



PHARMACOLOGICAL EVALUATION OF ANALGESIC ACTIVITY OF HYGROPHILA AURICULATA: INSIGHTS FROM PRECLINICAL ANIMAL MODELS

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ABSTRACT

Pain is a complex physiological and pathological condition that significantly affects quality of life and remains a major global health concern. Despite the availability of conventional analgesics such as nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids, their long-term use is often associated with adverse effects, tolerance, and dependency, highlighting the urgent need for safer and more effective alternatives. In this context, medicinal plants have gained considerable attention due to their historical use, therapeutic potential, and relatively lower incidence of side effects. Hygrophila auriculata, a traditionally used medicinal herb, has been widely recognized in various systems of medicine for its anti-inflammatory and analgesic properties. Rich in bioactive phytoconstituents such as flavonoids, alkaloids, and sterols, the plant has demonstrated promising pharmacological activities. Preclinical studies using established animal models, including the hot plate test, tail flick method, and acetic acid-induced writhing test, have consistently shown significant analgesic effects of Hygrophila auriculata extracts. These effects are believed to be mediated through both central and peripheral mechanisms, possibly involving inhibition of inflammatory mediators and modulation of pain pathways. In conclusion, existing preclinical evidence supports the analgesic potential of Hygrophila auriculata, suggesting its promise as a natural alternative for pain management. However, further studies, including clinical trials and mechanistic investigations, are necessary to validate its efficacy, safety, and therapeutic applicability in humans.

KEYWORDS: *Hygrophila auriculata*, Analgesic activity, Preclinical models, Medicinal plants and Pain management.

1. INTRODUCTION

Pain is an essential protective mechanism of the body, yet when persistent or poorly managed, it becomes a debilitating condition that significantly impairs physical, emotional, and social well-being. It is one of the most common reasons for seeking medical care and poses a substantial burden on healthcare systems worldwide. Despite considerable advancements in pharmacotherapy, effective and safe pain management remains a challenge, necessitating the exploration of alternative therapeutic strategies.^[1]

1.1. Definition and Classification of Pain

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It is a multidimensional phenomenon involving

physiological, psychological, and biochemical components.

- **Based on Duration:** Pain can be broadly classified into acute and chronic types.

Acute pain is short-lasting and usually arises suddenly in response to a specific injury or pathological condition. It typically resolves once the underlying cause is treated and serves a protective function by alerting the body to harm. In contrast, chronic pain persists for a prolonged duration, often extending beyond the normal healing period, typically more than three months. It may arise from ongoing tissue damage or may exist without a clear cause, often leading to significant psychological distress and reduced quality of life.^[2]

- **Based on Pathophysiology:** From a mechanistic perspective, pain is categorized into nociceptive and neuropathic pain. Nociceptive pain results from the activation of peripheral nociceptors due to tissue injury, inflammation, or mechanical damage. It is further subdivided into somatic and visceral pain depending on the tissue of origin. Neuropathic pain, on the other hand, arises due to damage or dysfunction of the nervous system. It is often characterized by burning, tingling, or shooting sensations and is typically more difficult to manage with conventional therapies.^[3]

1.2. Limitations of Conventional Analgesics

The pharmacological management of pain primarily relies on nonsteroidal anti-inflammatory drugs (NSAIDs), opioids, and adjuvant analgesics. While these agents are effective, their use is often limited by significant adverse effects and safety concerns.^[4]

NSAIDs are widely prescribed for mild to moderate pain and inflammatory conditions. However, their prolonged use is associated with gastrointestinal irritation, ulceration, renal toxicity, and increased cardiovascular risks. These limitations restrict their long-term applicability, particularly in vulnerable populations. Opioids are considered the gold standard for managing moderate to severe pain. Despite their potent analgesic effects, they are associated with serious drawbacks such as respiratory depression, sedation, constipation, tolerance, and physical dependence. The risk of misuse and addiction has further escalated concerns regarding their widespread use.^[5]

Additionally, many conventional analgesics provide only symptomatic relief without addressing the underlying cause of pain, thereby necessitating repeated or prolonged administration, which further increases the risk of adverse effects.

1.3. Growing Interest in Herbal Medicine for Pain Management

In recent years, there has been a resurgence of interest in herbal medicine as an alternative or complementary approach to pain management. Medicinal plants have been used for centuries in traditional systems of medicine such as Ayurveda, Unani, and Traditional Chinese Medicine for the treatment of various ailments, including pain and inflammation.^[6]

Herbal medicines are often perceived as safer due to their natural origin and long history of use. They contain a diverse array of bioactive compounds, including flavonoids, alkaloids, tannins, and terpenoids, which can exert analgesic and anti-inflammatory effects through multiple mechanisms. These may include inhibition of inflammatory mediators, modulation of neurotransmitters, and antioxidant activity.

Moreover, plant-based therapies often exhibit a synergistic effect due to the presence of multiple constituents, potentially enhancing therapeutic efficacy while minimizing toxicity. This multi-target approach is particularly advantageous in managing complex conditions such as chronic pain.^[7]

1.3.1. Rationale for Selecting *Hygrophila auriculata*

Hygrophila auriculata is a well-known medicinal plant widely distributed in tropical regions and traditionally used in various indigenous systems of medicine. It has been extensively utilized for its therapeutic properties, including anti-inflammatory, hepatoprotective, antioxidant, and analgesic activities.^[8]

The selection of *Hygrophila auriculata* for the present review is based on several scientific and ethnopharmacological considerations. First, traditional usage strongly supports its role in alleviating pain and inflammation, indicating its potential as a natural analgesic agent. Second, phytochemical investigations have revealed the presence of biologically active constituents such as flavonoids, sterols, and alkaloids, which are known to contribute to analgesic effects. Furthermore, preclinical studies employing established animal models have demonstrated significant analgesic activity of plant extracts, suggesting both central and peripheral mechanisms of action. These findings highlight its potential as a safer alternative to conventional analgesics.^[9]

Despite these promising results, a comprehensive evaluation of its analgesic potential, mechanisms of action, and translational relevance remains limited. Therefore, a detailed review of existing preclinical evidence is essential to consolidate current knowledge and identify future research directions.^[10]

This review aims to critically analyze the available preclinical studies on the analgesic activity of *Hygrophila auriculata*, with a focus on experimental models, mechanisms, and pharmacological outcomes. By integrating traditional knowledge with modern scientific evidence, the study seeks to provide a strong foundation for future research and potential clinical applications in pain management.

2. BOTANICAL PROFILE OF *Hygrophila auriculata*

Hygrophila auriculata (Schumach.) Heine is an important medicinal plant belonging to the family Acanthaceae. It is widely recognized in traditional systems of medicine for its diverse therapeutic properties. The plant is commonly known as “Kokilaksha” in Ayurveda and “Talimakhana” in Hindi.^[11] *Hygrophila auriculata* is known by several botanical synonyms, reflecting its wide geographical distribution and historical classification changes. The most commonly reported synonym is *Asteracantha longifolia* (L.) Nees. Other synonyms include *Hygrophila*

spinosa T. Anders, which is also frequently cited in pharmacognostic and phytochemical studies. The existence of multiple synonyms often creates confusion in the literature; however, *Hygrophila auriculata* is currently the accepted botanical name.^[12]



Fig. 1: *Hygrophila auriculata*.^[13]

Its taxonomic classification is as follows

Table 1: Botanical Classification of *Hygrophila auriculata*.

Taxonomic Rank	Classification
Kingdom	Plantae
Division	Angiosperms
Class	Dicotyledons
Order	Lamiales
Family	Acanthaceae
Genus	<i>Hygrophila</i>
Species	<i>Hygrophila auriculata</i>

2.1. Morphological Characteristics

Hygrophila auriculata is an erect, annual or perennial herb that thrives in marshy and wetland environments. The plant typically grows up to 60-120 cm in height and is characterized by a robust and spiny appearance. The stem is quadrangular, hairy, and swollen at the nodes, often exhibiting spines. Leaves are simple, sessile, and arranged in whorls, usually consisting of 6–8 leaves at each node. They are linear-lanceolate in shape with a slightly rough texture and may bear spines in the axils.^[14]

The plant produces attractive purple to violet-colored flowers, which are sessile and occur in dense axillary whorls. The calyx is linear and spiny, while the corolla is bilabiate, typical of the Acanthaceae family. The fruit is a linear capsule containing numerous small, flat, and brown seeds. These seeds are mucilaginous when soaked in water, a characteristic feature often used for identification.^[15]

2.2. Geographical Distribution

Hygrophila auriculata is widely distributed in tropical and subtropical regions of the world. It is commonly found throughout India, Sri Lanka, Nepal, and Southeast Asian countries. In India, the plant is abundantly distributed in states such as Uttar Pradesh, Bihar, West Bengal, Tamil Nadu, and Kerala. The plant predominantly grows in waterlogged areas, paddy fields, marshes, riverbanks, and ditches. It thrives in moist, fertile soils and is particularly prevalent during the monsoon season. Due to its adaptability, *Hygrophila auriculata* is also cultivated in certain regions for its medicinal and nutritional value.^[16]

2.3. Traditional Uses in Ayurveda and Folk Medicine

In Ayurveda, *Hygrophila auriculata* (Kokilaksha) is regarded as a potent medicinal herb with multiple therapeutic applications. It is classified under “Rasayana” drugs, which are believed to promote longevity and overall health. Traditionally, the plant has been used for the management of inflammatory conditions, pain, urinary disorders, and liver ailments. Its seeds are commonly used as a tonic and aphrodisiac, while the roots and leaves are utilized for their diuretic and anti-inflammatory properties.^[17]

In folk medicine, various parts of the plant are employed to treat conditions such as rheumatism, jaundice, edema, and wounds. Decoctions and extracts of the plant are often used to relieve pain and swelling, supporting its ethnopharmacological relevance as an analgesic agent. The widespread traditional use of *Hygrophila auriculata* in pain and inflammatory conditions provides a strong foundation for its scientific evaluation and justifies its selection for pharmacological studies.^[18]

Table 2: Traditional Uses of *Hygrophila auriculata* in Ayurveda and Folk Medicine.

System of Medicine	Plant Part Used	Preparation/Form	Traditional Uses	Therapeutic Category
Ayurveda	Whole plant	Decoction (Kwath)	Management of inflammatory conditions and generalized body pain	Anti-inflammatory, Analgesic ^[19]
Ayurveda	Seeds	Powder, tonic formulation	Used as aphrodisiac and general body tonic	Rejuvenative (Rasayana), Aphrodisiac
Ayurveda	Roots	Decoction	Treatment of urinary disorders and dysuria	Diuretic, Urinary health ^[20]
Ayurveda	Leaves	Paste, juice	Applied in swelling and local inflammation	Anti-inflammatory
Ayurveda	Whole plant	Various formulations	Management of liver disorders	Hepatoprotective ^[21]
Folk Medicine	Leaves	Paste	Applied topically for	Wound healing,

			wounds and skin infections	Antimicrobial
Folk Medicine	Whole plant	Decoction	Used in rheumatism and joint pain	Analgesic, Anti-rheumatic ^[22]
Folk Medicine	Seeds	Soaked or powdered form	Used to improve vitality and strength	General tonic
Folk Medicine	Roots	Decoction	Used in jaundice and edema	Hepatoprotective, Anti-edematous
Folk Medicine	Whole plant	Extract/infusion	Relief from pain and swelling	Analgesic, Anti-inflammatory ^[23]

3. PHYTOCHEMICAL COMPOSITION OF *Hygrophila auriculata*

The therapeutic potential of *Hygrophila auriculata* is largely attributed to its rich and diverse phytochemical profile. Various phytochemical investigations have revealed the presence of multiple classes of bioactive constituents that contribute to its pharmacological activities, particularly its analgesic and anti-inflammatory effects.^[24] These constituents are distributed across different parts of the plant, including leaves, roots, seeds, and the whole plant extract.

3.1. Major Bioactive Constituents

- **Alkaloids:** Alkaloids are nitrogen-containing compounds known for their significant pharmacological activities. In *Hygrophila auriculata*, alkaloids contribute to pain modulation by interacting with central nervous system receptors. They may influence neurotransmitter pathways, thereby exhibiting mild central analgesic effects.^[25]
- **Flavonoids:** Flavonoids are one of the important phytoconstituents present in *Hygrophila auriculata* and are primarily responsible for its analgesic and anti-inflammatory properties. These compounds act by inhibiting enzymes such as cyclooxygenase (COX) and lipoxygenase (LOX), thereby reducing the synthesis of prostaglandins and other inflammatory mediators. Additionally, their antioxidant activity helps in reducing oxidative stress associated with inflammation and pain.^[26]
- **Steroids (Phytosterols):** Plant steroids, including phytosterols like β -sitosterol, are known to possess anti-inflammatory properties. These compounds stabilize cell membranes and reduce inflammation, indirectly contributing to analgesic effects. They may also modulate immune responses, which play a role in chronic pain conditions.^[27]
- **Terpenoids:** Terpenoids are widely recognized for their anti-inflammatory and analgesic potential. In *Hygrophila auriculata*, terpenoids may act by inhibiting inflammatory pathways and reducing cytokine production. Their role in modulating peripheral pain mechanisms has been highlighted in several pharmacological studies.
- **Tannins and Saponins:** Tannins exhibit astringent and anti-inflammatory properties, which can help in reducing swelling and pain associated with tissue injury. Saponins are known for their membrane-permeabilizing and anti-inflammatory effects.

Together, these compounds contribute to the overall analgesic activity of the plant.^[28]

3.2. Reported Compounds Responsible for Analgesic Effects

Several specific phytoconstituents isolated from *Hygrophila auriculata* have been associated with analgesic activity. These include:

- **Apigenin and luteolin (flavonoids):** Known to inhibit inflammatory mediators and reduce nociceptive responses.^[30]
- **β -sitosterol (phytosterol):** Exhibits anti-inflammatory and membrane-stabilizing properties.
- **Lupeol (triterpenoid):** Demonstrates significant analgesic and anti-inflammatory effects through inhibition of pro-inflammatory cytokines.
- **Stigmasterol:** Contributes to anti-inflammatory and pain-relieving activity.^[29]

These compounds act through both **central and peripheral mechanisms**, including inhibition of prostaglandin synthesis, suppression of inflammatory cytokines, and modulation of pain signaling pathways. The presence of multiple bioactive compounds in *Hygrophila auriculata* suggests a **synergistic mode of action**, where different constituents target various pathways involved in pain perception and inflammation. This multi-target approach enhances therapeutic efficacy while potentially reducing side effects compared to single-compound synthetic drugs.^[31]

The phytochemical richness of *Hygrophila auriculata*, particularly its content of flavonoids, alkaloids, terpenoids, and phytosterols, provides a strong scientific basis for its traditional use as an analgesic agent. These constituents collectively contribute to its pain-relieving properties, supporting further pharmacological and clinical investigations.

4. TRADITIONAL AND ETHNOMEDICINAL USES RELATED TO PAIN

4.1. Role in the Treatment of Pain and Inflammatory Conditions

Hygrophila auriculata has long been recognized in traditional systems of medicine for its effectiveness in alleviating pain and inflammation. In Ayurveda, it is attributed with “Shothahara” (anti-inflammatory) and “Vedanasthapana” (analgesic) properties, indicating its dual role in reducing both the cause and perception of pain. The plant is frequently prescribed in conditions

characterized by swelling, redness, and discomfort, where inflammation plays a central role in the pathogenesis.^[32]

In the management of **arthritis and rheumatic disorders**, *Hygrophila auriculata* is particularly valued. These conditions involve chronic inflammation of joints, leading to pain, stiffness, and restricted mobility. Traditional practitioners use the plant to reduce joint swelling and improve flexibility, suggesting its influence on inflammatory mediators and synovial health. Its use extends to both osteoarthritis and inflammatory arthritis-like conditions described in classical texts.^[33]

The plant is also widely used for **generalized body pain**, including muscular aches, fatigue-induced pain, and post-exertional soreness. In such cases, it is believed to restore balance in bodily functions and improve circulation, thereby reducing discomfort. Additionally, its application in **localized inflammatory conditions**, such as swelling due to injury, soft tissue inflammation, and minor trauma, further supports its broad analgesic spectrum. The traditional use of *Hygrophila auriculata* indicates that it may act through both **peripheral mechanisms** (reducing inflammation at the site of injury) and **central mechanisms** (modulating pain perception), making it a versatile herbal remedy for pain management.^[34]

4.2. Preparation Forms Used in Traditional Medicine

The therapeutic efficacy of *Hygrophila auriculata* in traditional medicine is closely linked to the diverse methods of preparation employed to extract and deliver its bioactive constituents. These preparation techniques are refined through centuries of empirical knowledge to suit different types of pain and disease conditions.

- **Decoctions (Kwath):** Decoctions are among the most widely used preparations, especially for systemic inflammatory conditions and internal pain. The roots, leaves, or whole plant are boiled in water to extract water-soluble constituents such as flavonoids and tannins. This form is commonly administered in conditions like arthritis, rheumatism, and abdominal pain, where sustained therapeutic action is required.^[35]
- **Powders (Churna):** Dried plant materials, particularly seeds and roots, are pulverized into fine powders and consumed orally. These powders are often taken with water, milk, or honey, depending on the therapeutic need. They are preferred in chronic conditions such as long-standing joint pain and weakness, where gradual and sustained action is beneficial. Seed powders are also valued for their rejuvenating and strengthening properties.
- **Extracts and Infusions:** Alcoholic and aqueous extracts are used to concentrate the active phytoconstituents, enhancing potency and bioavailability. These preparations are especially useful in managing moderate to severe pain

conditions, where a stronger pharmacological effect is desired. Infusions, prepared by soaking plant material in hot water, are milder and often used for routine or preventive purposes.

- **Topical Applications:** External application is a key aspect of traditional therapy for localized pain. Fresh leaves are crushed into a paste and applied directly over inflamed or painful areas. This method is particularly effective in conditions such as joint swelling, muscular pain, sprains, and wounds. The topical route allows direct action at the site of inflammation, providing rapid relief and minimizing systemic exposure.^[36]

The diversity in preparation forms reflects the adaptability of *Hygrophila auriculata* in addressing different types and severities of pain.

4.3. Regional Medicinal Applications

The ethnomedicinal use of *Hygrophila auriculata* varies across different regions, influenced by local traditions, environmental availability, and cultural practices. Despite these variations, a consistent theme is its widespread application in pain and inflammatory conditions.

In **India**, particularly in rural and tribal communities, the plant is extensively used as a remedy for rheumatism, joint pain, and inflammatory swellings. Traditional healers often prescribe decoctions for internal use and leaf pastes for topical application. In Ayurvedic formulations, it is sometimes combined with other herbs to enhance its therapeutic efficacy in managing chronic pain disorders.^[37]

In **Sri Lanka and Nepal**, the plant is similarly used for musculoskeletal pain, including stiffness and swelling associated with inflammatory conditions. It is also employed in the treatment of edema, which is often linked with inflammatory processes. The seeds are consumed as a tonic to improve strength and reduce fatigue-related pain.

In parts of **Southeast Asia**, *Hygrophila auriculata* is incorporated into traditional remedies for both internal and external pain conditions. Its use in combination with other medicinal plants reflects a polyherbal approach, where synergistic effects are harnessed to improve therapeutic outcomes. These regional practices highlight the adaptability and cultural significance of the plant, reinforcing its role as a widely accepted natural analgesic.^[38]

4.4. Ethnopharmacological Significance

The extensive and consistent use of *Hygrophila auriculata* across diverse traditional systems underscores its strong ethnopharmacological relevance. The convergence of its applications in Ayurveda, folk medicine, and regional healing practices suggests a well-established therapeutic profile, particularly in the

management of pain and inflammation. Such traditional knowledge provides a valuable foundation for modern scientific research. The repeated use of the plant for similar indications across different cultures increases the credibility of its therapeutic claims and supports its selection for pharmacological evaluation.

Moreover, the plant's use in multiple forms both internal and external indicates a comprehensive approach to pain management, targeting various aspects of the condition. This aligns with modern concepts of multimodal analgesia, where different mechanisms are targeted simultaneously for improved outcomes. In conclusion, the ethnomedicinal evidence strongly supports the analgesic potential of *Hygrophila auriculata*, justifying further preclinical and clinical investigations to validate its efficacy, elucidate its mechanisms of action, and explore its potential as a safer alternative to conventional analgesics.^[39]

5. MECHANISMS OF PAIN AND ANALGESIA

Understanding the mechanisms underlying pain perception and its modulation is essential for evaluating the analgesic potential of medicinal plants such as *Hygrophila auriculata*. Pain involves complex interactions between peripheral tissues, the nervous system, and biochemical mediators.

5.1. Peripheral vs Central Pain Pathways

Pain perception begins with the activation of specialized sensory receptors known as nociceptors, which are located in peripheral tissues such as skin, muscles, and viscera. These receptors are stimulated by noxious stimuli including mechanical injury, heat, or chemical irritants.

- **Peripheral Pain Pathway:** In the peripheral pathway, tissue injury leads to the activation of nociceptors, which transmit signals through primary afferent neurons (A-delta and C fibers) to the dorsal horn of the spinal cord. This stage involves the release of local mediators that sensitize nociceptors, resulting in increased pain sensitivity (hyperalgesia). Peripheral analgesics primarily act at this level by inhibiting the generation or transmission of pain signals at the site of injury.^[40]
- **Central Pain Pathway:** From the spinal cord, pain signals are transmitted to higher brain centers, including the thalamus and cerebral cortex, where they are interpreted as pain. The central nervous system (CNS) also plays a role in modulating pain through descending inhibitory pathways involving neurotransmitters such as serotonin and norepinephrine. Central analgesics, such as opioids, act by altering pain perception and increasing the pain threshold within the CNS.^[41]

5.2. Role of Inflammatory Mediators

Inflammation is a key contributor to pain, particularly in conditions such as arthritis and tissue injury. The release of various biochemical mediators at the site of damage

leads to sensitization of nociceptors and amplification of pain signals.

- **Prostaglandins:** Prostaglandins, particularly PGE₂, are synthesized from arachidonic acid via cyclooxygenase (COX) enzymes. They play a central role in pain and inflammation by increasing the sensitivity of nociceptors to stimuli. This results in enhanced pain perception even in response to mild stimuli.^[42]
- **Cytokines:** Pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukins (IL-1β, IL-6), and others are released during inflammation. These molecules not only promote inflammatory responses but also contribute to pain by activating and sensitizing nociceptors.
- **Other Mediators:** Additional mediators such as histamine, bradykinin, and substance P also participate in pain signaling. They increase vascular permeability, promote inflammation, and directly stimulate pain receptors.

The combined action of these mediators leads to peripheral sensitization and, in chronic conditions, may also contribute to central sensitization, where the CNS becomes more responsive to pain stimuli.^[43]

5.3. Mechanisms of Action of Plant-Based Analgesics

Medicinal plants exert analgesic effects through multiple mechanisms, often targeting both peripheral and central pathways. This multi-target approach is a key advantage of plant-based therapies. One of the primary mechanisms involves the **inhibition of inflammatory mediators**. Many plant-derived compounds, particularly flavonoids and terpenoids, inhibit COX and lipoxygenase (LOX) enzymes, thereby reducing the synthesis of prostaglandins and leukotrienes. This leads to decreased inflammation and pain at the site of injury.^[44]

Plant constituents also exhibit **antioxidant activity**, which helps in reducing oxidative stress associated with inflammation. By scavenging free radicals, they prevent tissue damage and indirectly reduce pain. Another important mechanism is the **modulation of neurotransmitters** in the central nervous system. Certain alkaloids and flavonoids can interact with opioid receptors or influence serotonergic and adrenergic pathways, contributing to central analgesic effects. Additionally, plant-based compounds may **inhibit the release of pro-inflammatory cytokines**, thereby reducing both inflammation and nociceptor sensitization. Some compounds also stabilize cell membranes and reduce vascular permeability, further limiting inflammatory responses.^[45]

The analgesic activity of *Hygrophila auriculata* can be attributed to its rich phytochemical composition, including flavonoids, alkaloids, terpenoids, and phytosterols. These constituents are likely to act through a combination of mechanisms, such as inhibition of prostaglandin synthesis, suppression of inflammatory cytokines, and modulation of pain pathways. This

multimodal mechanism aligns with its traditional use and supports its potential as a natural analgesic agent with both peripheral and central actions.^[46]

6. PRECLINICAL STUDIES ON ANALGESIC ACTIVITY OF *Hygrophila auriculata*

A number of experimental studies have been carried out to scientifically validate the analgesic potential of *Hygrophila auriculata*. These studies have employed well-established animal models and reported quantitative outcomes, demonstrating both peripheral and central analgesic effects of different plant extracts.

6.1. Study Using Acetic Acid-Induced Writhing Model

An early pharmacological evaluation by Singh et al. (2006) assessed the analgesic activity of the ethanolic extract of the whole plant using the acetic acid-induced writhing test in mice. In this study, the control group exhibited an average of 65–70 writhes, indicating a strong nociceptive response. However, administration of the plant extract produced a significant and dose-dependent inhibition of writhing. At doses of 100 mg/kg, 200 mg/kg, and 400 mg/kg, the percentage inhibition was reported to be approximately 20–25%, 40–50%, and 60–70%, respectively.^[47]

At the highest dose, the effect of the extract was found to be nearly comparable to that of aspirin (100 mg/kg), which showed about 70–75% inhibition. This study clearly demonstrated that *Hygrophila auriculata* possesses significant peripheral analgesic activity, likely mediated through inhibition of prostaglandin synthesis and other inflammatory mediators.^[48]

6.2. Study Involving Both Peripheral and Central Models

Ahmed et al. (2008) conducted a comprehensive study using the methanolic extract of *Hygrophila auriculata* leaves, evaluating its effects in both acetic acid-induced writhing and hot plate models. In the writhing test, the extract produced a significant reduction in abdominal constrictions, with approximately 55–65% inhibition observed at a dose of 400 mg/kg.^[49]

In the hot plate test, which assesses central analgesic activity, the reaction time increased from about 3.5 seconds in the control group to nearly 6.8 seconds in treated animals. In comparison, morphine at a dose of 5 mg/kg produced a latency of approximately 8–9 seconds. Although the plant extract was less potent than morphine, the increase in reaction time clearly indicated involvement of central pain pathways. This study confirmed that *Hygrophila auriculata* exerts both peripheral and moderate central analgesic effects.^[50]

6.3. Study Using Formalin-Induced Paw Licking Test

Karthikeyan et al. (2010) evaluated the analgesic activity of aqueous and alcoholic extracts of the roots using the formalin-induced paw licking model in rats. The study

reported that the extract significantly reduced licking behavior in both phases of the test. In the early phase, which represents neurogenic pain, inhibition of approximately 30–40% was observed. In the late phase, associated with inflammatory pain, the inhibition was more pronounced, ranging between 55–65%. The greater suppression of the late phase indicates a strong anti-inflammatory component of the analgesic action, while the moderate effect in the early phase suggests additional central involvement.^[51]

6.4. Study on Carrageenan-Induced Inflammatory Pain

Reddy et al. (2012) investigated the anti-inflammatory and analgesic effects of *Hygrophila auriculata* using the carrageenan-induced paw edema model in rats. The study demonstrated a significant reduction in paw edema, particularly during the second phase of inflammation, which is primarily mediated by prostaglandins. At higher doses, the extract produced approximately 45–60% inhibition of paw swelling. This reduction in inflammation directly correlates with decreased pain perception, thereby supporting the role of the plant as a peripheral analgesic agent. The findings further reinforce the involvement of cyclooxygenase pathway inhibition.^[52]

6.5. Study in Chronic Pain Model (CFA-Induced Arthritis)

Kumar et al. (2014) extended the evaluation to chronic pain conditions using the Complete Freund's Adjuvant (CFA)-induced arthritis model. In this study, repeated administration of *Hygrophila auriculata* extract resulted in a significant reduction in paw swelling, hyperalgesia, and pain sensitivity over time. The treated animals showed marked improvement in behavioral responses to pain stimuli, along with a reduction in inflammatory markers. These findings suggest that the plant is not only effective in acute pain models but also possesses significant therapeutic potential in chronic inflammatory conditions such as arthritis.^[53]

Collectively, the available preclinical studies clearly demonstrate that *Hygrophila auriculata* exhibits significant and consistent analgesic activity across multiple experimental models. The effects are dose-dependent and involve both peripheral and central mechanisms. The strong inhibition observed in inflammatory models highlights the importance of its anti-inflammatory action, while the results from central models indicate modulation of pain pathways within the nervous system.^[54]

Table 3: Summary of Preclinical Studies on Analgesic Activity of *Hygrophila auriculata*.

Author (Year)	Plant Part & Extract	Animal Model Used	Dose (mg/kg)	Parameters Measured	Key Findings	Type of Analgesic Activity
Singh et al. (2006)	Whole plant (Ethanol extract)	Acetic acid-induced writhing test (mice)	100, 200, 400	Number of writhes	20–25%, 40–50%, and 60–70% inhibition observed in a dose-dependent manner; effect comparable to aspirin (70–75% inhibition) at higher dose	Peripheral analgesic (prostaglandin inhibition)
Ahmed et al. (2008)	Leaves (Methanolic extract)	Acetic acid writhing test and Hot plate test (mice)	Up to 400	Writhing count; Reaction time (sec)	55–65% inhibition in writhing test; reaction time increased from ~3.5 sec to ~6.8 sec (morphine: ~8–9 sec)	Peripheral + Central analgesic
Karthikeyan et al. (2010)	Roots (Aqueous & Alcoholic extracts)	Formalin-induced paw licking test (rats)	Not specified (effective doses used)	Paw licking time (early & late phase)	30–40% inhibition in early phase; 55–65% inhibition in late phase; stronger effect in inflammatory phase	Peripheral (dominant) + Central (moderate)
Reddy et al. (2012)	Whole plant extract	Carrageenan-induced paw edema (rats)	Higher doses (dose-dependent)	Paw volume (edema)	45–60% inhibition of paw edema, especially in second phase (prostaglandin-mediated)	Anti-inflammatory associated analgesic (Peripheral)
Kumar et al. (2014)	Whole plant extract	CFA-induced arthritis model (rats)	Repeated dosing	Paw swelling, hyperalgesia, behavioral pain response	Significant reduction in chronic inflammation, pain sensitivity, and improved behavioral responses; decreased inflammatory markers	Chronic analgesic + Anti-inflammatory

7. SAFETY AND TOXICOLOGICAL PROFILE OF *Hygrophila auriculata*

Evaluation of safety is a critical component in the development of any pharmacologically active plant. Although *Hygrophila auriculata* has a long history of traditional use, scientific validation through toxicological studies is essential to establish its safety margin and therapeutic applicability.

7.1. Acute Toxicity Studies

Acute toxicity studies conducted on *Hygrophila auriculata* extracts have generally demonstrated a **high margin of safety**. In experimental investigations using rodents, oral administration of aqueous and ethanolic extracts at doses up to 2000 mg/kg did not produce mortality or any significant behavioral abnormalities during the observation period (usually 24–72 hours). Animals treated with high doses showed no signs of toxicity such as tremors, convulsions, salivation, diarrhea, or lethargy. Additionally, no significant changes were observed in food intake, body weight, or general activity. Based on these findings, the median lethal dose (LD₅₀) is considered to be greater than 2000 mg/kg, indicating that the plant extract falls under the category of **relatively non-toxic substances**.^[55]

7.2. Sub-Acute Toxicity Studies

Sub-acute toxicity studies, typically conducted over a period of 14 to 28 days, have further supported the safety profile of *Hygrophila auriculata*. Repeated administration of plant extracts at varying dose levels did not result in any significant toxic effects in experimental animals. Biochemical parameters such as liver function markers (ALT, AST, ALP), renal function markers (urea, creatinine), and hematological indices remained within normal limits. Histopathological examination of vital organs, including liver, kidney, and heart, did not reveal any major structural abnormalities or tissue damage. These findings suggest that prolonged administration of *Hygrophila auriculata* at therapeutic doses is **well tolerated** and does not produce cumulative toxicity.

7.3. Reported Side Effects

Based on available preclinical studies and traditional usage, *Hygrophila auriculata* is generally considered safe, with **minimal or no reported adverse effects** at therapeutic doses. No significant toxic symptoms have been documented in animal studies, even at higher dose levels. However, it is important to note that systematic clinical data in humans are limited. Therefore, while traditional use indicates good tolerability, caution is advised in extrapolating these findings to long-term human use without further clinical evaluation.^[56]

7.4. Therapeutic Index

The therapeutic index (TI) is an important parameter that reflects the safety margin of a drug by comparing the effective dose to the toxic dose. In the case of *Hygrophila auriculata*, the absence of toxicity at doses significantly higher than those producing analgesic effects suggests a **wide therapeutic index**. For instance, analgesic activity has been reported at doses ranging from 100 to 400 mg/kg, whereas no toxicity was observed even at doses up to 2000 mg/kg. This large difference between effective and toxic doses indicates a favorable safety profile.

Overall, the available toxicological data indicate that *Hygrophila auriculata* is a **safe and well-tolerated medicinal plant** with a high therapeutic margin. Its low toxicity, combined with significant pharmacological activity, supports its potential for further development as a plant-based analgesic agent. However, comprehensive chronic toxicity studies and clinical trials are necessary to fully establish its long-term safety in humans.^[57]

8. LIMITATIONS OF CURRENT PRECLINICAL STUDIES

Despite promising findings, the existing preclinical evidence on the analgesic activity of *Hygrophila auriculata* presents several limitations that restrict its direct translation into clinical practice.

8.1. Lack of Standardized Extract Preparation

One of the major challenges in current studies is the absence of standardized extraction protocols. Different researchers have employed aqueous, ethanolic, and methanolic extracts prepared under varying conditions, leading to inconsistencies in phytochemical composition. Variations in plant part used, solvent polarity, extraction time, and geographical source of the plant further contribute to heterogeneity in results. This lack of standardization makes it difficult to compare findings across studies and to establish reproducible pharmacological outcomes.^[58]

8.2. Variability in Experimental Models

Another important limitation is the variability in experimental designs and animal models. Although commonly used models such as acetic acid-induced writhing, formalin test, and hot plate test are widely accepted, differences in protocols, animal species, dosing regimens, and evaluation parameters can influence the results. Such variability reduces the comparability of data and may lead to inconsistent conclusions regarding the efficacy and mechanism of action of the plant.

8.3. Limited Mechanistic Studies

While many studies have demonstrated significant analgesic effects, detailed investigations into the underlying molecular mechanisms remain limited. Most reports infer mechanisms based on observed outcomes rather than direct biochemical or molecular evidence. There is a lack of studies exploring specific targets such

as cyclooxygenase (COX) inhibition, cytokine modulation, receptor binding interactions, and signaling pathways. This gap limits a comprehensive understanding of how *Hygrophila auriculata* exerts its analgesic effect.

8.4. Absence of Clinical Validation

A critical limitation is the lack of well-designed clinical trials in humans. Although preclinical data and traditional usage strongly support its analgesic potential, there is insufficient clinical evidence to confirm its safety, efficacy, optimal dosage, and long-term effects in human populations. Without clinical validation, its therapeutic application remains largely experimental.^[59]

9. FUTURE PERSPECTIVES

To fully realize the therapeutic potential of *Hygrophila auriculata* as a natural analgesic agent, future research should focus on addressing the current limitations and advancing scientific understanding.

9.1. Need for Advanced Molecular Studies

Future investigations should emphasize detailed molecular and mechanistic studies to elucidate the exact pathways involved in its analgesic action. Techniques such as gene expression analysis, receptor binding studies, and enzyme inhibition assays can provide deeper insights into its interaction with pain pathways. Understanding these mechanisms will strengthen its pharmacological credibility and support drug development.

9.2. Isolation and Characterization of Active Constituents

Although several phytochemicals have been identified, there is a need to isolate, purify, and characterize the specific compounds responsible for analgesic activity. Advanced analytical techniques such as HPLC, GC-MS, and NMR spectroscopy can be employed for this purpose. Identification of active principles will enable the development of more targeted and standardized therapeutic agents.

9.3. Clinical Trials for Human Validation

Well-designed randomized controlled trials are essential to validate the efficacy and safety of *Hygrophila auriculata* in humans. Clinical studies should focus on different pain conditions, dosage optimization, pharmacokinetics, and long-term safety. Such evidence is crucial for its acceptance in modern medical practice.

9.4. Development of Novel Formulations

There is significant scope for the development of advanced drug delivery systems to enhance the bioavailability and therapeutic efficacy of *Hygrophila auriculata*. Novel formulations such as nanoparticles, liposomes, transdermal systems, and herbal gels can improve targeted delivery and patient compliance. Standardized formulations will also ensure consistency in therapeutic outcomes.

10. CONCLUSION

The present review highlights the significant analgesic potential of *Hygrophila auriculata*, supported by both traditional usage and preclinical scientific evidence. Various experimental studies have demonstrated that the plant exhibits notable analgesic activity across multiple models, including peripheral, central, and inflammatory pain pathways. The observed effects are largely attributed to its rich phytochemical composition, particularly flavonoids, alkaloids, terpenoids, and phytosterols, which act through inhibition of inflammatory mediators, modulation of nociceptive pathways, and antioxidant mechanisms. Importantly, *Hygrophila auriculata* has shown a dual mode of action, exerting both peripheral and central analgesic effects, which enhances its therapeutic relevance in managing different types of pain. Its significant anti-inflammatory activity further strengthens its role in treating pain associated with inflammatory conditions such as arthritis and tissue injury. Additionally, toxicological studies indicate a favorable safety profile with a wide therapeutic index, making it a comparatively safer alternative to conventional analgesics.

The importance of *Hygrophila auriculata* as a promising herbal analgesic lies in its long-standing ethnomedicinal use combined with emerging pharmacological validation. Unlike synthetic drugs, which are often associated with adverse effects and dependency risks, plant-based therapies offer a multi-targeted approach with improved tolerability. However, despite these promising findings, there remains considerable scope for further research. Future studies should focus on the isolation of active constituents, detailed mechanistic investigations, and well-designed clinical trials to confirm its efficacy and safety in humans. Moreover, the development of standardized extracts and novel drug delivery systems will be crucial for translating preclinical findings into clinical applications. In conclusion, *Hygrophila auriculata* holds substantial potential as a natural analgesic agent, and with continued scientific exploration, it may contribute significantly to the development of safer and more effective pain management therapies.

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