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Research Article

A Research Paper On Phytochemical Profiling And In-Vitro Antibacterial Evaluation Of Ethanolic Extract Of *GREWIA OPTIVA* (BHIMAL TREE)

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ABSTRACT

Medicinal plants continue to serve as valuable sources of biologically active compounds and provide opportunities for the discovery of natural products with therapeutic potential. Grewia optiva commonly known as the Bhimal tree, is an important multipurpose tree traditionally used in the Himalayan region for various medicinal and household purposes. Different parts of the plant have been reported to contain diverse secondary metabolites, suggesting that G. optiva may possess several pharmacological properties. However, the antibacterial potential of its bark, particularly in relation to its phytochemical composition, requires further systematic investigation. The present study is therefore designed to investigate the phytochemical profile and in vitro antibacterial activity of an ethanolic extract prepared from the bark of G. optiva. Bark samples of G. optiva will be collected from a suitable geographical location and authenticated by a qualified taxonomist. The authenticated bark will be cleaned, shade-dried, powdered and extracted using ethanol. The resulting extract will be concentrated and evaluated for percentage yield and preliminary phytochemical constituents. Qualitative phytochemical screening will be performed to determine the presence of major classes of secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, glycosides and carbohydrates. The antibacterial activity of the ethanolic bark extract will be evaluated against selected pathogenic bacterial strains using an appropriate outcomes. Without any doubt, pharmacovigilance is a very important element to be able to safely and sensibly use folic acid in clinical and public health contexts. in vitro assay, such as the agar well diffusion method. Different concentrations of the extract will be tested, and antibacterial activity will be assessed by measuring the zones of inhibition. A suitable standard antibacterial agent will be included for comparison.

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The study is expected to provide information regarding the major phytochemical groups present in G. optiva bark and their possible association with antibacterial activity. The findings may provide scientific support for the traditional importance of Bhimal bark and establish a foundation for further investigation of its bioactive constituents and potential development as a plant-derived antibacterial resource.

INTRODUCTION

Medicinal plants remain important sources of structurally diverse natural compounds and continue to be investigated for their potential therapeutic applications. The genus *Grewia* comprises several species that have traditionally been used as food, fodder and medicinal resources in tropical and subtropical regions. *Grewia optiva* Drummond ex Burret, commonly known as Bhimal, is a multipurpose tree of the Himalayan region and is particularly important in north-western Himalayan agroforestry systems. Various parts of the plant have been associated with nutritional, traditional and pharmacological applications, while studies on *Grewia* species have demonstrated the occurrence of numerous bioactive chemical groups, particularly flavonoids and other phenolic constituents. The increasing scientific interest in this genus provides a rationale for further investigation of comparatively less-explored plant parts of *G. optiva*. [1,2]

The bark of *G. optiva* represents a promising source for phytochemical investigation because previous chemical studies have demonstrated considerable structural diversity in its constituents. Phytochemical investigations of the stem bark have resulted in the isolation of triterpenes such as betulin, betulinic acid, oleanolic acid and ursolic acid, together with daucosterol. Other investigations have reported the isolation of the compounds grewialin and optivanin along with β-sitosterol, stigmasterol and lupeol. The identification of these compounds indicates that the bark contains chemically diverse secondary metabolites that may contribute to its biological properties. In addition, screening studies of *G. optiva* bark extracts have reported classes such as alkaloids, flavonoids, tannins, saponins and terpenoids, supporting the need for systematic phytochemical profiling of the bark extract. [3–5]

Bacterial infections remain an important public-health concern, and increasing antimicrobial resistance has intensified the search for new antibacterial substances from natural sources. Plant-derived secondary metabolites, including phenolics, flavonoids, tannins, terpenoids and related compounds, have been investigated for their ability to interfere with microbial growth through different biological mechanisms. Evidence summarized for the genus *Grewia* indicates antibacterial activity in some species and plant parts, although the available information is not uniform across species, extracts and bacterial strains. For *G. optiva*, existing research has concentrated more strongly on phytochemistry, antioxidant properties and other biological activities than on a systematic evaluation of ethanolic bark extracts against selected pathogenic bacteria. This creates a useful basis for further antibacterial investigation. [2,6]

Ethanol is widely used in plant extraction because it can solubilize a broad range of phytochemicals and is suitable for preliminary biological screening. Therefore, evaluation of an ethanolic bark extract of *G. optiva* can provide complementary information regarding its chemical profile and antibacterial potential. The present study is designed to perform preliminary phytochemical profiling of the ethanolic bark extract and assess its in vitro antibacterial activity against selected pathogenic bacterial strains. The findings may help establish a scientific basis for the traditional importance of Bhimal bark and

provide preliminary evidence for further isolation and characterization of potentially bioactive constituents. [3,4,6]

1. METHOD AND MATERIALS

Collection and Authentication of Plant Material

Fresh bark of *Grewia optiva* (Bhimal tree), belonging to the family Malvaceae, was collected from the Chail Chowk area of Mandi district, Himachal Pradesh, India, during the month of December. Healthy and mature trees were selected for collection of the plant material. The collected bark was carefully separated and washed thoroughly with running tap water followed by distilled water to remove adhering soil, dust and other extraneous materials.

The collected plant material was properly examined and authenticated by experts at the HP CDP JICA ODA, Mandi, Himachal Pradesh. After authentication, the bark material was subjected to further processing. The authenticated bark was subsequently used for the preparation of ethanolic extract and further phytochemical profiling and in-vitro antibacterial evaluation against selected pathogenic bacterial strains**.**

Preparation of Plant Material

The collected bark will be washed with running tap water followed by distilled water to remove surface contaminants. The material will then be cut into small pieces and dried under shade at room temperature in a well-ventilated area, avoiding direct exposure to sunlight in order to minimize possible degradation of heat- or light-sensitive constituents. Once completely dried, the bark will be pulverized using a clean mechanical grinder to obtain a coarse powder. The powdered material will be passed through a suitable sieve to obtain relatively uniform particle size and stored in a clean, dry, airtight container until extraction.

Preparation of Ethanolic Extract

A measured quantity of powdered *Grewia optiva* bark will be extracted using ethanol as the extraction solvent. The powdered material will be mixed with an appropriate quantity of ethanol and subjected to extraction by maceration with occasional shaking for adequate contact between the plant material and solvent. After completion of extraction, the mixture will be filtered through suitable filter paper to separate the plant residue. The resulting filtrate will be concentrated using a rotary evaporator or an appropriate low-temperature evaporation procedure until a concentrated extract is obtained. The percentage yield of the extract will be calculated using the weight of the dried extract relative to the initial quantity of powdered plant material. The prepared extract will be transferred into a properly labelled airtight container and stored under suitable conditions until further analysis.

Preliminary Phytochemical Screening

The ethanolic bark extract will be subjected to qualitative phytochemical screening to identify the major groups of secondary metabolites present in the extract. Standard chemical reactions will be performed for the detection of alkaloids, flavonoids, phenolic compounds, tannins, saponins, terpenoids, steroids, glycosides, carbohydrates and proteins. Appropriate reagents will be used for each test, and the formation of characteristic colour changes, precipitates or foam will be recorded as an indication of the corresponding phytochemical group. The results will be documented systematically to establish the preliminary phytochemical profile of the ethanolic bark extract.

Selection and Preparation of Bacterial Strains

Selected pathogenic bacterial strains will be used for evaluating the antibacterial potential of the ethanolic bark extract. Both Gram-positive and Gram-negative bacteria may be included to obtain a broader assessment of antibacterial activity. The bacterial strains will be obtained from an authorized microbiological laboratory or recognized microbial culture collection. Fresh cultures will be prepared before the experiment by subculturing the organisms on suitable microbiological media. A standardized bacterial inoculum will be prepared according to an appropriate laboratory standard to maintain comparable microbial concentrations during antibacterial testing.

In Vitro Antibacterial Evaluation

The antibacterial activity of the ethanolic extract will be investigated using the agar well diffusion method. Mueller–Hinton agar or another suitable antibacterial testing medium will be prepared according to the manufacturer's instructions and sterilized appropriately. The standardized bacterial suspension will be evenly inoculated over the surface of the prepared agar plates. Sterile wells of suitable diameter will then be made in the inoculated agar using a sterile cork borer. Different concentrations of the *Grewia optiva* bark extract will be introduced into the respective wells. A suitable standard antibacterial drug will be used as a positive control, while the solvent used for preparing the extract will serve as a negative control. Following incubation under appropriate conditions for the selected bacterial organisms, the plates will be examined for clear zones surrounding the wells. The diameter of each zone of inhibition will be measured in millimetres and recorded as an indicator of antibacterial activity.

Determination of Minimum Inhibitory Concentration

Where laboratory facilities permit, the minimum inhibitory concentration (MIC) of the ethanolic bark extract will be determined using a suitable broth dilution or microdilution method. Serial concentrations of the extract will be prepared in an appropriate sterile culture medium and inoculated with standardized bacterial suspensions. After incubation under suitable conditions, bacterial growth will be assessed by visual observation or an appropriate quantitative measurement. The MIC will be recorded as the lowest concentration of the extract that produces no visible bacterial growth compared with the corresponding growth control. This procedure will provide additional quantitative information regarding the antibacterial potency of the extract.

Experimental Controls and Replication

Appropriate positive and negative controls will be incorporated into the antibacterial assay to validate the experimental procedure. The standard antibacterial agent will serve as the positive control, whereas the extraction solvent without plant extract will be used as the negative control. Each experimental condition will be tested in suitable replicates to improve the reliability and reproducibility of the observations. The antibacterial results will be expressed as mean ± standard deviation where applicable.

Statistical Analysis

The experimental observations obtained from the antibacterial assay will be compiled and analyzed using an appropriate statistical method according to the experimental design. Results will be presented as mean ± standard deviation for replicate measurements. Statistical comparisons between the treatment groups and controls will be

performed using a suitable statistical test, and a value of $p < 0.05$ will be considered statistically significant where applicable. The final findings will be presented in tables and figures to facilitate comparison of the antibacterial activity of different concentrations of the ethanolic *Grewia optiva* bark extract.

2. RESULT

Test materials

In the present study, etahnolic extract of bark of tree *Grewia optiva* (GO) was prepared using Soxhlet apparatus and was evaluated for its antimicrobial activity against a gram positive and a gram-negative bacteria. A standard concentration of (1000 mg/ml) was prepared from crude plant extract in dimethyl sulfoxide (DMSO).

Microorganisms are used for antibacterial assay.

Standard strains of *Staphylococcus aureus* (MTCC-96) and *E. coli* (MTCC- 443) were used to evaluate the antibacterial potential of ethanolic extract of *Grewia optiva* (GO).

Antibacterial assay

Preparation of inoculum

The bacterial strains *Staphylococcus aureus* (MTCC-96) and *E. coli* (MTCC- 443) were freshly cultured on nutrient broth media. Inoculum of selected bacterial strains was prepared by transferring 2-3 loop-full of culture growth into 5 mL of sterile normal saline. The suspension was vortexed thoroughly to achieve a homogenous mixture. The turbidity of the suspension was then adjusted to match the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL. This standardized inoculum was subsequently used for the antibacterial assay.

Agar well diffusion method

The antibacterial potential of extract FB was investigated using agar well diffusion assay, based on previously reported method (Sharma et al., 2012) with a few modifications. Muller Hinton Agar (MHA) plates were prepared and spread plated with prepared bacterial inoculum using sterile cotton swab. Three equidistant wells (8 mm in diameter) were aseptically punched into each plate using a sterile cork borer. Subsequently, 100 µL of test material (GO- 1000 mg/ml of DMSO) was dispensed into respective wells in two different plates. The second well of each plate was filled with 100 µL of Dimethyl sulfoxide (DMSO), serving as the negative control. The third well received standard antibiotic (AB) Piperacillin/Tazobactam (PIT 100/10 µg), as the positive control. Plates were incubated at 37 °C for 24 hours and observed for the formation of zones of inhibition around the wells. Post-incubation, zones of inhibition were measured in millimetres and recorded comparative analysis.

Results and discussion

In the present study *in-vitro* evaluation of antibacterial potential of ethanolic extract of *Grewia optiva* (GO) was evaluated against *Staphylococcus aureus* and *E. coli*

Table 1. Details of test material along with controls used for antibacterial assay.

Sr. No.	Test material	Concentration used	Volume used for each assay

1.	Ethanolic extract of *Grewia optiva* (GO)	1000 mg/ml of DMSO	100 microliters
2.	Dimethyl Sulfoxide (DMSO) Negative control	100 % (v/v)	100 microliters
3.	Antibiotic (AB) Piperacillin/Tazobactam (Positive control)	(PIT 100/10 µg)	100 microliters

Antibacterial activity of the ethanolic extract of *Grewia optiva* (GO) against *Staphylococcus aureus* after 24 hrs of incubation. The formation of distinct zones of inhibition surrounding the wells indicates the antibacterial activity of the extract. Dimethyl sulfoxide (DMSO) served as the negative control, while AB served as the positive control on Mueller–Hinton agar (MHA) plates.

Antibacterial activity of the ethanolic extract of *Grewia optiva* **(GO)** against *E. coli* after 24 hrs of incubation. The formation of distinct zones of inhibition surrounding the wells indicates the antibacterial activity of the extract. Dimethyl sulfoxide **(DMSO)** served as the negative control, while **AB** served as the positive control on Mueller–Hinton agar (MHA) plates.

Table 2. Antibacterial assay of ethanolic extract of Grewia optiva (GO).

Test Samples	**Dimeter of Zone of inhibition (in mm)***	
	Staphylococcus aureus	***E. coli***
Ethanolic extract of *Grewia optiva* (GO)	25	35
Antibiotic (AB) Piperacillin/Tazobactam (Positive control)	32	30
Negative control (DMSO)	Nil	Nil

The antibacterial activity of the ethanolic extract of *Grewia optiva* (GO) against *Staphylococcus aureus* and *Escherichia coli* is presented in Table 2. The ethanolic extract demonstrated pronounced antibacterial activity against both test organisms, as indicated by the formation of distinct zones of inhibition. The inhibition zone produced by GO was 25 mm against *S. aureus* and 35 mm against *E. coli*. These findings indicate that the extract possesses broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria, although the degree of susceptibility differed between the two organisms. The comparatively larger inhibition zone against *E. coli* suggests that the tested *E. coli* isolate was more susceptible to the antibacterial constituents present in the extract than the tested *S. aureus* isolate.

The positive control, piperacillin/tazobactam, produced inhibition zones of 32 mm against *S. aureus* and 30 mm against *E. coli*. In the case of *S. aureus*, the inhibition zone produced by GO (25 mm) was smaller than that of the antibiotic control (32 mm), indicating lower inhibitory activity under the conditions of the present assay. In contrast, against *E. coli*, GO produced a zone of inhibition of 35 mm, which was slightly greater than that produced by piperacillin/tazobactam (30 mm). This observation demonstrates the substantial inhibitory potential of the extract against the tested *E. coli* isolate. Nevertheless, direct comparison between a crude plant extract and a conventional antibiotic based solely on inhibition-zone diameter should be interpreted cautiously because the two preparations differ substantially in their chemical composition, active-compound concentration, molecular characteristics, diffusion through agar, and mechanisms of antibacterial action.

The differential response of *S. aureus* and *E. coli* to GO may be related to differences in their cellular envelope structures and permeability characteristics. *S. aureus*, being Gram-positive, possesses a relatively thick peptidoglycan layer, whereas *E. coli*, a Gram-negative bacterium, possesses a thinner peptidoglycan layer surrounded by an outer membrane. Although the outer membrane of Gram-negative bacteria can restrict the entry of several antimicrobial compounds, plant-derived phytochemicals may interact differently with bacterial membranes depending on their polarity, molecular size, and chemical structure. The greater inhibition observed against *E. coli* in the present study may therefore reflect the particular susceptibility of the tested isolate to one or more constituents of GO. However, bacterial susceptibility is strain-dependent, and the present results should not be generalized to all *E. coli* or *S. aureus* strains without further investigation.

The antibacterial activity observed with the ethanolic extract may be attributed to the presence of different classes of secondary metabolites in *G. optiva*. Plant-derived phenolic compounds and flavonoids are known to interact with bacterial cell membranes and proteins and may interfere with essential metabolic processes. Tannins can potentially bind to cellular proteins and enzymes, alter membrane permeability, and interfere with microbial growth. Terpenoid constituents may also contribute to antimicrobial activity through interactions with bacterial membranes. Since ethanol is capable of extracting a broad range of moderately polar phytochemicals, the observed antibacterial activity may result from the combined or synergistic action of several constituents rather than from a single compound. Nevertheless, the present agar diffusion results alone cannot establish which phytochemicals are responsible for the observed inhibition, and chemical characterization of the active extract is therefore necessary.

The 25 mm inhibition zone against *S. aureus* demonstrates that GO retained considerable activity against the tested Gram-positive organism, despite producing a smaller zone than the antibiotic control. This finding is particularly relevant because *S. aureus* is an important bacterial pathogen associated with a variety of human and animal infections. Similarly, the 35 mm inhibition zone against *E. coli* represents the highest antibacterial response recorded for GO in the present assay. The stronger response against *E. coli* suggests that constituents present in the extract may have favourable interactions with the cellular targets of this organism. However, the magnitude of a zone of inhibition should not be interpreted directly as an indicator of bactericidal

potency because agar diffusion is influenced by factors such as extract concentration, compound solubility, diffusion coefficient, molecular weight, and stability in the agar medium.

The DMSO negative control produced no zone of inhibition (Nil) against either *S. aureus* or *E. coli*. This result is important because it demonstrates that the solvent used for preparation of the extract did not exert detectable antibacterial activity at the concentration employed in the assay. Therefore, the inhibition zones observed with GO can be attributed primarily to the constituents of the ethanolic extract rather than to the solvent. The inclusion of both positive and negative controls consequently strengthens the validity and interpretation of the antibacterial assay.

The results also suggest that *G. optiva* could be explored as a potential source of plant-derived antibacterial compounds. However, the present findings represent an initial assessment based on agar diffusion and do not provide information regarding the minimum concentration required to inhibit or kill the bacteria. Further quantitative evaluation through minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays would provide more precise information regarding the potency of the extract. In addition, testing different extract concentrations would help establish whether the antibacterial response is concentration-dependent. Evaluation against a larger panel of bacterial species and multiple clinical or environmental isolates would also help determine the spectrum and consistency of the antibacterial activity.

CONCLUSION

The present study demonstrates that the ethanolic bark extract of *Grewia optiva* possesses promising antibacterial potential against the selected pathogenic bacteria, including *Staphylococcus aureus* and *Escherichia coli*. The observed antibacterial response suggests that the bark contains biologically active phytoconstituents capable of interfering with bacterial growth. Among the tested organisms, the extract showed a comparatively stronger inhibitory effect against *E. coli*, with a maximum inhibition zone of 35 mm, indicating notable susceptibility of this bacterial strain to the extract. Activity against *S. aureus* further demonstrates that the antibacterial effect of the extract is not restricted to a single bacterial group.

The phytochemical profile of *G. optiva* bark may provide a possible explanation for the observed biological activity, as secondary metabolites such as flavonoids, phenolic compounds, tannins, terpenoids, alkaloids and saponins are known to contribute to antimicrobial effects in medicinal plants. However, the activity of a crude extract cannot be attributed to any single phytochemical constituent without further isolation and characterization studies.

Overall, the findings provide preliminary scientific support for the traditional importance of *G. optiva* and indicate that its bark may represent a valuable source of natural antibacterial compounds. Further studies involving fractionation, identification of active constituents, MIC determination, toxicity assessment and mechanistic investigations are recommended to establish its therapeutic potential more conclusively.

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