International Journal on Science and Technology (IJSAT)
E-ISSN: 2229-7677 ● Website: www.ijsat.org ● Email: editor@ijsat.org

In vitro Antimicrobial Activity of Aqueous Root Extract of Hygrophila auriculata against Staphylococcus aureus and Escherichia coli

Mr. Sourav Saha[1], Ms. Laxmipriya Baskey[2], Ms. Manmeet kaur[3], Ms. Anuta Sarkar[4]

[1]Assistant Professor, [2,3,4]Scholar
[1,2,3,4]Netaji Subhas Institute of Pharmacy
Netaji Subhas University

Abstract:
The growing emergence of antimicrobial resistance has created a need for the discovery of effective and safer alternatives from natural sources. The present study was undertaken to investigate the pharmacognostic characteristics, phytochemical constituents, and in vitro antibacterial activity of the root of Hygrophila auriculata (Kokilaksha), a medicinal plant widely used in traditional Indian medicine. The roots were collected, authenticated, dried, powdered, and subjected to physicochemical evaluation and aqueous extraction. Preliminary phytochemical screening was carried out to identify major classes of bioactive compounds. The antibacterial activity of the aqueous root extract was evaluated against Staphylococcus aureus and Escherichia coli using the agar well diffusion method, while the Minimum Inhibitory Concentration (MIC) was determined by broth dilution technique. The extract exhibited concentration-dependent antibacterial activity against both test organisms. The maximum zone of inhibition was observed at 100 mg/mL, while MIC values were found to be 75 mg/mL for S. aureus and 100 mg/mL for E. coli. The higher sensitivity of S. aureus indicated better activity against Gram-positive bacteria. The antimicrobial effect may be attributed to the presence of phytoconstituents such as flavonoids, tannins, saponins, alkaloids, and phenolic compounds, which are known to interfere with microbial growth through multiple mechanisms. Although the activity was lower than the standard antibiotic, the findings suggest that Hygrophila auriculata root possesses promising antibacterial potential and may serve as a valuable source for the development of plant-based antimicrobial agents. Further studies involving isolation and characterization of active constituents are recommended.

Keywords: Hygrophila auriculata, Kokilaksha, Antibacterial Activity, Medicinal Plant, Phytochemical Screening, Aqueous Root Extract, Agar Well Diffusion, Minimum Inhibitory Concentration (MIC), Staphylococcus aureus, Escherichia coli, Antimicrobial Agents, Pharmacognosy.

1. Introduction

1.1 Herbal Medicines and Their Importance

For thousands of years, herbal remedies have been utilized in medicine, and they are particularly

acknowledged as a useful and accessible healthcare resource. The pharmaceutical industry has shown a great deal of interest in herbal medicines and their preparations during the past few decades. Though the knowledge gained from their traditional use over time should not be disregarded, the majority of herbal medicines still require scientific investigation (Jacobson and Silverglade, 1999; Taylor et al., 2001).

A wider range of plants than those typically found in the human diet can be used to make botanical medications and dietary supplements. Phytopharmaceuticals, also known as botanicals, are highly ideal for prophylactic use in order to maintain our natural wellness and prevent disorders. The proper cultivation and collection of plant materials, followed by their extraction and isolation of the phytochemical entities to enable optimized bioactive compound production and subsequent therapeutic applications, are crucial to the screening and evaluation of medicinal plants. For multi-component medications and their standardized extracts, this is crucial to guaranteeing good quality and consistency from batch to batch (Mukherjee et al., 2006). One of the earliest civilizations was established on the Indian subcontinent.

Nearly 3,000 different plants were employed as medicines by prehistoric people. Since ancient times, humans have utilized plants for their remarkable therapeutic properties. Recently, there has been a renewed interest in herbal remedies among humans. Despite significant advancements in the field of synthetic medications and antibiotics over the 20th century, plants continue to be a key source of drugs used in both contemporary and traditional medicine worldwide. Even in contemporary medicine, 25% of medications are derived from plants. Because they include a variety of complex chemical compounds with varying compositions that are present in one or more plants as secondary plant metabolites, medicinal plants offer therapeutic qualities. The development of ancient India's Materia Medica has benefited greatly from the use of medicinal plants. The Charaksahlita (1000 B.C.), one of the oldest treatises on Indian medicine, describes the usage of more than 340 medications with vegetative origins. The existence of several complex chemical compounds with varying compositions, which are found as secondary plant metabolites in one or more portions of these plants, gives medicinal plants their therapeutic qualities (Chandrasekar et al., 2010).

Many novel synthetic medications have emerged as a result of scientific and technological advancements. Even though Western medicine and medications provide an instant cure for illnesses brought on by microorganisms, they also have numerous long-term side effects. Herbal-based medicines help reduce the health risks associated with synthetic medications. Drug resistance is spreading, making a growing number of illnesses resistant to therapy (Kumar, 1998).

Recent years have seen a global increase in interest in plant study, and a substantial amount of data has been gathered to demonstrate the enormous potential of medicinal plants employed in numerous traditional systems. The use of various portions of various medicinal plants to treat particular illnesses has been popular in India from ancient times. Human survival now depends on the dissemination and preservation of information about medicinal plants and their applications (Sofowora, 1984).

India is home to Hygrophila auriculata Schumach (Acanthaceae). It is a wild herb that can be found all throughout India in damp areas on the margins of tanks, ditches, and paddy fields. Numerous

additional nations, including Malasia, Burma, Sri Lanka, Nepal, Pakistan, and others, are also home to it (Sivarajan, 2017). It is a sturdy, upright annual herb. The stems have thicker nodes and are sub-quadrangular. The blooms are pale, purple, blue, and closely packed in axils; they are clearly two-lipped, with the upper being two-lobed and the lower being three-lobed. The leaves are oblanceolate and have a yellow spine in their axils. The fruits have four to eight seeds and are rectangular, linear capsules (Sivarajan,2017)

1.2 Chemical composition

Numerous bioactive phytochemicals, including triterpenoids like lupeol and betulin, phytosterols like stigmasterol, and flavonoids, tannins, saponins, polyphenols, and other steroidal compounds, can be found in the root of Hygrophila auriculata . These components are thought to have a part in the plant's documented pharmacological properties, which include diuretic, hepatoprotective, anti-inflammatory, and antioxidant actions. Lupeol is one of the main components of the root and has garnered interest due to its possible medicinal uses. The root of Hygrophila auriculata is a significant component of traditional herbal medicine and a topic of continuing phytochemical research due to the presence of these many secondary metabolites.

1.3 Therapeutic use

In Indian medicine, the roots, leaves, and seeds have been used as diuretics and to treat rheumatism, anasarca, jaundice, dropsy, and urinogenital tract disorders. Kokilaksha is a well-known treatment for arthritis in the Ayurvedic medical system. It is thought to increase hunger and strength as well as treat oedema, ascites, thirst, bladder stones, eye disorders, and dysentery. Its root decoction has a diuretic effect. Leucorrhoea can be treated with the leaves, roots, and flowers of Stuea frondosa. Additionally, impotence, spermatorrhea, and seminal debilities are known to benefit from it (Shanmugasundaram and Venkataraman, 2005).

1.4 Mechanisms of Antimicrobial Activity

Antimicrobial substances act through several biological pathways, including damage to microbial cell structures, inhibition of essential biosynthetic processes, disruption of metabolic functions, prevention of biofilm development, and induction of oxidative stress. Natural products derived from plants have gained significant interest because of their ability to inhibit a wide range of pathogenic microorganisms, including bacteria and fungi. Studies have shown that these agents exert their antimicrobial effects by targeting cell membranes, interfering with enzyme systems, affecting genetic material, and reducing microbial virulence.

1.5 Disruption of Cell Wall and Cell Membrane

The cell wall and cell membrane are crucial for maintaining the structural integrity and physiological functions of microorganisms. Many plant-derived compounds exert antimicrobial effects by altering these structures, leading to increased membrane permeability and leakage of intracellular components. Such damage results in the loss of vital cellular constituents, ultimately causing microbial death. Various phytochemicals, including phenolics, flavonoids, terpenoids, alkaloids, and essential oil constituents, interact with membrane lipids and proteins, compromising membrane stability. Compounds such as thymol, carvacrol, eugenol, quercetin, and catechin have demonstrated strong antimicrobial properties through their ability to disrupt microbial membranes.

1.6 Inhibition of Protein and Nucleic Acid Synthesis

Microbial growth and reproduction depend on continuous protein production and nucleic acid replication. Certain bioactive plant compounds interfere with these processes by targeting ribosomes, replication enzymes, and transcriptional machinery. Flavonoids, tannins, and alkaloids can bind to microbial DNA or inhibit enzymes involved in DNA replication, thereby restricting cell division and proliferation. Some phytochemicals also suppress the activity of DNA gyrase and topoisomerases, enzymes essential for maintaining DNA structure during replication. As a result, microbial development is inhibited, leading to either growth suppression or cell death. Examples of compounds reported to possess such activities include berberine, quercetin, apigenin, and gallic acid.

1.7 Interference with Metabolic Processes and Enzyme Function

Microorganisms require numerous enzymatic reactions and metabolic pathways to generate energy and sustain cellular activities. Antimicrobial phytochemicals can inhibit key enzymes involved in these pathways, thereby impairing microbial survival. Phenolic compounds and tannins may form complexes with proteins or enzymes, reducing their biological activity. Inhibition of these metabolic processes disrupts nutrient utilization, energy production, and synthesis of essential cellular components. Flavonoids, terpenoids, and phenolic acids have been widely reported to suppress microbial metabolism and enzyme activity, contributing to their antimicrobial efficacy.

1.8 Oxidative Stress Induction and Virulence Suppression

Another important antimicrobial mechanism involves the generation of reactive oxygen species (ROS) within microbial cells. Excessive ROS production causes oxidative damage to cellular proteins, lipids, and nucleic acids, leading to impaired cellular function and eventual cell death. In addition, several plant-derived compounds inhibit quorum sensing and biofilm formation, which are key factors associated with microbial pathogenicity and resistance. By reducing these virulence mechanisms, antimicrobial agents decrease the ability of pathogens to establish and maintain infections. Bioactive molecules such as resveratrol, curcumin, epigallocatechin gallate, and quercetin have been shown to interfere with microbial communication systems and promote oxidative stress-mediated antimicrobial effects.

Overall, plant-derived antimicrobial compounds exhibit their activity through multiple complementary mechanisms, including membrane disruption, inhibition of nucleic acid and protein synthesis, suppression of metabolic enzymes, induction of oxidative stress, and attenuation of microbial virulence. These diverse actions contribute to their broad-spectrum antimicrobial potential and support their application in the development of novel therapeutic agents against infectious diseases.

1.9 Antimicrobial Activity of Hygrophila auriculata Root

Microbial infections caused by pathogenic bacteria and fungi remain a major public health concern worldwide. The increasing prevalence of antimicrobial resistance has stimulated the search for alternative therapeutic agents from medicinal plants. Hygrophila auriculata (Schumach.) Heine, commonly known as Kokilaksha, has been widely used in traditional medicine for the treatment of various ailments. The root of H. auriculata contains several bioactive phytoconstituents, including lupeol, betulin, stigmasterol, flavonoids, tannins, saponins, and phenolic compounds, which contribute to its antimicrobial potential.

The antimicrobial activity of H. auriculata root is mainly attributed to its ability to interfere with microbial cell structure and metabolism. Phenolic compounds and flavonoids present in the root can disrupt the integrity of bacterial cell membranes, causing leakage of intracellular components and inhibition of essential cellular functions. Tannins are known to form complexes with microbial proteins and enzymes, thereby impairing microbial growth and survival. Saponins possess membrane-active properties that increase cell permeability and ultimately lead to microbial cell lysis.

Several studies have reported that extracts of H. auriculata exhibit significant antibacterial activity against both Gram-positive and Gram-negative bacteria. The root extracts have shown inhibitory effects against organisms such as Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa. The antimicrobial efficacy is often concentration-dependent and varies according to the extraction solvent and microbial strain tested. Methanolic and ethanolic root extracts generally exhibit stronger antimicrobial activity due to their ability to extract a broader spectrum of bioactive compounds.

In addition to antibacterial activity, H. auriculata root has demonstrated antifungal properties against various fungal pathogens. The presence of phenolic compounds and flavonoids contributes to the inhibition of fungal growth by disrupting cell membrane integrity and interfering with essential metabolic pathways.

The antimicrobial effects of H. auriculata root are also associated with its ability to inhibit microbial virulence factors and biofilm formation. Biofilms provide protection to microorganisms against environmental stress and antimicrobial agents. Phytochemicals present in the root can interfere with microbial adhesion, quorum sensing, and biofilm development, thereby reducing pathogenicity and enhancing susceptibility to antimicrobial treatment.

Overall, the root of Hygrophila auriculata possesses considerable antimicrobial potential due to the presence of diverse bioactive constituents, including flavonoids, tannins, saponins, phenolic compounds, and triterpenoids. These phytochemicals act through multiple mechanisms, such as disruption of microbial membranes, inhibition of enzyme activity, induction of oxidative stress, and suppression of biofilm formation.

2. Literature Review

2.1 Taxonomical Classification of Hygrophila auriculata

Kingdom: Plantae
Clade: Tracheophytes
Clade: Angiosperms
Clade: Eudicots
Clade: Asterids
Order: Lamiales
Family: Acanthaceae
Genus: Hygrophila
Species: Hygrophila auriculata
Synonym: Asteracantha longifolia
Common Name: Kokilaksha, Talimakhana

Figure 5.3: Hygrophila auriculata Plant

2.2 Plant Description

Hygrophila auriculata is an important medicinal herb belonging to the family Acanthaceae. It is commonly known as Kokilaksha or Talimakhana and is widely distributed in tropical and subtropical regions of Asia, Africa, and India. The plant typically grows in marshy and aquatic habitats such as ponds, wetlands, and irrigated fields, and is well adapted to waterlogged conditions.

It is an annual or perennial erect, much-branched herb that may reach a height of about 60–120 cm. The stem is quadrangular and often bears small spines at the nodes. Leaves are simple, opposite, and lanceolate in shape with a slightly rough surface. The plant produces purple to bluish flowers that are usually sessile or short-pedicelled and arranged in axillary whorls. The fruits are small capsules containing numerous seeds.

The species is well known for its medicinal importance in traditional systems of medicine such as Ayurveda, where different parts of the plant, especially the seeds and roots, are used for their diuretic, hepatoprotective, and anti-inflammatory properties. The root is considered particularly significant due to its rich phytochemical profile, including alkaloids, flavonoids, tannins, sterols, and triterpenoids.

2.3 Cultivation

Hygrophila auriculata is a widely distributed semi-aquatic herb that naturally grows in marshy lands, wetlands, ponds, riverbanks, and irrigated agricultural fields. It is commonly found throughout tropical and subtropical regions, particularly in India, Sri Lanka, Bangladesh, and parts of Southeast Asia. The plant shows strong adaptability to waterlogged and swampy conditions and can survive in environments with fluctuating water levels. It grows best in warm, humid climates and prefers loamy to clayey soil rich in organic matter. Propagation generally occurs through seeds and sometimes through stem cuttings under favorable conditions. Germination and growth are enhanced during the rainy season when moisture availability is high. The plant requires full sunlight for proper vegetative development and flowering. Although it is a hardy species, it is sensitive to frost and low-temperature conditions, which can significantly reduce its growth and survival rate.

2.4 Botany

Kokilaksha is an erect, branched herb belonging to the family Acanthaceae, typically attaining a height of about 60–120 cm depending on environmental conditions. The stem is quadrangular in shape, green

to purplish in color, and often bears small spines at the nodes, which is a characteristic feature of the species. Leaves are simple, opposite, and lanceolate with an acute apex and slightly rough surface; prominent venation is clearly visible on both surfaces. The flowers are small, tubular, and range in colour from light purple to bluish violet, usually arranged in axillary whorls or clusters. The fruit is a small, two-celled capsule containing numerous tiny seeds that aid in propagation. Morphologically, the plant exhibits typical characteristics of the family Acanthaceae, including opposite phyllotaxy, bilabiate flowers, and capsule-type fruit formation.

2.5 Toxicity

Hygrophila auriculata is generally regarded as safe when used within traditional medicinal limits. It has been extensively used in Ayurvedic and folk medicine without major reports of severe toxicity. However, scientific studies on long-term safety and high-dose exposure are still limited. Excess consumption of crude extracts may occasionally lead to mild gastrointestinal disturbances such as nausea or discomfort. As with many medicinal plants, the safety profile may vary depending on the extraction method, dosage, and duration of use. Therefore, further detailed toxicological investigations are required to establish its complete safety profile and therapeutic index.

2.6 Constituents

The phytochemical composition of Hygrophila auriculata is diverse and contributes significantly to its biological activities. Different parts of the plant, particularly the root, contain a variety of secondary metabolites such as flavonoids, alkaloids, tannins, saponins, phenolic compounds, sterols, and triterpenoids. Important bioactive molecules including lupeol, betulin, and stigmasterol have been reported from the plant, which are known for their anti-inflammatory, antioxidant, and antimicrobial properties. In addition, the presence of polyphenolic compounds enhances its free radical scavenging potential. Preliminary phytochemical screening of various extracts has also confirmed carbohydrates, glycosides, and steroidal constituents, indicating a rich and complex chemical profile responsible for its pharmacological activities.

2.7 Uses

In traditional medical systems, Hygrophila auriculata holds significant therapeutic value. It is widely used as a diuretic, anti-inflammatory, hepatoprotective, antipyretic, and rejuvenating agent. The roots and seeds are considered particularly important in Ayurveda for the management of urinary tract disorders, liver dysfunction, edema, and general debility. The plant is also used to improve vitality, enhance strength, and support overall body metabolism. In several indigenous medicinal practices, it is employed for conditions such as jaundice, rheumatic pain, inflammation, and weakness associated with chronic diseases. Its broad traditional usage reflects its importance as a multipurpose medicinal herb.

2.8 Ethnomedicinal Uses

The ethnomedicinal applications of Hygrophila auriculata vary across different regions but primarily focus on its role in treating metabolic, urinary, and inflammatory disorders. The seeds, commonly known as “Talimakhana,” are valued as a nutritive and restorative tonic. Traditional practitioners use the plant for conditions such as diabetes, liver disorders, urinary tract infections, and inflammatory swellings. The root is particularly valued for its renal and hepatic protective effects. In folk medicine, it is also used to manage conditions like jaundice, fever, rheumatism, and general fatigue. The wide range

of traditional uses highlights its importance in ethnomedicine and supports its continued scientific investigation for pharmacological validation.

2.9 Phytochemical Review

Hygrophila auriculata is reported to possess a rich and diverse phytochemical profile that is responsible for its wide range of biological activities. Various parts of the plant, especially the root, seeds, and leaves, contain multiple secondary metabolites such as flavonoids, alkaloids, tannins, saponins, phenolic compounds, sterols, and triterpenoids. Important bioactive constituents identified from the plant include lupeol, betulin, and stigmasterol, which are known for their antioxidant, anti-inflammatory, and antimicrobial properties. In addition, polyphenolic compounds contribute significantly to its free radical scavenging potential. Preliminary phytochemical screening of different solvent extracts has also confirmed the presence of carbohydrates, glycosides, and steroidal compounds, indicating a complex chemical composition. The variation in phytoconstituents depends on factors such as plant part used, geographical location, and extraction solvent, which may influence its biological activity.

2.10 Pharmacological Review

Hygrophila auriculata has been extensively studied for its pharmacological properties in both in vitro and in vivo experimental models. The plant exhibits significant anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, diuretic, and antidiabetic activities. Its antioxidant potential is mainly attributed to phenolic compounds and flavonoids, which help in scavenging free radicals and reducing oxidative stress-related cellular damage. The antimicrobial activity is associated with the ability of its phytoconstituents to disrupt microbial cell membranes, inhibit enzyme systems, and interfere with microbial growth. Hepatoprotective effects have been observed in experimental models where plant extracts reduce liver enzyme levels and improve hepatic architecture, indicating protection against toxin-induced damage. Diuretic activity is linked to enhanced renal function and increased urine output, supporting its traditional use in urinary disorders. Furthermore, antidiabetic studies suggest that the plant may improve glucose metabolism by influencing insulin sensitivity, enhancing glucose uptake, and regulating carbohydrate metabolizing enzymes. Overall, the pharmacological profile of the plant supports its traditional medicinal applications and highlights its potential for further drug development studies.

3. Aim and Objectives

1.1 Aim of the Work

1. To carry out the pharmacognostic and phytochemical investigations on the root of Hygrophila auriculata
2. To evaluate the in-vitro anti-microbial potential of the root.

1.2 Objectives of the Study

The main objectives of our study are:

1. To identify and establish the taxonomical profile of the plant by studying various pharmacognostic as well as phytochemical parameters.
2. To prepare extracts of the Hygrophila auriculata root and evaluate the anti-microbial activity.

1.3 Plan of Work

1. Collection, selection, and authentication of roots of Hygrophila auriculata from the identified region.
2. Cleaning, drying, and coarse powder preparation of the collected root material.
3. Macroscopic and microscopic evaluation of the root for pharmacognostic characterization.
4. Determination of physicochemical parameters such as moisture content, total ash value, acid insoluble ash, and extractive values.
5. Preparations of aqueous root extract using suitable extraction technique.
6. Preliminary phytochemical screening of the aqueous extract for identification of major phytoconstituents.
7. Preparation and standardization of bacterial cultures of Staphylococcus aureus and Escherichia coli.
8. Evaluation of in vitro antimicrobial activity of the aqueous extract using agar well diffusion method.
9. Determination of Minimum Inhibitory Concentration (MIC) by broth dilution method.
10. Measurement of zone of inhibition and comparison with standard antibiotic.

4. Methodology

4.1 Materials and Method

The present section describes in detail the materials used and experimental procedures followed for the evaluation of in vitro antimicrobial activity of Hygrophila auriculata root. The study is organized under the following headings.

4.1.1 Collection of Plant Material, Chemicals and Microorganisms

The roots of Hygrophila auriculata were collected from naturally growing plants in marshy and wetland areas and authenticated by a qualified botanist of the institution. The collected plant material was washed thoroughly with running water to remove soil and debris, followed by shade drying at room temperature. Standard microbial strains (both Gram-positive and Gram-negative bacteria and selected fungal strains) were obtained from a certified microbiology laboratory. All chemicals and reagents used in the study were of analytical grade and procured from authorized suppliers. Required instruments and laboratory facilities were provided by the institutional research laboratory.

4.1.2 Physicochemical Analysis

Physicochemical parameters such as moisture content, total ash value, acid-insoluble ash, and extractive values were determined according to standard procedures recommended by the Indian Pharmacopoeia and WHO guidelines for quality control of medicinal plant materials. These parameters were used to evaluate the purity and quality of the crude drug.

4.1.3 Extraction Procedure (Soxhlet Extraction Method)

1. Approximately 50 grams of the root powder was accurately weighed and packed into a thimble made of filter paper, which was then placed inside the Soxhlet extraction apparatus.
2. Distilled water was used as the extraction solvent and poured into a round-bottom flask attached to the Soxhlet apparatus.
3. The assembly was heated using a heating mantle, allowing the aqueous solvent to boil and evaporate. The vaporized solvent condensed in the condenser and continuously percolated through the plant material present in the extraction chamber.

4. The extraction process was continued for about 6–8 hours until the siphon tube solvent became colorless, indicating complete extraction of the phytoconstituents.
5. After completion of extraction, the aqueous extract collected in the round-bottom flask was filtered through Whatman No. 1 filter paper to remove any insoluble plant particles.
6. The filtrate was concentrated using a thermostatic water bath maintained at 45°C to evaporate excess water, resulting in a dry, greenish-brown amorphous herbal extract.
7. The dried extract was collected and stored in an airtight desiccator until further use

4.2 Physicochemical Screening of the Extract

Physicochemical parameters such as moisture content, total ash value, acid-insoluble ash, and extractive values were determined according to standard procedures recommended by the Indian Pharmacopoeia and WHO guidelines for quality control of medicinal plant materials. These parameters were used to evaluate the purity and quality of the crude drug.

4.2.1 Total Ash

A known quantity of powdered root material was accurately weighed and placed in a pre-weighed silica crucible. The sample was incinerated gradually at a temperature not exceeding 450°C until it became free from carbon. The crucible was then cooled in a desiccator and weighed. The process was repeated until a constant weight was obtained. The total ash content was calculated as a percentage of the air-dried sample.

4.2.2 Acid Insoluble Ash

The total ash obtained was boiled with dilute hydrochloric acid solution and filtered through ashless filter paper. The residue was washed with hot water, transferred to a crucible, ignited, cooled, and weighed. The acid-insoluble ash was expressed as percentage of air-dried plant material.

4.2.3 Water-Soluble Extractive Value

A known quantity of powdered root was macerated with distilled water for 24 hours with intermittent shaking. After filtration, a measured portion of the filtrate was evaporated to dryness in a pre-weighed dish and dried at 105°C. The residue obtained was weighed, and the water-soluble extractive value was calculated.

4.2.4 Alcohol-Soluble Extractive Value

The powdered root was macerated with ethanol (95%) under similar conditions as above. After filtration, a portion of the filtrate was evaporated and dried to constant weight. The percentage of alcohol-soluble extractive value was calculated with reference to air-dried material.

4.3 Phytochemical Screening of the Extract

The prepared extracts were subjected to qualitative phytochemical analysis to detect the presence of major classes of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, steroids, triterpenoids, glycosides, carbohydrates, proteins, and phenolic compounds. Standard chemical tests were performed for each class of compound using established protocols.

4.3.1 Tests for Carbohydrates

Molisch's test, Fehling's test, and Benedict's test were used to detect the presence of carbohydrates based on characteristic color reactions and precipitate formation.

4.3.2 Tests for Alkaloids

Alkaloids were identified using Dragendorff's, Mayer's, Wagner's, and Hager's reagents, indicated by formation of characteristic precipitates.

4.3.3 Tests for Proteins and Amino Acids

Biuret test, Ninhydrin test, Xanthoproteic test, and lead acetate test were performed to confirm the presence of proteins and amino acids in the extract.

4.3.4 Tests for Tannins and Phenolic Compounds

Ferric chloride test and potassium dichromate test were used, where color change or precipitation indicated the presence of phenolic compounds and tannins.

4.3.5 Tests for Flavonoids

Shinoda test was performed using magnesium turnings and concentrated hydrochloric acid, indicated by development of red or pink coloration.

4.3.6 Tests for Steroids and Triterpenoids

Liebermann–Burchard and Salkowski tests were used to detect steroidal and triterpenoid compounds based on characteristic color changes.

4.3.7 Tests for Saponins

Foam test was performed by shaking the extract with water; persistent froth formation indicated the presence of saponins.

4.3.8 Tests for Glycosides

Legal test, Baljet test, Borntrager's test, and Keller–Killiani test were used to identify different types of glycosides.

4.3.9 Tests for Fixed Oils

Spot test and saponification test were used to confirm the presence of fixed oils based on oil stains and soap formation.

Table 4.3: Summary of Materials and Methods

S. No.	Experiment	Method
1	Collection of plant material	Roots of Hygrophila auriculata collected from natural habitat and authenticated by botanist

2	Cleaning and drying	Roots washed, shade-dried at room temperature, and powdered
3	Physicochemical analysis	Determination of moisture content, total ash, acid-insoluble ash, and extractive values
4	Ash value determination	Total ash and acid-insoluble ash determined using standard IP/WHO methods
5	Extractive value	Water and alcohol-soluble extractives determined by maceration method
6	Preparation of extract	Root powder extracted using ethanol/methanol (maceration or Soxhlet), filtered and concentrated
7	Phytochemical screening	Qualitative tests for alkaloids, flavonoids, tannins, saponins, steroids, glycosides, etc.
8	Tests for carbohydrates	Molisch, Fehling, and Benedict tests
9	Tests for alkaloids	Dragendorff's, Mayer's, Wagner's, Hager's tests
10	Tests for proteins	Biuret, Ninhydrin, Xanthoproteic, Lead acetate tests
11	Tests for phenolics/tannins	Ferric chloride and potassium dichromate tests
12	Tests for flavonoids	Shinoda test
13	Tests for steroids/triterpenoids	Liebermann–Burchard and Salkowski tests
14	Tests for saponins	Foam test
15	Antimicrobial assay	Agar well diffusion method against bacterial and fungal strains
16	Evaluation	Measurement of zone of inhibition and comparison with standard drugs

4.4 Pharmacological Studies

4.4.1 Evaluation of Antimicrobial Activity

The antimicrobial activity of the root extract was evaluated using the agar well diffusion method against selected bacterial and fungal strains. Sterile agar plates were inoculated with microbial cultures, and wells were filled with different concentrations of plant extract. After incubation, zones of inhibition were measured in millimeters and compared with standard antibiotics to assess antimicrobial potential.

4.4.2 Microorganisms Used

The antibacterial activity was evaluated against the following standard bacterial strains:

- Staphylococcus aureus
- Escherichia coli

Both strains were obtained from a microbiology laboratory and maintained on nutrient agar slants at 4°C.

4.4.3 Preparation of Nutrient Broth

Nutrient broth medium was prepared for the cultivation and maintenance of bacterial strains used in the study. The medium was used for the preparation of bacterial inoculum prior to antimicrobial evaluation.
Approximately 1.3 g of nutrient broth powder was weighed accurately and dissolved in 100 mL of distilled water in a conical flask. The solution was mixed thoroughly to obtain a uniform medium. The pH of the medium was adjusted to 7.2 ± 0.2 using 0.1 N NaOH.
The prepared nutrient broth was then distributed into culture tubes or conical flasks and sterilized by autoclaving at 121°C and 15 psi pressure for 15 minutes. After sterilization, the broth was allowed to cool at room temperature under aseptic conditions.
The sterile nutrient broth was used for inoculation of test organisms such as Staphylococcus aureus and Escherichia coli to prepare standardized bacterial suspensions for antimicrobial studies.

4.4.4 Preparation of Test Tubes

A total of six sterile test tubes were taken and labeled properly for each bacterial strain.
Each tube was filled with 5 mL of sterile nutrient broth.

4.4.5 Preparation of Extract Concentrations

The aqueous root extract of Hygrophila auriculata was prepared in different concentrations by serial dilution method. The following concentrations were used:

- 25 mg/mL
- 50 mg/mL
- 75 mg/mL
- 100 mg/mL

4.5 Experimental design

The tubes were arranged as follows:

Table 4.5: Summary of Materials and Methods

Tube	Content	Purpose
T1	Nutrient broth + inoculum	Growth control
T2	Nutrient broth only	Sterility control
T3	Broth + inoculum + 25 mg/mL extract	Test
T4	Broth + inoculum + 50 mg/mL extract	Test
T5	Broth + inoculum + 75 mg/mL extract	Test

T6	Broth + inoculum + 100 mg/mL extract	Test

4.6 Incubation
All test tubes were incubated at 37°C for 24 hours under aerobic conditions.

4.7 Observation
After incubation, the tubes were visually examined for turbidity:

- **Turbid solution = bacterial growth present**
- **Clear solution = inhibition of growth**

The lowest concentration of the aqueous root extract that showed no visible turbidity was recorded as the MIC value.
The experiment was performed in triplicate and mean values were recorded for accuracy.

Table 4.7: Observation Table

Organism	25 mg/mL	50 mg/mL	75 mg/mL	100 mg/mL	MIC (mg/mL)
S. aureus	+	+	–	–	75
E. coli	+	+	+	–	100

Key
(+) Growth observed
(–) No growth observed

5. Results and Discussion
5.1 Phytochemical Screening
The qualitative phytochemical screening of the aqueous extract of Hygrophila auriculata root was performed to confirm the presence of key therapeutic secondary metabolites. The results of these chemical tests are summarized in the table below.

Table 5.1: Qualitative Phytochemical Analysis of Aqueous Root Extract

Test Parameter	Specific Test Performed	Observation Recorded	Inference
Carbohydrates	Molisch's Test	Violet ring formed	+
Alkaloids	Dragendorff's Test	Orange precipitate	+
Flavonoids	Shinoda Test	Pink-red coloration	+
Tannins	Ferric Chloride Test	Dark blue-green color	+
Phenolic Compounds	Ferric Chloride Test	Bluish-black coloration	+
Saponins	Foam Test	Persistent froth observed	+

Glycosides	Keller-Killiani Test	Brown ring formed	+
Steroids	Liebermann-Burchard Test	Green coloration	+

Discussion of Phytochemical Results

The qualitative analysis revealed that the aqueous extract contains multiple classes of phytoconstituents known to possess pharmacological significance. The occurrence of flavonoids and phenolic compounds indicates potential antioxidant and antimicrobial properties, whereas tannins and saponins may contribute to microbial growth inhibition through interactions with cellular proteins and membranes. The presence of alkaloids, glycosides and steroids further supports the therapeutic potential of the plant and justifies its traditional medicinal use.

5.2 Antibacterial Activity (Agar Well Diffusion Method)

The aqueous root extract of Hygrophila auriculata was evaluated for its antibacterial potential against Staphylococcus aureus and Escherichia coli using the agar well diffusion technique.

The study showed that the extract produced measurable inhibition zones against both organisms, and the activity increased gradually with rising concentrations. This clearly indicated a concentration-dependent antibacterial effect.

Table 5.2: Antibacterial Activity of Aqueous Root Extract (Zone of Inhibition)

Sample	Concentration	S. aureus (mm)	E. coli (mm)
Control (Distilled Water)	—	0.0	0.0
Standard (Ciprofloxacin)	10 µg/mL	26.2 ± 0.48	24.5 ± 0.44
Extract	25 mg/mL	10.1 ± 0.28	9.0 ± 0.26
Extract	50 mg/mL	13.4 ± 0.32	11.9 ± 0.30
Extract	75 mg/mL	16.6 ± 0.36	15.0 ± 0.35
Extract	100 mg/mL	19.5 ± 0.40	17.4 ± 0.38

Interpretation of Results (Zone of Inhibition)

- The strongest inhibition was recorded at **100 mg/mL**
- The weakest activity appeared at **25 mg/mL**
- The standard antibiotic showed significantly higher inhibition than the plant extract
- Overall, the extract showed **moderate antibacterial potential**

5.3 MIC (Minimum Inhibitory Concentration) Results

MIC was determined using broth dilution technique to identify the lowest concentration that prevented visible microbial growth.

Table 5.3: MIC of Aqueous Root Extract

Bacterial Strain	25 mg/mL	50 mg/mL	75 mg/mL	100 mg/mL	MIC (mg/mL)
S. aureus	Growth	Growth	No growth	No growth	75

Bacterial Strain	25 mg/mL	50 mg/mL	75 mg/mL	100 mg/mL	MIC (mg/mL)
E. coli	Growth	Growth	Growth	No growth	100

MIC Interpretation

The MIC study revealed that:

- S. aureus was inhibited at a lower concentration (75 mg/mL)
- E. coli required a higher concentration (100 mg/mL) for inhibition

This indicates that Gram-positive bacteria were more responsive to the extract compared to Gram-negative bacteria.

Discussion

The present investigation demonstrated that the aqueous root extract of Hygrophila auriculata possesses noticeable antibacterial activity against both tested organisms, although the degree of inhibition varied depending on bacterial type and extract concentration.

The increase in zone size with rising concentration suggests that the antibacterial effect is directly linked to the amount of bioactive compounds present in the extract.

Greater sensitivity of Staphylococcus aureus can be explained by its simpler cell wall architecture, which consists mainly of thick peptidoglycan without an outer lipid membrane. This structural feature allows easier penetration of phytochemicals.

In contrast, Escherichia coli possesses an additional outer membrane containing lipopolysaccharides. This layer acts as a protective barrier and limits entry of many antimicrobial agents, resulting in comparatively reduced susceptibility.

The observed antimicrobial effect may be associated with the presence of phytochemical constituents such as flavonoids, tannins, saponins, alkaloids, and phenolic compounds. These compounds are known to act through several mechanisms including:

- Damage to bacterial cell membranes
- Inhibition of essential bacterial enzymes
- Precipitation of microbial proteins
- Disruption of metabolic pathways

Among these, phenolic compounds and flavonoids are particularly important due to their ability to interact with bacterial cell walls and alter membrane permeability, leading to leakage of intracellular components.

Tannins may contribute by binding to proteins and forming complexes that reduce microbial activity, while saponins can increase membrane permeability, enhancing antibacterial action.

Although the standard drug ciprofloxacin exhibited superior activity, the plant extract still showed meaningful inhibition, especially considering it is a crude aqueous preparation. This suggests that purification or isolation of active compounds could further enhance antimicrobial efficacy.

REFERENCES:

1. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman & Hall; 1998.
2. Trease GE, Evans WC. Pharmacognosy. 16th ed. Saunders Elsevier; 2009.
3. Kokate CK. Practical Pharmacognosy. Vallabh Prakashan; 2010.
4. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments. Nirali Prakashan; 2011.
5. Evans WC. Trease and Evans Pharmacognosy. 15th ed. Elsevier; 2002.
6. WHO. Quality Control Methods for Medicinal Plant Materials. World Health Organization; 2011.
7. Indian Pharmacopoeia Commission. Indian Pharmacopoeia. Govt. of India; 2018.
8. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standards Institute; 2020.
9. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by standardized single disc method. Am J Clin Pathol. 1966.
10. Cappuccino JG, Sherman N. Microbiology: A Laboratory Manual. Pearson; 2014.
11. Prescott LM, Harley JP, Klein DA. Microbiology. McGraw Hill; 2017.
12. Pelczar MJ, Chan ECS, Krieg NR. Microbiology. Tata McGraw Hill; 1993.
13. Cheesbrough M. District Laboratory Practice in Tropical Countries. Cambridge University Press; 2006.
14. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests; 2018.
15. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity. J Pharm Anal. 2016.
16. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999.
17. Nostro A, Germano MP, D'Angelo V, Marino A, Cannatelli MA. Extraction methods and antimicrobial activity. Lett Appl Microbiol. 2000.
18. Parekh J, Chanda S. Antibacterial activity of medicinal plants. Turk J Biol. 2007.
19. Dabur R et al. Antimicrobial activity of some Indian medicinal plants. Afr J Biotechnol. 2007.
20. Sahoo S, et al. Antimicrobial screening of medicinal plants. Int J Pharm Sci Rev Res. 2010.
21. Gurib-Fakim A. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Mol Aspects Med. 2006.
22. Fabricant DS, Farnsworth NR. The value of plants in drug discovery. Environ Health Perspect. 2001.
23. Newman DJ, Cragg GM. Natural products as sources of new drugs. J Nat Prod. 2007.
24. Ríos JL, Recio MC. Medicinal plants and antimicrobial activity. J Ethnopharmacol. 2005.
25. Rang HP, Dale MM. Pharmacology. Elsevier; 2015.
26. Brunton LL, Hilal-Dandan R, Knollmann BC. Goodman & Gilman's Pharmacological Basis of Therapeutics. 2018.
27. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. Nirali Prakashan; 2012.
28. Sharma PV. Plant Taxonomy. Tata McGraw Hill; 2009.
29. Jain SK. Dictionary of Indian Folk Medicine and Ethnobotany. Deep Publications; 1991.
30. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. Springer; 2007.
31. The Wealth of India. Council of Scientific and Industrial Research (CSIR); 2005.
32. Dey P, et al. Pharmacognostic studies on medicinal plants. J Pharmacogn Phytochem. 2014.

33. Nayak BS et al. Antimicrobial screening of plant extracts. J Ethnopharmacol. 2003.
34. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books; 1993.
35. Rani S, et al. Phytochemical and antimicrobial studies of medicinal plants. Int J Pharm Biol Sci. 2011