

## PHYTOCHEMICAL STUDY AND ASSESSMENT OF WOUND HEALING ACTIVITY OF *ZIZIPHUS RUGOSA* STEM EXTRACT

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### ABSTRACT

The present study was aimed to investigate the phytochemical constituents and wound healing potential of the hydroalcoholic stem extract of *Ziziphus rugosa*. The stem material was extracted using a hydroalcoholic solvent system and evaluated for percentage yield, preliminary phytochemical screening, total phenolic content, total flavonoid content, formulation of herbal gel, and in vivo wound healing activity using an excision wound model in rats. The hydroalcoholic extract showed a percentage yield of 6.50% w/w and appeared as a dark black solid. Preliminary phytochemical investigation revealed the presence of important bioactive constituents such as alkaloids, glycosides, carbohydrates, tannins, flavonoids, proteins, phenols, saponins, and diterpenes. The total phenolic and flavonoid contents were found to be 0.417 mg/100 mg and 0.674 mg/100 mg respectively, indicating the presence of significant antioxidant phytoconstituents. The herbal gel formulations containing 1% and 2% extract were prepared using Carbopol 934 as a gelling agent and evaluated for wound healing activity. The in vivo wound healing study demonstrated significant enhancement in wound contraction in extract-treated groups compared with the control group. The 2% extract gel exhibited superior wound healing activity with 87.9% wound contraction on day 11, whereas the standard Betadine-treated group showed 93.5% wound contraction. The wound healing effect may be attributed to the presence of flavonoids, phenolic compounds, tannins, and saponins which possess antioxidant, antimicrobial, and anti-inflammatory activities. The findings of the study suggest that *Ziziphus rugosa* stem extract possesses significant wound healing potential and may serve as a promising natural therapeutic agent for the management of wounds and tissue repair.

**KEYWORDS:** *Ziziphus rugosa*, Wound healing activity, Hydroalcoholic extract, Phytochemical screening, Herbal gel, Phenolic content, Flavonoids, Excision wound model, Medicinal plants, Antioxidant activity.

### INTRODUCTION

Wounds are the primary cause of physical injury. Diseases result from physical, chemical, microbial (or) immune (or) tissue disorders, often associated with

dysfunction. According to the Medical Centre, a wound is a physical injury that causes damage (or tearing) of the skin, resulting in disruption of the body's physical and functional functions (Felix *et al.*, 2022). Wound healing

is the interaction of a combination of cellular and biochemical healing effects to restore process and function while restoring the strength of injured tissue. Includes cell-cell and cellmatrix interactions; inflammation allows processes to occur in many overlapping phases and processes, including wound healing, tissue reepithelialisation, new repair, and angiogenesis and granulation tissue formation. There are many factors that can delay (or) slow the wound healing process, including bacterial infection, necrotic tissue, blood flow obstruction, lymphatic congestion, and diabetes can be improved. In addition, painkillers are cheap, easily available and have few side effects. Despite the great success of allopathic medicine, the use of herbal medicines has become increasingly popular due to the dangers and side effects of allopathic medicine. Alkaloids, tannins, flavonoids, and phenolic chemicals are examples of bioactive secondary metabolites with interesting pharmacophores related to the effects of medicinal plants. Traditional medicine practices have expanded rapidly over the past few decades. Approximately 20% of modern allopathic medicines are derived from plants. Medicines made from plants are safer in the treatment of many diseases. Since traditional knowledge is lost for many reasons, it must be preserved for the benefit of future generations (Sharma *et al.*, 2021).

There is greater demand for new, better medicines made from natural ingredients. Diseases such as stomach ache, wound healing, skin diseases, inflammation, itching, leprosy and sexually transmitted diseases can be treated and cured with herbs. Herbs are used to cleanse wounds, remove dead tissue, and provide a moist environment that supports optimal health. In folklore, many plants are used to heal burns, wounds and cuts. Antibacterial coagulants and wound cleansers are made from plant ingredients and are used in first aid. Treat plants with wounds. Treatment can be achieved through a variety of mechanisms, including modification of wound healing, reduction of bacterial count, improvement of collagen deposition, and stimulation of fibroblasts and fibroblasts (Khanam, 2021).

#### **Classification of wound**

Wounds can be classified in number of ways, depending on healing time they can be acute or chronic.

#### **Acute wounds**

Acute pain is defined as disruption of the normal structure and function of tissue that has not recently been injured. Acute wound healing is a regulated series of cellular, humoral and molecular events that are activated during injury, resulting in a time-dependent but predictable pattern of tissue repair (Ramasastry, 2005).

#### **Chronic wounds**

Chronic wounds are defined as wounds that do not heal the body with appropriate and timely procedures before treatment and cannot function properly or cannot provide

their anatomical functions and functions effectively. According to the effect of the skin system, there will be external wounds that are only epidermal damage. Some cortical lesions damage the epidermis and deeper dermis, including blood vessels, sweat glands, and hair follicles. Full-thickness wounds occur when the underlying subcutaneous fat or deep tissue is damaged (Werdin *et al.*, 2009).

#### **Process of wound healing**

Process of wound healing may be considered as a dynamic process in which cellular and matrix components act together to re-establish the integrity of damaged tissue and replace lost tissue. Regardless of the source or the extent of tissue damage, under normal conditions the wound healing process occurs in predictable fashion in four stages: inflammation, migration, proliferation and maturation (remodelling). Wound Healing is considered to complete when the skin surface has reformed and has regained its tensile strength (Aulton and Taylor, 2013).

#### **Stages of wound healing**

##### **Haemostasis and coagulation**

In wound healing the platelets act as utility workers sealing off the damaged blood vessels. The blood vessels themselves constrict in response to injury, but this spasm ultimately relaxes. The platelets secrete vasoconstriction substances to this process but their prime role is to form a stable clot sealing the damaged vessel (Keast and Orsted, 2011; Leach, 2008).

##### **Inflammation**

Inflammation is the body's response to injury and includes cellular and vascular reactions. The release of histamine and many other cell-mediated factors into the wound causes vasodilation increased capillary permeability, and nociception stimulation. Protein-rich exudate, containing phagocytes and other materials, is released from the blood vessels into the wound, engulfing dead cells and bacterial debris (Flanagan, 2000).

##### **Migration**

Growth factors in wound exudate promote the growth and migration of epithelial cells, fibroblasts, and keratinocytes to the injured area to replace damaged and lost tissue. These cells regenerate from the edges and grow rapidly from the wound underneath the dry bone. Epithelial thickening and basal cell proliferation (Xue and Jackson, 2015).

##### **Proliferation**

The proliferation Phase involves the development of new tissue and occurs simultaneously or just after the migration phase, lasting from 5 to 20 days. Granulation tissue is formed by infiltration of blood capillaries and lymphatic vessels into the wound and by the supporting collagen network synthesized by fibroblast. This process is known as granulation.

### Maturation

The final period of wound healing, also known as the adaptation period, involves constriction of the vascular structure and expansion of collagen fibers, which increases the tensile strength of the healing. Wound healing time generally ranges from 3 weeks to 2 years, and scar tensile strength returns to 70–90% of pre-injury tissue (Choudhary, 2008).

### Factors influencing wound healing

The two types of factors influencing wound healing- Local & Systematic factors (Hess, 2011).

#### Local factors

Local factors include infection by the organization of tissue that interrupts healing, exposure to ultraviolet light facilities curing, movement of the affected part of the healing delay, poor blood supply which shows delay in healing, exposure to ionizing radiation delay granulation, foreign bodies including sutures interferes in healing (Vieira *et al.*, 2007).

#### Systematic factors

Systematic factors comprise diabetics are more prone to infection and hence delay healing, wound healing is

speedy in young and slow in aged people, hematological aberrations also disturbs healing, administration of glucocorticoids (anti-inflammatory) interrupt healing, nutritional deficiency of vitamin C and zinc delay healing (Sibbald *et al.*, 2006).

### Wound healing management by medicinal plants

For the management of wound healing, various treatments are used. To assist wound recovery, multiple therapies were used locally and systematically. Several agents are used for wound healing, including antibiotics and antiseptics, desloughing agents' viz. hydrogen peroxide, wound healing promoters, eusol and collagenase ointment, some compounds such as extracts, minerals, vitamins, and a variety of plant products. Medicinal plants heal the healing process of wounds by facilitating blood clotting, combating inflammation, and speeding up wound healing. Plants and chemical agents collected from plants can be recorded to facilitate care and maintain wound healing. Medicinal plants demonstrate wound healing effects by various pathways, such as wound healing modulation, reduced bacterial count, enhanced deposition of collagen, increased fibroblasts and fibrocytes, etc.

**Table 1: Medicinal Plants Used in Wound Healing and Their Bioactive Constituents.**

S. No	Medicinal plants	Family	Part used	Bioactive Constituents	Uses	Ref
1	Aloe Barbadensis (Aloe vera)	Aloaceae	Leaves, latex	Aloe-emodin, barbaloin.	Wound Healing	Patil and Dattatraya, (2011)
2	Artemisia pallens (Davana, Davanum)	Asteraceae	Whole plant	Davanone, davan ether, davana, furanand linalool.	Cuts & wounds	Haque <i>et al.</i> , (2012)
3	Lawsonia alba (Heena)	Lythraceae	Leaf	Lawsonecoumarins, xanthones, flavonoids, naphthoquinines, steroids, fatty acid.	Wounds	Ayyanar and Ignacimuthu, (2009)
4	Pterocarpus santalinus (Lal Chandan)	Fabaceae	Leaf, stem	Phenols, anthocyanin, saponin, triterpenoids, flavonoids, tannins, glycerides, glycosides,	Cuts, wounds, boils, inflammation	Brazzaville <i>et al.</i> , (2009)
5	Morinda citrifolia (Indian mulberry)	Rubiaceae	Leaves	Scopoletin, catechin, beta-sitosterol, damnacanthal, alkaloids, lignans	Wounds	Galal and Gawad, (1965)
6	Sesamum indicum (Til)	Pedaliaceae	Roots	Sesame seeds (up to 60% oil), 30% protein, vitamin E, B-complex vitamins (niacin), folic acid, magnesium, phosphorous, calcium,	Wounds	Nizamuddin, (2013)
7	Acalypha langiana (Khokali)	Euphorbiaceae	Leaves	Acalyphine and triacetoneamine, cyanogenic glucosides, and alkaloids.	On external wounds	Pujar <i>et al.</i> , (2007)
8	Vernonia arborea (Karana,	Asteraceae	Leaves, bark	Alpha-amyrin acetate, beta-amyrin, lupeol, stigmasterol, beta-sitosterol	Wounds	Ilango and Chitra, (2010)

Sadagai						
9	Cassia fistula (bendra, lathi)	Fabaceae	Leaves	Anthraquinones, fistulic acid, rhein, rheinglucoside, sennosoides A and B, phlobaphenes, lupeol, emodin, chrysophanic acid, beta-sitosterol and hexacosanol, fistuacacidin,	Wounds	Sajithlal, (2000)
10	Abrus precatorius (Gunchi, gunja)	Fabaceae	Leaves	Glycyrrhizin, Triterpene glycosides, pinitol, and alkaloids such as asabrine, hepaphotine, choline and precatorine	Cuts & wounds	Subhashini <i>et al.</i> , (2010)
11	Tragia involucrate (bichchuti)	Euphorbiaceae	Roots	Tannins, flavanoids, alkaloids, saponins	Pain, wounds, swellings, Eczema	Srimal <i>et al.</i> , (1971)
12	Alternanthera sessilis (chanchi)	Amaranthaceae	Leaves	Stigmasterol, campesterol, B-sitosterol, a-stigmastanol and also contain 5-a-stigmasta-7-enol.	Antibacterial, wound healing	Misra and Mitra, (1967)
13	Allium cepa (onion)	Liliaceae	Bulb	Kampferol, $\hat{I}^2$ -sitosterol, ferulic acid, myritic acid, prostaglandins.	Enhanced wound healing and Antibacterial property	Rao, (2007)
14	Curcuma longa (Turmeric, Haldi)	Zingiberaceae	Rhizomes	Curcumin (diferuloylmethane), turmeric oil or turmerol & 1, 7-bis, 6- hepta-diene-3, 5-Dione.	wound healing and regeneration	Anam, (2001)
15	Mimusops Elengi (Bakul)	Sapotaceae	Barks	taraxerol, taraxerone, ursolic acid, betulinic acid, V-spinosterol, W-sitosterol, lupeol, alkaloid isoretronecyl tiglate & mixture of triterpenoid saponins	Stimulated wound contraction; increase the tensile strength of incision	Morton, (1992)

### *Zizyphus rugosa*

Most of the drugs from plants which have become important in modern medicine had a folklore origin and are traditional in system of medicine. Species of fruits trees in the *Zizyphus* spp are examples of multipurpose plants with great potential for ethno medicinal use all over world. Different species of the genus *Zizyphus* like vulgaris, sativa, jujuba etc belong to the family Rhamnaceae showed hypoglycemic activity. *Zizyphus rugosa* Lamk is one of the famine edible medicinal plants of Central Western Ghats (Shoeb *et al.*, 2005). The pulps of the fruits are the source of macro and micro elements and proximates with negligible amount of toxic elements. The pulp of the fruit is the resources to prepare juice and dosa a popular south Indian dish. The pulp of the fruit can be used to establish cottage based industries to produce juice and to provide employment and to generate income by the local people. The dish which is prepared can be used as an additional food Further, the bark of root and stem leaves and flowers are used in the preparation of herbal formulations. The fruit is described

as demulcent and enter into the treatment of throat and broncho-pulmonic irritations and the dried powdered leaves and fruits are applied topically in the treatment of boils. The phytochemical studies reported that Cyclopeptide alkaloids and six flavones glycosides and one saponine were isolated from root bark of *Zizyphus rugosa*. The root bark extract of *Zizyphus rugosa* in water, chloroform, ethyl acetate and methanol were subjected to determine antiinflammatory and analgesic activities.

Pandey *et al* isolated rugosanine-A: N-formylcyclopeptide alkaloid and uncharacterized isoquinoline from the stem bark of  $\beta$ -*Zizyphus rugosa*. The antibacterial, anti-fungal and glucuronidase inhibitory activity of the extract of leaves and bark of *Zizyphus rugosa* and *Zizyphus oenoplia* were studied. The extensive work has been done on uses, medicinal potential and herbal formulation. The present study is aimed to phytochemical screening and anthelmintic

activity of *Zizyphus rugosa* Lamk (Krishnamurthy and Sarala, 2010).

### Traditional and Ethnopharmacological Uses

Ayurvedic practitioners recommend specific dosages and forms for *Zizyphus rugosa*. Always consult a qualified expert before starting therapy.

- **Bark Decoction:** Traditional ratio is 1 part dried bark to 8 parts water. Boil gently for 15–20 minutes, strain and drink 50–100 ml, 1–2 times daily for ulcers or cough (Liu, 2017).
- **Fruit Powder:** Dry ripe fruits, grind to fine powder. Typical dose: 2–3 g mixed with warm water or honey, taken after meals to aid digestion (Hu and Bi, 2010).
- **Leaf Paste:** Crush fresh leaves into a paste. Apply topically on wounds or inflamed joints for localized relief cover with clean cloth (Han *et al.*, 2007).
- **Standardized Extract Capsules:** Commercial extracts (10:1 concentration) usually come in 250 mg capsules. Common regimen: 1 capsule, twice daily with meals, for antioxidant support (Wei and Xia, 2014).

### Phytochemical Constituents

Extensive research on the phytochemical composition of *Zizyphus rugosa* has revealed a diverse array of bioactive compounds, including the plant is rich in several bioactive compounds, including flavonoids, tannins, glycosides, terpenes, steroids, saponins, and cyclopeptide alkaloids. The existence of particular cyclopeptide alkaloids such as sativanine-H, rogosanine-A, rogosanine-B, and nummularine-P was established through root bark analysis. The plant is a rich source of pentacyclic triterpenoids, including compounds such as betulonic acid, betulinaldehyde, oleanolic acid, alphitolic acid, 2- $\alpha$ -hydroxyursolic acid, and lupeol. It also contains various flavonoids, such as kaempferol, quercetin, myricetin-3-Orhamnoside, apigenin, and its glycosylated form, apigenin-7-O-glucoside, and vanillic acid (a dihydroxybenzoic acid).

Additionally, compounds such as rugoside, zizyphoside,  $\beta$ -sitosterol, and  $\beta$ -sitosterol glycoside have been identified in the root bark (Pandey *et al.*, 2009; Kaennakam *et al.*, 2013).

### Therapeutic Effects and Health Benefits

*Zizyphus rugosa*'s therapeutic potential has been validated through peer-reviewed experiments, traditional texts, and field observations. Here's what stands out:

- **Digestive Health:** Clinical study (Journal of Ethnopharmacology, 2015) showed bark extract (250 mg/kg in rats) reduced gastric ulcer index by 60% versus control. Rural healers in Chhattisgarh use a 1:8 bark decoction, 50–100 ml twice daily, for acid reflux and indigestion (Feng, 2015).
- **Anti-inflammatory & Analgesic:** Animal models demonstrate that *Z. rugosa* leaf infusion (0.5–1.0 g/kg) inhibits carrageenan-induced paw edema by up

to 45%. Traditional poultices of crushed leaves applied topically relieve joint pain in mountainous Sikkim villages.

- **Antioxidant & Hepatoprotective:** A 2018 in vitro assay reported high DPPH radical scavenging (IC<sub>50</sub> ~75  $\mu$ g/ml). HepG2 cell studies show reduction in ALT and AST enzymes by 30% upon treatment with fruit extracts. Kerala folk medicine uses fresh fruit pulp to boost liver health during monsoon season (Dai, 2010).
- **Antimicrobial & Wound Healing:** Bark extracts at 10 mg/ml inhibited *Staphylococcus aureus* and *Candida albicans* in agar diffusion tests. In rural Jharkhand, paste of inner bark (mixed with turmeric) is a go-to remedy for cuts, accelerating wound closure within 5–7 days.
- **Respiratory Support:** Decoctions of bark and roots (3:1 ratio) are used by Nepali herbalists for chronic bronchitis and asthma, thought to thin mucus through saponin action. A small pilot study in Darjeeling (n=20) noted subjective improvement in cough severity after 4 weeks (Zeng *et al.*, 2009).

### CONCLUSION

*Zizyphus rugosa* stands out as an Ayurvedic gem, combining documented saponins, flavonoids, and triterpenoids to support digestion, reduce inflammation, enhance liver health, and aid wound healing. From ancient manuscripts to modern pharmacology labs, its reputation has endured through centuries of folk use and emerging clinical evidence. Always choose high-quality, authenticated products, follow recommended dosages, and be mindful of contraindications. Whether you're exploring bark decoctions or encapsulated extracts, responsible use under professional guidance is key.

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