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Review Article

LATICIFEROUS PLANT PROTEASES IN WOUND CARE

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ABSTRACT

Traditionally, different parts of plants such as bark, stem, and leaves have been used in wound healing. Around 10% of the angiosperm plants produce a natural polymer from specialized laticiferous cells called latex. The major role played by the latex is in wound healing and defensive mechanism against infectious diseases in plants. In the past 20 y, with biochemical and pharmacological characterization of plant latex it has come to light that proteases are involved in wound healing. Some have been extensively studied with characterization while some are yet to be explored. This review conglomerates the reports of different families of laticiferous plants studied scientifically for wound care. The review also emphasizes on the methodology to be adopted in accessing the proteases studied for procoagulant and thrombolytic activities. Emphasis was given on the all possible reports on laticiferous plants in wound healing with thorough literature survey from sources viz., PubMed and Google Scholar. However, research on latex protease is still in budding stage. Adopting the proteases having promising applicability in wound care needs to be focussed.

Keywords: Wound healing, Plant latex, Proteases, Coagulation, Laticiferous plants.

INTRODUCTION

The physiological event associated with loss of functional capacity of a living cell or tissue due to trauma caused by physical, chemical, microbial or other factors is called a wound. This damage immediately attracts the attention of various cellular and non-cellular factors for its repair. This complex cascade of biochemical events that ultimately achieves restoration of an original structure, function and composition of cell/tissue is termed as "wound healing". Wound healing is a well-orchestrated mechanism that occurs within a defined period of time. It mainly involves four phases–hemostasis phase (within minutes), inflammatory phase (24 to 48 h may be up to 2 w), proliferative phase (2 d to 3 w) and remodeling phase (3 w to 2 y) [1]. When this normal mechanism fails, external aid becomes a necessity. Throughout the world, plant sources such as leaves, stem, bark, root, latex, and whole plant have been extensively used in order to achieve wound healing. This paper mainly concentrates upon summarizing the up-to-date scientific research in wound care carried out using plant latex and their proteases present in it.

Proteases (3.4.21) are the enzymes involved in the catabolism of protein into peptides and amino acids. Based on the catalytic residues, proteases are classified into aspartate proteases, cysteine proteases, glutamic acid proteases, serine proteases, threonine proteases, and metalloproteases. The major proteases that are involved in the blood coagulation cascade and thereby in wound healing are the serine proteases and matrix metallo proteases (MMPs). [2] This paper mainly concentrates upon summarizing the up-to-date scientific research in wound care carried out using plant latex and their proteases present in it.

Regulation of the proteases is known to be an important factor in the wound healing mechanism. The wounded environment includes the active keratinocytes, fibroblasts and endothelial cell which are involved in the regulation of protease levels at the wounded site. This is the innate mechanism of wound healing and when this fails the external application of proteases becomes a must. The proteases involved in the wound healing also must be capable of breaking down the collagens, elastin, and proteoglycans [2]. The degradation of newly formed extracellular matrix is because of excessive wound proteases that disturb the balance between tissues break down and repairs [3]. Hence protease action can impair wound healing process.

The role of proteases can be assessed based on the chart mentioned in (fig 1). Both fibrinogenolytic and fibrinolytic property needs to be assessed thereby ascertaining the protease to be either procoagulant or anticoagulant respectively. Procoagulant activity also called as hemostatic activity indicates that it aids in blood clot formation or in other words fibrin clot formation, which is essential to stop bleeding and enable the wound healing process. Otherwise, some proteases possess fibrinolytic activity also known as anticoagulant activity which indicates that they aid in degradation of the fibrin clot or thrombus formed in blood circulation. Thus it is established that proteases play as major pharmaceuticals in wound care.

Latex is a natural plant polymer secreted by highly specialized cells known as laticifers. Plant latex is a mixture of secondary metabolites, and bioactive components like acetogenins, alkaloids, resins, phytosterols tannins, terpinoides, and enzymes [4, 5] Laticiferous plants belonging to families Altingiaceae, Amaranthaceae, Apocyanaceae, Asclepiadaceae, Asteraceae, Caricaceae, Diptero-carpaceae, Euphorbiaceae, Lamiaceae, Moraceae, Papaveraceae, Plumbaginaceae, and Solanaceae have been traditionally used for various purposese including wound healing, some of which have been extensively studied.

Bioactives of plant latex possess anti-carcinogenic, anti-proliferative, anti-inflammatory, vasodilatory, antioxidant, antimicrobial, antiparasitic, insecticidal and wound healing properties [4]. Also provides the methodical approach towards studying the wound healing property of the protease. This paper mainly concentrates upon summarizing the up-to-date scientific research in wound care carried out using plant latex and their proteases present in it. Also we provide the methodical approach towards studying the wound healing property of the proteases.

Plants with latex proteases involved in wound healing

Argemone mexicana

Argemone mexicana, also known as Mexican poppy, is a species of Poppy, found mainly in Mexico. It belongs to the family Papaveraceae. It has bright yellow coloured latex. Though the latex is poisonous to grazing animals it is considered to be medically useful. Latex of *Argemone mexicana* is used to treat boils, as wound dressing agent and is also used to treat dermatitis. Latex of this plant is used externally to disinfect open wounds. The *in vivo* wound healing activity of the extract and latex of *Argemone mexicana* was compared to that of established drugs. The tensile strength of the extract-treated-group was found to be higher than the latex-treated-group of animals on 12th post-wounding day [6-8].

Asclepias curassavica

Asclepias curassavica is a tropical evergreen perennial subshrub. It is native to American tropics and belongs to the family of Apocynaceae. It is commonly known as tropical milkweed. The stem of this plant produces latex when subjected to superficial incisions. This latex produced, contains many proteins and exhibits proteolytic activity. It acts as an emetic and vermifuge and can be used against gonorrhoea.

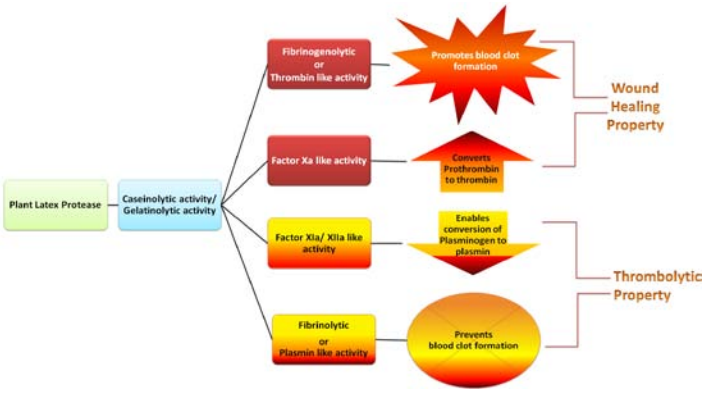

Fig. 1: General chart representing the assessment of protease for wound healing and thrombolytic properties

The study was conducted mainly to identify the clotting factors present in the latex and to analyse their thrombin like activity. Various assays like proteolytic assay, the thrombin time assay, fibrinogen polymerisation assay, inhibitor assay and concentration dependent coagulation assay were carried out. It was observed that the plasma clotting time reduced from 143 seconds to 56 seconds in the presence of crude latex extract. Further detailed studies like in-silico studies–docking studies showed that cysteine proteases interacted with the clotting factors. Thus it was concluded that cysteine proteases found in the latex of *Asclepias curassavica* were capable of accelerating the blood coagulation process and were also responsible for thrombin like activity [9].

Atrocarpus heterophyllus

Artocarpus heterophyllus is a tree belonging to Moraceae family. It is popularly known as Jackfruit and its fruit is widely consumed. Though the origin of this species is yet unknown, it is believed to be indigenous to the rainforests of India and Southeast Asia. *Artocarpus heterophyllus* is a latex producing plant and research concerning this latex has led to the discovery of a serine protease with fibrinogenolytic and fibrinolytic properties.

AMP48, a 48kDa antimicrobial protease, was isolated and purified from the latex and its human fibrinogenolytic and fibrinolytic activity were studied. The serine protease effectively hydrolysed the subunits of human fibrinogen as well as human fibrin clot. The extent of fibrin clot degradation by AMP48 enzyme was studied using attenuated total reflection Fourier transform infrared spectroscopy. These studies revealed that an incubation period of 60 min, at a temperature of 37 °C was required by the protease to effectively digest all three subunits of human fibrinogen. The hydrolysis of fibrin clot was brought about by the alteration of the secondary structure of β-sheet and α-helix resulting in the conversion of solid state of the clot to partial liquid state. Thus, considering these properties it was concluded that the serine protease enzyme, AMP48, can be used as an effective antithrombotic agent [10].

Calotropis gigantea

Calotropis gigantea also known as Crown flower is a laticiferous plant belonging to the family Apocynaceae, subfamily Asclepiadaceae and is known for its medical properties. It is native to India, Sri Lanka, China, Malaysia and tropical Himalayas. It is a large shrub sporting clusters of either white or lavender waxy flowers.

Literature reveals that the latex from *Calotropis gigantea* is commonly used on fresh cuts to stop bleeding and is also used as an inflammatory agent. The latex of this plant has proteases in abundance that aid several pharmacological properties exhibited by *Calotropis gigantea*. It was observed that the latex hydrolysed human fibrinogen bringing about coagulation of plasma and subsequent plasmin like activity [11].

The study involved testing for caseinolytic activity, human fibrinogenolytic activity, coagulant activity, fibrinolytic activity, human blood clot hydrolysing activity, human plasma clot hydrolysing activity and haemorrhagic activity. Their study was supported by the results that at a low concentration of 1µg of crude latex with 60 min incubation, 95% of human fibrinogen were hydrolysed. Also at a concentration of 30µg, the time take for clot formation reduced from 150 to 47 seconds. Along with clot formation, a dose dependant clot digesting activity of this protease was also observed. Evidenced by the inhibitory action of IAA, the study was concluded with the characterisation of the latex protease to be cysteine protease. The study was concluded by stating that the proteases extracted from the latex of *Calotropis gigantea* belonged to the class of cysteine proteases and that their procoagulant and clot dissolving properties were responsible for the observed physiological action of the latex.

In yet another study, animal experiments were carried out to evaluate the wound healing potential of *Calotropis gigantea*. It was observed from the study that the latex of this plant significantly improved wound contraction [12]. Also, in this study wounds were deliberately created on the rats and were treated with the latex of this plant in a periodic and systemic manner. Application of latex as ointment showed a significant 90% wound contraction in the animals in 14 d. The study was concluded that the latex of *Calotropis gigantea* greatly influenced wound healing and had the potential for being a therapeutic agent.

Similar results were obtained in another study where incision and excision wound models were used and the treatment with the latex of *Calotropis gigantea* showed significant improvement in wound healing process. It was observed that with an administration of 200 mg/kg/day of latex of this species, there was an increase in the reduction in wound area by 83.42% when compared with the control group (76.22%). The mode of administration adopted here was topical application. Thus the conclusion was drawn that the latex could be used as an effective therapeutic agent [13].

Calotropis procera

Calotropis procera commonly known as milkweed is a shrub or a small tree belonging to the laticiferous family Apocynaceae and subfamily Asclepiadaceae. It is native to Africa and Southern China. The latex of this plant has been used in the treatment of various diseases and is also known to possess anti-inflammatory properties [14].

A study was conducted on the inflammatory properties of the proteases present in the latex of *Calotropis procera* [14]. Study includes proteolytic activity, activated partial thromboplastin time, prothrombin time, fibrinogen agarose plate assay, the fibrinogen polymerisation assay, fibrinogenolytic activity and human plasma clot hydrolysing activity. The results of these experiments were obtained by performing animal experiments involving Swiss mice maintained under monitored conditions. Sepsis was deliberately induced in these organisms to determine the platelet count and proteolytic activity whereas human blood samples were used for experimenting on different wound healing mechanisms.

Finally, it was concluded that the cysteine proteases present in the latex of this plant possessed various properties that aided clotting of blood in wounded regions. It was also deduced that, along with the clot forming property, the proteases in the latex possessed clot dissolving property as well. This was exhibited by two of the isolated subfractions, LPp11 and LPp111, which demonstrated thrombin-like and plasmin-like properties. The effect was such that the clotting time was reduced by 50% by these fractions as observed in the APTT assay. Thus the therapeutic potential and the ability to maintain coagulation haemostasis of protein fractions derived from latex of *Calotropis procera* was determined.

Table 1: **Activities of different laticiferous plants**

Family	Plant Name	Common name	Role	References
Altingiaceae	*Liquidambar orientalis Mill*	Oriental sweet or Turkish sweet gum	Excision wounds	[7]
Amaranthaceae	*Achyranthes aspera L*	Prickly chaff flower, Devil's horsewhip	Latex of the plant applied on the wound.	[31]
Apocyanaceae	*Alafia multiflora*	-	Applied to wounds used to cure stubborn wounds.	[32]
	Alstonia scholaris R. Br	Devil tree; White cheese wood; Black board tree; pagoda tree.	The latex is applied to wounds and boils	[31]
	Asclepias curassavica	Tropical milkweed	Acts as an emetic and vermifuge and can be used against gonorrhoea.	[9]
	Calotopis gigantean	Crown flower	Effective therapeutic agent	[13]
	Ervatamia coronaria	East Indian Rosebay	Anti-inflammatory activity	[18]
	Holarrhena floribunda	False rubber tree	Applied to snake bite.	[32]
	Pergularia extensa	Trellis-vine	Anti-inflammatory, anti-cancer, antioxidant, antifungal	[17]
	Plumeria rubra	Red jasmine	Anti-inflammatory and wound healing activities	[28]
	Strophanthus sarmentusus	Spider tresses	Used in wounds	[32]
	Voacanga thouarsii	Wild frangipani	Wound healing	[32]
	Wrightia tinctoria	Ivory wood or Pala indigo	Topical applications on fresh wounds	[30]
Asclepiadaceae	*Holostemmaada-kodien Schultes*	Holostemma	Applied on Blisters	[33]
Asteraceae	*Tragopogon dubius*	Yellow salsify	Latex is applied on heel wounds	[34]
Caricaceae	*Carica candamarcensis*	Mountain papaya	Healing properties	[15]
	Carica papaya	Papaya	Favour wound healing	[16]
Dipterocarpaceae	*Vateria indica Linn*	Sarja	Latex used for wound healing.	[35]
Euphorbiaceae	*Euphorbia antiquorum*	Thoroughwort	Stem latex is applied on burn wounds	[36]
	Euphorbia caducifolia	Leafless Milk Hedge and Leafless Euphorbia	Treatment of bleeding wounds, cutaneous eruptions and other skin diseases	[19]
	Euphorbia cuneala	-	Applied to warts, wounds and sores.	[32]
	Euphorbia grantii	African Milk Bush	Used as folk medicine for open wounds and promotes blood clotting and tissue healing.	[32]
	Euphorbia granulate	-	Applied to snake bites and scorpion stings.	[32]
	Euphorbia helioscopia	Sun spurge	Latex applied on skin eruptions	[34]
	Euphorbia hirta	Asthma weed or Pill-Bearing Spurge	Potential industrial and the therapeutic applications	[20]
	Euphorbia neriifolia	Common Milk Hedge, Hedge Euphorbia, Oleander Spurge	Treatment of wounds in the form of topical application	[21]
		Leafy Milk Hedge	Anti-inflammatory and wound healing properties	[22]
	Euphorbia pilosa	Downy spurge, Hairy spurge, Wolfsmilch	Plant latex is applied on wounds	[37]
	Euphorbia primulifolia	-	Applied to syphilitic sores.Applied on skin parasites and warts.	[32]
	Euphorbia scordiifolia	-	Applied as an analgesic to tsetse fly bites	[32]
	Hevea brasiliensis	Rubber tree	Enhanced vascular permeability and angiogenic activity	[24]
	Jatropha curcas	Purging Nut	Used in the treatment of ulcers, bleeding gums, tumours and wounds.	[25]
	Jatropha gossypifolia L.	Cotton-leaf physic nut bush, Red physic nut	Whole plant latex applied on wounds, Latex and leaf juice are used to treat ulcer, skin disease (leprosy) and gum infections	[38] [39]
	Pedilanthus tithymaloides	Devil's Backbone, Japanese Poinsettia, Slipper Spurge, Redbird Cactus, Christmas Candle	Latex of the plant applied on the wound.	[40]
	Ricinus communis L	Castor bean, African coffee tree	Latex of the plant applied on the wound	[40]
	Synadenium grantii	African Milk Bush	Influence wound healing	[29]
Lamiaceae	*Clerodendrum viscosum Vent*	Glory tree	Leaf latex applied in fresh cuts and wounds to check bleeding	[41]
Moraceae	*Artocarpus heterophyllus*	Jackfruit	An effective antithrombotic agent	[10]
	Ficus benghalensis L	Indian banyan	The latex is applied on the navels	[42]
	Ficus glomerata	Cluster fig. Tree, Indian fig. Tree or Goolar (Gular)	Treat boils, wounds	[43]
	Ficus hispida	-	Treatment of ulcers, haemorrhage, diabetes, wounds and boils	[23]
Papaveraceae	*Argemone Mexicana*	Mexican poppy	Treat boils, as wound dressing agent, treat dermatitis, disinfect open wounds	[8]
Plumbaginaceae	*Plumbago zeylanica L*	Chitu, Teete,Fire plant	Milky latex is applied on affected parts of scabies	[41]
Solanaceae	*Datura stramonium L.*	Jimson weed, Devil's snare, or datura	Latex of the leaves was applied on the wound	[40]

Table 2: Different classes of proteases found in various Laticiferous species

Name	Species name	Class of protease	References
Artocarpin	*Artocarpus heterophyllus*	Serine protease	[10]
Asclepian c I	*Asclepias curassavica*	Cysteine protease	[9]
-	*Calotropis gigantean*	Cysteine protease	[11]

Procerain	*Calotropis procera*	Cysteine protease	[14]
Ervatamin A,B,C	*Ervatamia coronaria*	Cysteine protease	[18]
Nivulian-II and Nivulian-I	*Euphorbia nivulia*	Cysteine protease	[22]
Benghalensin	*Ficus bengalhensis*	Serine protease	[42]
Protease	*Ficus hispida*	Cysteine protease	[23]
Hevains A,B,L	*Hevea brasiliensis*	Serine protease	[24]
Curcain	*Jatropha curcas*	_____	[25]
Pergularian e I	*Pergularia extensa*	Cysteine protease	[17]
Plumerin-R	*Plumeria rubra*	_____	[28]
Wrightin	*Wrightia tinctoria*	Serine protease	[30]

Carica candamarcensis

Commonly known as mountain papaya, the papaya-like *Carica candamarcensis* is a plant belonging to the Caricaceae family. It is native of the Andean range from Panama to Bolivia. The immature fruit of this species has latex which is found to contain large amounts of cysteine proteinase. A fraction, P1G10, containing these cysteine proteinase enzyme was studied with respect to their activity in wound healing. The effect of latex proteinase of *Carica candamarcensis* was studied on heat induced third degree burns using a rodent model. The experiments were carried out were to observe the influence of the latex fraction on epithelialization of the burn. The results showed that 0.1% P1G10 was more effective in accelerating epithelialisation than 1% or 0.01% P1G10. Further histological analyses of the burn-tissue section were conducted to support the obtained results. Positive results thus concluded that the latex possesses healing properties and has potential clinical applications [15].

Carica papaya

Commonly known as papaya, it is a giant herbaceous plant that belongs to the family Caricaceae. It originated in Central Asia. The leaves and stem of this plant produces huge amount of white milky latex. The latex present in fruit, specifically unripe fruit, contains a mixture of different cysteine endopeptidases.

A study concerning the healing efficiency of papaya latex was performed where in the latex was used to treat deliberately wounded mice and its efficiency was evaluated based on the hydroxyproline content, wound contracting and epithelialization time. It was observed that the latex increased hydroxyproline content and wound contraction and decreased epithelialization time. Administration of 100 mg/mouse of 1% and 2.5% of latex to wounded animals indicated an increase in hydroproline content. Increased wound contraction was observed in the same set of animals on the 20th day after the application of latex. Decrease in epithilialization time from 32 d to 24-22 d further proved the beneficial aspects of the use of this latex. Thus, it was concluded that the latex of papaya favoured wound healing [16].

Cynanchum puciflorum

Cynanchum puciflorum is basically a herbaceous climber with slender stem. Typically it belongs to milk weed family and the whole plant contains milky juice. It is commonly called as coated swallow–wort, few–flowered cynanchum. These are usually found in the forests of Western Ghats. *Cynanchum puciflorum* latex showed thrombin like and plasmin like activities of cysteine proteases. Fibrin clotting assay followed by its spectrophotometric analysis and SDS-PAGE analysis of plasma clot hydrolysis were carried out. It was observed that direct incubation of latex enzyme with fibrinogen resulted in the formation of fibrin clots, whereas prolonged incubation resulted in the degradation of the fibrin clots. Following appropriate incubation conditions indicated the requirement of 10µg and 60s to effectively bring about fibrinogenolysis. These led to the conclusion that it has thrombin like and plasmin like activities respectively. Thus this study deduced the presence of protease activity in the latex of *Cynanchum puciflorum*. It was also observed that these proteases belonged to the class of cysteine proteases which also helped in reducing bleeding [17].

Ervatamia coronaria

Ervatamia coronaria is a small perennial shrub of Apocynaceae family, commonly known as East Indian Rosebay. It is believed to be indigenous to India and extensive studies have been carried out regarding its latex and its effect on wound healing. A highly stable 25kDa cysteine protease, Ervatamin, was purified from the latex of *Ervatamia coronaria*. This study was initiated by the anti-inflammatory effect exhibited by the latex on wounds and the subsequent casienolytic activity which led to the discovery of the cysteine protease enzyme. Thus, the area for research related to the latex, its biochemical compounds and its applications has considerably broadened [18].

Euphorbia caducifolia

Euphorbia caducifolia is a cactus-like, indoor growing Euphorbia. It is a Euphorbiaceae species that is native to Rajasthan of India, but is also found in rocky desert areas of Pakistan. Some of its common names are Leafless Milk Hedge and Leafless Euphorbia and is found to be very similar to *Euphorbia nivulia*. The latex of this plant is used by the local inhabitants for various medicinal purposes such as treatment of bleeding wounds, cutaneous eruptions and other skin diseases. *In vitro* tests, clotting of platelet-free plasma and angiogenesis in the chick chorioallantoic membrane, were carried out in order to determine the effect of *Euphorbia caducifolia* on the mechanism of wound healing. Excision and incision wound models were used and the effect of latex on clotting and angiogenesis was studied by observing the wound contraction, tensile strength and hydroxyproline and DNA content.

This study showed increased angiogenesis and favoured clotting mechanism. Thereby, it was concluded that *Euphorbia caducifolia* latex can be effectively used in wound healing [19].

Euphorbia hirta

Euphorbia hirta is a weed belonging to family Euphorbiaceae and is found distributed in the tropical regions of major continents such as Africa, Asia and America. It is possibly native to India and is commonly known by the names Asthma weed or Pill-Bearing Spurge. Since the plant belongs to a laticiferous family, the sap of the plant contains latex that is toxic as well as an irritant. However, further investigations have revealed that the constituents of latex can be beneficial as well.

A 34kDa serine protease in the latex of *Euphorbia hirta* was named Hirtin and was found to possess fibrinolytic and fibrinogenolytic activities. Thus the study was concluded saying that hirtin is a thrombin like serine protease and may have potential industrial and the therapeutic applications [20].

Euphorbia neriifolia Linn

Euphorbia neriifolia is a succulent shrub native to India, Myanmar, Pakistan, New Guinea and the Malay Archipelago. It belongs to the laticiferous family of Euphorbiaceae and was first described by Carl Linnaeus. The common names provided for *Euphorbia neriifolia* are Common Milk Hedge, Hedge Euphorbia, Oleander Spurge and so on. The latex of this plant has been widely used in the treatment of wounds in the form of topical application. This fact has been supported by a study where the aqueous extract of latex of this plant was evaluated for its wound healing activity. Surgically produced cutaneous wounds in guinea pigs were used for the study.

The wounds were treated with 0.5% and 1% of euphorbia latex separately. Upon doing this, it was observed that 0.5% topical treatment had no significant effect, while 1% latex treatment decreased the wound area by 18% and 30% on day 4 and day 7 respectively and also increased the tensile strength by 80% on 7th day of application. Similarly, epithelial regeneration, increased DNA and hydroxyproline content were seen on the use of 1% latex. The increase in tensile strength, DNA content, epithelialisation and angiogenesis was observed and it was concluded that the latex of *Euphorbia neriifolia* has the significant positive effect over wound healing [21].

Euphorbia nivulia

Euphorbia nivulia is commonly known as Leafy milk hedge, a spiny, wild, deciduous plant that belongs to the family Euphorbiaceae. The production of latex is the characteristic feature of this plant. This latex is further characterised by the presence of proteolytic enzymes in abundance. It is native to Northern and Central India.

The protease in the latex was found to belong to the class of cysteine proteases. It was observed that they reduced bleeding/clotting time and whole blood coagulation time, thereby reducing the rate of bleeding from fresh wounds. The results obtained were as such-a reduction in clotting and bleeding time from 57 and 60s to 8 and 14s, respectively on topical application of the latex and an increase in wound contraction by 99.29% on the 16th post wounding day. Thus it was concluded that the latex of *Euphorbia nivulia* is a promising haemostatic agent and wound healing promoter [22].

Ficus hispida

Ficus hispida belongs to the family Moraceae. It is a moderate sized tree and is native to India and other tropical countries. It is considered to be traditionally valuable plant due to its pharmacological activities. Different parts of this plant are used for the treatment of ulcers, haemorrhage, diabetes, wounds and boils. The plant also produces milky and sticky latex which has a vital role in wound healing. A study concerning the effect of proteases on haematological value of mice, wound healing response and anti-inflammatory conditions revealed that the clotting time and an erythrocyte sedimentation rate increased when treated with various dosages of isolated proteases. In case of the effect of proteases on wound healing responses, it was observed that the ointment containing 1% (w/w) of proteases showed better healing quality. The proteases also showed mild anti-inflammatory activity [23].

Hevea brasiliensis

Most commonly known as the rubber tree, *Hevea brasiliensis* belongs to the family Euphorbiaceae. It is a native to Brazil and Guinas. It belongs to a laticiferous family and hence produces milky latex which is the major raw material for natural rubber.

Using *Hevea brasiliensis* as the source of latex [24], its effect on vascular permeability and angiogenesis, two critical events of wound healing were observed. The serum showed enhanced vascular permeability and angiogenic activity, thus facilitating wound healing. Animal models were used for the purpose of study and the effect of serum on blood vessels was found to be more prominent at a concentration of 7.5 µg, showing an increase in the growth of blood vessels by 50-60%. However, the rate of wound healing significantly reduced when serum treated with proteases was used. Thus, it was concluded that a protein present in the serum influenced wound healing and that the activity of proteases was detrimental to the process.

Jatropha curcas

Jatropha curcas is a small flowering species with soft bark and milky latex. It belongs to the family of Euphorbiaceae and is commonly known as Purging Nut. It is native to an American tropic. *Jatropha curcas* is considered as a valuable plant as its seeds, leaves, flowers, nuts, bark, roots and latex have got various potential applications. The latex of this plant is used in the treatment of ulcers, bleeding gums, tumours and wounds [25].

When the latex of *Jatropha curcas* is applied directly on the wound, the bleeding soon stops. Studies showed that the latex of this plant has got coagulating activity. In the experiments carried out using the latex, it was noted that there was a significant reduction in the clotting time of human blood [26]. Another detailed study revealed that a proteolytic enzyme called curcain present in the latex of *Jatropha curcas* is associated with wound healing. The latex also exhibits anti-inflammatory property [27].

Pergularia extensa

Pergularia extensa is a hispid, perennial vine which belongs to the family Apocynaceae and sub family Asclepidaceae. It is native to Burma, India, Sri Lanka, Pakistan and Afghanistan and is generally known as Trellis-vine. This plant has got multiple pharmacological activities such as anti-inflammatory, anti-cancer, antioxidant, antifungal and so on. A study mainly concentrating on the cysteine proteases present in the latex of *Pergularia extensa* that exhibit thrombin like and plasmin like activities where in various assays were conducted which included caseinolytic activity, fibrinogen clotting assay and plasmin clot hydrolysis. The latex sample was assessed for proteolytic activity using denatured casein as substrate. Under these conditions, the latex proteases of *Pergularia extensa* showed an activity of about 4.5 U/mg/min and at a concentration of 10µg, indicated the need for 70s to convert fibrinogen to fibrin. Confirmation of presence of cysteine proteases was done by performing an inhibitor assay involving Iodoacetic acid. This study explained the involvement of cysteine proteases of *Pergularia extensa in* blood coagulation and fibrinolysis, thereby providing scientific validation for the use of *Pergularia extensa* latex to stop bleeding [17].

Plumeria rubra

Plumeria rubra Linn commonly known as red jasmine is a laticiferous tree belonging to the family Apocynaceae. It is native to Mexico and is found throughout India [28]. The latex of this tree has proteolytic enzymes in abundance. The extent of involvement of these proteases in wound healing was studied by [28]. The aim of their study was to investigate the anti-inflammatory and wound healing activities of the protease isolated from the latex of *Plumeria rubra* Linn.

The study involved the anti-inflammatory effects of the Plumerin-R, a protease isolated from the latex of this plant by acetone precipitation method. On administering Plumerin-R, either directly or in the form of ointments to rats with carrageenan-induced paw oedema, a significant increase in the rate of wound healing or decrease in the time taken for wound healing was observed. The results of the study showed that, administration of 80 mg/kg of Plumerin-R reduced carrageenan induced paw odema by 48.8% and application of 0.5% Plumerin-R ointment showed an increase in wound healing in an excision wound model when compared with the effect of the standard drug. Thus the study was concluded stating that the protease, Plumerin-R, extracted from latex of *Plumeria rubra* had significant anti-inflammatory and wound healing properties.

Synadenium grantii

Synadenium grantii, belonging to the Euphorbiaceae family, can be described as a succulent shrub with milky latex and is found to originate in eastern Africa. Hence the name African Milk Bush. The study on the latex of this plant has yielded contradictory results. While the latex is generally considered to be poisonous, some of its constituents, on the other hand, have proven to be beneficial.

A 34kDa latex glycoprotein was observed to possess various properties that could influence wound healing. At an incubation period of 240 min and concentration of 1.5µg, the latex glycoprotein shows maximum fibrinogenolytic activity. Also, while the latex glycoprotein fails to induce clot formation in purified fibrinogen, it acts effectively on citrated human plasma and significantly reduces the re-calcification time from 165s to 30s. Further, on performing an inhibitor assay, the latex glycoprotein was concluded to be a serine protease. The latex glycoprotein with protease activity exhibited heat-dependant fibrinogenolytic behaviour leading to the conclusion that the latex is a mixture of specific and non-specific proteases of which majority are heat labile. The study also suggested that, along with being involved in the blood coagulation cascade, the protease also exhibited clot dissolving property. These facts thus evidence the use of this latex in folk medicine for wound healing [29].

Wrightia tinctoria

Wrightia tinctoria also known as Ivory wood or Pala indigo plant is a small deciduous tree that belongs to the same laticiferous family Apocynaceae. It is native to India and Burma. It is a popularly used folk medicinal plant. The latex of this plant has been used extensively in the treatment of psoriasis, blisters, and mouth ulcers and also for topical applications on fresh wounds. A comparative study was carried out between the effects of latex proteases of *Wrightia tinctoria* and Neosporin, a standard drug on wound healing revealed the presence of thermo stable serine proteases, which were inhibited by Phenylmethylsulfonyl Fluoride (PMSF) but were relatively more effective in wound healing. Thus the study supported the topical application of *Wrightia tinctoria* latex on fresh wounds for enhanced wound healing [30].

CONCLUSION

Wound healing is a natural process occurring in the human body. The process of wound healing mainly takes place as a result of four precisely and highly programed phases namely haemostasis, inflammation, proliferation and remodelling. Various external agents can aid the natural process of wound healing. Plant kingdom is rich in chemical constituents that play a major role in the healing process. This review mainly concentrates on the role of latex of various plants that help in wound healing process. Most of the plants in this review are those with wound healing potential. The plants mentioned in this review can be

furthermore subjected to animal and human studies to determine their effectiveness. Intensive research related to protease will help in developing ointments or cosmetic creams containing the plant extracts which can be employed in wound healing.

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CONFLICT OF INTERESTS

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