, antimigration, and apoptotic effects (Al-Nemari *et al*., 2020).

Water and methanol extracts of *A. squamosa* leaf exhibited the highest antibacterial activity against 6 selected standard strains of intestinal gram-negative bacteria like *Salmonella typhi* (MTCC 3216), *Klebsiella pneumonia* (MTCC 7028), *Salmonella paratyphi* (MTCC 735), *Proteus mirabilis* (MTCC 3310), *Escherichia coli* (MTCC 46) and *Vibrio cholera* (MTCC 3906) in comparison with the standard antibiotics (Gowdhami *et al*., 2014).

The antibiofilm and antibacterial characteristics of ethanol leaf extract of *A. squamosa* made without and with a pulsed electric field aided technique were studied in comparison. When compared to ethanolic *A. squamosa* leaf extract, pulsed electric field - ethanolic *A. squamosa* leaf extract inhibited *Listeria monocytogenes, Pseudomonas aeruginosa*, *Bacillus subtilis, and Escherichia coli* the greatest abundant spoilage bacteria detected in squid rings. With increasing concentrations, the antibiofilm capabilities of both extracts against bacteria were enhanced. Overall, pulsed electric field - ethanolic custard apple leaf extract have shown promising antibacterial activity, suggesting that it might be used as a biopreservative agent to suppress bacterial development in squid rings during refrigerated storage (Olatunde *et al*., 2021).

Several bacterial strains of *E. faecalis, S. aureus* and *K. pneumonia* were utilised to test the antibacterial action of methanol extracts of *A. squamosa* leaves. With MIC values of 78, 78, and 39 g/ml, *A. squamosa* leaves have shown considerable antibacterial activity against *S. aureus, K. pneumoniae,* and *E. faecalis*. The leaves of *A. squamosa* showed considerable biofilm breakup, quick time-dependent kinetics of bacterial killing, enhanced membrane permeability, and considerably decreased cell numbers and viability. This study confirmed that *A. squamosa* leaves have a great phytomedicinal potential, exhibiting antibacterial activity *via* bacterial membrane destabilisation (Pinto *et al*., 2017).

The leaves of *A. squamosa* have traditionally been used to treat diarrhoea and other related illnesses. Ethanol extraction and column chromatography were used to partially purify active components. The extract's MICs were seen against *Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus, Campylobacter jejuni*. The existence of oxophoebine and reticuline was revealed by LC-MS analysis. The MIC assay demonstrated that the action was heat-labile, with the greatest loss of action occurring at high temperatures but remaining quite stable at refrigerator temperatures. As *A. squamosa* has broad-spectrum yet heat-labile action against foodborne bacteria, as well as bactericidal action against *C. jejuni* and *B. cereus*, it has the potential to be developed as a feed additive (Dholvitayakhun *et al*., 2013).

6.5 Antiviral effect

In 2013, *A. squamosa* leaf extract was tested for anti-dengue virus type-2 activity in Surabaya, Indonesia. The antiviral efficacy of *A. squamosa* leaf extract against dengue virus type-2 in vero cells was assessed using the viral ToxGloTM Assay. *A. squamosa* leaf extract suppressed dengue virus type-2 multiplication in vero cells with IC_{50} = 73.78 g/ml and SI = 4.49 when cells were treated two days after virus infection, however, its cytotoxicity to vero cells was 331.54 g/ml. Thus, *A. squamosa* showed antiviral effectiveness against dengue virus type 2 with low toxicity and a high potential as a therapeutic candidate. As a result, it could be developed as an effective antiviral against type-2 dengue virus after *in vivo* testing (Ansori *et al*., 2021).

6.6 Antifungal effect

All *A. squamosa* leaves extracts were tested for antifungal effect against five different strains of fungi (*Microsporum canis, Fusarium solani, Candida albicans, Aspergillus niger,* and *Alternaria alternata*) using the agar well diffusion method, and the MIC of individual extract was tested for antifungal susceptibility using the broth microdilution method. In both broth dilution and agar well diffusion procedures, organic and aqueous extracts were reported to exhibit dose-dependent inhibition. The phytochemical examination of extract revealed the presence of tannins, glycosides, saponins, phenols, flavonoids, *etc*. Thus, *A. squamosa* leaf extracts in methanol, chloroform, and aqueous form have antifungal activity (Kalidindi *et al*., 2015).

6.7 Antitumour effect

A. squamosa leaves demonstrated promising cytotoxicity with Jurkat and HL60 cell lines, with CI50 values of 4.2 and 6.4 g/ml, correspondingly. *A. squamosa* leaves inhibited VERO cell growth and lowered clonogenic endurance in MCF-7 and HCT-116 lineages at higher concentrations (IC_{90}). UPLC-ESI-MS/MS examination recognized the alkaloids asimilobine, nornuciferine, liriodenine, anonaine, corypalmine, and reticuline in the extracts. Thus, leaves of *A. squamosa* have antitumour properties (Pinto *et al*., 2017).

The antitumor properties of Lebanese *A. squamosa* leaf extracts were evaluated. Methanolic extracts produced *via* UAE demonstrated a significant anti-proliferative action against the HCT116 cell line in the WST-1 assay, with IC_{50} between 0.18 to 0.88 g. ml^{-1}. Also, the western blot experiment revealed that these extracts may decrease HCT 116 cell growth by triggering cell cycle arrest via activation of the p21 pathway (Awada *et al*., 2023).

7. Conclusion

Since last few years, an explosion of research is seen in diabetic wound treatment. A variety of variables contribute to diabetic wound healing impairment. Natural product-based approaches can be beneficial due to their phytochemical compositions. The wound healing, antioxidant, antibacterial, and anti-inflammatory actions of *A. squamosa* leaves makes it an indispensable choice for further research of developing therapeutics for quicker healing of diabetic wounds. Strong *in vivo* and *in vitro* research is required to identify the role of distinct phytochemicals from *A. squamosa* leaves acting at different stages of wound healing in diabetes. When combined with a nanocarrier, *A. squamosa* leaves can aid to heal diabetic wounds. Improving phytochemical distribution at specific sites may aid in enhancing the degree of wound healing in diabetes.

Abbreviations

ABTS:2,22 azino bis (3 ethylbenzothiazoline 6 sulfonic acid)

AKT/PKB: Protein kinase B

CX3CL1: (C-X3-C motif) ligand 1

DPP-4: dipeptidyl peptidase-4

DPPH:(2,2-Diphenyl-1-picrylhydrazyl)

E2f3: transcription factor 3

ECM: extracellular matrix

GC/MS: Gas chromatography-mass spectrometry

GC-FID: GC-flame ionisation detection

HCT-116: human colorectal carcinoma cell

HepG2: hepatoblastoma cell line

HPLC analysis: High performance liquid chromatography analysis.

HPLC-DAD: High-Performance Liquid Chromatography with Diode-Array Detection.

HSPs: heat shock proteins

IGF-1: Insuline -like growth factor -1

IGF-16: insulin-like growth factor

IL-10: Interleukin 10

IL-12- Interleukin- 12

IL-64- Interleukin-62

KGF-24: Keratinocyte Growth Fackor-24

LC-MS: Liquid chromatography-mass spectrometry

MCF-7: Michigan Cancer Foundation -7

MIC: Minimal inhibitory concentration.

MMP: Matrix Metalloproteinase

MTCC: Microbial Type Culture Collection & Gene Bank

MTT :3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide

NMR: nuclear magnetic resonance

p38-MAPK: p38 mitogen-activated protein kinases

PDGF: Platelet-derived growth factor

T2DM: Type 2 diabetes mellitus

TBARS: Thiobarbituric acid reactive substances

TGF-7: Transforming growth factor -7

TGF-á: Transforming growth factor-á

THP-1: human leukemia monocytic cell

TIMPs29: Tissue inhibitors of metalloproteinases 29

TLC: Thin Layer Chromatography

TLR4: Toll-like receptor 4

TLRs-2: Toll-like receptors-2

TLRs22: Toll-like receptors 22

TNF -3: Tumor Necrosis Factor

UAE: Uterine artery embolization

UPLC-ESI-MS/MS: Ultra-performance liquid chromatography-electrospray ionization-tandem mass spectrometry.

UPR: Unfolded protein response

VEGF: Vascular endothelial growth factor

VEGF-5: Vascular endothelial growth factor -5

VEGFR: Vascular endothelial growth factor receptor-1

WST-1: Water-soluble tetrazolium salt.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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