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EVALUATION OF ANTIBACTERIAL ACTIVITY OF ETHONOLIC EXTRACT OF ABUTILON INDICUM STEM BARK

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ABSTRACT

The present study focuses on the preparation and evaluation of the ethanolic extract of *Abutilon indicum* leaves for its phytochemical composition and antimicrobial potential. Dried leaf material was subjected to methanolic extraction using a Soxhlet apparatus, followed by concentration under reduced pressure and vacuum drying to obtain the crude extract. Phytochemical screening revealed the presence of several bioactive constituents, including alkaloids, flavonoids, tannins, terpenoids, saponins, steroids, phenolic compounds, and glycosides. Physicochemical parameters such as ash values, extractive values, crude fiber content, and loss on drying were within acceptable limits, confirming the quality and stability of the extract. Antimicrobial studies demonstrated significant inhibitory activity of the ethanolic extract against both Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Neisseria meningitidis*. Additionally, the extract exhibited antifungal activity against *Candida albicans* and *Aspergillus niger*, with a dose-dependent increase in zone of inhibition. These findings support the traditional medicinal use of *Abutilon indicum* and highlight its potential as a natural source of antimicrobial agents.

1. INTRODUCTION

The emergence and rapid spread of antibiotic-resistant bacterial strains have become a global health concern, driving an urgent need for novel antimicrobial agents, especially from natural sources. Medicinal plants, long recognized in traditional medicine systems such as Ayurveda, Siddha, and Unani, have gained renewed scientific interest due to their diverse bioactive compounds and relatively low toxicity profiles. Among these, *Abutilon indicum* (family: Malvaceae), commonly known as Indian mallow or "Atibala" in Ayurvedic texts, stands out as a versatile medicinal herb with a broad spectrum of pharmacological properties. *Abutilon indicum* is a perennial shrub found in tropical and subtropical regions, including India, Sri Lanka, and Southeast Asia. Traditionally, various parts of the plant—leaves, roots, flowers, and seeds have been used for treating ailments such as bronchitis, inflammation, piles, ulcers, and urinary disorders. Modern phytochemical investigations have revealed that *Abutilon indicum* contains a wide range of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, glycosides, phenolic compounds, and steroids, many of which are known for their antimicrobial and antioxidant properties. Recent studies have focused on the ethanolic extract of *Abutilon indicum* leaves, which has shown promising antibacterial activity against both Gram-positive and Gram-negative bacterial strains. The plant extract has demonstrated significant zones of inhibition in standard in vitro assays such as the Cup and Plate method, particularly against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, and *Neisseria meningitidis*. These effects are believed to be mediated by the disruption of bacterial cell wall synthesis, inhibition of nucleic acid replication, or interference with key metabolic pathways, mechanisms commonly associated with plant-derived antimicrobials.

2. MATERIAL AND METHODS

2.1 Collection and authentication of *Abutilon indicum* plant

The botanical garden at Chaudhary Charan Singh (CCS) University, Meerut, provided the Abutilon indicum plant material used in this study. Renowned botanist Professor Vijay Malik of CCS University confirmed and examined the plant after collection. Morphological identification and verification using herbarium specimens were part of the authentication procedure used to confirm the taxonomic identity of the plant. Thanks to Professor Malik's expertise, the Abutilon indicum sample used in this study was accurately identified as a member of the Acanthaceae family.

2.2 Preparation of extract

A coarse powder was made from Abutilon indicum leaves after they were air dried and ground. The next step included extracting the material from the powder using a Soxhlet apparatus using methanol as the solvent. In order

to remove any solid particles, the extract was filtered through muslin fabric after continuous extraction. To increase the concentration of the filtrate, we evaporated the solvent using a rotary evaporator at a lower pressure. After that, the crude ethanolic extract was solidified for further research by means of vacuum drying.

2.3 Physiochemical constant

The shade-dried and crushed leaves of *Asparagus racemosus* were utilized to determine various physicochemical parameters, following the standard protocols outlined by the World Health Organization (WHO) guidelines for the quality control of medicinal plant materials.

2.3.1 Value of Ash

Determining the ash value is an important step in checking the purity and quality of crude pharmaceuticals by looking at how much inorganic material they include. The method includes burning a known amount of air-dried plant material in a silicon crucible at a temperature of roughly 500–600°C until the sample turns white or almost white, which means that there is no carbon in it. Then, the residue is chilled in a desiccator and weighed. Using certain techniques, this total ash may be split into acid-insoluble ash and water-soluble ash. These values assist find impurities, contaminants, or too much inorganic materials in herbal products.[9]

2.3.2 Total Ash

Total ash is the inorganic substance that is left over after plant material has been completely burned. It shows the overall quantity of minerals, which includes both physiological ash (from the plant itself) and non-physiological ash (from things like soil or sand that get into the plant). To find out how much ash is in something, a known amount of the air-dried sample is burned in a crucible at 500–600°C until it is carbon-free. The residue is weighed when it has cooled in a desiccator. This number is useful for checking the purity and quality of crude pharmaceuticals and finding out if they have been mixed with inorganic chemicals.[10]

2.3.3 Water-soluble ash

Water-soluble ash is the part of total ash that dissolves in water. It shows how much inorganic material is water-soluble. To find out, boil the complete ash with distilled water, filter the solution, and burn the rest of the insoluble materials. The water-soluble ash value is the difference in weight between the total ash and the residue after the water treatment. This test is crucial for checking the quality and purity of herbal materials since it can find water-soluble contaminants or additional chemicals in crude medications.[11]

2.3.4 Ash that dissolves in water

To find out how much water-soluble ash there is, first find out how much ash there is in the sample. After that, boil the complete ash with 25 mL of distilled water for 5 minutes. Use ashless filter paper to filter the mixture and catch the stuff that doesn't dissolve. Use hot water to wash away the residue, then burn it in a crucible that has already been weighed. Let it cool in a desiccator and then weigh it. Take the weight of the ash and subtract the weight of the residue from it. The difference shows how much water-soluble ash is in the sample. This helps find out whether there are any water-soluble inorganic components or adulterants in the sample.[12]

2.3.5 Ash with Sulphur

Sulphated ash is the inorganic residue that is left behind after organic content in a substance has been completely oxidized with the help of sulfuric acid. This is a frequent way to find out how much inorganic material is in a sample, especially in drugs. The technique involves moistening the sample with strong sulfuric acid and slowly burning it at 600 ± 25°C until all the carbon is gone and a white or almost white residue is left. After that, the residue is cooled and weighed. By assessing the non-volatile inorganic composition of pharmaceuticals, sulphated ash determination helps find adulterants and check their purity.[13]

2.3.6 The Value of Extraction

Extractive value tells you how much active ingredients were taken from a plant material using a certain solvent. This tells you about the plant's chemical composition and quality. To find out what it is, a known amount of the air-dried, powdered medicine is mixed with a determined amount of solvent (such water, alcohol, or a combination of the two) for a certain amount of time, generally 24 hours, with some shaking. After that, the mixture is filtered, and a certain amount of the filtrate is dried on a water bath. Weigh the residue once it has dried. To find the extractive value, divide the weight of the residue by the weight of the original sample. This test helps figure out how many active components that can dissolve are in the crude medication.[14]

2.3.7 Value of Soluble Extracts

The soluble extractive value tells you how many active ingredients in a crude medication can be taken out using a certain solvent, such water, alcohol, or another solvent. To find out, you soak a known weight of the powdered medicine in the chosen solvent for a defined amount of time, filter the solution, and then let a measured amount of the filtrate dry. Weigh the residue that is left after evaporation and figure out the extractive value as a percentage of the weight of the original sample. This number helps figure out how strong and good the herbal material is by assessing the amount of soluble bioactive chemicals.[15]

2.3.8 Alcohol Extractive that dissolves

Alcohol soluble extractive is the quantity of active ingredients in a plant material that can be taken out using alcohol, generally 70% ethanol. It gives an idea of how many bioactive chemicals, such alkaloids, glycosides, and flavonoids, are soluble in alcohol. To find out, a known amount of powdered medicine is soaked in

alcohol for a certain amount of time, filtered, and then a part of the filtrate is dried by evaporation. Weigh the residue and figure out the extractive value as a percentage of the original sample. This test is vital for figuring out how good herbal medications are and how much alcohol-soluble phytochemicals they contain.[16]

2.3.9 Steps for Finding the Fixed Oil Content

The Soxhlet extraction method is a standard way to find out how much fixed oil is in a plant sample. A known weight of the dried, powdered sample is put into a thimble and extracted with a non-polar solvent, such as petroleum ether or hexane, for a few hours. The solvent breaks down the oils and fats that are already in the sample. The solvent is evaporated after extraction, leaving behind the residue of fixed oil. The residue is weighed, and the amount of fixed oil is figured out as a percentage of the weight of the original sample. This approach gives a precise reading of the oil content in seeds, nuts, and other oily plant products.[17]

2.3.10 Ether-soluble volatile extract

Volatile ether soluble extract is the part of a plant that dissolves in volatile solvents like diethyl ether. The main things in this extract are non-polar substances such fixed oils, fats, waxes, and certain volatile oils. To find it, a known amount of the powdered material is taken out with diethyl ether using either maceration or Soxhlet extraction. Then, the solvent is allowed to evaporate, and the remaining material is weighed. The extractive value is shown as a percentage of the weight of the original sample. This test helps figure out how much ether-soluble stuff there is, which is helpful for figuring out how good plant materials with oils and other chemicals are.[18]

2.3.11 How to Find the Loss on Drying (LOD)

Loss on drying tells you how much moisture and volatile chemicals are in a sample by weighing it before and after it dries. You put a known weight of the material in a pre-weighed, dry container or crucible to perform the test. The sample is then put in an oven at a certain temperature (generally between 100°C and 105°C) for a certain amount of time, usually 2 to 5 hours, until its weight stays the same. After drying, the sample is put in a desiccator to cool down and then weighed again. The difference between the first and last weights shows the loss on drying as a percentage of the original sample weight. This test helps figure out how stable and how much moisture is in crude pharmaceuticals or pharmaceutical materials.[19]

2.3.12 Foaming Index

Foaming index is a qualitative test that looks for saponins in plant extracts and tries to figure out how much of them are there. When shaken with water, saponins can make stable foam. To find the foaming index, you make a series of dilutions of the water extract and shake them hard in test tubes. After a predetermined amount of time, generally 15 minutes, the height of the foam is measured. The foaming index is the maximum dilution that still makes the foam rise at least 1 cm. This test helps figure out how much saponin is in the sample. This is essential since saponins have therapeutic qualities, such as an expectorant and an antibiotic.[20]

2.3.13 Index of Swelling

The swelling index shows how efficiently a powdered medicine can absorb water and swell, which means it has hydrophilic components like polysaccharides and mucilage. To find it, a known amount of the powdered sample is put in a graduated cylinder, then distilled water is added. The combination is left alone for a set amount of time, generally between 1 and 24 hours, without being shaken. After settling, the swelling causes the volume to go up, and this is recorded. The swelling index is the percentage increase in volume compared to the powder's initial volume. This test helps figure out what herbal medications with mucilaginous qualities are and how good they are.[21]

2.4 Inoculums and test organisms

Staphylococcus aureus), Streptococcus pyogenes and Escherichia coli and Neisseria meningitidis were among the Gram-positive and Gram-negative bacterial strains active in this training. Among the fungal strains that were also included were Aspergillus niger and Candida All of the microbial strains were donated by the Department of Microbiology at Chaudhary Charan Singh (CCS) University in Meerut, Uttar Pradesh.

2.7 Standard Drugs

Standard antibacterial agents, Ampicillin (each at a concentration of 30 µg/disc), and the antifungal agent ketoconazole (30 µg/disc) were obtained as gift samples from Akums Drugs and Pharmaceuticals, Haridwar, Uttarakhand. These standard discs were secondhand as positive controls in the evaluation of antimicrobial activity.

2.8 Preparation of Nutrients Agar media

The bacterial culture in this study was grown on nutrient agar prepared from dehydrated powder following standard laboratory procedures. The medium contained peptone (organic nitrogen source), beef extract (vitamins and minerals), sodium chloride (osmotic balance), and agar (solidifying agent). A total of 28 g of dehydrated nutrient agar was dissolved in 1000 ml of distilled water using a water bath to prevent degradation. The solution was sterilized by autoclaving at 121°C and 15 psi for 15 minutes, ensuring its suitability for microbial cultivation and maintaining consistency and sterility for accurate bacterial studies.

3.9 Preparation of Test Organisms

The test organisms were initially maintained on nutrient agar slants and subcultured weekly to preserve viability and purity. For inoculum preparation, bacteria were transferred from slants using sterile saline and cultured on nutrient agar at 37°C for 24 hours. Post-incubation,

bacterial cells were washed off using 50 ml distilled water to create a suspension. The suspension was standardized using a spectrophotometer to achieve 25% light transmission at 520 nm. Preliminary trials determined the optimal concentration for use in agar or broth media. Prepared cultures were stored under refrigeration until further use.

2.10 Experimental procedure

The antimicrobial activity of the ethanolic extract of *Abutilon indicum* stem bark was assessed using the Cup and Plate method. Nutrient agar was poured into sterile 100 mm Petri dishes, and five wells (6 mm each) were made in each plate. A test solution (10 mg/ml in 1% DMSO) was added (0.1 ml) into each well. Ampicillin (30 µg/disc) and ketoconazole (30 µg/disc) served as standard antibacterial and antifungal controls, respectively. After one hour of diffusion at room temperature, the plates were incubated at 37°C for 24 hours. Antimicrobial activity was evaluated by measuring the zones of inhibition around each well.

3. RESULTS AND DISCUSSION

3.1 Collection and authentication of *Abutilon indicum* plant

The plant material of *Abutilon indicum* castoff in this study was composed from the Botanical Garden of Chaudhary Charan Singh (CCS) University, Meerut. After collection, the plant was carefully examined and authentic by Professor Vijay Malik, a renowned botanist associated with CCS University. The authentication process involved morphological identification and comparison with herbarium specimens to confirm the taxonomic identity of the plant. Professor Malik's expertise ensured that the specimen used in this study was correctly identified as *Abutilon indicum* , a member of the Acanthaceae family.

3.2 Preparation of extract

To make a coarse powder, we ground the dried leaves of Abutilon indicum. The next step was to extract the powdered material using a Soxhlet apparatus using methanol as the solvent. In order to remove any solid particles, the extract was filtered through muslin fabric after continuous extraction. The filtrate was concentrated by evaporating the solvent at low pressure by resources of a rotary evaporator. Afterwards, the crude ethanolic extract was solidified for further examination by means of vacuum drying.

3.4 Phytochemical examination

Table 1: Results of phytochemical examination of Abutilon indicum.

S. No.	Name of phytochemicals	Observation
1	Alkaloids	+
2	Saponin	+
3	Tannin	+
4	Flavonoids	+
5	Terpenoids	+
6	Phenolic Compound	+
7	Steroids	+
8	Glycoside	+
9	Carbonyl	-
10	Polysaccharide	+

3.5 Physiochemical constant examination

Table 2: Results of Physicochemical contents examination of *Abutilon indicum*.

S. no.	Parameters	Percentage w/w
Ash value		
1	Total ash	8.07±0.22
2	Water soluble ash	1.50±1.28
3	Acid soluble ash	1.09±0.11
4	Sulphate ash	9.33±0.86
Extractive value		
5	Ethanol solubility extractive	15.67±1.21
6	Water soluble extractive	21.87±0.89
7	Ether insoluble non-volatile	6.90±0.22
8	Ether insoluble volatile	1.01±0.19
9	Loss on drying	7.04±0.32
10	Crude fiber content	17.58±0.78
11	Foaming index	Less than 100

4.6 Antibacterial activity

Table 3: Antibacterial activity gram positive of ethanolic extract of *Abutilon indicum*.

S. No.	Drug	Concentration	Zone of inhibition value (mm) ± SD	
			Staphylococcus aureus	***Streptococcus pyogenes***
1	Ethanolic extract of *Abutilon indicum*	10 (mg/ml)	17.08±0.09	16.34±0.98
2	Ethanolic extract of *Abutilon indicum*	20 (mg/ml)	22.09±0.50	23.16±0.39
3	Ampicillin	30 (µg/disc)	24.85±0.34	25.09±0.12

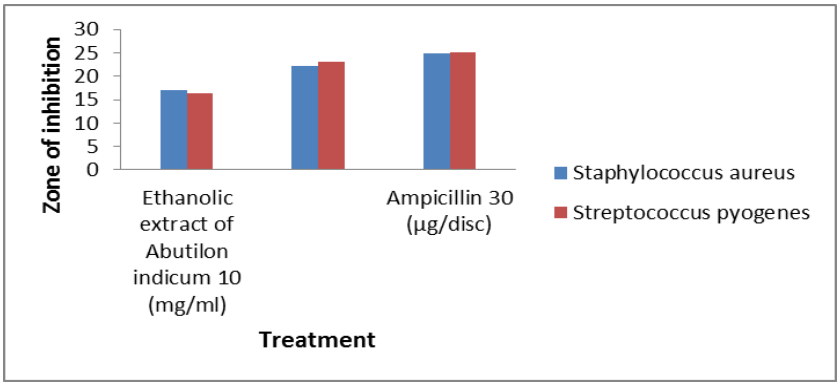

Figure 1: Antibacterial activity gram positive of ethanolic extract of *Abutilon indicum*.

Table 4: Antibacterial activity gram negative of ethanolic extract of *Abutilon indicum*.

S. No.	Drug	Concentration	Zone of inhibition value (mm) ± SD	
			Escherichia coli	***Neisseria meningitidis***
1	Ethanolic extract of *Abutilon indicum*	10 (mg/ml)	14.04±0.05	15.21±0.26
2	Ethanolic extract of *Abutilon indicum*	20 (mg/ml)	19.02±0.33	17.44±0.11
3	Ampicillin	30 (µg/disc)	21.21±0.24	21.02±0.32

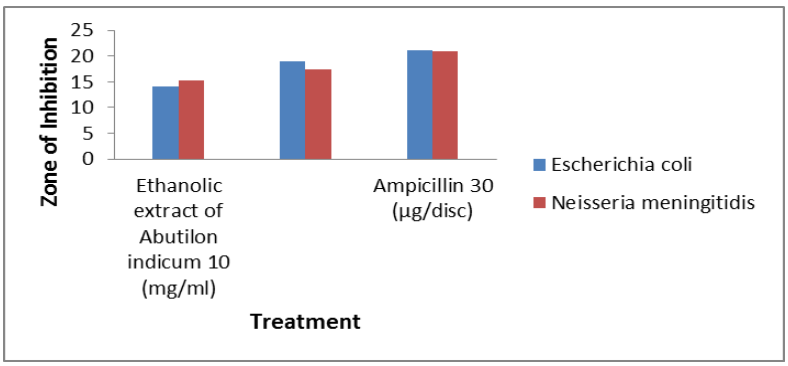

Figure 2: Antibacterial activity gram negative of ethanolic extract of *Abutilon indicum*.

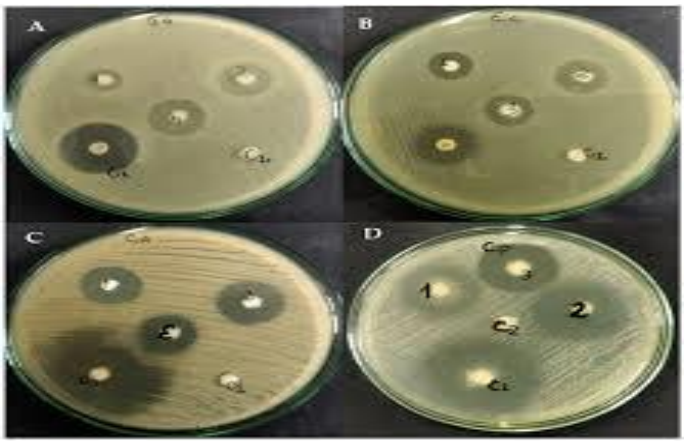

Figure 4.3: Antibacterial activity of ethanolic extract of *Abutilon indicum on (A) Staphylococcus aureus, (B) Streptococcus pyogenes, (C) Escherichia coli and (D)Neisseria meningitides.*

Table 5: Antifungal activity of ethanolic extract of *Abutilon indicum*.

S. No.	Drug	Concentration	Zone of inhibition value (mm) ± SD	
			Candida albicans	*Aspergillus niger*
1	Ethanolic extract of *Abutilon indicum*	10 (mg/ml)	17.03±0.08	13.09±0.05
2	Ethanolic extract of *Abutilon indicum*	20 (mg/ml)	21.03±0.14	19.22±0.18
3	Ketoconazole	30 (µg/disc)	22.85±0.18	23.02±0.84

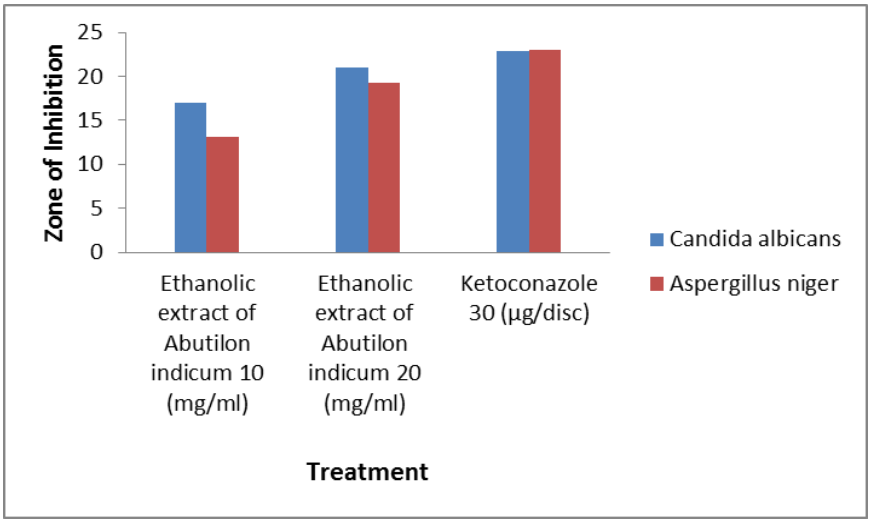

Figure 4.5: Antifungal activity of ethanolic extract of *Abutilon indicum*.

DISCUSSION

The present study focused on the preparation, phytochemical profiling, physicochemical evaluation, and antimicrobial screening of the ethanolic extract of *Abutilon indicum* leaves. The extract was obtained using Soxhlet extraction with methanol, a polar solvent known to effectively isolate a broad range of bioactive constituents. The extract was concentrated under reduced pressure and dried to yield a stable crude extract suitable for further testing. Phytochemical screening confirmed the presence of several important secondary metabolites, including alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, glycosides, phenolic compounds, and polysaccharides. The presence of these compounds supports the traditional use of *Abutilon indicum* in herbal medicine, particularly for its antimicrobial and anti-inflammatory properties. Notably, carbonyl compounds were absent, suggesting the selective nature of the extraction process. Physicochemical analysis revealed acceptable levels of total ash, acid-insoluble ash, and water-soluble ash, indicating minimal contamination and high purity of the raw plant material. The ethanol and water-soluble extractive values were 15.67% and 21.87%, respectively, suggesting good solubility of active constituents in both solvents. The low foaming index (less than 100) indicates minimal saponin content in terms of surfactant activity, aligning with the qualitative phytochemical test. The loss on drying (7.04%) and crude fiber content (17.58%) were within acceptable pharmacognostic limits, supporting the sample's stability and identity. Antimicrobial activity of the extract demonstrated a dose-dependent inhibition against both Gram-positive and Gram-negative bacteria. The ethanolic extract exhibited significant antibacterial activity against *Staphylococcus aureus* and *Streptococcus pyogenes*, with inhibition zones comparable to that of ampicillin at higher concentrations. Similarly, against Gram-negative organisms such as *Escherichia coli* and *Neisseria meningitidis*, the extract showed moderate to good activity, though slightly less than the standard antibiotic. In terms of antifungal activity, the extract was effective against *Candida albicans* and *Aspergillus niger*, especially at 20 mg/ml, where inhibition zones approached those of the standard antifungal drug ketoconazole. This suggests that *Abutilon indicum* may possess broad-spectrum antimicrobial properties, potentially due to the synergistic effects of its phytochemicals.

CONCLUSION

The methanolic extraction of *Abutilon indicum* leaves yielded a crude extract rich in diverse phytochemicals, including alkaloids, flavonoids, tannins, terpenoids, phenolic compounds, steroids, and glycosides. These bioactive constituents likely contribute to the plant's observed therapeutic potential. The physicochemical analysis confirmed acceptable purity and quality parameters, with extractive values indicating efficient solubility of phytoconstituents Antibacterial and antifungal assays demonstrated significant antimicrobial activity of the extract against both Gram-positive and Gram-negative bacteria, as well as common fungal strains. The activity was concentration-dependent and, at higher doses, showed inhibition zones comparable to standard drugs like ampicillin and ketoconazole.