



HYGROPHILA AURICULATA (SCHUMACH.) HEINE: A REVIEW OF UNANI INSIGHTS, ETHNOPHARMACOLOGICAL USES, PHYTOCHEMICAL PROFILE, TOXICOLOGICAL EVALUATION & MODERN PHARMACOLOGICAL EVIDENCE

Ameera Ayyub^{1*}, Nazish Siddiqui², Shamshad Alam³, Ariz Ayyub⁴

^{1*}P.G. Scholar, Department of Ilmul Advia (Unani Pharmacology),

²Associate Professor, Department of Ilmul Advia (Unani Pharmacology),

³Assistant Professor, Department of Ilmul Advia (Unani Pharmacology),

⁴P.G. Scholar, Department of Amraz-e-Jild wa Zohrawiya (Unani Dermatology),

Faculty of Unani Medicine, Aligarh Muslim University, Aligarh, 202002, (U.P.) INDIA

***Corresponding author:** Dr. Ameera Ayyub

P.G. Scholar, Department of Ilmul Advia (Unani Pharmacology), Faculty of Unani Medicine,

A.M.U., Aligarh, 202002, (U.P.) INDIA **Email id:** ameeraayyub1234@gmail.com

Mobile No.: 8938968572

Abstract

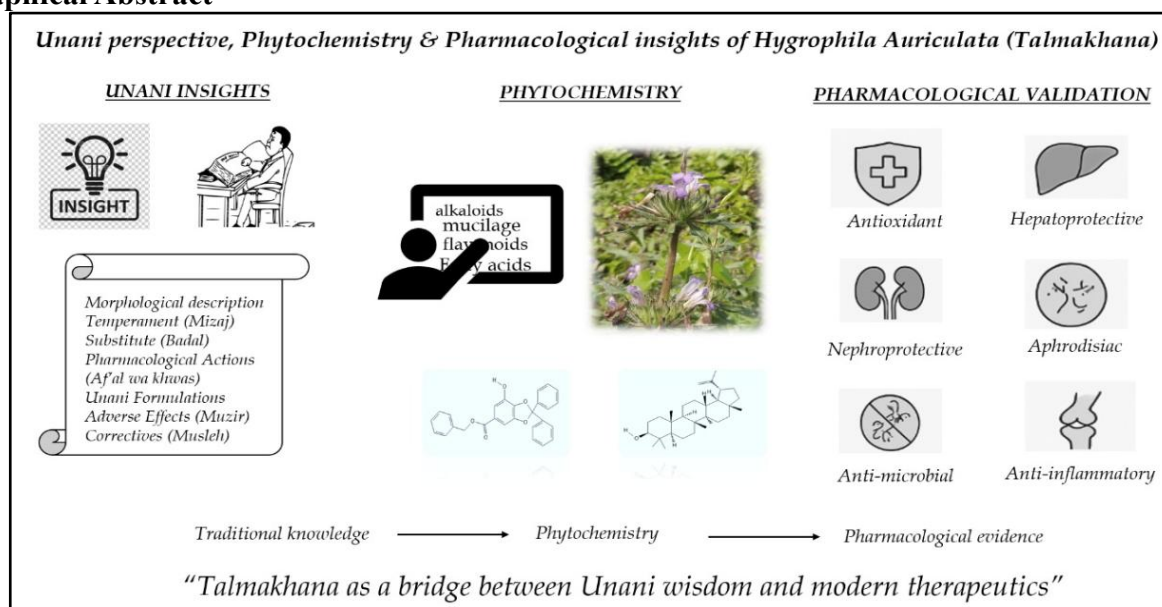
Herbal medicines remain a cornerstone of traditional and complementary healthcare, offering bioactive compounds with significant therapeutic potential. Among these, *Hygrophila auriculata* (Schumach.) Heine, belonging to the family *Acanthaceae*, commonly known as Talmakhana in Unani medicine, stands out as an exemplary botanical with diverse therapeutic applications. The plant's utilization spans multiple traditional medical systems—including Unani, Ayurveda, Siddha, and homeopathy. This review aims to provide a comprehensive account of the medicinal value of *H. auriculata* (Schumach.) Heine, highlighting its Unani insights, ethnomedicinal relevance, pharmacognostic features, phytochemical composition, toxicology and modern pharmacological activities. An extensive survey was conducted from 1941-2025 using various literatures such as related books, Unani classical texts, review articles, research papers via scientific databases & relevant studies reporting this information, then the content was systematically reviewed and synthesized. Findings reveal that *H. auriculata* (Schumach.) Heine, contains diverse bioactive compounds, including flavonoids, phenolic acids, glycosides, and alkaloids, which contribute to its broad-spectrum biological activities such as antioxidant, antimicrobial, anti-inflammatory, analgesic, and anticancer properties. Ethno-medicinal records support its use in treating respiratory disorders, digestive ailments, wounds, and inflammatory conditions. Pharmacognostic studies further authenticate its identity and quality. Overall, the compiled data highlight the therapeutic versatility of *H. auriculata* (Schumach.) Heine, supported by both traditional knowledge and modern scientific validation. Integrating traditional wisdom with contemporary scientific inquiry offers promising avenues for novel drug discovery. However, to fully harness its potential, future research should prioritize clinical trials, bioavailability studies, dosage standardization, and formulation development to establish its role in evidence-based medicine.

Keywords: *Hygrophila auriculata*; Unani medicine; Traditional medicine; Phytochemical; Pharmacological; Antioxidant; Talmakhana

Abbreviations

AD	Anno Domini
BC	Before Christ
cm	Centimeter
CE	Common Era
mm	Millimeter
g	Grams
ml	Milliliter
RP-HPLC	Reversed-Phased High-Performance Liquid Chromatography
HPTLC	High-Performance Thin-Layer Chromatography
GCMS	Gas Chromatography-Mass Spectrometry
µm	Micrometer
nm	Nanometer
°C	Celsius
LOD	Limit of detection
LOQ	Limit of Quantification
µg	Microgram
RSD	Relative Standard Deviation
DPPH	2,2-Diphenyl-1-picrylhydrazyl
NO	Nitric oxide
FRAP	Ferric Reducing Antioxidant Power Assay
IC ₅₀	Half maximal inhibitory concentration
FTC	Ferric Thiocyanate
TBA	Thiobarbituric Acid
CCl ₄	Carbon tetrachloride
AST	Aspartate aminotransferase
ALT	Alanine transaminase
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
mg	Milligram
Kg	Kilogram
RBC	Red Blood Corpuscle
i.p.	Intraperitoneal
DMBA	7,12-Dimethylbenz(a)anthracene
CYC	Cyclophosphamide
Hb	Haemoglobin
HbA1c	Glycated haemoglobin
SOD	Superoxide Dismutase
CAT	Catalase
GSH	Glutathione
CP	Cisplatin
MDA	Malondialdehyde
LPS	Lipopolysaccharide
IL	Interleukin
TNF	Tumor necrosis factor
LPO	Lipid peroxidation
p.o.	per orally
tGCI	Transient Global Cerebral Ischemia
T.S.	Transverse section

Graphical Abstract



1. Introduction

Natural products have long supported human life by providing essential sources of food and medicine. Over the past few centuries, there has been a growing interest in medicinal plants, especially in developing countries, as plant-based therapies are often considered safer and associated with fewer side effects compared to synthetic drugs [1]. For thousands of years, traditional medical systems have relied on plants as their primary source of treatment. Even today, plants continue to serve as a vital resource for the discovery and development of new medicines. The use of plant-based treatments is largely rooted in empirical knowledge accumulated over centuries [2]. Currently, Unani medicine plays a significant role within the traditional medical systems and has been used since ancient times for the management of various diseases [3].

Ancient Greeks were the first to develop the Unani medical system, which was subsequently improved and extended by Roman, Arabic, Spanish, Iranian, and Indian academics. Consequently, it is sometimes called Greco-Arab Medicine. It is a comprehensive medical system that provides preventative, curative, rehabilitative, and promotional treatment, addressing all states of health and sickness [4]. The teachings of eminent Greek and Roman physicians, such as Buqrat (Hippocrates, 460–370 BC) and Jalinoos (Galen, 129–210 AD), as well as renowned Arab and Persian scholars, such as Al-Razi (Rhazes, 850–925 AD), Ibn Sina (Avicenna, 980–1037 AD), Al-Zahrawi (Abulcasis, 936–1036 AD), and Ibn Nafis (1213–1288 AD), have contributed to the development of Unani medicine into a structured medical science [5]. The four humours—blood, phlegm, yellow bile, and black bile—each linked to distinct temperamental traits—hot and moist, cold and moist, hot and dry,

and cold and dry, respectively—are the basis of the Hippocratic idea of the four humours. The seven fundamental parts of the human body—elements, temperament, humors, organs, pneuma, faculties, and functions—are outlined in Unani doctrine. Any of these components that are missing or out of balance endanger life and cause illness. The approach places a strong emphasis on the function of *Medicatrix Naturae*, the body's innate healing ability that controls physiological functions, confers resistance to sickness, and promotes healing [6]. The diagnostic and therapeutic approaches of Unani medicine are grounded in scientific reasoning and holistic principles, considering the entire personality rather than isolating individual symptoms [7]. While some of its concepts differ from Western medicine and may not always align with its principles, the Unani system presents a coherent and rational framework for understanding health and disease. A wide range of single and compound remedies are employed within the system to address various pathological conditions [8].

Hygrophila auriculata (Schumach.) Heine, belonging to the family *Acanthaceae*, is recognized as the source of the Unani medicinal drug Talmakhana. Talmakhana comprises small, ovate, compressed dried seeds of *H. auriculata* (Schumach.) Heine, which are pale brown in colour [9, 10]. The plant itself is an unbranched, stout, erect herb that can grow up to approximately 150 cm in height. Its flowers are bright blue, while the roots are creamy yellow, characterized by a distinct marshy odour and a slimy, cooling taste [10,11]. The seeds are rich in mucilage, which rapidly forms a thick coating in the mouth, adhering tenaciously to the tongue and palate upon contact [12]. *H. auriculata* (Schumach.) Heine is also known by various synonyms, including *Asteracantha longifolia* (L.) Nees, *Hygrophila spinosa* T. Anderson, *Barleria auriculata* K. Schum, *Hygrophila schulli* (Ham.) Almeida, and *Ruelia longifolia* (L.) Roxb. The name *Hygrophila auriculata* (Schumach.) Heine, has been verified to be acceptable on the “Plants of the World Online” & “World Flora Online” websites [13, 14, 15]. The plant is widely distributed across tropical and subtropical regions, including India, Sri Lanka, Indonesia, Myanmar, Burma, Malaysia, Nepal, and parts of Africa, particularly thriving in moist and marshy areas [16]. The plant has been extensively utilized in various traditional medical systems such as Unani, Ayurveda, and Siddha. It holds a significant place in Hindu Materia Medica [17]. In Ayurveda, different parts of the plant are used as a tonic, aphrodisiac, hypnotic, and diuretic, and are prescribed for conditions such as arthritis, diarrhoea, and sexual dysfunctions like impotency [18, 19]. In Siddha medicine, a leaf decoction is considered beneficial in managing hypertension [20]. Similarly, in homeopathy, *H. auriculata* (Schumach.) Heine is employed to treat jaundice, urogenital disorders, arthritis, and constipation [21]. In Unani medicine, since its origin from Greek medical traditions, various parts of *H. auriculata* (Schumach.) Heine have been used for multiple therapeutic purposes. Classical Unani literature extensively documents the plant’s pharmacological actions, including its use as a cooling agent, diuretic, stimulant, resolvent, and lithotriptic. It has been particularly recommended for dropsical conditions and kidney stones, and externally as a poultice or embrocation for rheumatism [9, 22]. According to Unani practices, *H. auriculata* (Schumach.) Heine is believed to enhance adaptability, vitality, and overall body function, helping to correct dysfunctions within the system. The seeds are considered nourishing, aphrodisiac, and tonic, and are widely used in treating blood disorders and conditions of biliousness. They are especially recommended for ailments such as nocturnal emission, seminal weakness, spermatorrhoea, and erectile dysfunction [23, 24, 25]. Additionally, “Pharmacopoeia of India” and European sources attest to the exceptional diuretic properties of the plant’s roots. The seeds and roots, sold in dried form in local markets, are highly regarded by native populations for their diuretic effects in managing dropsical conditions, a claim supported by European medical observations [11].

2. Methodology

The data for the present study were compiled from authoritative and relevant Unani medical texts, like *Muheet-e-Azam* by Hakim Azam Khan (1813–1902 AD) and *Khazainul Advia* by Najmul Ghani (1859 CE), along with other classical references. Supplementary information was obtained from contemporary scientific literature, including review articles and original research papers accessed through databases such as PubMed, ScienceDirect, Google Scholar, Research gate as well as the other

Google search engines. The botanical identity and synonyms of *Hygrophila auriculata* (Schumach.) Heine were cross-verified using authentic reference books and indexed journals. Specific keywords such as *Hygrophila auriculata*, Talmakhana, Unani medicine, traditional medicine, phytochemistry, pharmacological, antioxidant, hepatoprotective, and toxicity were employed to retrieve relevant data. Information pertaining to Unani perspectives, botanical description, ethnopharmacological applications, phytochemical profiles, pharmacological studies, and toxicity assessments was systematically collected from 1941 to 2025, critically analysed, and synthesized for this review. The accepted botanical nomenclature was further confirmed through “World Flora Online” (www.worldfloraonline.org) & “Plants of the World Online” (<https://powo.science.kew.org/>), while chemical structures were generated using PubChem.

3. Taxonomical Classification of *Hygrophila auriculata* (Schumach.) Heine [14, 15]

Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Lamiales
Family	Acanthaceae
Genus	<i>Hygrophila</i>
Species	<i>auriculata</i> (Schumach.) Heine

4. Vernaculars of *Hygrophila auriculata* (Schumach.) Heine in various languages

Vernacular names are the local or regional terms used to identify plants across various languages and cultural settings. These names highlight the deep-rooted awareness and utilization of plants by different communities over time [26]. Beyond being mere identifiers, vernacular names embody generations of traditional knowledge regarding the therapeutic properties of plants. In systems of traditional medicine, they play a vital role in facilitating the transfer of knowledge, improving communication between practitioners and patients, supporting biodiversity preservation, enabling accurate scientific research, and fostering culturally sensitive healthcare practices. Documenting and safeguarding these names acknowledge and respects the contributions of communities that have preserved and transmitted medicinal knowledge long before the advent of modern medicine [27].

The herb *Hygrophila auriculata* (Schumach.) Heine is known by various names in different languages and traditional systems. In English, it is commonly referred to as Long Leaved Barleria, Hygrophila, Marsh Barbel, and Asteracantha [28, 29]. Within Unani medicine, it is called Talmakhana, while in Urdu it is known as Talimakhana [9,12]. In Hindi, the plant is identified by several names including Talimakhana, Talmakhana, Gokshura, Kamtakaliya, Phulmakhana, Ik-chura, Talmakhana-ka-pair, and Talmakhara [28, 30]. In Persian, it is known as Makhana, and in Latin it is referred to as Asteracantha, Talmakhana, or Talpukhra. The Sanskrit names include Kokilaksha, Ikashugandha, Ikshura-Gokantaka, Kolistha, and Rakshogandha [22,30]. Additionally, the plant is recognized as Supadan in Burma and Nirmulli in the Sidha system, while in Germany it is called Langblattriger Sterndorn [31].

5. Distribution & Botanical Description of *Hygrophila auriculata* (Schumach.) Heine

5.1 Habitat & Distribution

Hygrophila auriculata (Schumach.) Heine, an annual marsh-dwelling herb, belongs to the family Acanthaceae. It is characterized by its unbranched, stout, and erect structure, reaching up to approximately 150 cm in height. This species predominantly thrives in wet environments. The genus *Hygrophila* comprises around 75 species, with about 11 species occurring in India [11, 32]. *H. auriculata* (Schumach.) Heine is commonly found in marshes, ditches, paddy fields, roadside areas, and along the banks of tanks and canals across the plains of India [33, 34]. The plant propagates through seeds, with flowering and fruiting typically occurring in October [35]. It is also frequently

found in aquatic habitats in the tropical Himalayan region [36]. Globally, the species is distributed in regions such as Sri Lanka, Indonesia, Myanmar, Burma, Malaysia, Nepal, and tropical areas of South Africa, often favouring moist and marshy locales as well as uncultivated land patches [12, 16].

5.2 Plant Description

Hygrophila auriculata (Schumach.) Heine is an erect, stout, semi-woody annual herb that typically grows to a height of 3–4 feet. The plant is characterized by unbranched, slightly quadrangular stems that are densely covered with coarse hairs and feature swollen, clustered nodes [33]. The leaves are simple, greenish-brown, nearly sessile, and oppositely arranged in apparent whorls of six at each node. Among these, the two outer leaves are larger, measuring 15–18 cm in length and 2.5–3.5 cm in width, while the four inner leaves are considerably smaller, with lengths of 3–3.8 cm [30]. The leaf surfaces are sparsely covered with stiff hairs, with acute tips, finely serrated margins fringed with trichomes, and tapering ends. The venation pattern is pinnate and reticulate, with a lamina size ranging from 11 to 16 cm. Straight, sharp spines, 2.5–4.5 cm long and yellowish-brown in colour, arise from the axils of each whorl [28]. The flowers are arranged in axillary clusters of eight at each node, forming four pairs. The lanceolate, hairy, and ciliate bracts are approximately 2.5 cm long, while the linear-lanceolate bracteoles, measuring 1.5–2 cm, have hyaline and hairy margins. The calyx is divided into four segments, with the upper sepal being larger (1.6–2 cm) compared to the other three segments (1.3 cm), all of which are linear-lanceolate, hairy on the dorsal side, and bordered with translucent cilia [12,37]. The corolla is bilabiate, purple-blue in colour, about 3.2 cm long, with a tubular section of 11–13 mm that swells near the top. The two lips of the corolla are nearly equal, each measuring 1.6 cm. The flower possesses four didynamous stamens with smooth filaments; each pair consists of one longer and one shorter filament fused at the base. The anthers are dorsifixed, and the style is slender, slightly pubescent, and thread-like [29, 38]. The ovary is bilocular, containing four ovules. The fruits are linear-oblong capsules, 5–7 mm in length, containing 4–8 seeds. The seeds are small, ovate, flattened, pale brown, and covered with a hygroscopic, mucilaginous layer. They measure approximately 0.2–0.25 cm in length and 0.1–1.5 cm in width, with a truncate base [34]. The roots are biennial, tapering, and extensively branched with numerous fine rootlets. They are cylindrical, measuring 3–8 mm in diameter, with a rough surface that is longitudinally grooved and often marked by transverse cracks and circular scars from fallen lateral roots [39]. The different parts of the plant are depicted in Fig. 1.



Plant



Flowering

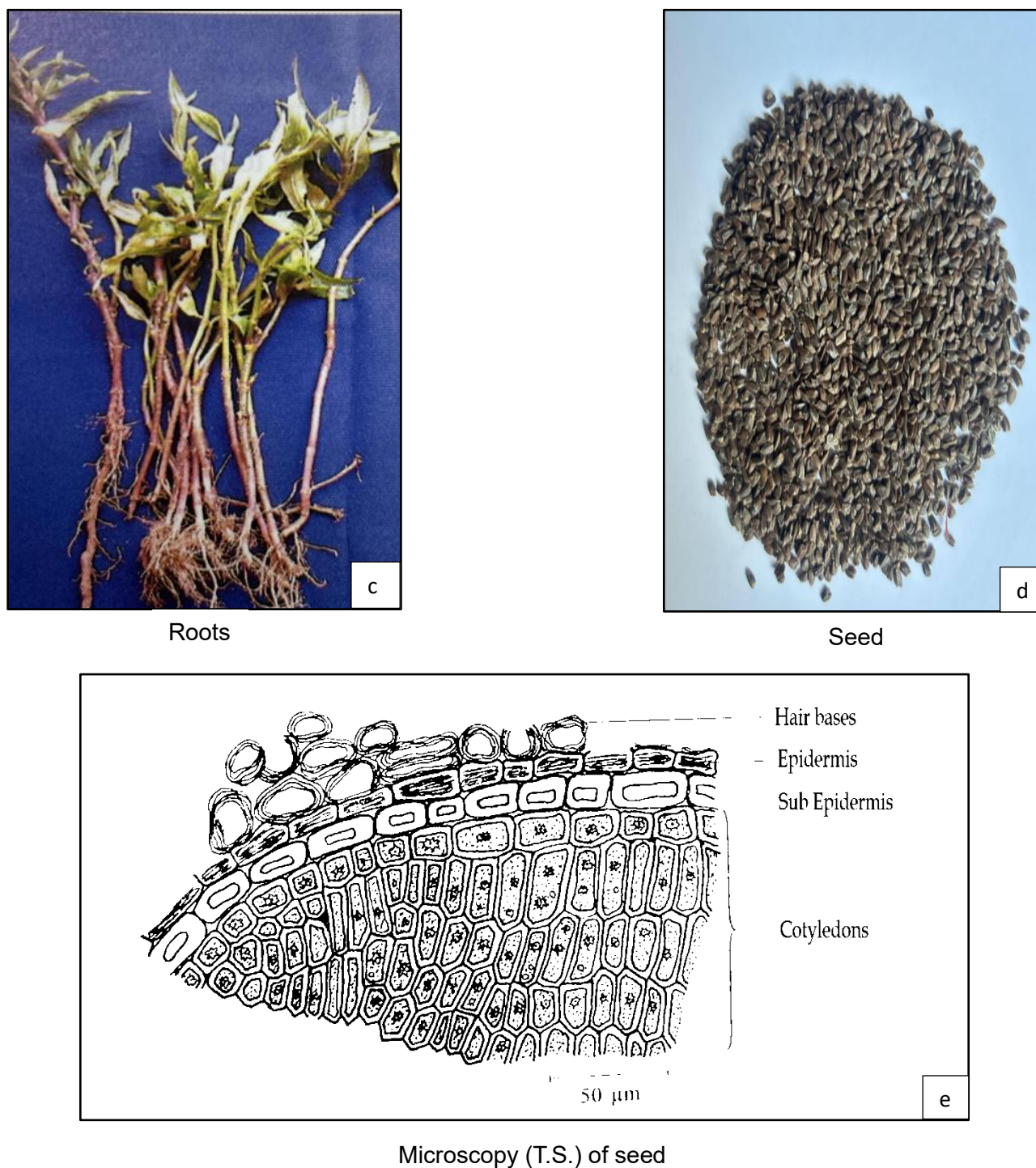


Fig. 1 *Hygrophila auriculata* (Schumach.) Heine: [a] Whole Plant <https://powo.science.kew.org/taxon/49646-1#synonyms> [b] Flowering Shoot : <https://powo.science.kew.org/taxon/49646-1#synonyms> [c] Roots [33] [d] Seeds obtained from Dawakhana Tibbiya College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India [e] Microscopy (T.S.) of seed [10]

6. Description of *Hygrophila auriculata* (Schumach.) Heine in Unani Ancient Literatures

6.1 Morphological Description

Various Unani scholars have provided detailed botanical descriptions of *Hygrophila auriculata* (Schumach.) Heine, highlighting its morphological features and medicinal relevance. In *Khazainul Advia* (Unani pharmacopeia), *Najmul Ghani* (1859 CE), a renowned Unani physician, describes

Talmakhana plant as an erect herbaceous plant reaching 2–4 feet in height, characterized by a quadrangular stem. The leaves occur in whorls of six at each node, with a sharp spine approximately one inch long situated between the stem and the leaves. Both stem and leaves are covered with fine white hairs [22]. Similarly, *Hakim Muhammad Azam Khan* (1815-1920CE), a prominent physician in Unani medicine and other Unani scholars in their classical texts further noted that its leaves resemble those of *Cichorium intybus* leaves but are coarser in texture and distinctly hairy. The flowers are purplish, occasionally tending toward violet-blue, with a distinct white center. These flowers are smaller in size compared to those of *Cichorium intybus* and typically occur in whorls of four at each node. Each flower bears four petals, which are relatively broader [23, 40, 41]. They further noted that its stem possesses rigid nodes resembling those of *Cichorium intybus*, but each node bear spines similar to those of *Acacia nilotica*. This feature distinguishes it from *Cichorium intybus*, whose nodes lack spines. The root system is characterized by numerous thread-like branches that are grooved and ridged [22, 23, 42]. The different parts of the plant are depicted in Fig. 1.

In Unani literature, *Talmakhana* is specifically identified with the seeds of *H. auriculata* (Schumach.) Heine. These seeds are typically pale brown, small, elongated, slightly broad, and display a faint gloss. They possess mucilaginous properties comparable to those of *Lepidium iberis* and show morphological resemblance to the seeds of *Blepharis edulis* [9, 22]. Regionally, the plant has also been referred to by various vernacular names, such as *Prwamoli* (attributed to the reddish root), *Rachoor* (due to its superficial similarity to sugarcane), *Patoka* or *Asthool Kantaka* (in reference to its long spines), and *Kokla Jhka* (associated with the appearance of its thin, pale brown seeds) [22, 23, 24]. Shelf life of seeds is 3 years after that bitterness produces in them and they get decompose [40]. The macroscopic and microscopic attributes of the seed are systematically shown in Table 1a and Table 1b, respectively & also illustrated in Fig. 1 d & e. The descriptions given by Unani scholars provide important information about the botanical features and properties of *H. auriculata* (Schumach.) Heine and its seeds, known as *Talmakhana*, supporting their use in medicinal applications.

Table 1a Macroscopic Characteristics of the Seed [10, 43]

Parameter	Description
Shape	Oblong to broadly ovate
Size	Length: 1–3 mm; Width: 1–2 mm
Surface	Covered with long white hairs; appears smooth due to dense mucilage
Texture	Thin, flat or compressed; thick-walled
Base	Truncate
Apex	Slightly obliquely pointed, with hairs directed towards it
Hilum	Present at the angle of the depression at the pointed end
Micropyle	Located adjacent to the hilum
Colour	Dark brown
Moisture	Upon soaking, the hairs form a filamentous sheath that spreads over the surface
Taste	Highly mucilaginous; slightly bitter and acrid
Odour	Not distinct

Table 1b Microscopic Characteristics of the Seed [10, 28]

Parameter	Description
Testa (Hairy Outer Coat)	Composed of oval to hexagonal lignified and sclerenchymatous cells with thin-walled, tangentially elongated cells; outer surface covered by a pigmented cuticle and attached hairs
Embryo (General)	Consists of oval to polygonal parenchymatous cells containing oil globules

Longitudinal Section (LS)	Shows outer dark brown testa attached to hairs; embryo large and white with two thick, straight cotyledons and a small radicle pointing toward the micropyle
Transverse Section (TS)	Displays outermost testa layer of collapsed, thick-walled, tangentially elongated epidermal cells (dark brown), with circular hair bases externally
Sub epidermis	Layer beneath epidermis, made of oval to rectangular tangentially elongated cells
Cotyledons	Inner tissue shows radially elongated parenchyma cells; epidermal cells are squarish to rectangular
Cell Contents	Cotyledon cells contain aleurone grains, fixed oils, and micro-rosette crystals of calcium oxalate

6.2 Part used

Hygrophila auriculata (Schumach.) Heine serves as the primary source of the Unani drug *Talmakhana* (seeds). In addition to its seeds, the entire plant, along with its roots, leaves, and even the plant ash, are employed for various medicinal applications [9,10, 22,23,30,31,34,40].

6.3 Temperament (Mizaj)

The Unani system of medicine emphasizes the principle of temperament (mizaj) in therapeutic interventions. In this framework, treatment is tailored by considering the patient's inherent temperament, the predominant humour responsible for the ailment, the functional capacity of the affected organ, and the intensity of the condition. Medications are classified according to their specific temperament (mizaj) and are further ranked from grade I to IV based on their strength, with higher grades associated with an increased likelihood of side effects [44].

In Unani medicine, *Talmakhana* (seeds) are considered to have a temperament that is cold (barid) in second degree and wet (ratab) temperament in first degree [9,22,23,40,41,42]. Some scholars says that *Talmakhana* has a hot (haar) and a dry (yabis) temperament in spite of having *Ratobat-e-Fuzliya* (excess moisture) in it [22,23,24,45].

6.4 Therapeutic dose and Substitute (Badal)

The recommended therapeutic dose of *Hygrophila auriculata* (Schumach.) Heine is 3–6 g in powder form [29], while its root decoction is prescribed at 3–6 ml [33]. For *Talmakhana* (seeds), the dosage in powdered form generally ranges from 5–7 g [9,23,40,41,45], though some references suggest 3.5–7 g, or even up to 14 g when required [10, 22].

Various substitutes (*badal*) are recommended for *Talmakhana* seeds due to their comparable pharmacological effects. These include the roots of *Orchis latifolia* (Salab Misri), *Asparagus racemosus* (Satawar), *Asparagus adscendens* (Moosli), the seeds of *Cheiranthus cheiri* (Todri), and the pods of *Helicteres isora* (Marorphali) [9,22,23,24,41,45].

6.5 Pharmacological actions (Af'al wa khwas)

Unani physicians traditionally employ various parts of *Hygrophila auriculata* (Schumach.) Heine such as its seeds, leaves, roots for various therapeutic purposes, considering it to be as Mufarreh (Exhilarant), Musammin-e-Badan (Adipogenous), Muqawwi-e-Badan (General Tonic), Muqawwi-e-Aza (Tonic to organs of the body), Muqawwi-e-Jigar wa Meda (Liver Tonic & Stomachic) & Muqawwi-e-Bah (Aphrodisiac) [23, 41]. It was also recognized for its Musakkin (Analgesic), Qabiz (Astringent), Muhalil (Resolvent), Mubarid (Refrigerant) properties, as well as its role as Mudir-e-Bol (Diuretic), Muffat-e-Urook (Vasodilator), Mulattif-e-Mawad (Demulcent), Jali (Detergent), Mudir-e-Arq (Diaphoretic) [22,24,40]. Additionally, it was noted for its Muffat-e-Hisat (Lithotriptic), Mushil (Purgative), Mulayyin (Laxative), Moalid-e-Sheer (Lactogenic) properties [9, 46]. Furthermore, *H. auriculata* (Schumach) Heine was acknowledged as Mufarreh Qalb (Exhilarant to Heart), Moalid-e-Mani (Semen Procreative), Mugaliz-e-Mani (Inspissant to Semen), Mumsik

(Avoricious), Moaddil-e-Dam wa Sauda wa Safra (Alterative), Mugarre (Glutinous), Muqawwi-e-Asaab (Nervine Tonic), Muqawwi-e-Basar (Ophthalmic), Man-e-Haml (Arresting abortion) in classical literatures of Unani medicine [23,42,45,47].

6.6 Ethnopharmacological Significance in Traditional medicine

The rich heritage of Unani pharmacopoeias, along with other traditional systems such as Ayurveda, provides substantial evidence for the use of *Hygrophila auriculata* (Schumach.) Heine in diverse medicinal forms, including its seeds, leaves, roots, ashes, and the entire plant. In Indian traditional medicine, the roots, leaves, and seeds are primarily employed as diuretics and are also used to treat conditions such as jaundice, dropsy, rheumatism, anasarca, and disorders of the urinary and genital systems [28,30,32,35,42,48]. The dried roots are commonly sold in Indian markets and are highly regarded for their diuretic effects in cases of dropsy, with similar benefits reported by European practitioners [11].

In the Unani system, the root is often administered as a decoction by boiling one ounce of the root in a pint of water for ten minutes, straining, and consuming it in divided doses throughout the day [23,12,49,50]. The root considered as both tonic and diuretic are used to manage ascites, urinary gravel, liver disorders including jaundice, stomach tumors, and even snakebite [22,28,42,51,52]. For the treatment of urinary calculi, a decoction of the root in doses of approximately 70 ml twice daily has shown beneficial effects [22]. The seeds, particularly when ground into a paste, are applied topically to alleviate pain from rheumatic joints and lumbago [30, 52]. Among tribal communities, the seeds are also regarded as a sexual tonic [35]. Hakims frequently prescribe powdered seeds, alone or in combination with other substances like sugar, milk, or wine, in doses ranging from one to three dirhems to address conditions such as impotence, gonorrhoea, and spermatorrhoea, as the seeds are believed to have aphrodisiac properties [9,12,22,23,31]. Additionally, the seeds are used in treating blood disorders like anaemia and conditions associated with vitiated Safra [12,32]. For asthmatic complaints, powdered seeds mixed with honey and ghee are recommended, while ground seeds administered in buttermilk are known to be effective for diarrhoea [22, 31]. In the Unani system, *H. auriculata* (Schumach.) Heine is believed to enhance adaptability, stamina, and energy by improving bodily functions. The seeds, combined with *Tribulus terrestris* and *Asparagus adsendens*, are administered with cow's milk and sugar to treat general debility [23, 31]. Moreover, a confection composed of its seeds and other aphrodisiac, demulcent, and nutritive substances is traditionally used to address impotence, premature ejaculation, nocturnal emissions, and semen-related weaknesses [22, 31]. In Unani a specific formulation is recommended for leucorrhoea, which includes Talamakhana seeds, gums from *Butea frondosa* and *Bombax malabaricum*, seeds of *Sida cardifolisa*, *Nardostachys jatamansi*, *Pistachia terebinthes*, poppy seeds, *Curculigo orchoides*, *Shorea robusta*, and a tuber of *Orchis latifolia*, to be powdered and administered with cow's milk in doses of 5–9g [31].

The leaves of the plant are used as pot-herbs and, when boiled in vinegar, serve as a diuretic treatment for ascites [23,24,51,52]. Baboo Kanny Lall Dey recommended an acetum preparation by macerating dried leaves in vinegar for dropsy treatment, administered in doses of one to three tablespoonfuls in water thrice daily [31,49,50]. Topically, leaf paste is applied for conditions like gleet, lumbago, and joint pains, with vapours from its decoction used for symptomatic relief [12,35,23,24]. In some regions such as the Gold Coast, boiled leaves are used for bathing to treat fever [12]. The leaves are also used to address a wide array of ailments including cough, urethral discharge, polydipsia, vesical calculi, dysentery, renal calculi, diarrhoea, jaundice, anaemia, constipation, and anuria [12,32]. Boiled leaf water is beneficial as a mouthwash for toothache [23]. Additionally, the ashes from the burnt plant are used as a diuretic in dropsy [12,31,50]. A tincture prepared with alcohol (1 in 3 ratio) of whole plant has been used in doses of 20 to 30 drops three times daily to treat urinary infections, particularly dysuria and painful urination [31]. The fruits are also used to manage menorrhagia in women [28]. A seed paste prepared with sheep's milk, applied to the palm's multiple times daily, has been beneficial in alleviating winter-related itching caused by black bile disorders. Leaf paste is additionally applied in the treatment of piles [22].

In Ayurveda, *H. auriculata* (Schumach.) Heine is recognized for its therapeutic potential in diabetes and dysentery [53, 54]. The seeds are used to treat arthritis and blood disorders and are believed to have aphrodisiac and tonic properties, also serving as a uterine sedative during pregnancy. Seeds are often given with sugar or milk to treat impotency, gonorrhoea, and spermatorrhoea, while a paste mixed with buttermilk is used to manage diarrhea [18,53]. Root decoctions are used for rheumatism and as diuretics, while leaves are utilized for their tonic, hypnotic, and aphrodisiac effects [19,53,55]. In Siddha medicine, a leaf decoction is prescribed twice daily to control blood pressure [20, 53]. In homeopathy, the plant is used in the management of jaundice, urinary tract disorders, arthritis, and constipation [21, 53]. Ethnopharmacological Applications of *Hygrophila auriculata* (Schumach.) Heine are depicted in Table 2.

Table 2 Ethnopharmacological Applications of *Hygrophila auriculata* (Schumach.) Heine

Traditional Ethno-pharmacological uses	Part used	Dosage form	Method of administration	References
Dropsy	Roots	Decoction	Taken Orally in divided doses during the day	[12,23,49,50]
Renal & vesical calculi	Roots	Decoction	Taken orally about 70ml twice a day	[22]
Rheumatism & Lumbago	Seeds, Leaves	Paste	Applied topically	[12,24,30,52]
Gonorrhoea, Impotence & Spermatorrhoea	Seeds	Powder	Taken orally with sugar, milk or wine in doses of 1–3 dirhems	[9,12,22,23,31]
Asthma	Seeds	Powder	Taken orally mixed with honey and ghee	[22,31]
Diarrhoea	Seeds	Paste	Taken orally mixing it in buttermilk	[22,31]
General debility	Seeds	Powder	Taken orally combined with <i>T. terrestris</i> and <i>A. adsendens</i> , with cow's milk & sugar	[23,31]
Pre-mature ejaculation, Nocturnal Emission	Seeds	Confection	Taken orally as per need with milk	[22,31]
Leucorrhoea	Seeds	Powder	Taken orally with other ingredients with cow's milk, dose 5–9g	[31]

Ascites	Leaves	Decoction	Taken orally in boiled vinegar	[23,24,51,52]
Arthritis & Arthralgia	Leaves	Decoction	Vapours of decoction applied locally	[12,23,24,35]
Fever	Leaves	Boiled in water	Used for bathing	[12]
Toothache	Leaves	Boiled in water	Used as mouthwash	[23]
Dropsy	Whole plant	Ashes	Taken orally with water	[12,31,50]
Urinary Infections	Whole plant	Tincture (1:3 alcohol)	20–30 drops thrice daily	[31]
Sores on palm	Seeds	Paste in sheep's milk	Applied topically on palms 5–6 times daily for 2 weeks	[22]
Piles	Leaves	Paste	Applied topically	[22]

6.7 Unani formulations and pharmaceutical products

In Unani medicine, the seeds of *Hygrophila auriculata* (Schumach.) Heine, commonly known as Talmakhana, are utilized in powdered form, either mixed with milk or combined with other appropriate formulations, to enhance sexual health and performance [9,22,23,40,42,45]. The pharmacopeial preparations *Majoon-e-Piyaz* [9,22,23,41] and *Safoof-e-Kalan* [9,22,23,41] are widely recognized Unani formulations that incorporate Talmakhana as a principal ingredient. Additionally, commercially available products containing *H. auriculata* (Schumach.) Heine are used in the management of various health conditions. These include *Speman* and *Afrodet Plus®* for promoting spermatogenesis [56, 57], *Uricitral* for childhood urinary tract infections [58], *Livergen* for gastrointestinal and hepatic regulation [59], *UCEX01* as an antiurolithic agent [60], and *Talmakhana* and *Neermulli* for their anabolic-androgenic effects [19].

7. Total metabolites & Phytochemical Report of *Hygrophila auriculata* (Schumach.) Heine

The chemical composition of *Hygrophila auriculata* (Schumach.) Heine has been extensively studied by various researchers. The plant has been found to contain a diverse range of bioactive compounds including Alkaloids, Flavonoids, Triterpenoid alcohols, Fatty acids, Sterols, Minerals, Amino acids, Mucilage, and Essential oils.

Aerial Parts

The aerial parts of the plant are rich in several important constituents:

Major Compounds: Lupeol, Lupenone, Stigmasterol, and Betulin have been identified as predominant components [53,61,62].

Other Constituent:

Chopra and co-workers, isolated potassium salts and sugars. The diuretic and the soothing properties of the plant are probably due to the potassium salts and to the large quantities of mucilage present in the plant. Decoction of whole plant as also the water extract of its ash showed diuretic action in rats [11,61]. Alkaloids such as Asteracanthine and Asteracanthinine have also been reported and these were further isolated from seeds [16]. Other compounds include Hydrocarbons, Aliphatic esters such

as 25-oxo-henriacantanyl acetate, methyl-8-n-hexyl tetracosanate, 3-methylnonacosane, 23-ethylcholesta-11(12),23(24)-dien-3 β -ol [29,62]. Screening studies have reported the presence of Flavonoids, Phenolics, Carbohydrates, Sterols, Steroids, Amino acids, Proteins, Saponins, Oils, Gums, Mucilage, Tannins, Reducing and Non-reducing sugars, Ellagic acid, Gallic acid, and Quercetin [18,63,64,65,66,67,68,69,70,71]. The plant is also rich in minerals such as Calcium (Ca), Magnesium (Mg), Potassium (K), Iron (Fe), and trace elements like Copper (Cu), Zinc (Zn), Manganese (Mn), Cobalt (Co), Chromium (Cr), Lead (Pb), Cadmium (Cd), Nickel (Ni), Sodium (Na), Aluminium (Al), and Strontium (Sr) estimated by Flame photometer, Atomic Absorption Spectrometer and Inductively Coupled Plasma [16,72,73].

Seeds

The seeds have been analysed for their lipid, mineral, and other bioactive constituents:

Oil Content: Seeds contain approximately 23% oil, classified as semidrying oil [11]. The fatty acid profile is dominated by linoleic acid (80.1%) and oleic acid (11.9%), with minor amounts of palmitic (7.2%), stearic (0.8%), and myristic acids (6%) [74,75].

Other Constituents: Seeds also contain mucilage, albuminoids, traces of alkaloids, and potassium salts [30]. The ash content (6.4%) includes potassium oxide (K₂O, 3.3%) and phosphorus pentoxide (P₂O₅, 2.1%) [52]. The unsaponifiable fraction yielded sterol-like compounds, and enzymes such as diastase, lipase, and protease were also reported. Alkaloids including Asterol I–IV, Asteracanthine, and Asteracanthicine have been isolated [76]. Polysaccharides consisting mainly of D-xylose, with traces of D-glucose, L-arabinose, and D-mannose were identified along with β -sitosterol. Amino acids such as histidine (11.41 mg/g), lysine (4.11 mg/g), and phenylalanine (16.31 mg/g), as well as uronic acid, have been reported [10,16].

Roots

The roots are a rich source of sterols and other bioactive substances:

Major Compounds: Hygrosterol, lupeol, lupenone, and β -sitosterol have been prominently reported. The HPTLC estimation of lupeol and sitosterol in various part like root, leaves, seeds and stems was reported [77,78].

Other Constituents: The roots also yield a trace amount of essential oil, a yellowish-green wax, sticky gum, and significant amounts of maltose. The ash from the roots is predominantly potassium salts [33]. A crystalline substance described as cauliflower-like nodules was isolated through alcoholic extraction and found to dissolve in concentrated sulfuric acid with a yellow coloration. An alkaloidal principal named cholesterol or phytosterol, with the empirical formula (C₂₈H₄₆O) and melting point 194°C, was named Hygrosterol [11,31].

Flowers

The flowers were found to contain flavonoids such as apigenin 7-O-glucuronide and traces of apigenin-7-O-glucoside [79].

Leaves

The leaves are reported to contain Triterpenes such as lupeol and hydrocarbons like hentriacontane. Flavonoids including luteolin and luteolin-7-O-rutinoside have also been isolated [31] (Fig. 2 a & b).

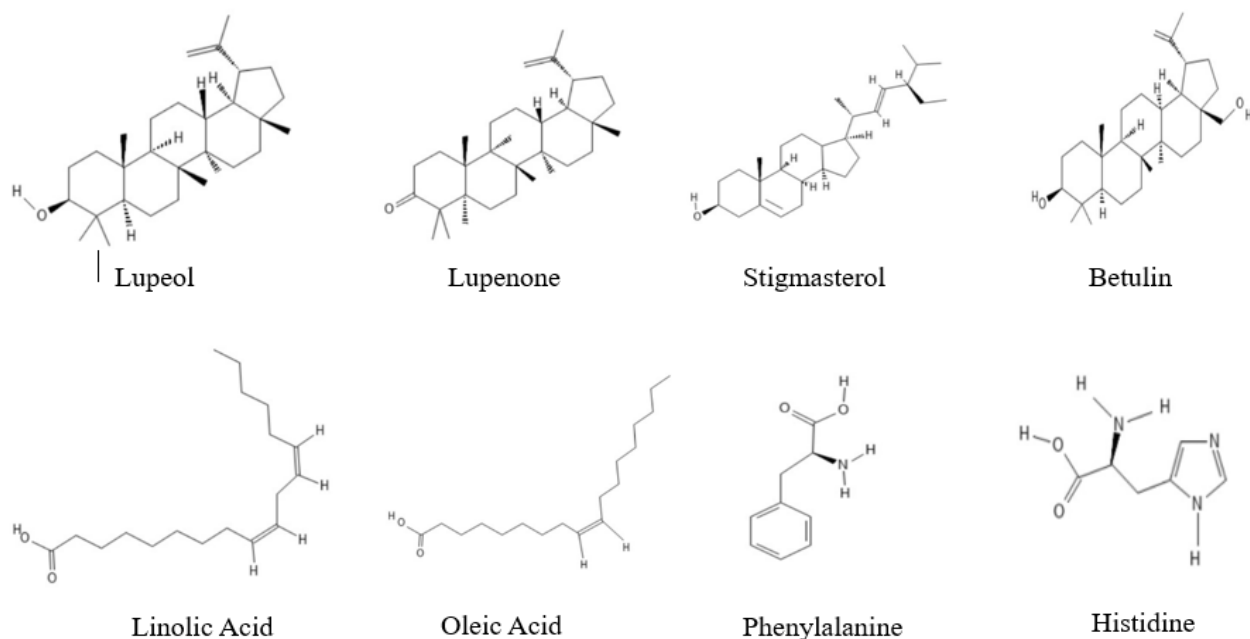


Fig. 2a Major Phytochemical Constituent of *H. auriculata* (Schumach.) Heine

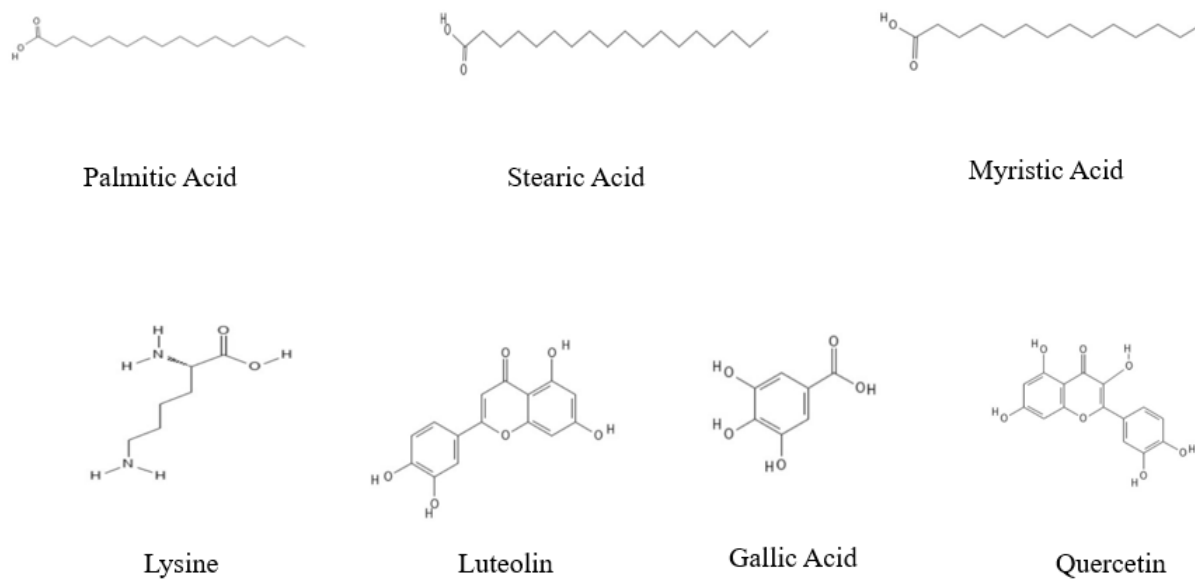


Fig 2b Minor Phytochemical Constituent of *H. auriculata* (Schumach.) Heine

Analytical methods for determination of phytochemicals in *Hygrophila auriculata* (Schumach.) Heine

8.1 GC-MS ANALYSIS

In the GC-MS analysis of the ethanolic seed extract of *Hygrophila auriculata* (Schumach.) Heine, Revathi & Manonmani [80], identified the presence of several bioactive compounds. The detailed composition of these compounds is presented in Table 3. The detection of these bioactive constituents supports the traditional use of *H. auriculata* seeds in the management of various ailments by indigenous practitioners. Similarly, Salve & Bhukter [81], conducted GC-MS analysis of the methanolic root extract of the plant and reported the presence of compounds such as 2-furancarboxaldehyde, 5-(hydroxymethyl), oleic acid, elaidic acid, isopropyl ester, 5-(hydroxymethyl)-2-(dimethoxymethyl) furan, and methyl 2,6-difluorobenzoate. Furthermore, Jebamalai et al. [82], investigated the phytochemical profile of the methanolic leaf extract through GC-MS, identifying approximately 20 compounds, including Butane, 1,1-diethoxy-3-methyl (8.23%), Pentane, 1,1-diethoxy (7.51%), 3,3-Diethoxy-2-butanone (0.50%), Propane, 1,1,3-triethoxy (23.59%), and 1,1,3-triethoxybutane (1.29%).

Table 3: Identified Phytochemicals from Seed Extract of *Hygrophila auriculata* (Schumach.) Heine by GC-MS Analysis [80]

Retention Time (min)	Name of the Compound	Molecular Formula	Molecular Weight
4.717	1,3-Dioxane, 4,4-dimethyl-	C ₆ H ₁₂ O ₂	116.14
15.861	1-Tetradecene	C ₁₄ H ₂₈	196.37
19.996	1-Hexadecene	C ₁₆ H ₃₂	224.42
20.555	1,3-Benzenedioxiole,4,5-dimethoxy-6-(2-propenyl)-	C ₁₂ H ₁₄ O ₄	222.24
23.209	Tetradecanoic Acid	C ₁₄ H ₂₈ O ₂	228.37
23.72	1-Nonadecene	C ₁₉ H ₃₈	266.5
26.46	1,2-Benzenedicarboxylic Acid, bis(2-methoxyethyl) ester	C ₁₄ H ₁₈ O ₆	282.2891
26.71	n-Hexadecanoic Acid	C ₁₆ H ₃₂ O ₂	256.4
27.08	Hexadecanoic Acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284.5
28.642	9, 12-Octadecadienoic Acid (Z, Z)-, methyl ester	C ₁₉ H ₃₄ O ₂	294.5
29.468	9,12-Octadecadienoic acid (Z, Z)-	C ₁₈ H ₃₂ O ₂	280.4
29.518	Octadec-9-enoic acid	C ₁₈ H ₃₄ O ₂	282.5
29.665	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	308.5
29.806	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284.5
30.165	Octadecanoic acid, ethyl ester	C ₂₀ H ₄₀ O ₂	312.5
31.472	Carbamic acid, 2-(dimethylamino)ethyl ester	C ₅ H ₁₃ N ₂ O ₂	168.6
32.203	Oxiraneoctanoic acid, 3-octyl, cis-	C ₁₈ H ₃₄ O ₃	298
32.3	13-Tetradecenal	C ₁₄ H ₂₆ O	210.3
35.025	Butyl 9,12-octadecadienoate	C ₂₂ H ₄₀ O ₂	336.6
38.248	Squalene	C ₃₀ H ₅₀	410.7
31.986	Z, -z -8,10-Hexadecadien-1-ol		
32.203	9,12-Octadecadienoic acid (z, z),	C ₁₈ H ₃₂ O ₂	280
32.618	2-Aminoethanol, hydrogen sulphate (ester)	C ₂ H ₇ NO ₃ S ₂	157
36.824	9,12-Octadecadienoic acid (z, z)-2,3-dihydroxypropyl ester	C ₂₁ H ₃₈ O ₄	354

8.2 HPTLC

A study reported the HPTLC-based determination of lupeol and sitosterol in various plant parts—including roots, leaves, seeds, and stems—using a solvent mixture composed of toluene, ethyl acetate, and methanol in the ratio of 15:3:1.5 (v/v/v) [78]. In a separate investigation by Hussain et al. [83] a method employing HPTLC for the concurrent analysis of lupeol and stigmasterol was described, where the mobile phase consisted of toluene, methanol, and formic acid in a proportion of 7.0:2.7:0.3 (v/v/v), and detection was performed at a wavelength of 530 nm. Additionally, another research work by Hussain et al. [84] detailed a densitometric HPTLC technique for the simultaneous measurement of biologically significant compounds, gallic acid and quercetin, at 270 nm using a solvent system made up of toluene, ethyl acetate, and formic acid mixed in a ratio of 5:4:1 (v/v/v).

8.3 HPLC

Maji [85] developed a reversed-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous quantification of betulin, lupeol, and stigmasterol in *Asteracantha longifolia* (Synonym: *Hygrophila auriculata* (Schumach.) Heine) extract. Chromatographic separation was performed using a Luna C18 column (5 µm, 250 × 4.6 mm) with an isocratic mobile phase consisting of acetonitrile and 0.1% acetic acid in water (94:6, v/v) at a flow rate of 1.0 mL/min. Detection was carried out at a wavelength of 215 nm, while the column temperature was maintained at 25°C with a total run time of 30 minutes. The method was validated for various parameters including linearity, specificity, system suitability, accuracy, limit of detection (LOD), limit of quantification (LOQ), precision, and ruggedness. Calibration curves demonstrated excellent linearity within concentration ranges of 5–200 µg/mL for betulin ($r^2 = 0.9994$), and 10–800 µg/mL for both lupeol ($r^2 = 0.9995$) and stigmasterol ($r^2 = 0.9986$). The LODs were determined to be 0.33 µg/mL for betulin, 1.0 µg/mL for lupeol, and 3.0 µg/mL for stigmasterol, whereas the LOQs were 2.0, 5.0, and 8.0 µg/mL, respectively. The mean recovery ranged between 98.92% and 100.11%, and the %RSD for intra-day and inter-day precision was less than 2%. System suitability parameters were within acceptable limits, confirming the robustness of the method.

9. Pharmacological studies

A detailed evaluation was conducted to explore the pharmacological properties of extracts and isolated constituents derived from *Hygrophila auriculata* (Schumach.) Heine. Key outcomes from preclinical studies, including both *in vitro* and *in vivo* models, highlighting the therapeutic effects of this plant are presented in Table 4.

Table 4 Pharmacological studies (*in vitro/in vivo*) conducted on *Hygrophila auriculata* (Schumach.) Heine

Pharmacological activity	Study	Part used	Test Extract	Method/Model	Control/Standard	Results	References
Antioxidant Activity	<i>In vitro</i>	Aerial parts & roots	Hydroethanolic Extract	DPPH Radical Scavenging, NO, H ₂ O ₂ & FRAP Assays	Standard: Ascorbic acid	The plant extracts exhibited dose-dependent antioxidant activity with the lowest IC ₅₀ values and significant ferric reducing ability in the FRAP assay	[86]

	<i>In vitro</i>	Roots	Aqueous extract	Ferric Thiocyanate (FTC) & Thiobarbituric Acid (TBA) methods	Standards: Vitamin E & C	Significant antioxidant activity was demonstrated by inhibiting linoleic acid oxidation in both FTC & TBA assays	[64]
	<i>In vitro</i>	Whole Plant	Aqueous, Alcoholic, Petroleum ether, Chloroform, Ethyl acetate & n-butanol fractions (alcoholic extract)	DPPH, Hydroxyl & NO free radicals scavenging assay	Standard: Vitamin E	n-Butanol (terpenoid-rich) fraction showed the highest activity with IC ₅₀ values of 7.5, 20.5, 17, and 19.5 µg/mL, respectively	[87]
Hepatoprotective Activity	<i>In vivo</i>	Whole Plant	Methanolic Extract	Wistar rats of either sex were used as a CCl ₄ -induced hepatotoxicity model. Hepatoprotective activity of the extract was evaluated by measuring serum AST, ALT, and histopathological changes	Negative control: CCl ₄ ; Standard: Silymarin	Pre-treatment with methanol extract (600–1200 mg/kg) significantly inhibited CCl ₄ -induced rise in serum ALT & AST, showing superior hepatoprotective activity compared to silymarin, as confirmed by histopathological analysis	[88]
	<i>In vitro & in vivo</i>	Leaves	Total alkaloid fraction of the methanol extract	CCl ₄ -induced hepatotoxicity was studied in Wistar albino rats (n=24) and in vitro using rat hepatocytes and HepG2 cells. Hepatoprotective effects were assessed via biochemical parameters, histopathology & MTT assay in HepG2 cells	Negative control: CCl ₄ ; Standard: Silymarin; Positive control: Olive oil	The total alkaloid fraction showed antihepatotoxic effects in rat hepatocytes at 40–80 µg/ml & dose-dependent protection in CCl ₄ -exposed HepG2 cells. In vivo, 80 mg/kg was as effective as 250 mg/kg silymarin, significantly reducing serum ALT, AST, ALP, total protein & bilirubin	[21]
Erythropoietic activity	<i>In vivo</i>	Aerial parts	Ethanollic extract	Iron in plant extracts was determined spectrophotometrically. Haloperidol-induced anaemia in male albino rats was used to study	Negative control: Haloperidol	Ethanollic extract (100 & 200 mg/kg, i.p) restored haematological parameters, serum iron, and protein & normalized	[89]

				haemoglobin, haematocrit & RBC count		microcytic, anisocytotic & hypochromic RBCs, likely due to its iron content	
Antitumor/Anticancer Activity	<i>In vivo</i>	Whole plant	Methanolic extract	Anticancer activity of the extract was evaluated in DMBA-induced mammary tumors in Sprague Dawley rats by measuring tumor size, histopathological and biochemical analyses were performed post-sacrifice	Negative control: DMBA; Standard: CYC	<i>A. longifolia</i> (Synonym: <i>Hygrophila auriculata</i> (Schumach.) Heine) extracts significantly ($P < 0.05$) reduced tumor size, lipid peroxidation & increased superoxide dismutase & catalase levels, indicating antioxidant activity	[90]
Aphrodisiac Activity	<i>In vivo</i>	Seeds	Ethanollic extract	Cyproterone acetate (CPA) induced male infertility was studied	Negative control: CPA	Ethanollic extract restored sperm parameters, seminiferous tubule structure, body weight, organ indices, and liver enzymes, showing androgenic and antioxidant effects without metabolic toxicity	[91]
Hypoglycaemic Activity	<i>In vitro</i>	Seeds	Water, Methanol, Ethanol, Chloroform	In vitro anti-diabetic activity was assessed via α -amylase inhibition & glucose diffusion studies using dialysis membranes	Standard: Acarbose	The methanolic extract showed the highest antioxidant activity, α -amylase inhibition & glucose diffusion inhibition	[92]
	<i>In vivo</i>	Leaves	Aqueous extract	Alloxan-induced diabetic male Wistar rats were used. Parameters: glucose, insulin, Hb, HbA1c, liver enzymes, cholesterol, pancreatic antioxidants	Negative control: Alloxan	Extract treatment in alloxan-induced rats reduced plasma glucose, HbA1c, liver enzymes while increasing	[93]

				(SOD, CAT, GSH) & pancreas histology		insulin, Hb, pancreatic antioxidant enzymes. Pancreatic histology improved, indicating antidiabetic and antioxidant efficacy	
Diuretic Activity	<i>In vivo</i>	Seeds	Alcoholic extract	Diuretic activity in Wistar rats (n=6) evaluated by 24 h urine output & sodium, potassium & chloride levels	Positive control: Frusemide; Placebo control: Normal saline	<i>H. auriculata</i> alcoholic extract showed significant diuretic activity, with both 300 and 500 mg/kg doses increasing electrolyte excretion, but only 500 mg/kg significantly increased urine volume	[94]
Nephroprotective Activity	<i>In vivo</i>	Aerial parts	Methanolic extract	Cisplatin (CP)-induced acute renal failure male Wistar albino rats were used. Parameters: blood urea, serum creatinine, MDA, GSH, CAT, SOD, GSH-Px activities & histopathological changes	Negative control: CP Standard: Sylmarin	Extract pretreatment reduced CP-induced renal damage, oxidative stress, and improved histopathology	[95]
	<i>In vivo</i>	Whole Plant	Ethanollic extract	Gentamicin-induced nephrotoxicity in male Sprague-Dawley rats was assessed via serum creatinine, urea, SOD, catalase, lipid peroxidation, and GSH	Negative control: Gentamicin	Ethanollic extract (50–250 mg/kg) dose-dependently scavenged free radicals, lowered serum creatinine & urea & protected against gentamicin-induced tubular necrosis	[70]
Antiurolithiatic Activity	<i>In vivo</i>	Whole plant	Aqueous extract	Ethylene glycol-induced male albino rats were used for preventive & curative antilithiatic effects	Standard: Cystone; Negative control: Ethylene glycol	<i>H. spinosa</i> (Synonym: <i>Hygrophila auriculata</i> (Schumach.) Heine) extract reduced urinary ions/protein, increased magnesium, lowered	[96]

						serum creatinine & improved kidney histology in lithiatric rats	
Anti-inflammatory Activity	<i>In vivo</i>	Whole Plant	Terpenoid fraction (alcoholic extract)	Edema was induced in Wistar rats via 0.1 ml of 1% carrageenan in the hind paw, and septic shock via LPS (100 µg/kg, i.p.). Serum levels of IL-1β, IL-6, TNF-α, SOD, LPO & NO were measured. Molecular docking was performed using Auto Dock 4.2	Negative control: carrageenan & LPS	TF significantly restored serum cytokines, LPO, NO & SOD in LPS-challenged rats, with TNF-α identified as the key anti-inflammatory target in silico	[97]
Antipyretic Activity	<i>In vivo</i>	Leaves & Root	Alcoholic extract	Antipyretic activity was evaluated using Brewer's yeast induced pyrexia in rats	Negative control: Brewer's yeast; Standard: Paracetamol	Both extracts (200 & 400 mg/kg) significantly reduced rectal temperature, starting 3 h post-treatment. At 6 h, root extract was slightly more effective than leaf extract, compared to paracetamol, showing antipyretic activity	[98]
Antinociceptive Activity	<i>In vivo</i>	Leaves	Petroleum ether, Chloroform, Alcohol, Aqueous extracts	Analgesic activity was evaluated in albino mice using hot plate, tail flick & acetic acid-induced writhing tests	Standard: Aspirin; Negative control: Acetic acid	Chloroform, alcoholic, & aqueous extracts (200–400 mg/kg) dose-dependently reduced acetic acid-induced writhing & increased thermal pain threshold in mice, comparable to aspirin (100 mg/kg)	[67]
Anti Motility Effect	<i>In vivo</i>	Leaves	Petroleum ether, Chloroform, Alcohol, Aqueous extracts	Antimotility activity was studied by charcoal meal feeding method in albino mice of either sex	Standard: Atropine sulphate	Leaf extracts (200 & 400 mg/kg p.o.) showed significant antimotility activity in albino mice in the charcoal meal test, with the 400 mg/kg alcoholic	[67]

						extract reducing intestinal transit by 53.9%, comparable to atropine sulphate (0.1 mg/kg i.p.)	
Neuroprotective Activity	<i>In vivo</i>	Plant	Terpenoid rich fraction (TF)	Male Wistar rats underwent transient global cerebral ischemia (tGCI). Neuroprotection was assessed via neurological & sensorimotor deficits, brain antioxidants (SOD, CAT, MDA) & CA1 hippocampal histopathology	Vehicle: carboxy methyl cellulose (CMC); Positive control: vitamin E	TF improved motor coordination, restored SOD/GSH, reduced MDA & protected CA1 neurons after tGCI	[99]
Anticataract Activity	<i>In vitro</i>	Whole Plant	Various fractions isolated from the methanolic extract	Goat eye lenses were divided into five groups: Group I (5.5 mM glucose, normal control), Group II (55 mM glucose, toxic control), and Groups III–V (55 mM glucose with 4 mg/ml of fractions A, B, or F) & evaluated for opacity	Negative control: Glucose	The opacity grades in groups I–V were 0, +, ++, and +++, respectively. Fraction A effectively prevented glucose-induced cataract, supporting the goat lens model for anticataract testing	[100]
Anthelmintic Activity	<i>In vivo</i>	Leaves	Petroleum ether, chloroform, alcoholic & aqueous extract (10, 25, 50, 100 mg/ml) respectively	The activity was evaluated on 6 adults <i>Pheretima posthuma</i> worms, each exposed to the extracts. Paralysis and death times were recorded based on immobility & discoloration	Standard: Piperazine citrate; 1% Tween 80 in normal saline as control	The alcoholic extract (100 mg/ml) showed significant anthelmintic activity, while chloroform & aqueous extracts showed moderate activity & petroleum ether extract showed the least	[101]

9.1 Antimicrobial Study

Various biological activities, including antimicrobial, anti-inflammatory, and antioxidant effects, have been reported from extracts and isolated compounds derived from medicinal plants. Antimicrobial agents from these plants may inhibit the growth of bacteria, fungi, viruses, and protozoa through mechanisms that differ from those employed by conventional antimicrobial drugs, potentially offering important therapeutic options for treating resistant strains of microorganisms [102]. In a study conducted by Patra et al. [101], successive extracts i.e., petroleum ether, chloroform, alcohol, and water prepared from the leaves of *Hygrophila auriculata* (Schumach.) Heine at a concentration of 100

mg per disc, demonstrated *in vitro* antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. The antibacterial effect was assessed using the disc-diffusion method, and the inhibition zones (in mm) were measured and compared with the standard antibiotic, ofloxacin, as presented in Table 5. Among the extracts, the chloroform and alcoholic extracts showed pronounced antibacterial activity, while the aqueous extract exhibited moderate activity, and the petroleum ether extract displayed the least effectiveness against the tested pathogens.

Table 5 Antibacterial activity in terms of Zone of Inhibition (ZoI) expressed in mm of *Hygrophila auriculata* (Schumach.) Heine leaves [101]

Test Organism	Different Extracts of the Leaf (100 mg/disc)				Ofloxacin (5 µg/disc)
	Petroleum ether	Chloroform	Alcoholic	Aqueous	
<i>E. coli</i>	10	13	17	13	23
<i>S. aureus</i>	9	14	13	12	25
<i>B. subtilis</i>	8	16	16	11	24
<i>P. aeruginosa</i>	8	15	16	12	26

Perumal Samy [103] evaluated the antibacterial potential of methanolic extracts from various plants at a concentration of 30 mg/ml using the disc diffusion method. The study tested the extracts against both Gram-negative bacteria—*Escherichia coli*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*—and Gram-positive bacteria—*Staphylococcus aureus* and *Burkholderia pseudomallei* (strains 1 and 2). Among the extracts, *Asteracantha longifolia* (Synonym: *Hygrophila auriculata* (Schumach.) Heine) demonstrated the most significant antibacterial activity, particularly against *E. aerogenes*, *S. aureus*, and *B. pseudomallei* (strains 1 and 2), as detailed in Table 6.

Table 6 Antibacterial activity of studied plant methanol extracts in terms of Zone of Inhibition (ZoI) expressed in mm [103]

Plant	Methanol-extract (µg/ml)	E. c.	E. a.	P. v.	P. m.	P. a.	B. p. (1)	B. p. (2)	S. a.
<i>A. longifolia</i>	30	—	7.6	—	—	—	20.2	22.4	24.0
<i>C. acida</i>	30	—	—	—	—	—	24.3	20.1	19.9
<i>S. microsperma</i>	30	—	—	25.2	—	—	19.4	17.8	—
<i>T. cordifolia</i>	30	—	10	8	11	—	—	—	—
<i>Z. officinale</i>	30	—	12.3	—	—	—	10.5	9.2	7.4
Chloramphenicol	15	16	17	19	18	16	20	23.6	22.1
Ceftazidime	15	19.1	22.1	20.1	17.6	19.3	24.5	23.7	25.3

*Gram (–): E. c. = *Escherichia coli*, E. a. = *Enterobacter aerogenes*, P. v. = *Proteus vulgaris*, P. m. = *Proteus mirabilis*, P. a. = *Pseudomonas aeruginosa*.

*Gram (+): S. a. = *Staphylococcus aureus*, B. p. = *Burkholderia pseudomallei* (strains 1 and 2).

*“_” = No activity.

10. Toxicological effects of *Hygrophila auriculata* (Schumach.) Heine

Several studies have been conducted to evaluate the toxicological profile of *Hygrophila auriculata* (Schumach.) Heine, and no significant acute or severe adverse effects have been reported. Nevertheless, mild side effects may arise when the plant is consumed in doses higher than recommended or over extended periods, potentially impacting the liver, kidneys, and metabolic processes. According to Unani practitioners, the seeds of *H. auriculata* (commonly known as Talmakhana) may cause gastrointestinal disturbances, as they are difficult to digest and can lead to flatulence [9,22,23,24,40,41,45]. Additionally, its consumption is considered harmful for individuals with a hot temperament [47]. To counter these undesirable effects, Unani physicians recommend the use of corrective substances known as *Musleh*, which are either mixed with or administered alongside *H. auriculata* seeds. These include candy sugar and raw sugar [22, 23], honey [9,24,40], milk [9,24,41], or black pepper [40]. For individuals with hot temperaments, the consumption of cold-natured foods is advised as a corrective measure [45].

A toxicity assessment conducted by Mazumdar et al. [104] on *Hygrophila spinosa* (Synonym: *Hygrophila auriculata* (Schumach.) Heine) petroleum ether extract indicated no toxic effects at low to moderate daily doses (2 and 4 mg/kg) and low weekly doses (20 mg/kg). However, higher weekly doses (≥ 40 mg/kg) and elevated daily doses (8 mg/kg) resulted in significant disturbances in liver, kidney, metabolic, and haematological parameters in mice. In another study, Ahmed et al. [105] observed that extracts from the seeds and leaves exhibited high toxicity, inhibiting the growth of *Microtermes obesi* by arresting its movement, thereby demonstrating antitermite activity. An acute toxicity study carried out by Neharkar et al. [106] aimed to establish LD50 values and determine a safe dose range for *H. auriculata*. The investigation involved administering a methanolic extract of the plant's leaves to albino rats at a dose of 2000 mg/kg body weight. Following a stepwise sighting study (5, 50, 300, 2000 mg/kg), the selected dose was given, and the animals were monitored for 14 days. The results showed no mortality or significant changes in body weight, food and water intake, or organ weights. Apart from triglyceride levels, no significant biochemical differences were observed compared to the control group. Furthermore, a multicenter, double-blind, homeopathic pathogenetic trial conducted by Rakshit et al. [107] involving 48 patients suggested that *H. auriculata* could be safely used in the treatment of various conditions, including urticaria, frontal sinusitis, conjunctivitis, stomatitis, gastroenteritis, nausea (morning sickness), and intermittent fever.

11. Discussion

The rich legacy of medicinal plants use across human history has been a cornerstone for healthcare systems worldwide, with plant-based remedies offering safer and more holistic alternatives to synthetic drugs [1]. Among these, *Hygrophila auriculata* (Schumach.) Heine, commonly known as Talmakhana in Unani medicine, stands out as an exemplary botanical with diverse therapeutic applications [23]. The plant's utilization spans multiple traditional medical systems—including Unani, Ayurveda, Siddha, and Homeopathy—underscoring its ethnopharmacological significance and broad geographical distribution [17].

In Unani medicine, Talmakhana is highly valued for its adaptability to treat a wide array of ailments. The seeds are primarily employed as a tonic, aphrodisiac, diuretic, and stimulant, while the roots and leaves are used for managing conditions such as dropsy, kidney stones, jaundice, rheumatism, and reproductive disorders [22]. The pharmacological actions attributed to the plant reflect its multifaceted role in enhancing vitality, correcting humoral imbalances, and promoting natural healing processes through its cold and moist temperament, as described in Unani classical texts. The documented temperament of Talmakhana seeds as cold and moist aligns with its role in managing inflammatory and heat-related disorders such as Headaches, Fevers, Strokes & many more [9,23,44]. Furthermore, traditional Unani formulations like *Majoon-e-Piyaz* and *Safoof-e-Kalan* continue to harness Talmakhana's therapeutic benefits in combination with other herbal ingredients [9,22,41], while

modern pharmaceutical products such as Speman, Afrodet Plus®, and Uricitral have extended its application into contemporary healthcare [56,57,58].

The botanical characteristics of *H. auriculata* (Schumach.) Heine, including its mucilaginous seeds and rich phytochemical profile, align with its widespread use across regions like India, Sri Lanka, and parts of Africa. Furthermore, scientific validation through pharmacological studies complements its ethnomedical applications. The plant's seeds are recognized for their mucilage content and hygroscopic nature, which aids in gastrointestinal and renal regulation. Documentation from ancient and modern sources affirms the plant's pharmacological action as diuretic, lithotriptic, anti-inflammatory, laxative, and nervine tonic properties, which are consistent with its traditional applications in treating urinary, hepatic, and reproductive disorders. Notably, its seeds are considered as general as well as sexual tonic and used in various sexual health formulations, for treating a wide range of ailments such as seminal weakness, nocturnal emissions, erectile dysfunction & in general debility while its roots serve as effective diuretics and tonics for liver and kidney ailments. The consistency of its use across cultures further highlights the plant's therapeutic relevance [12,31].

Hygrophila auriculata (Schumach.) Heine, a widely studied medicinal plant, exhibits a rich phytochemical profile and diverse pharmacological activities, validating its traditional use in various systems of medicine. Comprehensive investigations have revealed that its aerial parts, seeds, roots, flowers, and leaves are rich sources of bioactive compounds such as alkaloids, flavonoids, triterpenes, sterols, fatty acids, essential oils, and minerals [53]. Notably, major compounds like lupeol, lupenone, stigmasterol, betulin, and hygrosterol have been isolated, while other constituents include potassium salts, mucilage, albuminoids, carbohydrates, proteins, saponins, tannins, and trace minerals like calcium, iron, and zinc. These compounds are believed to contribute to its diverse therapeutic properties [11,61,63].

The seeds, constituting about 23% oil, are rich in essential fatty acids, primarily linoleic and oleic acids, along with sterol-like compounds and enzymes such as diastase and protease. Polysaccharides and amino acids like histidine, lysine, and phenylalanine further enhance their nutritional and therapeutic potential [16,30]. The roots contain high amounts of sterols, maltose, and potassium salts, with the isolation of hygrosterol—a phytosterol—highlighting their importance. Flowers and leaves are rich in flavonoids and triterpenes, contributing to antioxidant and anti-inflammatory activities [11,33,79].

Analytical techniques such as GC-MS, HPTLC, and HPLC have confirmed the presence of numerous phytochemicals in different plant parts. These methods have facilitated the accurate quantification of bioactive compounds like lupeol, sitosterol, stigmasterol, and betulin, supporting quality control and standardization efforts in herbal formulations [78,80,85].

Pharmacological studies conducted through both *in vitro* and *in vivo* models have further demonstrated the therapeutic potential of *H. auriculata* (Schumach.) Heine. Antioxidant, hepatoprotective, erythropoietic, anticancer, aphrodisiac, hypoglycaemic, diuretic, nephroprotective, antiurolithiatic, anti-inflammatory, antipyretic, antinociceptive, antimotility, neuroprotective, anticataract, and anthelmintic activities have been extensively documented. Many of these studies indicate dose-dependent efficacy, with some extracts showing comparable or superior results to standard drugs like silymarin, paracetamol, or Atropine sulphate [67,88,98].

Additionally, the plant's antimicrobial properties have been highlighted, especially its effectiveness against resistant bacterial strains. Methanolic and chloroform extracts, in particular, exhibited strong antibacterial effects, providing alternative treatment avenues against infectious diseases [102]. Despite its wide-ranging benefits, toxicological assessments have underscored the importance of dosage. While acute toxicity studies reported no significant adverse effects at moderate doses, higher doses or prolonged use may result in gastrointestinal disturbances, liver and kidney stress, and metabolic imbalances [9,104]. Traditional Unani medicine recommends corrective agents such as honey, milk, and sugar to mitigate these effects, especially in individuals with a hot temperament [22,23,45].

Overall, the compiled data affirm the therapeutic versatility of *H. auriculata* (Schumach.) Heine, supported by both traditional knowledge and modern scientific validation. Despite differences in diagnostic frameworks and treatment philosophies between Unani and Western medicine, the system's reliance on holistic principles and temperamental classification offers a structured approach to patient care. The empirical knowledge accumulated over centuries, as seen in ethnobotanical records and clinical applications, forms a basis for exploring *H. auriculata* (Schumach.) Heine, potential in integrative and complementary therapies [22,23,50].

12. Conclusion

Herbal medicines constitute an indispensable component of nature's pharmacopeia, offering a reservoir of bioactive compounds that contribute to health promotion and disease management. The therapeutic rationale underlying herbal medicine is founded on the premise that plants contain pharmacologically active molecules with significant therapeutic potential. Among these, *H. auriculata* (Schumach.) Heine, occupies a noteworthy position as a medicinal plant of considerable importance. This review delineates its pharmacognostic features, ethnomedicinal relevance, phytochemical composition, and pharmacological activities. Evidence from contemporary studies indicates that *H. auriculata* (Schumach.) Heine exhibits a broad spectrum of pharmacological activities, including antioxidant, hepatoprotective, nephroprotective, antiurolithiatic, diuretic, aphrodisiac, anti-inflammatory, anticancerous, hypoglycaemic, anthelmintic, anticataract, antinociceptive, neuroprotective and antimicrobial effects. Comprehensive investigations have revealed that its aerial parts, seeds, roots, flowers, and leaves are rich sources of bioactive compounds such as alkaloids, flavonoids, triterpenes, sterols, fatty acids, essential oils, and minerals. Moreover, its longstanding application in the Unani system of medicine reinforces its traditional value and supports its potential role in the prevention and treatment of various pathological conditions.

13. Future Implications

Overall, the compiled data highlight the therapeutic versatility of *H. auriculata*, supported by both traditional knowledge and modern scientific validation. To fully harness its potential, future research should prioritize clinical trials, bioavailability studies, dosage standardization, and formulation development. Rigorous evaluation of its bioactive constituents, mechanisms of action, and safety profiles is essential to ensure effective and safe use, particularly in vulnerable populations. Integrating traditional wisdom with contemporary scientific inquiry offers promising avenues for novel drug discovery, expanded clinical applications, and its establishment as a valuable component of integrative medicine.

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CRedit authorship contribution statement

Ameera Ayyub

Conceptualization; Methodology; Data curation; Writing – original draft; Writing – review and editing; Formal analysis; Final approval.

Nazish Siddiqui

Investigation; Supervision; Writing – review and editing; Validation; Final approval.

Shamshad Alam

Supervision; Validation; Final approval.

Ariz Ayyub

Data curation; Formal analysis; Writing – review and editing; Final approval.

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