

REVIEW ON PHARMACOLOGICAL ACTIVITIES OF HYGROPHILA AURICULATA

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Abstract: *Hygrophila auriculata*, also known as *Aster acantha longifolia*, a member of the Acanthaceae family, is a common plant found growing in marshy and waterlogged places. It is also called "Neermulli, Talmakhana, Kokilaksha, and Iksura." The plant is a well-known medicinal herb that grows throughout India. The herb has significant medicinal significance, which the ancient medical literature has acknowledged and valued. The Ayurvedic system outlines the plant's medicinal properties, as well as those of its seeds, roots, leaves, and panchang (Pancha means "five" and ang means "parts," including stem, roots, flowers, fruits, and leaves burned together). Alkaloids, steroids, tannins, proteins, carbohydrates, lipids, terpenoids, sterols, palmitic, stearic, oleic, and linoleic acid are all present in the plant. There are other nutrients including copper, calcium, riboflavin, and β -carotene. The extracts and bioactive compounds of the plant are known to have anti-microbial, anti-inflammatory, hepatoprotective, anti-termite, nephroprotective, anti-cancer, anti-diabetic, anti-nociceptive, anti-cataract, antioxidant, hematopoietic, diuretic, anti-endotoxin, central nervous system protective, antipyretic, antifungal, aphrodisiac, antimotility, neuroprotection, and anti-urolithiasis activity. The many pharmacological activities and therapeutic qualities of *Hygrophila auriculata* are represented in the current review.

Keywords: *Hygrophila Auriculata*, Morphology, Pharmacological activities, Ayurveda, Home remedies.

I. INTRODUCTION (HEADING 1)

Worldwide, traditional medicines play a vital role in treating health issues. Traditional and modern medicine continues to benefit from the useful therapeutic compounds offered by medicinal plants. Traditional medicines are becoming more significant due to the negative impacts of modern medicine, and research is currently being done to determine the scientific basis of these remedies for therapeutic effects. The amount of research on medicinal plants has increased, and knowledge about these plants has been shared. This study is expected to reduce reliance on synthetic medications and advance scientific investigation into medicinal plants for human benefit.^[1]

Plants have been considered the most dependable and tried-and-true source of medicine since their inception. Ancient texts such as the Charaka Samhita, the Ayurvedic medical system, the Chinese medical system, and the Sushruta Samhita, among others, have beautifully documented the adaptable qualities and extraordinary restorative capabilities of a wide range of medicinal Plants.^[2]

Hygrophila auriculata (Schum) Heine, also known as *Hygrophila spinosa*, *Aster acantha longifolia* Nees, *Barleria auriculata* schum, and *Barleria longifolia* linn., belongs to the Acanthaceae family and. The plant is extensively dispersed in India, Sri Lanka, Myanmar, Malaysia, and Nepal. In Ayurvedic medicine, it is referred to as Seethaveryam or Mathuravipaka and is used to treat premeham (diabetes) and athisaram (dysentery). This plant is a sub-shrub that thrives in swampy areas near waterways.^[3]

It is referred to as Ikshura, Ikshagandha, and Kokilasha in Ayurvedic literature. Its eyes resemble those of a kola or Indian cuckoo. The various other common names/vernacular names of the plant are Kakilakshya, Ikshugandha, Kokilanayana, Kshura, Kshuraka, Vajra, Gokhulajanum, Katreiriki, Ikkiri, Tal-makhana, Talimakhana, Gokhulakanta, Gokshura, Talimkhana, Kuilirakha, Koillekha, Koilrekha, Kolist, Talimakhana, Kolsunda, Talimkhana, Kuliakhara, Kantakalika, Nirmalli, Vayalchulli, Nirmulli, Neremulli, Nirumalli, Kettu, Nirguvireru, Nerugobbi, Neerugubbi, Nirguviveru, Kokilaksamu, Kantakulika, Kalavankabija, Eyitror, Ekharo, Dayingiwa, Kolavalike, Kolavali, Kolarind, Soopadan, Long-leaved barleria etc.^[4] Herbs, 40–100 cm tall, hispid with long hairs, unbranched, subquadrangular stems with many fasciculate, swollen nodes. At each node, there are six sub-

sessile, lanceolate leaves that are sharp, hairy, and measure 6-15 x 1.5-3 cm. The two outer leaves are significantly bigger than the four inner ones. The leaves' axils contain sharp, 2-3 cm long, yellowish-brown thorns. Eight flowers in axillary clusters at each node are arranged in four pairs. Bracteoles are linear-lanceolate, 1.5–2 cm long, with hyaline borders in the lower portion; they are hairy and ciliate with long white hairs, just like the bracteolate leaves. Four partite calyx; upper sepals unequally larger and longer than the other three; all linear-lanceolate, 1.2-2 cm long; edge hyaline ciliate; hairy on back. Purple-blue, 2-3 cm long, blipped corolla; 11–13 mm long, enlarged at the tip tube; 4 didynamous stamens; glabrous filaments. Two-celled, four-ovule, linear-oblong capsules with four pointed, 5-7 mm-long seeds are found in the ovary. Ovoid, compressed, hairy, hygroscopic, black seeds.^[5]

It has therapeutic characteristics including anticancer, hypoglycemic, aphrodisiac, antibacterial, anti-oxidant, lipid peroxidation, hepatoprotective, and hematopoietic. The composition includes lupeol, stigmasterol, bulletin, fatty acids, and alkaloids. Commercially, it is utilized in over-the-counter (OTC) preparations for treating liver disorders and general tonics.^[6]

II. PLANT PROFILE

Table 1: Taxonomical Classification^[7]

Kingdom	Plantae – plantes, Planta, Vegetal, plants
Sub-kingdom	Viridiplantae
Infrakingdom	Streptophyta land plants
Superdivision	Embryophyta
Division	Tracheophyta vascular plants, tracheophytes
Subdivision	Spermatophytina–spermatophytes, seed plants, phanerogames
Class	Magnoliopsida
Superorder	Asteranae
Order	Lamiales
Family	Acanthaceae – acanthacées
Genus	Hygrophila R. Br. – swamp weed
Species	Auriculata

Hygrophila auriculata is an annual herb that grows to a height of 60 cm. The plant stem is square, hairy, and thicker at its nodes. The bark is dark brown, and the leaves are elliptic-lanceolate and hispid. The blossoms of this herb are violet and somewhat purple-blue. The fruit is oblong, linear, and glabrous, measuring about 1cm in length and bearing spherical hairy brown seeds.^[7]

Figure 1A & 1B: Flowers of *Hygrophila auriculata*



Table 2: Vernacular Names^[8]

English	Hygrophila, Marsh Barbel
Hindi	Talimkhana, Gokulakanta
Sanskrit	Kokilaksha, Ikoura
Gujarati	Ekhro, Gokhru
Marathi	Talimkhana, Vikhra, Kolsunda
Bengali	Shulamardan, Kantakalika
Kannada	Kalavankabija, Kolavali
Tamil	Nirumuli, Golmidi
Malayalam	Voyal-chili, Culli
Telugu	Kokilakshi, Nirguviveru

III. MORPHOLOGY

1)Seed- The capsule is 7.5 mm long and shorter than the calyx, with 4-8 seeds on stiff retinacula that are flat and white hairy when moist. The seeds are orbicular and are 0.3 mm wide.^[9]

2)Stem- The plant is a vigorous herb or under-shrub that can grow up to 80cm tall. It has sparsely sub-hispid hairy stems with whorled spines at the nodes, which are 1.3cm long and often six in a whorl.^[9]

3)Flower- The flowers are purple-blue, with whorled axillary fascicles. There are 8 at each node. The length of the flowers is 3.0 to 3.7 cm. The bracts are lanceolate and the bracteoles are linear. The calyx is silky and has 4 lobes. The posterior lobes of the calyx are silky. The interior of the calyx is 2-toothed. The corolla tube is 1.2 to 1.5 cm in length. It is cylindrical below and enlarged above. The upper lip is two-lobed. The lower lip is trilobed and the palate is crested with two crested folds. The stamens are didynamous and all fertile. The ovule is oblong and the ovule is 4-ovuled. The style is filiform. The flowering and fruiting season is August to March.^[9]

4)Root- The root consists of a continuous rhizodermis (epidermis) and two layers of tangentially oblong compact outer cortex. The inner cortex is broad and parenchymatous. Air chambers are formed by thin, uniseriate partition filaments composed of parenchyma cells. Some partition cells have thick walls, are dilated, and are squarish-rectangular. The vascular cylinder consists of a thin endodermal layer and a pericyclic layer. The xylem has five exarch strands and a few large angular vessels, while the phloem is divided into five tiny groups that alternate with the principal strands. The middle portion of the flower is thin and parenchymatous.^[10]

5)Leaves- The leaves are sparsely hispid on both sides, tapering at the base, sessile, and in verticals of 6 at a node. The two outer leaves of the whorl are larger, reaching 18 by 1.3-3.2 cm and oblong-lanceolate or oblanceolate, while the four inner leaves (two on each side) reach about 3.8 cm long. Each of the six leaves has a nearly straight sharp yellow spine 2.5-4.5 cm long in its axis.^[10]

IV. CULTIVATION AND COLLECTION OF PLANT

The Greek words *hygros*, which means "moist" or "humid," and *philos*, which means "love" or "friend," are combined to form the name *Hygrophila*, which means "water-loving" and describes the genus's preferred habitat of moist areas and settings. The Latin term for "little ear" is the source of the species name *auriculata*, which means "eared" or "having an earlike appendage."

Hygrophila auriculata is a fast-growing, easy-to-grow plant that may be propagated via both stem cuttings and seeds. When the plant reaches the end of its August flowering cycle, seeds must be collected. This plant species' seeds have minimal viability, which makes it difficult to grow them outside of their natural habitat because their germination rate is so low. The seeds ought to be grown in their native environment, which has enough of sunshine and nutrient-rich soil, for an improved germination rate. The most effective way is growing the plant from stem cuttings.^[11]

V. PHYTOCONSTITUENTS

GC-MS analysis of the ethanolic extract of *Hygrophila auriculata* (Schum) Heine's seed revealed the existence of many bioactive chemicals, as demonstrated by the studies on the bioactive components of the extract.

Table 3: Constituents of ethanolic extract of *Hygrophila auriculata* (Schum) Heine's seed^[12]

Retention Time	Name of the Compound	Molecular Formula	Molecular Weight
4.717	1.3-DIOXANE , 4.4DIMETHYL	C6H12O2	116.6
15.861	1-TETRADECENE	C14H28	196.37
19.996	1-HEXADECENE	C16H32	224.42
20.555	1.3- BENZODIOXOLE.4.5DIMETHOXY6(2PROPENYL)-	C12H14O4	222.24
23.209	TETRADECANDIC ACID	C14H28O2	228.37
23.72	1-NONADECENE	C19H38	266.5
26.46	1.2- BENZENEDICARBOXYLIC ACID,BIS(2- METHOXYETHYL ESTER	C14H18O6	282.2891
26.71	N-HEXADECANOIC ACID	C16H32O2	256.4
27.08	HEXADECANOIC ACID ETHYL ESTER	C18H36O2	284.5
28.642	9,12-OCTADECADIENOIC ACID(z,z)-METHYL ESTER	C19H34O2	294.5
29.468	9,12-OCTADECADIENOIC ACID (ZZ)-	C18H32O2	280.4
29.518	OCTADEC-9-ENOIC ACID	C18H34O2	282.5
29.665	LINOLEIC ACID ETHYL ESTER	C20H36O2	308.5
29.806	OCTADECANOIC ACID	C18H36O	284.5
30.165	OCTADECANOIC ACID, ETHYL ESTER	C20H40O2	312.5

31.472	CARBAMIC ACID, 2 (DIMETHYLAMINO)ET HYLESTER	C5H13CN2O2	168.6
32.203	OXIRANEOCTANOIC ACID, 3-OCTYL CIS	C18H34O3	298
32.3	1,3-TETRADECENAL	C14H26O	210.3
35.025	BUTYL9,12- OCTADECADIENOATE	C22H40O2	336.6
38.248	SQUALENE	C30H50	410.7
31.986	Z,z-8,10-Hexadecadien-1-ol		
32.203	9,12-Octadecodienoie acid(z,z)	C18H32O2	280
32.618	2-Aminoethanethiol hydrogen sulfate(Ester)	C2H7NO3S2	157
36.824	9,12-Octadecadienoic acidi(z,z)-2,3- Dihydroxypropyl Ester.	C21H38O4	354

The Methanolic extract of leaves of *Hygrophila auriculata* was screened for various phytoconstituents through GC-MS analysis. The 20 components from the methanolic extract of leaves were screened by the GC-MS analysis findings.

Table 4: Constituents of ethanolic extract of *Hygrophila auriculata* (Schum) Heine's Leaves^[13]

Retention Time	Name of the Compound	Molecular Formula	Molecular Weight
5.818	Butane,1,1-diethoxy3- methyl-	C9H20O2	160
5.849	Pentane, 1,1- diethoxy-	C9H20O2	160
6.032	3,3-Diethoxy-2- Butanone	C8H16O3	160
7.851	Propane, 1,1,3- tri ethoxy-	C9H20O3	176
8.284	1,1,3- Triethoxybutane	C10H22O3	190

10.395	Benzene, [Ethoxy(1Propenylox y	C12H16O2	192
12.788	Nonane, 3,7- Dimethyl-	C11H24	156
15.591	Diethyl Phthalate	C12H14O2	222
17.725	Octadecanoic acid, 2-oxo- methyl ester	C19H36O3	312
18.097	Isopropyl myristate	C17H34O2	270
18.267	2-Hexadecan-1-ol, 3,7,11,15-tetram	C20H40O2	296
18.333	2,6,10-trimethyl, 14- Ethylene- 14-Pe	C12H24	168
18.539	2,6,10-trimethyl, 14- Ethylene- 14-Pe	C20H38	278
18.746	7-Octadecyne,2methyl-	C19H36	264
19.915	Heptadecanoic acid, Ethyl ester	C19H38O2	298
21.227	Phytol Isomer	C20H40O	296
22.402	Phytol, acetate	C22H42O2	338
24.901	Tridecanol, 2-ethyl-2 - methyl-	C16H34O	242
26.752	Squalene	C30H50	410
28.808	1,2- Benzenedicarboxylic	C24H38O4	390

VI. PHARMACOLOGICAL ACTIVITIES

1] Diuretic activity

For the activity, the procedure outlined by lipschitz et al. Was used. The coarse powder (500 g) was extracted with distilled water and 95% w/v alcohol separately by cold maceration process to produce an alcoholic and aqueous extract of h.auriculata. Then the alcoholic extract was fractionated with petroleum ether, chloroform, ethyl acetate, and n-butanol to produce fractions of each of the solvents. For the experiment, male wistar albino rats weighing 150–200 g were utilized. The animals were split up into many groups: the first group was administered with frusemide (10 mg/kg, p.o.); the control group was administered with normal saline (25 ml/kg body weight); the remaining groups received doses of extracts/fractions in normal saline (200 mg/kg each). After five hours, the volume of urine collected was quantified, and the concentrations of na⁺, k⁺, and cl⁻ in the urine as well as the total urine volume were ascertained. The total urine volume and the contents of na⁺, k⁺, and cl⁻ in the urine were significantly increased by the alcoholic extract and n-butanol fraction of h. Auriculata at doses of 200 mg/kg in rats.^[14]

2] Antinociceptive property

Using both chemical and thermal methods of nociception in mice, the antinociceptive properties of the aqueous extract of the aerial portions (haa) and root (har) were evaluated. The acetic acid writhing test was conducted chemically, and the hot plate and tail flick tests were conducted thermally. *Hygrophila auriculata* powdered aerial parts and roots were macerated in separate batches with distilled water for a whole day. The extracts were then dried, evaporated, and filtered. The root and aerial portions produced a brownish residue that was extracted. Six groups of six animals each were formed out of the animals.

Group i served as the normal control group and was given distilled water (1 ml/kg, p.o.). Group ii served as the reference group and received aspirin (100 mg/kg/p.o.). Groups iii–vi served as the treatment groups, where groups v & vi received har and group iii & iv received haa at doses of 100 and 200 mg/kg/p.o., respectively.

Acetic acid-induced writhing test

An intraperitoneal dose of 1 ml/kg body weight of acetic acid (1% v/v) was given to each group 60 minutes after the test chemicals were administered. After acetic acid injection, the number of writhes was counted for ten minutes to determine anti-nociception. Full extension of the hind limb and constriction of the abdomen are signs of writhing. In a dose-dependent way, har and haa dramatically decreased the amount of hindlimb stretching and abdominal constrictions caused by acetic acid injections.

Hot plate test :

Using eddy's hot plate, which was kept at $55 \pm 1^\circ\text{C}$, the test was conducted. Every animal's baseline response time to thermal heat was noted. The study included the animals that responded with forepaw licking or jumping within 6 to 8 seconds. The animals in each of the six groups were individually exposed to a heated plate kept at 55°C for 60 minutes following the injection of the test and reference chemicals. Reaction time was measured as the number of seconds required for forepaw licking or jumping. To protect the paws, a 15-second cut-off time is followed.

The pain inhibition percentage was calculated according to the following formula:

Pain inhibition percentage = $((t_1 - t_0) / t_0) \times 100$ t_1 is post-drug latency and t_0 is predrug latency.

Haa showed a pain inhibition percentage of 36.5% and 67.3%, respectively whereas har showed a pain inhibition percentage of 40 and 70%, respectively.

Tail flick test :

By placing the tip of the tail final 1-2 centimeters on the radiant heat source, the basal reaction time of the animals to the heat was measured. The endpoint is considered to be the tail's withdrawal from the heat (flicking response). The animals chosen for the investigation were those that displayed a flicking reaction in three to five seconds. To protect the tail, a 15-second cut-off time is followed. Thirty and sixty minutes after the medications were administered, the tail flick equipment was used to measure the withdrawal period. Har (200 mg/kg/p. O.) Exhibited a greater pip of 77.14%. From the results, it could be concluded that the extracts exhibit antinociceptive activity^[15]

3] Erythropoietic activity

In this study, erythropoietic activity was tested in experimental rats exposed to an ethanolic extract of al by the use of haloperidol-induced iron deficiency anemia to examine the hematological, serum iron, and serum protein profiles.

The 350 gm of powdered aerial components were extracted successively over the course of 24 hours in a Soxhlet device using 95% ethanol. The extract was then concentrated and dried under low pressure.

Haloperidol (0.2 mg/kg body weight) administered intraperitoneally to rats over the course of four days resulted in iron deficiency anemia, which was accompanied by a significant decrease in serum iron and serum protein levels as well as a drop in hemoglobin concentration and erythrocyte count.

There were five groups of six rats each for the test animals. Group i received only an administered vehicle as control; group ii received haloperidol at a dose of 0.2 mg/kg body weight intraperitoneally; group iii received 200 mg/kg body weight i.p. Of ethanolic extract of al alone; group iv received both haloperidol and ethanolic extract of al at a dose of 0.2 mg/kg and 100 mg/kg body weight i.p.; and group v received both haloperidol and ethanolic extract of al at a dose of 0.2 mg/kg and 200 mg/kg body weight intraperitoneally.

On day 4, using a hematology cell counter, blood samples were taken from the rat eye's retro-orbital plexus vein and analyzed for hematological parameters (erythrocyte count, leukocyte count, hemoglobin count, and hematocrit value). The direct biuret test method and the ferrozine method were used to estimate serum protein and iron levels. Excellent erythropoietic activity was demonstrated by the results of the ethanolic extract. Serum iron and serum protein levels were also seen to rise, along with a notable increase in the count of erythrocytes, hemoglobin content, leukocytes, and hematocrit values, among other parameters.^[16]

4] Mosquitocidal activity

In the current study, the egg hatchability and larvicidal qualities of the plant extract of *h. Auriculata* against *a. Stephens*, a malarial vector that is medically significant, were assessed. To assess mosquitocidal activity, larvicidal assays and ovicidal bioassays were carried out.

Ovicidal bioassay

The ovicidal activity was tested using a slightly altered version of su and mulla's methodology. We gathered freshly laid eggs. A minimum of one hundred eggs were utilized. The rate of egg hatching was evaluated. Based on the fact that eggs with unopened opercula do not hatch, the percentage of egg mortality was computed. Eggs subjected to varying amounts of methanolic extract hatched at a considerably lower rate than control eggs.

Larvicidal assay

The standard method was used to assess the larvicidal activity of the methanol extract of *h. Auriculata*. Only the early third instar larvae (0–6 h old) of the target mosquitoes were used to test the entire bioassay. 25 larvae of each test species were used in a single concentration and were duplicated four times. Using abbot's formula, the percent mortality was adjusted for control mortality. Finney's description of probit analysis was used to calculate the lc_{50} values, chi-square values, and other statistical metrics. Mortality of larvae and pupae was recorded at 24 and 48 hours in the experiment used to measure pupicidal activity. The larvicidal activity was demonstrated by the prolonged duration of larval instars and overall developmental time at all dosages.

The findings implied that the methanolic extract of *hygrophila auriculata* possesses mosquito-killing properties.^[17]

5] Anxiolytic activity

The current study set out to assess the anxiolytic activity of seeds from the *hygrophila auriculata* plant. The powdered seeds of *hygrophila auriculata* were extracted using two distinct solvents: ethanol and water. The standard utilized was diazepam, and the extracts were given orally at a dose of 300–600 mg/kg of body weight. The elevated plus maze, light-dark model, open field test for examine anxiolytic behavior, rotarod for motor coordination testing, and photo-actinometer for locomotor activity was utilized the total amount of time that each mouse spent in the maze's open and closed arms was recorded for five to seven minutes in the elevated plus maze model. Both extracts demonstrated a significant increase in the number of entries and percentage of time spent in the open arm. The duration of time spent in the compartments, the frequency of crossings between compartments, the avoidance and latency to entering the light area, and other factors are interpreted as anxiety-like behaviors in the light-dark model. Time spent in the light area increased significantly as a result of the extracts.

In the open field test, the animals were put in the middle of the field 30 minutes after the treatment, and the number of squares crossed in 5 minutes was recorded. The extract revealed a notable rise in the quantity of squares crossed, all of which are indications of anxiolytic behavior.

The animals were positioned with all four paws on the bar in the rotarod model, and the fall-down latency was measured. As the mice's time spent on the revolving rod decreased, the extracts generated showed a significant decline in motor coordination scores, suggesting that they had anxiolytic-like effects. When an animal blocks one or more light beams in a photo actinometer, its locomotive activity is recorded. As compared to the diazepam-treated standard group, the extract-treated animals showed lower cut-off numbers in locomotor activity as measured by a photo actinometer. The outcome was an ethanolic extract of *hygrophila auriculata* with high anxiolytic action at a dose of 600 mg/kg.^[18]

6] Antimotility activity

The goal of this work was to investigate the antimotility properties of several *h. Spinosa* leaf extracts. With atropine sulfate as the standard medication, antimotility action was investigated using the charcoal meal feeding method at a dose of 0.1 mg/kg (i.p.).

Soxhlet apparatus was used to extract the powdered plant material using petroleum ether, chloroform, and alcohol in succession. The process of decoction was used to create the aqueous extract. The extracts were filtered, and the resulting filtrates were evaporated using a rotating vacuum evaporator to produce various extracts. The study used albino mice, 20–25 grams in weight, of either sex.

The gastrointestinal motility of charcoal meal was used to investigate antimotility activity. The extracts were administered to the animals at doses of 200 and 400 mg/kg of body weight after they had been separated into separate groups. The positive control group had atropine sulfate intraperitoneally at a dose of 0.1 mg/kg of body weight, while the control group received 1% v/v tween 80 in water at a dose of 10 ml/kg of body weight. Each animal received 0.3 ml of charcoal meal, which included 10% charcoal and 5% gum acacia, orally after receiving the medication for 30 minutes. After thirty minutes, the animals were slaughtered, and the movement of charcoal meal that had moved from the pylorus to the caecum was measured. *Hygrophila spinosa*'s alcoholic extract has the highest level of antimotility activity.^[19]

7] Aphrodisiac activity

This study aimed to evaluate the spermatogenic and aphrodisiac activities of the plant's alkaloid-enriched fraction of seeds using both in vitro and in vivo methodologies. The alkaloidal fraction of *hygrophila auriculata* was prepared by extracting the 4 kg of *hygrophila auriculata* seed powder using a process known as soxhlet extraction and chloroform. The trials employed 250–350 g male wistar rats in good health. Testicles were aseptically dissected after male animals were slaughtered. Collagenase dispersion was used to isolate testicular leydig cells. Using 3 β hydroxysteroid dehydrogenase (hsd) positive staining, the purity of the separated leydig cells was examined. Using the trypan blue cell exclusion method, the viability of the cells was examined.

The in vitro experiment was divided into four groups. 1) blank 2) control 3) positive standard dehydroepiandrosterone (dhea) 4) test (alkaloidal fraction).

The cells were incubated for 3 h and then subjected to hptlc analysis to estimate the amount of testosterone. The data was integrated using wincats software. The results indicated dose dose-dependent increase in testosterone concentration in test groups. In vitro studies showed that the fraction might act locally in testis on leydig cells and stimulate testosterone synthesis.

In vivo

In vivo studies were performed by dividing the animals into five groups, each of six rats. Group i served as control and received 0.3% gum acacia suspension for 28 days.

Group ii served as positive control and was administered with 0.5 mg/kg b.w. Dose of testosterone propionate in arachis oil intramuscularly twice a week for 28 days.

Group iii received 10 mg/kg,

Group iv received 25 mg/kg

Group v received 50 mg/kg of body weight of alkaloidal fraction suspended in 0.3% gum acacia suspension p.o.

Alteration in the amount of cholesterol and testosterone in the serum of test animals total sperm count, and sexual organ weight were selected as parameters for in vivo activity. Results of these studies showed increased levels of serum cholesterol and serum testosterone in treated groups. As cholesterol is a precursor in testosterone synthesis. The alkaloidal fraction showed an increase in the number of sperm present in the epididymis as compared to the control. Animals were sacrificed and the weights of secondary sexual organs were determined. The results showed that the weight of organs was not increased significantly except for the weight of testes in the treatment group, at all dose levels. Secondary sexual organs like testes, prostate, seminal vesicle, and epididymis are sensitive to hormones. Their weights are increased as a function of steroidal stimulus.^[20]

8] Nephroprotective activity

The present investigation aimed to assess the nephroprotective potential of *hygrophila auriculata* leaves using several drug-induced animal models, including cisplatin, gentamycin, and paracetamol. For this purpose, wistar albino rats were used. Ethanolic extract of *hygrophila auriculata* leaf was made by employing soxhlet extraction. 200 ml of ethanol and about 1000 grams of leaf powder were used in the extraction process 4.0% w/w was found to be the extract yield. The potential for nephroprotection was assessed by biochemical indicators such as serum and tissue parameters.

Increases in creatinine, uric acid, sgot, sgpt, alkaline phosphatase, and bun in the disease control group are indicative of renal damage caused by cisplatin administration. Serum biomarker levels were significantly lower in animal groups that received extract pre-treatment at 250 and 500 mg/kg p.o.

Likewise, gentamicin paracetamol treated animal groups that received extract pre-treatment at 250 and 500 mg/kg p.o. Demonstrated a substantial decrease in serum biomarker levels, whereas the disease control group showed a significant spike in serum marker levels. There is great potential for the extract to cure renal damage because several phytochemicals have been demonstrated to have a significant impact on correcting drug-induced kidney damage.^[21]

9] Cardioprotective activity

The current study examines the ability of leaves of *hygrophila auriculata* to protect against cardiotoxicity caused by doxorubicin was investigated. Using a soxhlet equipment, methanol was used to extract the dried powder of leaves. To prepare petroleum ether soluble fraction, n-butanol soluble fraction, chloroform soluble fraction, and ethyl acetate soluble fraction, this methanolic extract of *hygrophila auriculata* was further fractionated with petroleum ether, n-butanol, and ethyl acetate.

Doxorubicin (25 mg/kg i.p.) Administration caused cardiomyopathy by significantly raising serum levels of creatine kinase (ck), lactate dehydrogenase (ldh), triglycerides, cholesterol, and lipid peroxidation activities while lowering tissue homogenate levels of sod, cat, and gsh. The administration of a methanolic extract of *hygrophila*

auriculata leaves (100, 200, and 400 mg/kg) along with its various fractions, such as petroleum ether, n-butanol, chloroform, and ethyl acetate (100 and 200 mg/kg), orally before doxorubicin has been shown to reduce mortality and restore altered cardiac marker enzymes. The preventive qualities of *hygrophila auriculata* leaves were further validated by histological investigations, which demonstrated normal cardiac muscle bundles and decreased zone of necrosis.^[22]

10] Hypoglycemic activity

Evaluating the hypoglycemic and antioxidant properties of *hygrophila auriculata* aerial parts in diabetic patients was the study's main goal. *Aster acantha longifolia* aqueous extracts have been shown to have hypoglycemic activity. Fernando et al. (1991) conducted preliminary investigations and discovered that the extract, at a therapeutic dose equivalent to 5 g/kg of the starting material, significantly lowers the fasting blood glucose level and significantly improves the glucose tolerance of rats.

Hygrophila auriculata aerial pieces were dried and roughly ground into powder. 50% aqueous ethanol was used to extract the powder that was obtained. It was concentrated, filtered, and freeze-dried for 24 hours to get the final extract, which was a 1.8% yield. Male sprague-drawley rats were given 50 mg/kg of streptozocin to induce hyperglycemia and glycosuria. By monitoring the fasting blood glucose level in stz rats, diabetes was proven.

Streptozotocin-induced diabetic rats showed a significant decrease in blood glucose, thiobarbituric acid reactive substances (tbars), and hydroperoxide in both the liver and kidney after three weeks of treatment with an ethanolic extract of the aerial parts of *hygrophila auriculata* (100 and 250 mg/kg body weight). Comparing the treated group to the control, the ethanolic extract of *hygrophila auriculata*'s aerial parts markedly raised the levels of glutathione (gsh), glutathione peroxidase (gpx), glutathione s-transferase (gst), and catalase (cat). Rats on glibenclamide also showed a decrease in lipid peroxidation linked with an ethanolic extract of *hygrophila auriculata* administered to the rats. Superoxide dismutase (sod) and catalase activity have both risen. According to the study's findings, in diabetic model organisms, an ethanolic extract of *hygrophila auriculata*'s aerial portions had strong antidiabetic and antioxidant properties.^{[23][24]}

11] Hepatoprotective activity

This study was intended to investigate the in vitro and in vivo hepatoprotective effects of the total alkaloid fraction of the leaves of *h. Auriculata*.

The fresh mature leaves (260 g) were extracted with methanol using soxhlet extraction and then the extract was concentrated. A dark green semisolid mass is formed. The standard drug was silymarin powder. Liver cells were isolated by a modified procedure of seglen by using pentobarbital as an anesthetic agent. Freshly isolated rat hepatocytes were exposed to ccl4 (1%) along with/without various concentrations of the total alkaloid fraction (80–40 µg/ml). After 60 min tissue or hepatocyte necrosis which is an indication of the altered concentration of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, triglycerides, and lactate dehydrogenase was measured using ecoline diagnostic kits isolated hepatocytes which were treated with a total alkaloid fraction of *h. Auriculata* showed significant restoration of the altered biochemical parameters toward the normal. The screening of hepatoprotective activity was based on the protection of human liver-derived hepg2 cells against ccl4-induced damage [9] determined by estimating mitochondrial synthesis using the tetrazolium assay. Dose-dependent increase in the percentage viability was observed when ccl4exposed hepg2 cells were treated with different concentrations of the total alkaloid fraction. In vivo, the hepatoprotective effect was investigated in colony-bred wistar adult albino rats (150–200 g) of either sex. Then after treatment with standard and test drugs animal blood was collected serum was separated and the concentration of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, triglycerides, and lactate dehydrogenase was estimated. Its in vivo hepatoprotective effect at 80 mg/kg body weight was comparable with that of the standard silymarin at 250 mg/kg body weight. All biochemical findings were positively supported by the histopathological results.^[25]

12] Anticancer activity

This study was designed to assess the potential anti-tumor effect of *hygrophila spinosa*. 1000 gm of powdered plant material was macerated in ethanol-water by solution and dried to prepare a hydroalcoholic extract of *hygrophila spinosa*. The chemical carcinogen, 7,12-dimethylbenz[a]anthracene (dmba) induced mammary gland tumor in rodents was used to evaluate the anti-tumor effect. The effect is significant as hydroalcoholic extract decreases the size of tumors induced by the carcinogen. It was also found that the percentage of era positive and pr-positive tumors was significantly lower in animals receiving hydroalcoholic extract of *hygrophila spinosa* concerning control. The results showed that the hydroalcoholic extract of *hygrophila spinosa* possesses anti-tumor activity.^[26]

13]Antibacterial activity

The present work aims to study the in-vitro antibacterial activities of the leaf, stem, and root extract of *hygrophila auriculata* against clinical isolate of methicillin-resistant staphylococcus aureus (mrsa) from throat-infected patients by using agar well diffusion method and broth serial dilution method. It was compared with the standard drug- rifampicin (positive control). Butanol, ethyl acetate, methanol, and ethanol were used as the negative control. The resistance pattern of the strain was checked by the agar well diffusion method and confirmed to be an mrsa strain. The prepared plates were incubated and results were evaluated by measuring the zone of inhibition - zoi (in mm.) Of the drug extract. Mic and mbc were also determined for the resistant strain. All the experiments were conducted in triplicates and in sterilized conditions. The butanol extract of *hygrophila auriculata* is found to have low mic & mbc values of 0.5mg/ml. Hence, the result of the present study suggests that the leaf extract of *hygrophila auriculata* exhibits antimicrobial properties against mrsa a resistant strain.^[27]

Home remedies

The powdered seeds of kokilaksha are used in the following ways:

- 1) the powder mixed with water as prescribed by your ayurvedic physician can be used to treat ra kokilaksha and other ingredients like masha, atmagupta, godhuma, sali, sashtika, vidari, and sarkara are powdered, added to milk, and ghee and prescribed to men for improving sexual health.^[28]
- 2) the ash should be administered preferably with cow's urine in doses of 1.5 to 3 grams for dropsy^[29]
- 3) the mucilage obtained by infusing the seeds in water is also prescribed for gonorrhoea and urinary diseases and as a tonic.^[29]
- 4) about 60 grams of the root is boiled in half a liter of water for 20 to 30 minutes in a dosed vessel. About 30 to 60 ml of this preparation is given two or three times daily for the liver disorder.^[29]
- 5) the decoction of the young leaves is taken orally for two consecutive weeks on an empty stomach to treat anemia.^[30]
- 6) take 1/4-1/2 teaspoon of kokilaksha powder. B. Add honey or milk to it. C. Have it after lunch and dinner. D. Continue for at least 1-2 months for better results. Malnutrition.^[31]
- 7) dried leaf powder mixed with castor oil is applied twice a day till the recovery of the affected parts to cure skin disease.^[32]
- 8) the decoction of the root of *aster acantha longifolia* is given in a dose of 10 ml to treat jaundice and swelling of the body.^[33]
- 9) the ash prepared by burning the dried plant (kshara) is given with a decoction of *tribulus terrestris* to treat renal calculi.^[33]
- 10) the cold infusion of the seed of *aster acantha longifolia* is given in a dose of 25-30 ml to treat hepatomegaly and bloating of the abdomen.^[33]
- 11) root decoction for pedal edema and joint pain: lukewarm decoction of the roots of kokilaksha is administered in a dose of 20- 30 ml twice daily. This is effective in joint pain and pedal edema.^[33]

MARKETED FORMULATION OF HYGROPHILLA AURICULATA

1] Kokilaksha Kashaya (Decoction)

uses; Jaundice, Anemia, Atrophic arthritis, Gout, and Hepatic conditions.^[8]

2] Chopachinyadi churn (Powder)

uses; Sting, Atrophic arthritis, and Gout arthritis.^[8]

3] Biogest capsule (Capsule)

uses; Improves immunity.^[8]

4] Ashwamed capsule (Capsule)

causes; Male sexual dysfunction, Ejaculation, and Erection problems.^[8]

5] Rathi capsule (Capsule):

Uses; Premature ejaculation, tones male sex organs and functions, enhances virile power, and reduces sexual weakness.^[8]

6]Cardiraksh capsule (Capsule)

uses; Hypertension, lower the lipid profile level.^[8]

7] Speman capsule (Capsule)

uses; Improves sperm count^[8]

VII. CONCLUSION

Hygrophila auriculata is extensively utilized in Indian Ayurveda. *H. auriculata* is abundant in various bioactive components (fatty acids, minerals, polyphenols, proanthocyanins, alkaloids, enzymes, amino acids, terpenoids, vitamins, and glycosides) which help treat diseases such as blood disorders, kidney-related diseases, diabetes, cancer, etc. The extract can be made from almost any section of the plant. This review mainly highlighted the chemical constitution, pharmacological features, and therapeutic benefits of *Hygrophila auriculata*, a potential herbal drug known for its efficacy and safety. Nevertheless, many research investigations are currently ongoing. Given these various aspects, this might be considered a natural source for the development of future drugs. Various investigations have found that *hygrophila auriculata* can be used as a natural crude medicine. It is expected to be used in the development of innovative drugs.

Considering the promising health benefit features of the plant, it may be concluded that the miraculous healing properties of the plant are one kind of blessing to mankind and in the future a lot more medicinal values are expected to be explored from the plant.

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