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

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ABSTRACT

The present study was undertaken to investigate the phytochemical constituents and wound healing activity of the hydroalcoholic stem extract of *Ziziphus rugosa*. Medicinal plants are widely recognized for their therapeutic importance due to the presence of bioactive phytoconstituents with minimal adverse effects. The hydroalcoholic extract of *Ziziphus rugosa* stem was prepared and evaluated for extractive value, 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 extraction yielded 6.50% w/w dark black solid extract, indicating the successful extraction of phytoconstituents. Preliminary phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, tannins, phenols, carbohydrates, proteins, saponins, and diterpenes. The total phenolic content and total flavonoid content were found to be 0.417 mg/100 mg and 0.674 mg/100 mg respectively, suggesting appreciable antioxidant potential of the extract. Herbal gel formulations containing 1% and 2% extract concentrations were prepared using Carbopol 934 as the gelling agent. The wound healing activity demonstrated significant enhancement in wound contraction compared to the control group. The 2% extract gel showed superior wound healing activity with 87.9% wound contraction on day 11, while the standard Betadine-treated group exhibited 93.5% healing. The enhanced wound healing activity may be attributed to the antioxidant, anti-inflammatory, antimicrobial, and tissue regenerative properties of the phytoconstituents present in the extract. The findings of the study scientifically support the traditional use of *Ziziphus rugosa* in wound management and suggest its potential as a promising natural wound healing agent.

Keywords: *Ziziphus rugosa*, Wound healing, Hydroalcoholic extract, Herbal gel, Flavonoids, Phenolic compounds.

INTRODUCTION

Medicinal plants have been used since ancient times as an important source of therapeutic agents for the prevention and treatment of various diseases. Herbal medicines play a significant role in traditional healthcare systems due to their effectiveness, accessibility, affordability, and relatively fewer side effects compared to synthetic drugs (Shakya, 2016). In recent years, there has been growing scientific interest in medicinal plants because of their diverse pharmacological activities and the presence of biologically active phytoconstituents such as alkaloids, flavonoids, tannins, phenolics, glycosides, terpenoids, and saponins. These

phytochemicals possess a wide range of biological properties including antioxidant, anti-inflammatory, antimicrobial, anticancer, antidiabetic, hepatoprotective, and wound healing activities (Houghton, 1995).

Wound healing is a complex and dynamic biological process that involves a series of overlapping phases including hemostasis, inflammation, proliferation, collagen synthesis, epithelialization, and tissue remodeling. Proper wound healing is essential for restoration of the structural and functional integrity of damaged tissues (Rodrigues *et al.*, 2019). Delayed or impaired wound healing may occur due to microbial infection, oxidative stress, diabetes, malnutrition, inflammation, or poor vascularization, leading to chronic wounds and tissue damage. Conventional wound healing agents such as antibiotics, antiseptics, and corticosteroids are commonly used for wound management; however, prolonged use of these synthetic agents may produce adverse effects including allergic reactions, microbial resistance, skin irritation, and delayed tissue regeneration. Therefore, there is increasing demand for safer and more effective natural wound healing agents derived from medicinal plants (Guo and DiPietro, 2010).

Among various medicinal plants, *Ziziphus rugosa* is an important ethnomedicinal plant belonging to the family Rhamnaceae. The plant is widely distributed in tropical and subtropical regions and has been traditionally used for the treatment of wounds, inflammation, fever, skin disorders, ulcers, diarrhea, and microbial infections. Different parts of the plant including leaves, bark, roots, stems, and fruits are reported to possess various therapeutic properties due to the presence of diverse secondary metabolites. The stem of *Ziziphus rugosa* is particularly rich in phytoconstituents that may contribute to its medicinal value and pharmacological activities (Imran *et al.*, 2023).

Phytochemical constituents present in *Ziziphus rugosa* such as flavonoids, tannins, phenolic compounds, alkaloids, glycosides, and saponins are known to exhibit antioxidant, antimicrobial, anti-inflammatory, and tissue regenerative activities. Antioxidants help neutralize reactive oxygen species and reduce oxidative stress at the wound site, thereby accelerating tissue repair and collagen synthesis. Flavonoids and phenolic compounds also enhance fibroblast proliferation, angiogenesis, and epithelialization, which are essential steps in the wound healing process. Tannins promote wound contraction by forming a protective layer over damaged tissues and preventing microbial contamination. In addition, antimicrobial properties of the plant help prevent infection and support faster healing.

Despite its traditional medicinal importance, limited scientific studies are available regarding the phytochemical composition and wound healing potential of *Ziziphus rugosa* stem extract. Therefore, systematic phytochemical evaluation and biological assessment are necessary to validate its traditional claims and establish its therapeutic significance. The present study was undertaken to investigate the phytochemical constituents present in the hydroalcoholic stem extract of *Ziziphus rugosa* and to evaluate its wound healing activity using suitable experimental models. The study aims to provide scientific evidence for the traditional use of the plant and explore its

potential as a natural wound healing agent for future pharmaceutical applications.

MATERIAL AND METHODS

Material

Fresh stems of *Ziziphus rugosa* were collected and authenticated for the present study. Chemicals and reagents used during the investigation included Carbopol 934, propylene glycol, glycerin, methyl paraben, propyl paraben, triethanolamine, ethanol, methanol, ferric chloride, lead acetate, copper acetate, gelatin, sodium hydroxide, Benedict's reagent, Fehling's solution, Folin–Ciocalteu reagent, gallic acid, quercetin, and other analytical grade chemicals procured from standard suppliers. Betadine ointment was used as the standard drug for wound healing evaluation. Experimental rats were used for the in vivo wound healing study under standard laboratory conditions.

Methods

Extraction by maceration method

Powdered plant materials were weighed (50 gm) and packed in (250 ml) air tight glass Bottle. The plant materials were subjected to extraction by hydroalcoholic (ethanol: water; 65:35) as solvent for about 24 hrs (Kokate, 1994). The liquid extract was collected in a tarred conical flask. The solvent removed from the extract by evaporation method. The extracts obtained with hydroalcoholic solvent were weighed to a constant weight and percentage w/w basis was calculated.

Determination of percentage yield

The percentage yields of extract were calculated by using following formula:

Percentage yield = Weight of Extract/ Weight of powder drug Taken x 100

Phytochemical analysis

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the different extracts of stem of *Ziziphus rugosa*, were subjected to the phytochemical tests as per standard methods. Phytochemical screening was revealed for the presence of alkaloids, glycosides, carbohydrates, tannins, resins, flavonoids, steroids, proteins and amino acids (Audu *et al.,* 2007).

Quantitative estimation of phenols and flavonoids

Estimation of total phenol content

Estimation of total phenol content Total phenol content of the extracts was evaluated with folin-ciocalteu method (Mishra *et al.*, 2017). Samples containing polyphenols are reduced by the folin-ciocalteu reagent there by producing blue colored complex. The phenolic concentration of extracts was evaluated from a gallic acid calibration curve. To prepare a calibration curve, 2 ml aliquots of 5, 10, 15, 20, and 25 µg/ml gallic acid solutions were mixed with 1ml folin–ciocalteu reagent (diluted ten-fold) and 1 ml (7.5 g/L) sodium carbonate. After incubation at 25°C for 10 min, the quantative phenolic estimation was performed at 765 nm against reagent blank by UV Spectrophotometer. The calibration curve was constructed by putting the value of absorbance vs. concentration. A similar procedure was adopted for the extract (1000µg/ml) as above described in the preparation of calibration curve. All determinations were performed in triplicate. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) mg per 100mg of extract.

Estimation of total flavonoids content

The aluminum chloride colorimetric method was modified (Campolo *et al.,* 2022).

Quercetin was used to make the calibration curve. Ten milligrams of quercetin was dissolved in 10 ml methanol and then diluted for 5-25 µg/ml. The diluted standard solutions (2 ml) were separately mixed with 1 ml of 2% aluminum chloride. After incubation at room temperature for 10 min, the absorbance of the reaction mixture was measured at 420 nm with a UV spectrophotometer. Similarly, extracts solutions (1000 µg/ml) were reacted with aluminum chloride for determination of flavonoid content. Total flavonoid content was expressed as milligrams of Quercetin equivalent (QE) mg per 100mg of extract.

Formulation of gel

Method of Preparation

In order to prepare the gel formulation, first dissolve carbopol 940 in distilled water. Next, add methyl paraben, propyl paraben, and glycerine, and let the mixture sit overnight. Consider the leaf extracts in propylene glycol, to which polymer dispersion was added later. After that, the remaining water was added, then triethanolamine was added while stirring constantly to get the pH down to 7.

Table 1: Formulation of Gel

S. No.	Ingredients	F1	F2
1	*Ziziphus rugosa* Extract (%)	1	2
2	Carbopol 934 (%)	1	1
3	Propylene Glycol (ml)	8	8
4	Methyl paraben (%)	0.4	0.4
5	Propyl paraben (%)	0.2	0.2
6	Triethanolamine (ml)	qs	qs
7	Glycerin (ml)	1	1
8	Distilled water (ml)	Upto 100 ml	Upto 100 ml

***In vivo* wound healing activity of gel**

Animals

Wistar rats weighing between 180-200 g with no prior treatment were used for the study. Animals were housed in polypropylene cages maintained at 22 ± 2°C temperature with 12 h light and 12 h dark cycle. The animals were fed on a standard pelleted diet and had free access to water throughout the experiment. Animals that are described as fasting were deprived of food for at least 16 h but allowed free access to drinking water. The animal study was approved by the Institutional Animal Ethics Committee, as stated by prescribed guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Acute dermal toxicity

Acute dermal toxicity was determined, according to OECD 404, in rats (OECD, 2002) Approximately 24 hours before the test, fur was removed by closely clipping the dorsal area of the trunk on both flanks using a suitable depilatory material. Special attention was given not to abrade the skin. Skin was washed with sterile water a day before the toxicity test procedure. A limit test dose of 2,000 mg/Kg of the 10% (w/w) gel was applied on the shaved back of the rats within a range that was approximately 10% of the body surface area and held in contact to the skin with a gauze patch to facilitate good contact and uniform distribution of the test chemical on the skin for the duration of the exposure period (24 hours). The test was first conducted in a single rat for initial testing, and then after observing the response of the first rat for 24 hours a confirmatory test was done on another two rats simultaneously.

At the end of the exposure period, the gel was removed and the animals were observed for 14 days for the development of any adverse skin reactions like inflammation, irritation, or redness. Behavior, general condition, posture and reflexes, attitude towards food, and water were also evaluated.

Experimental model

Male Sprague-Dawley rats were divided into four groups of six rats each and housed separately in individual cages. Before the creation of wounds, the animals were anaesthetised with ketamine (30 mg/kg, i.m.) and xylazine (3 mg/kg, i.m.). To create the wound, an electrical shaver was employed to shave the dorsal neck of each rat. A mark was made in the dorsal thoracic region, one centimetre away from the vertebral column and five centimetres from the ear. An excision wound was created by cutting out the full thickness of skin using a circular stamp with a diameter of two centimetres (Latif *et al.*, 2015).

Experimental designs

Wounds in the control group, designated as (Nayak *et al.*, 2006):

Group 1, were treated with normal saline (0.2 ml) twice daily.

The reference standard control, labelled as Group 2, involved rats that were given 0.2 ml of Betadine twice daily.

Rats in Groups 3 and 4 received *Ziziphus rugosa* extract gel (1% and 2%, respectively) twice daily, and no dressing was applied to the wound site.

Assessment of degree of wound healing

Progressions of the wound healing were assessed on days 0, 3, 7, and 11 post-wound creation. Wound area was traced by a transparent tracing paper placing over the wound, which was then placed on a sheet of graph paper (2 mm^2) to count the number of squares within the wound area (Ponrasu *et al.*, 2020). The percentage of wound closure was measured using the following formula:

Wound Closure (%) = [(Wound Area at Day 0 - Wound Area at Later Day) / Wound Area at Day 0] X 100

Data Analysis

The results were analyzed using one-way Analysis of Variance (ANOVA), which is a statistical method used to compare the mean values of more than two groups simultaneously. One-way ANOVA helps in determining whether there is any statistically significant difference among the experimental groups with respect to the measured biological and biochemical parameters.

RESULTS AND DISCUSSION

The present study was carried out to evaluate the phytochemical constituents and wound healing activity of the hydroalcoholic extract of *Ziziphus rugosa*. The results obtained from extraction, phytochemical screening, estimation of total phenolic and flavonoid content, formulation of herbal gel, and *in vivo* wound healing studies demonstrated promising therapeutic potential of the plant extract.

The hydroalcoholic extraction of *Ziziphus rugosa* yielded a dark black solid extract with a percentage yield of 6.50% w/w as shown in Table 2. The hydroalcoholic solvent system was found suitable for extracting both polar and moderately non-polar phytoconstituents from the plant material. The dark coloration of the extract may be attributed to the presence of phenolic compounds, flavonoids, pigments, and other secondary metabolites concentrated during the extraction process.

The obtained extractive value indicates the presence of appreciable amounts of soluble bioactive compounds, which may contribute to the pharmacological activity of the plant.

The preliminary phytochemical screening presented in Table 3 revealed the presence of several important phytoconstituents including alkaloids, glycosides, carbohydrates, tannins, flavonoids, proteins, phenols, saponins, and diterpenes in the hydroalcoholic extract. Positive results observed in Wagner's test confirmed the presence of alkaloids, while Legal's test indicated glycosides. Flavonoids were strongly present as evidenced by positive Lead acetate and Alkaline reagent tests. Phenolic compounds were confirmed by Ferric chloride test, whereas saponins and diterpenes showed positive reactions in Froth and Copper acetate tests respectively. These phytoconstituents are known to possess antioxidant, antimicrobial, anti-inflammatory, and wound healing activities, which may be responsible for the observed biological effects of the extract.

The quantitative estimation of total phenolic content and total flavonoid content further supported the phytochemical screening results. As shown in Table 4, the hydroalcoholic extract exhibited total phenolic content of 0.417 mg/100 mg, whereas the total flavonoid content was found to be 0.674 mg/100 mg as shown in Table 5. Phenolic compounds and flavonoids are well known for their potent antioxidant properties and ability to scavenge free radicals. Oxidative stress is considered one of the major factors responsible for delayed wound healing and tissue damage. Therefore, the presence of appreciable amounts of phenolic and flavonoid compounds suggests that the extract possesses strong antioxidant potential, which may contribute significantly to tissue regeneration and wound repair.

The herbal gel formulations containing *Ziziphus rugosa* extract were successfully prepared using Carbopol 934 as a gelling agent, as shown in Table 1. Carbopol-based gels are widely used in topical formulations because of their good viscosity, spreadability, stability, and patient acceptability. Propylene glycol and glycerin were incorporated to improve consistency, moisturizing properties, and drug permeation, while methyl paraben and propyl paraben were used as preservatives. Triethanolamine was added to adjust the pH and obtain a suitable gel consistency. Two formulations containing 1% and 2% extract concentrations were prepared for evaluation of wound healing activity.

The wound healing activity of *Ziziphus rugosa* extract gel was evaluated using an excision wound model in rats, and the results are presented in Table 6. The control group treated with normal saline exhibited slow wound contraction throughout the experimental period, showing only 58.2% wound healing on day 11. The standard group treated with Betadine showed rapid wound closure with 93.5% healing on day 11, confirming the effectiveness of the standard treatment.

The extract-treated groups demonstrated significant wound healing activity in a dose-dependent manner. The 1% extract gel formulation showed 81.3% wound healing on day 11, whereas the 2% extract gel produced 87.9% wound contraction during the same period. The higher concentration of extract exhibited better wound healing activity, indicating enhanced therapeutic efficacy due

to increased availability of bioactive constituents. The progressive increase in wound contraction in treated groups suggests accelerated tissue regeneration, collagen synthesis, fibroblast proliferation, and epithelialization.

The significant wound healing activity observed in the study may be attributed to the synergistic action of phytoconstituents present in the extract. Flavonoids and phenolic compounds possess antioxidant properties that help neutralize reactive oxygen species generated at the wound site, thereby reducing oxidative stress and promoting tissue repair. Tannins may facilitate wound contraction by precipitating proteins and forming a protective layer over the wound surface. Saponins are reported to enhance collagen formation and angiogenesis, while alkaloids and diterpenes may contribute to antimicrobial and anti-inflammatory effects, thereby preventing infection and reducing inflammation at the wound site.

The findings of the present study scientifically validate the traditional use of *Ziziphus rugosa* in wound management and demonstrate that the hydroalcoholic stem extract possesses significant phytochemical and wound healing potential. The results suggest that *Ziziphus rugosa* extract gel may serve as a promising natural topical formulation for the treatment and management of wounds.

Table 2: Extractive values obtained from *Ziziphus rugosa*

S. N.	Extract	Colour	% Yield (w/w)
1.	Hydroalcoholic	Dark black solid	6.50

Table 3: Preliminary phytochemical screening of *Ziziphus rugosa*

S.N.	Phyto-constituents	Test Name	Hydroalcoholic extract
1	Alkaloids	Mayer's Test	-ve
		Dragendorff's Test	-ve
		Wagner's Test	**+ve**
		Hager's Test	-ve
2	Glycosides	Raymond's Test	-ve
		Killer Killani Test	-ve
		Legal Test	**+ve**
3	Carbohydrates	Molisch's Test	-ve
		Fehling's Test	**+ve**
		Benedict's Test	**+ve**
4	Tannins	Vanillin- HCl Test	-ve
		Gelatin Test	**+ve**
5	Flavonoids	Lead acetate Test	**+ve**
		Alkaline Reagent Test	**+ve**
6	Resins	Turbidity Test	-ve
7	Steroids	Libermann-Bur chard Test	-ve
		Salkowski Reaction	-ve
8	Proteins & Amino acids	Biuret Test	**+ve**
		Precipitation test	-ve

9.	Phenols	Ferric chloride test	**+ve**
10.	Saponins	Froth Test	**+ve**
11.	Diterpenes	Copper acetate Test	**+ve**

[+ve = positive; -ve = negative]

Table 4: Total phenol content of hydroalcoholic extract of *Ziziphus rugosa*

S. N.	Extract	Total phenol content (mg/100mg)
1	Hydroalcoholic extract	0.417

Table 5: Total flavonoid content of hydroalcoholic extract of *Ziziphus rugosa*

S. N.	Extract	Total Flavonoid content (mg/100mg)
1	Hydroalcoholic extract	0.674

Table 6: Evaluation of wound healing activity of *Ziziphus rugosa* extract gel on rats

	% Wound Healing			
Groups	**Day 0**	**Day 3**	**Day 7**	**Day 11**
I (Normal saline)	0.0 ± 0.0	16.8 ± 2.1	39.5 ± 2.8	58.2 ± 3.2
II (Betadine)	0.0 ± 0.0	35.4 ± 2.4	72.6 ± 2.6	93.5 ± 2.1***
III (*Ziziphus rugosa* extract gel 1%)	0.0 ± 0.0	24.6 ± 2.7	56.8 ± 3.0	81.3 ± 2.8*
IV (*Ziziphus rugosa* extract gel 2%)	0.0 ± 0.0	29.7 ± 2.5	64.2 ± 3.1	87.9 ± 2.5**

A one-way ANOVA was used for testing comparison with controls, and significance was assessed. Significance was defined as $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$. All values are expressed as mean ± SD.

CONCLUSION

The present study concluded that the hydroalcoholic stem extract of *Ziziphus rugosa* possesses significant phytochemical constituents and promising wound healing activity. The extract showed the presence of important bioactive compounds such as flavonoids, phenols, tannins, alkaloids, saponins, and glycosides, which may contribute to its therapeutic effects. The herbal gel formulation exhibited significant wound contraction and accelerated healing in rats, particularly at the 2% extract concentration. The findings scientifically support the traditional use of *Ziziphus rugosa* in wound management and suggest its potential as a natural wound healing agent for future pharmaceutical applications.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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