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Development and Investigation of a Herbal Extract for Diabetic and Non-Diabetic Wound Healing

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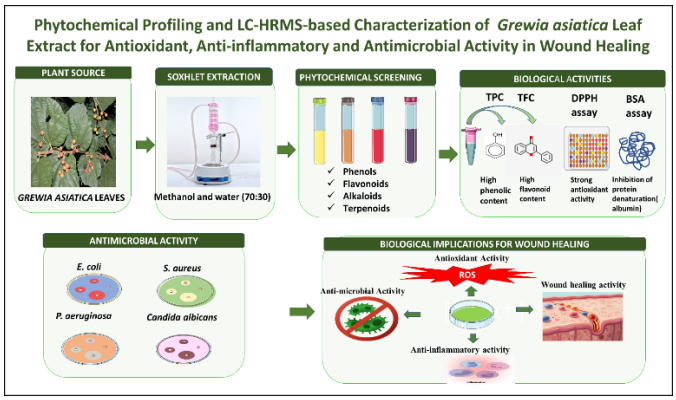
ABSTRACT: Grewia asiatica L. leaves were processed and extracted using a hydroalcoholic (70:30 v/v) solution. Phenolic compounds, flavonoids, tannins, alkaloids, terpenoids, glycosides, saponins, proteins, and carbohydrates were detected with a yield of 12.46% (w/w). The total flavonoid and phenolic contents, obtained using the Folin–Ciocalteu and AlCl3 assays, respectively, were substantial. The extract providing evidence for robust DPPH radical scavenging activity increasing with concentration. The results revealed a substantial anti-inflammatory effect in the bovine serum albumin protein denaturation assay, which was more optimal than that of diclofenac at comparable concentrations. Antimicrobial activity was tested using the agar diffusion method, and the zones of inhibition encompassed a broad spectrum of activity, with Pseudomonas aeruginosa remaining the most resistant, with zones of inhibition of 12.50 ± 0.50 and 12.17 ± 0.29 mm at the concentrations of 200 and 100 mg/mL, respectively. In contrast, other microorganisms, such as Escherichia coli, Staphylococcus aureus, and Candida albicans, were more susceptible. The polyphenolic antioxidants, anti-inflammatory , and antimicrobial activities observed in G. asiatica leaf extract can excavate probabilities for its use in topical wound healing products, particularly for diabetic and infected wounds.

Graphical Abstract



1 INTRODUCTION

Wound repair is an active and complex cellular process involving hemostasis, inflammation, proliferation, and tissue remodeling. Long-term conditions, such as diabetes, are characterized by impaired wound healing and often exhibit high levels of oxidative

stress, chronic inflammation, microbial infections, and reduced levels of angiogenesis and collagen production. The phytochemical components of Grewia asiatica L., such as antioxidants, anti-inflammatory agents, antibacterial compounds, and bioactive substances, play a pivotal role in the process of healing. Grewia asiatica L. is a small tree or deciduous shrub, 3-5 m tall, belonging to the Malvaceae family. It is traditionally used throughout South Asia for its medicinal benefits, in addition to yielding edible fruits popularly called Phalsa. The plant is characterized by ovate, serrated leaves, yellow flowers, and small purple fruits containing anthocyanins. Its ethnomedicinal applications include treating skin diseases, gastrointestinal diseases, respiratory system diseases, wound healing, and fever.(1)

Phytochemical studies on Grewia asiatica L. have identified a variety of bioactive natural phytochemicals including phenolic acids, anthocyanins, flavonoids, tannins, alkaloids, glycosides, terpenoids, and saponins. The major constituents are gallic acid, chlorogenic acid, ferulic acid, derivatives of cyanidin, catechin, kaempferol, and quercetin, all of which exhibit high antioxidant activity. The studies have shown that Grewia asiatica L. possesses antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective, cardioprotective, anticancer, and wound healing activities. These properties can reduce the risk of wound infections, mitigate oxidative injury, and promote tissue regeneration. The wound healing properties of G. asiatica may promote collagen deposition, stimulate fibroblast proliferation, enhance angiogenesis, and inhibit proinflammatory mediators. Together, these effects result in epithelial regrowth and the production of the extracellular matrix, suggesting it could be a natural ingredient in topical wound healing formulations.(2)

In the literature, it is found that Grewia asiatica L. confers the most marked antibacterial activity against Klebsiella pneumoniae, exhibiting an MIC of 3.90 μg/mL. The antifungal activity was prominently observed with two fungal species, Aspergillus fumigatus and Candida albicans, with a recorded MIC value of 31.2 μg/mL. Existing research has focused on the individual effects of specific plants on wound healing properties. Few studies has investigated their use in topical delivery in-situ gels, with the objective to optimize their duration of stay on the wound and improve contact time with the skin.(3) Their significance in diabetic wound healing has hardly been explored to address the need to treat multiple components of oxidative stress plus microbial load, and impaired collagen synthesis.

2 MATERIALS AND METHODS

2.1 Plant Material

In October–November 2024, *Grewia asiatica* L. leaves were collected and identified by the Indian Council of Agricultural Research, Central Institute for Subtropical Horticulture, Lucknow (ICAR-CISH). The identification was conducted in association with Dr. Sushil Kumar Shukla (Principal Scientist).

2.2 Extraction

The leaves from the plant were dried in a shaded location for a week at room temperature to prevent the loss of volatile substances. The dried leaves of *Grewia asiatica* L. were crushed and triturate using a mortar and pestle. Subsequently, a 60 mesh sieve was selected to grade the dry powders, which were then stored at a cool temperature for later use.(4)The powdered leaves were continuously extracted by means of Soxhlet extractor with methanol and water (70:30) at a temperature of 65 °C. The extracted value of the selected plant materials was quantified using the following formula: (Weight of extract/weight of dried plant material) x 100.

2.3 Phytochemical screening

Using traditional chemical methods, a preliminary qualitative screening of *Grewia asiatica* L. leaf extract was conducted to screen for the principal group of secondary metabolites. Tests for monosaccharide carbohydrates (Barfoed's test; orange-red precipitate), proteins and amino acids (Biuret and xanthoproteic tests; dark greenish-black coloration), and a mild reducing sugar reaction (Fehling's test; green coloration) were used. Alkaloids were detected using Mayer's reagent (faint green coloration) and Dragendorff's reagent (orangish coloration).(5)Lead acetate and ferric chloride tests indicated positive reactions for flavonoids. When oil was added, saponins formed a stable emulsion and a persistent froth in the froth test. Lead acetate (white precipitate), 10% NaOH (orangish-brown coloration), and ferric chloride (green precipitate) were used to confirm the presence of tannins and phenolics. In the Salkowski test, a greenish-black upper chloroform layer indicated the presence of terpenoids or sterols. Cardiac glycosides were suggested by orangish-yellow responses in the Baljet and Keller–Killiani tests. Results are noted as (−) for absence and (+) for presence.(6)

2.4 Total Phenolic Content determination

Total Phenolic Content (TPC) was determined using a modified Folin-Ciocalteu method. Briefly, 1.0 mL of *Grewia asiatica* L. leaf extract was mixed with 0.5 mL of Folin-Ciocalteu reagent and allowed to stand for 3-5 min at room temperature. Subsequently, 1.5 mL of 7% (w/v) sodium carbonate (Na_2CO_3) solution was added and the final reaction volume was adjusted to 10.0 mL using distilled water. The mixture (Figure 1A) was shielded from the light and incubated for 30 min at room temperature (25 ± 2°C), after which the absorbance was recorded at wavelength 765 nm against a reagewnt blank using a UV-Visible spectrophotometer. TPC was quantified using a linear standard calibration curve of gallic acid (20-100 μg mL^{-1}) and reported as milligrams of gallic acid equivalents per gram of extract (mg GAE g^{-1}).(7) (8)(9)

2.5 Total Flavonoid Content determination

Total Flavonoid Content (TFC) was determined using the aluminum chloride ($AlCl_3$) colorimetric method with minor modifications. Briefly, 1.0 mL of *Grewia asiatica* L. leaf extract (prepared in 20% methanol) was mixed with 4.0 mL of distilled water and 0.3 mL of 5% (w/v) sodium nitrite ($NaNO_2$). After incubation for 5 min at room temperature, 0.3 mL of 10% (w/v) aluminum chloride ($AlCl_3$) solution was added and the mixture (Figure 1B) was allowed to stand for an additional 6 min. Subsequently, 2.0 mL of 1 M sodium hydroxide (NaOH) was added, and final reaction volume was adjusted using 10.0 mL with distilled water. The absorbance was measured at 510 nm against a reagent blank using a UV-Visible spectrophotometer. A quercetin standard linear calibration curve (0–100 μg mL^{-1}) was used for quantification, and the results were reported as milligrams of quercetin equivalents per gram of extract (mg QE g^{-1} extract). (10)(11)

2.6 DPPH Radical Scavenging test

The DPPH Radical Scavenging activity was determined using the DPPH assay. A freshly prepared 0.1 mM DPPH solution was prepared in 40% methanol and stored in amber vials protected from light. Stock solutions of ascorbic acid (1 mg mL^{-1}) and *Grewia asiatica* L. leaf extract were prepared in 40% methanol (containing ≤1% DMSO when required) and diluted to concentrations of 10,20,40,60,80 and 100 μg mL^{-1}. For each concentration, 1.0 mL of the sample or standard solution was mixed together with 1.0 mL of 0.1 mM DPPH fresh solution to achieve a final reaction volume of 2.0 mL (final DPPH concentration: 0.05 mM). A control containing 1.0 mL of DPPH solution and 1.0 mL of solvent, and a sample blank containing 1.0 mL of sample solution and 1.0 mL of solvent were prepared. The reaction mixtures (Figure 1C) was shielded from the light and incubated for 30 min at room temperature (22–25°C), after which the optical density was recorded at wavelength of 517 nm using a UV-Visible spectrophotometer and a 1 cm path-length cuvette. The extract's DPPH radical scavenging potential was Quantified using the following equation:

DPPH radical scavenging activity = $\{(A_{control} - A_{sample}) / A_{control}\} \times 100$

where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance of the test sample.

2.7 Bovine serum albumin assay (anti-inflammatory activity)

The anti-inflammatory activity of *Grewia asiatica* L. leaf extract was evaluated using the bovine serum albumin (BSA) protein denaturation assay. A stock solution (1 mg mL^{-1}) of the extract was prepared in 20% DMSO and diluted with the appropriate solvent to obtain working concentrations of 50, 100, 150, 200, and 250 μg mL^{-1}, ensuring that the final DMSO concentration in the reaction mixture did not interfere with the assay. A freshly prepared 0.5% (w/v) BSA solution was prepared using distilled water and kept chilled until use. Fresh phosphate buffer (0.2 M, pH 6.4–6.8) was also prepared.

For each assay, 50 μL of the extract or standard solution was mixed with 450 μL of 0.5% BSA solution and incubated at 37°C for 20 min. The reaction mixture (Figure 1D) was then heated at 57°C for 3 min to induce protein denaturation, cooled to room temperature, and diluted with 2.5 mL of phosphate buffer (0.2 M, pH 6.4–6.8). The optical density was recorded at wavelength of 660 nm using a UV-Visible spectrophotometer against the appropriate blank.(12) The extract mediated inhibition of protein denaturation was determined using the following equation:

$$\% \text{ Inhibition} = \{(A_{control} - A_{sample}) / A_{control}\} \times 100$$

where $A_{control}$ is the absorbance intensity of the control sample (BSA with solvent) and A_{sample} is the absorbance of the reaction containing the extract.

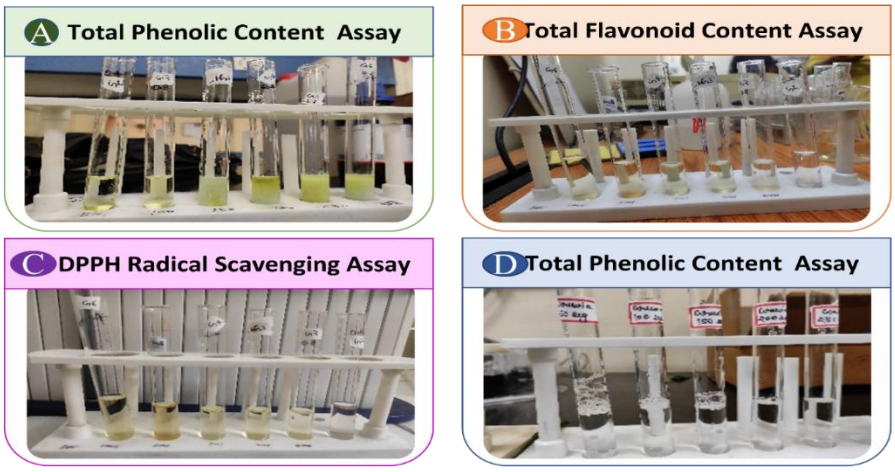


Figure.1 Representative Images of *In-vitro* Biological Assays

2.8 Antimicrobial activity of the plant extract

For antimicrobial activity, Mueller-Hinton agar (MHA) and Sabouraud dextrose agar (SDA) were prepared following the standard procedure provided by the manufacturer (HiMedia Laboratories, India). Briefly, the appropriate amount of dehydrated medium was suspended in distilled water was subjected to heating with continuous stirring until completely dissolved, and sterilized by autoclaving at 121°C (15 psi) for 15 min. After sterilization, the media were cooled to approximately 45–50°C and poured aseptically into autoclaved Petri plates.

The antimicrobial activity of *Grewia asiatica* L. leaf extract was measured using the agar well diffusion method against *Escherichia coli* (MTCC 452), *Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeruginosa* (MTCC 424), and *Candida albicans* (MTCC 183). Microbial inoculum were adjusted to the turbidity of a 0.5 McFarland standard (approximately 1.5×10^8 CFU mL^{-1} for bacterial isolates).(13)
An aliquot of 500 µL of each standardized inoculum (microbial suspension) was evenly spread over the surface of solidified Mueller-Hinton agar plates for bacteria and Sabouraud dextrose agar plates for *C. albicans* to obtain confluent growth. Circular Wells of 8 mm diameter were aseptically created into the agar plates using a sterile cork borer. Each well was carefully loaded with 50 µL of the leaf extract at concentrations of 50, 100, and 200 mg mL^{-1}. Twenty percent DMSO served as the negative control, while ciprofloxacin (250 µg mL^{-1}) and itraconazole (350 µg mL^{-1}) were served as a reference positive control for bacteria and fungi, respectively. The bacterial plates were incubated at 37°C for 24 h, whereas the fungal plates were incubated at 25°C for 120 h. After incubation, the diameters of inhibition zones were recorded in millimeters(mm). The results were expressed as mean ± SD from 3 independent experiments.(14)

3 RESULTS AND DISCUSSION

Grewia asiatica L. leaves were collected, shade-dried, and pulverized into a coarse powder. The powdered leaves were subjected to extraction using a Soxhlet apparatus with a methanol: water mixture (70:30, v/v) at 65°C. The solvent was subsequently removed to obtain the crude extract, with a yield of 12.46% (w/w) as visualized in Figure 2.

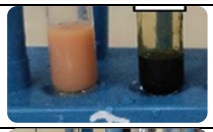
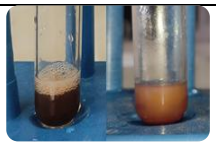
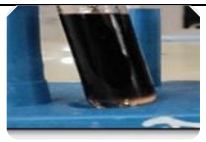
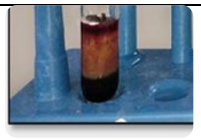
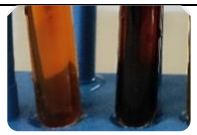
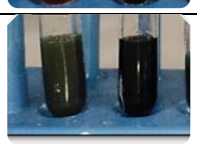
Figure 2. *Grewia asiatica L.* Leaf extract

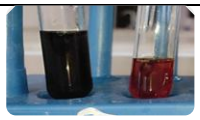
3.1 Phytochemical screening

Qualitative Phytochemical Screening of *Grewia asiatica* L. leaf extract confirmed the presence of several major classes of bioactive secondary metabolites (Table 1). Alkaloids were detected by both Mayer's (+) and Dragendorff's (+++) tests, indicating a moderate to strong presence of alkaloidal compounds. Flavonoids were strongly detected by the lead acetate test (+++), while phenolic compounds exhibited a moderate positive reaction using the ferric chloride test (++). Saponins showed strong positive reactions in both the foam and emulsion tests (+++), suggesting their abundance in the extract. Similarly, tannins were strongly detected by both the lead acetate and 10% sodium hydroxide tests (+++). Terpenoids were confirmed by a strong positive reaction in the Salkowski test (+++).

Cardiac glycosides were detected by both the Baljet's and Keller-Killiani tests, producing moderate (++) and weak (+) positive reactions, respectively. Proteins and amino acids exhibited strong positive reactions in the Biuret and xanthoproteic tests (+++). Carbohydrates were also detected, as observed by the positive Barfoed's test (+++), suggesting the presence of monosaccharides. Overall, the results of qualitative phytochemical evaluation showed that *Grewia asiatica* leaf extract is rich in biologically active constituents, particularly flavonoids, saponins, tannins, terpenoids, phenolic compounds, alkaloids, proteins, amino acids, cardiac glycosides, and carbohydrates.

Table 1 Phytochemical screening of *Grewia asiatica L.*

S.N.	Chemical constituent	Tests	*Grewia asiatica* leave's extract(A)	Observation
1	Alkaloids	Mayer's test	+	
		Dragendorff's test	+++	
2	Flavonoid	Lead Acetate Examination	+++	
		Ferric Chloride Examination	++	
3	Saponin	Foam test	+++	
		Emulsion Test	+++	
4	Tannins	Lead Acetate	+++	
		10% NaOH test	+++	
5	Terpenoids	Salkowski test	+++	
6	Phenolic compounds	Ferric Chloride Examination	++	
7	Glycosides	Baljet's Cyanogenic Glycosides Test	++	
		Keller-Killiani test	+	
8	Proteins and amino acids	Biuret test	+++	
		Xanthoproteic test	+++	

9	Carbohydrates	Barfoeds test (presence of disaccharides)	+++	
		Fehling's test	+	

3.2 Total Phenolic Content determination

The Total Phenolic Content (TPC) of *Grewia asiatica* L. leaf extract was determined using the using a modified Folin-Ciocalteu colorimetric method and gallic acid serve as the reference standard. The gallic acid calibration curve showed excellent linearity over the concentration range of 10–100 µg mL^{-1} with the regression equation Y = 0.0114x + 0.0015 (Figure 3). The phenolic content of the extract was estimated from the calibration curve and recorded as milligrams of gallic acid equivalents per gram of dry extract (mg GAE g^{-1}). The Grewia asiatica L. leaf extract exhibited a Total Phenolic Content of 15.21 mg GAE g^{-1} extract (Figure 4), signifying a substantial presence of phenolic compounds. These phenolic constituents have been reported for their antioxidant properties, which may play a crucial role in wound healing activities.

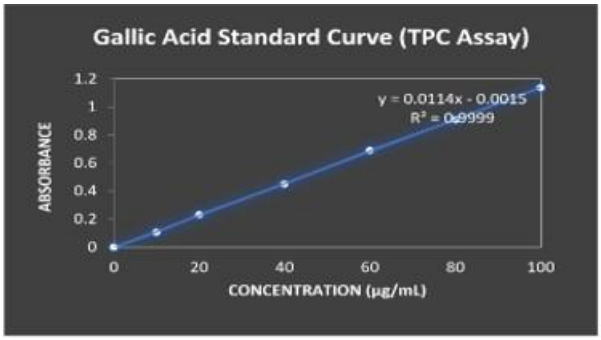


Figure 3. Standard Curve of Gallic Acid For TPC Assay

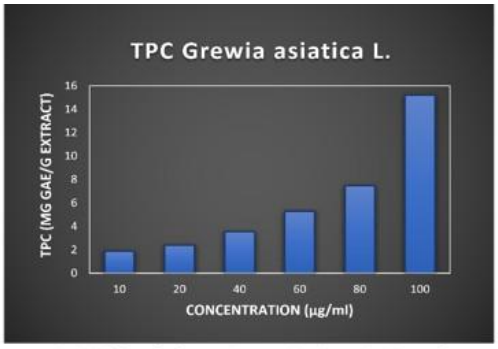


Figure 4. TPC of *Grewia asiatica* L. Leaf extract

3.3 Total flavonoid content determination

The TFC of *Grewia asiatica* L. leaf extract was determined by using the aluminum chloride colorimetric method with quercetin as the reference standard and recorded as milligrams of quercetin equivalents per gram of extract (mg QE g^{-1} extract). The quercetin calibration curve showed excellent linearity over the concentration range of 10–100 µg mL^{-1}, with the regression equation y=0.0009x+0.0004 and R^2=0.9995 value (Figure 5). The TFC of the extract was determined to be [102.97] mg QE g^{-1} extract, (Figure 6), signifying the presence of a considerable amount of flavonoids.

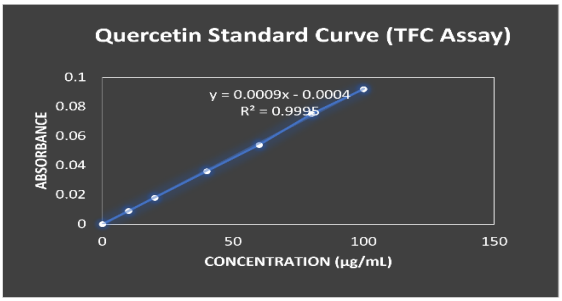


Figure 5. Standard curve of Quercetin for TFC assay

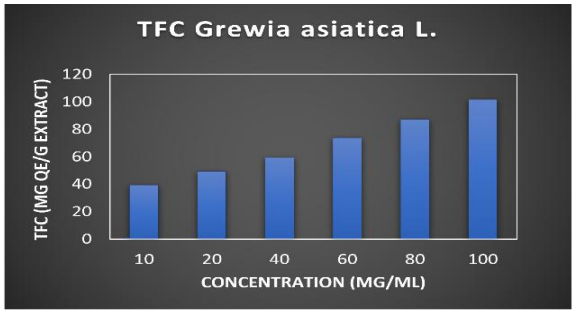


Figure 6. TFC of *Grewia asiatica* L. Leaf extract

3.4 Anti-oxidant Activity

The DPPH radical scavenging method was conducted to determine the antioxidant potential of *Grewia asiatica* leaf extract, and its activity was benchmrked against that of quercetin, the standard antioxidant. Quercetin exhibited consistently high antioxidant activity, with %RSA values ranging from approximately 97% to 98% across the tested concentrations (10-100 µg/mL). The *Grewia asiatica* leaf extract also displayed remarkable free radical scavenging activity, with %RSA increasing from approximately

87% at 10 µg/mL to 96% at 100 µg/mL, indicating a clear concentration-dependent response as depicted in Figure 7. Although the antioxidant activity of the extract marginally lower than that of quercetin at all concentrations, the difference decreased as the concentration increased. The remarkable anti-oxidant activity of the extract may be attributed to the presence of bioactive phytoconstituents such as flavonoids and phenolic compounds, which are known for their antioxidant properties. These findings suggest that *Grewia asiatica* leaf extract possesses substantial antioxidant potential and may represent a promising natural source of antioxidants, supporting its potential therapeutic and wound-healing applications.

3.5 Anti-inflammatory Activity

The Anti-inflammatory activity of *Grewia asiatica* L. leaf extract was assessed using the bovine serum albumin (BSA) protein denaturation assay in reference to the standard anti-inflammatory drug, diclofenac. The *Grewia asiatica* leaf extract exhibited marked inhibition of protein denaturation in a concentration-dependent manner, with percentage inhibition increasing from approximately 80% at 50 µg/mL to 92% at 250 µg/mL. In comparison, diclofenac showed a lower inhibitory effect under the same experimental conditions, with percentage inhibition increasing from approximately 9% at 50 µg/mL to 70% at 250 µg/mL. At all tested concentrations, the *Grewia asiatica* extract demonstrated substantially higher anti-inflammatory activity than diclofenac as illustrated in Figure 7. The pronounced inhibition of protein denaturation suggests that the extract possesses potent anti-inflammatory properties, which may be results of the presence of bioactive phytochemicals such as flavonoids and phenolic compounds. These observation indicates that *Grewia asiatica* leaf extract is a promising natural anti-inflammatory agent and may have promising therapeutic applications in the management of inflammatory disorders.(15)

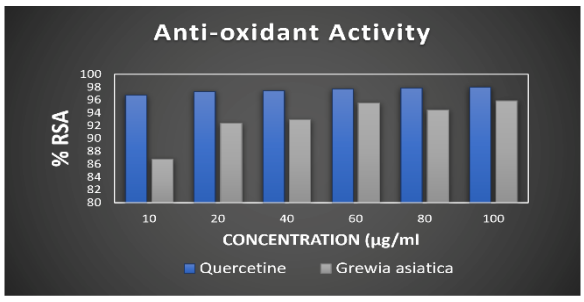


Figure 7. Antioxidant activity of *Grewia asiatica L.* leaf extract and Quercetin

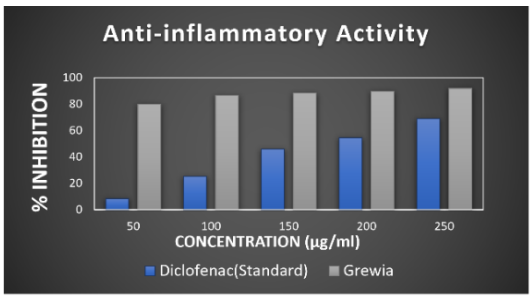


Figure 8. Anti-inflammatory activity of *Grewia asiatica L.* leaf extract and Diclofenac

3.5 Anti-microbial Activity

The anti-microbial activity of *Grewia asiatica* L. leaf extract against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Candida albicans* was evaluated at three concentrations (200, 100, and 50 mg/mL) using the agar well diffusion method.

The outcomes of anti-bacterial study of *Grewia asiatica* L. leaf extract against E. coli are shown in Table 2 and Figure 9. At 200 mg/mL concentration, mean zone of inhibition of 8.33 ± 0.29 mm was observed, which decreased slightly to 7.83 ± 0.58 mm at 100 mg/mL. No zone of inhibition was observed at 50 mg/mL, indicating that the extract was ineffective against E. coli at this concentration under the experimental conditions. The positive control, (ciprofloxacin), depicted a significantly larger zone of inhibition of 30.00 ± 0.00 mm, in contrast, the negative control (20% DMSO) showed no zone of inhibition, demonstrating that the solvent had no antibacterial effect.(16)

The extract revealed moderate antibacterial activity against *Staphylococcus aureus* (Table 3 and Figure 10) at both 200 and 100 mg/mL, as mean zones of inhibition of 6.17 ± 0.29 mm and 6.50 ± 0.50 mm, respectively was recorded. No inhibition at 50 mg/mL concentration indicating extract was ineffective against *S. aureus* under the experimental conditions. The positive control, illustrated a considerably larger mean zone of inhibition of 28.83 ± 0.29 mm, confirming the susceptibility of the test organism. In contrast, the negative control showed no antibacterial activity.(17)

The extract shown appreciable antibacterial activity against *Pseudomonas aeruginosa*. The observations of studies are recorded and presented in Table 4 and Figure 11. The mean zone of inhibition produced by extract at 200 mg/mL was 12.50 ± 0.50 mm, while 12.17 ± 0.29 mm almost the same was observed at 100 mg/mL. No zone of inhibition was detected at 50 mg/mL, indicating ineffectiveness against *P. aeruginosa* at this concentration. The positive control, exhibited 28.17 ± 0.29 mm zone of inhibition and negative control showed no antibacterial activity.(18)

The extract displayed weak antifungal activity against *C. albicans*. The observations recorded during the study are summarized in Table 5 and Figure 12. The mean zone of inhibition at 200 mg/mL concentration was 6.17 ± 0.29 mm. No zone of inhibition was observed at 100 mg/mL and 50 mg/mL, showing ineffectiveness against *C. albicans* at these lower concentrations under the experimental conditions. The positive control, itraconazole (350 ppm), showed a substantially larger mean zone of inhibition of 20.33 ± 0.58 mm, confirming the susceptibility of the test organism and validating the assay. In contrast, the negative control (20% DMSO) showed no zone of inhibition, demonstrating that the solvent had no antifungal effect. Although the antifungal activity of *Grewia asiatica* leaf extract was markedly lower than that of itraconazole, the inhibition observed at 200 mg/mL suggests the presence of phytochemicals with antifungal potential.

Table 2 Anti-bacterial activity study of *Grewia asiatica* L. leaf extract against E. coli

Observation							
S.No.	**Sample**	**Test organism**	**Conc. (mg/ml)**	**Zone of inhibition (mm)**			
				R1	**R2**	**R3**	**Mean ± S.d.**
1.	*Grewia asiatica* L.	E. coli	C1(200)	8.5	8	8.5	8.33±0.29
			C2(100)	7.5	8.5	7.5	7.83±0.58
			C3(50)	Nil	Nil	Nil	Nil
2.	Positive Control (Ciprofloxacin)		250ppm	30	30	30	30.00±0.00
3.	Negative Control (20%DMSO)		-	Nil	Nil	Nil	Nil

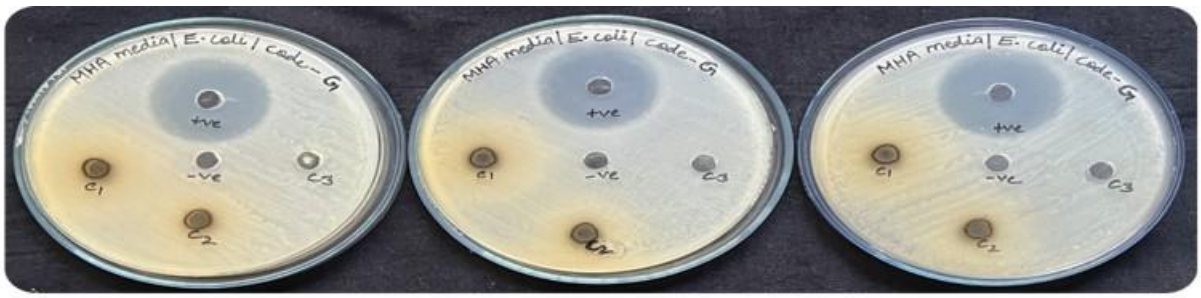


Figure 9. Anti-bacterial activity of *Grewia asiatica L.* against E. coli

Table 3 Anti-bacterial activity study of *Grewia asiatica L* against S. aureus

Observation							
S.No.	**Sample**	**Test organism**	**Conc. (mg/ml)**	**Zone of inhibition (mm)**			
				R1	**R2**	**R3**	**Mean ± S.d.**
1.	*Grewia asiatica* L.	S. aureus	C1(200)	6	6.5	6	6.17 ± 0.29 mm
			C2(100)	7	6.5	6	6.50 ± 0.50 mm
			C3(50)	Nil	Nil	Nil	Nil
2.	Positive Control (Ciprofloxacin		250ppm	28.5	29	29	28.83±0.29
3.	Negative Control (20%DMSO)		-	Nil	Nil	Nil	Nil

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Figure 10. Anti-bacterial activity of *Grewia asiatica L.* against S. aureus

Table 4 Anti-bacterial activity study of *Grewia asiatica L.* against P. aeruginosa

Observation							
S.No.	**Sample**	**Test organism**	**Conc. (mg/ml)**	**Zone of inhibition (mm)**			
				R1	**R2**	**R3**	**Mean ± S.d.**
1.	*Grewia asiatica* L.	P. aeruginosa	C1(200)	13	12	12.5	12.50 ± 0.50 mm
			C2(100)	12	12.5	12	12.17 ± 0.29 mm
			C3(50)	Nil	Nil	Nil	Nil
2.	Positive Control (Ciprofloxacin		250ppm	28.5	28	28	28.17±0.29
3.	Negative Control (20%DMSO)		-	Nil	Nil	Nil	Nil

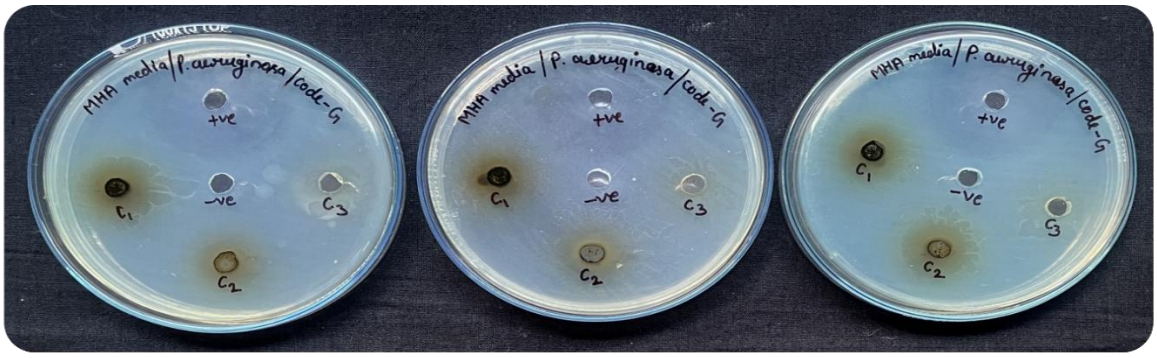

Figure 11. Anti-bacterial activity of *Grewia asiatica L.* against P. aeruginosa

Table 5 Antifungal activity of *Grewia asiatica L.* against C. albicans using the well diffusion method.

Observation							
S.No.	**Sample**	**Test organism**	**Conc. (mg/ml)**	**Zone of inhibition (mm)**			
				R1	**R2**	**R3**	**Mean ± S.d.**
1.	*Grewia asiatica* L.	C. albicans	C1(200)	6.5	6	6	6.17 ± 0.29
			C2(100)	Nil	Nil	Nil	Nil
			C3(50)	Nil	Nil	Nil	Nil

2.	Positive Control (Itraconazole)		350ppm	20	20	21	20.33±0.58
3.	Negative Control (20%DMSO)		-	Nil	Nil	Nil	Nil

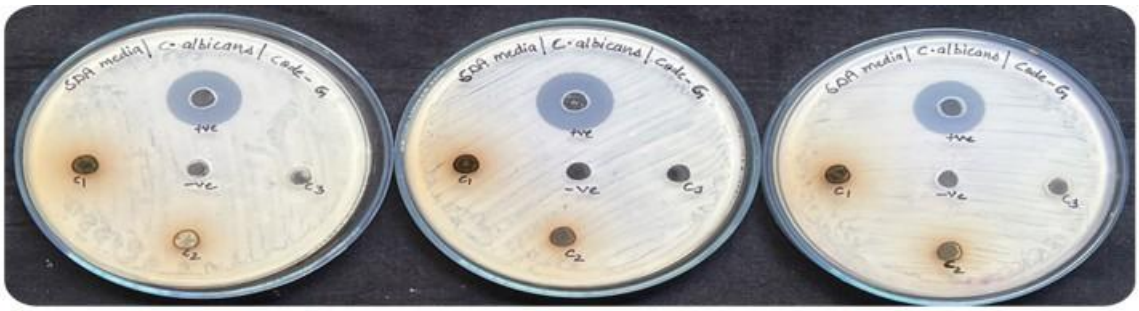

Figure 12. Anti-fungal activity of *Grewia asiatica L.* against C. albicans

The antimicrobial activity of *Grewia asiatica* L. leaf extract varied among the tested microorganisms. The diameter of the zone of inhibition was maximum at 12.50 ± 0.50 mm for P. aeruginosa, 8.33 ± 0.29 mm for E. coli, 6.17 ± 0.29 mm for S. aureus, and 6.17 ± 0.29 mm for *C. albicans*. The results demostrates extract possesses noteworthy antibacterial activity against *P. aeruginosa*, *E. coli* and *S. aureus*. The extract demonstrated weak antifungal activity against *Candida albicans*. This activity may be attributed to the presence of phytochemicals such as flavonoids, phenolic compounds, tannins, and other secondary metabolites that are known to exhibit antimicrobial properties.

4 CONCLUSION

The present investigation reveals that the leaf extract of G. asiatica is abundant bioactive phytoconstituents with significant therapeutic potential. The hydroalcoholic extract possessed high phenolic and flavonoid content, high dose-dependent anti-oxidant activity, and potent anti-inflammatory activity with comprehensive range of antimicrobial activity, suggesting its use in wound management.. Overall result represents that the leaf extract of *Grewia asiatica* L. is a potential natural source for the formulation of topical products, particularly in the context of diabetic and infected wound healing.

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Conflicts of interest

The authors confirms that they have no conflicts of interest.

Disclosure of Generative AI and AI-Assisted Technologies

Microsoft PowerPoint was used to draft the graphical abstract and all illustrations were sourced from Servier Medical Art and NIAID NIH BioArt Source were used. The authors critically reviewed, edited, and verified all AI-generated and third-party content and take full responsibility for the accuracy, originality, and integrity of the final manuscript.

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