Chapter 3

Hygrophila auriculata (K. Schum) Heine

(Acanthaceae)

3.1. Introduction

Hygrophila auriculata (K. Schum) Heine (synonym: Asteracantha longifolia Nees, Barleria auriculata Schum, Barleria longifolia Linn), is described in the Ayurvedic literature as Ikshura, Ikshagandha and Kokilasha having eyes like the kokila or the Indian cuckoo. It is classified in the Ayurvedic system of medicine as seethaveryam, mathuravipaka and is used for the treatment of a number of conditions including premeham (diabetes) and athisaram (dysentery).

❖ Vernacular name

- English : Long-Leaved Barlevia
- Hindi : Talmalkhana, Talmakhana
- Kannada : Kolavalike
- Oriya : Koillekha
- Sanskrit : Kokilasha
- Tamil : Nirmulli

❖ Distribution

The plant is a wild herb commonly found in moist places on the banks of rivers, ditches and paddy fields throughout India, Sri Lanka, Burma, Malaysia and Nepal.

❖ Morphological Characters

The plant is a sub shrub, usually growing in marshy places along water courses. The stem is reddish brown and the shoot has 8 leaves and six thorns at each node (Figure 1. A, B and C). The leaves occur in whorls, the outer pair of leaves is larger, lanceolate, scalerous, margins, minutely dentate, subsessile, thorns strong straight or curved. Flowers occur in axillary whorls, bract and bracteoles leafy. Calyx four lobed, lobes unequal.
corolla, - 5 petals gamopetalous, unequally 2- lipped, middle lobe of the lower lip with yellow palate; corolla purple coloured; stamens - four, in two pair, filaments unequal; anthers divergent; ovary two celled; four ovules in each cell; fruit a dehiscent capsule [1, 2].

![Image of H. auriculata](image)

**Figure 3.1.** (A, B and C) Flowering branches of *H. auriculata*

Shoots showing axillary flowers, throns, and cluster leaves.

**Ethnobotanical uses**

A survey of the ethnobotanical literature shows that the roots, seeds, and aerial parts of the plant are widely used in the traditional system of medicine for the treatment of jaundice, hepatic obstruction, rheumatism, inflammation, pain, urinary infection, edema, gout, malaria, impotence and as an aphrodisiac [3].

The seeds are used as ingredients in various aphrodisiacs and tonic confections, and in the treatment of blood disorders, biliousness, gonorrhoea, spermatorrhea and fever. The seeds are ground into a paste and given in buttermilk to cure diarrhea. AKSIR-UL-IMRAZ, a preparation having Talamkhana (seeds) as one of the ingredients, is used to prevent leukorrhea. The ashes of the plant are also used against dropsy and gravel. A tincture of the whole plant is beneficial in urinary affections, dysuria, and painful micturition. A root decoction drunk to combat rheumatism, gonorrhea, and hepatic obstruction. The leaves are diuretic, sweet, tonic, aphrodisiac, hypnotic and useful in the treatment of cough, diarrhoea, thirst, urinary calculi, urinary discharges, inflammations,
joint pain, eye diseases, pains, ascites, anemia, and abdominal disorders. An aqueous extract of the herb is taken orally as diuretic, spasmodlytic and hypotensive. The herb exhibits antihepatotoxic activity in dogs. The oil extracted from the whole plant is antibacterial [4–7].

3.2. Phytoconstituents reported in H. auriculata

Flavonoids

Bairaj and Nagarajan (1982) isolated apigenin 7-O-glucuronide from the flowers of H. auriculata along with traces of apigenin 7-O-glucoside [8].

Alkaloids

Parashar and Harikishan Singh (1964) isolated alkaloidal fraction from the alcoholic extract of aerial parts of H. auriculata. Two alkaloids asteracanthine and asteracanthicine were reported from the seeds [9].

Triterpenes

Govindachari et al., (1957) reported the presence of lupeol in roots leaves and stem, and a hydrocarbon, hentricontane in leaves and stems [10]. Betulin was isolated from the methanolic extract of aerial parts of H. auriculata [11]. Nair et al., (1965) reported the presence of luteolin and luteolin-7-O-rutinoside in the leaves of H. auriculata [12].

Aliphatic esters


Sterols

Quasim and Dutta (1967) reported the presence of stigmasterol in the roots of H. auriculata [13]. Patra et al., (2012) isolated the stigmast-5-en -3β-ol (β-sitosterol) from the chloroform extract of leaves of H. spinosa [14].
Minerals

Choudhary and Bandyopadhyay (1998) identified the presence of high concentration of Fe, Cu, and Co in all organs of *H. spinosa*. Thanki and Thaker (1980) studied the amino acid composition of the seeds of *H. auriculata* and reported that the seed proteins of *H. auriculata* have been found to contain all the essential amino acids and are comparable to those of groundnut protein [15].

Essential oils

Essential oils were isolated from the root and aerial parts of *A. longifolia* and tested for antibacterial activity [19].

- Lupeol
- Leutolin
- Betulin
Apigenin 7-O- glucoside

Apigenin 7-O- glucuronide

Stigmasterol

25-Oxo-hentriacontyl acetate  Methyl 8-\textit{n}-hexyltetraicosanoate
<table>
<thead>
<tr>
<th>Phytoconstituent</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
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<td>Luteolin 7-O-glucosides</td>
<td>H</td>
<td>7-O-β-D-glucopyranosides</td>
<td>OH</td>
</tr>
</tbody>
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Henetriacotane

Stig mast-5-en-3β-ol

**Figure 3.2** Structures of selected some phytoconstituents previously isolated from *H. auriculata*
3.3. Reported bioactivities of *H. auriculata*

The both aqueous and alcoholic extracts have LD$_{50}$ 4g/kg. The extract bears the LD$_{50}$ 3019.95 mg/kg p.o [16].

**Anti-tumor Activity**

- Ahmed *et al.*, (2001) reported the anti-tumor activity of seeds of *A. longifolia* against experimental hepatocarcinogenesis in rats. They also stated that the seeds significantly ameliorated the activities of antioxidant enzymes glutathione peroxidase and catalase in a dose dependant manner [17].
- Mazumdar *et al.*, (1997) reported that the petroleum ether extract of the roots of *H. spinosa* exhibited anti-tumour activity in *Ehrlich ascites* carcinoma and sarcoma-180 bearing mice [18].
- An hydroalcoholic extract of whole plant of *H. spinosa* at a dose of 300mg/kg body weight showed significant anti-tumour activity against 7, 12-dimethylbenz(a)anthracene (DMBA) induced mammary tumours in female rats comparable with tamoxifen as a standard drugs [16].
- Balasubramaniam *et al.*, (2012) reported that the effect of *H. auriculata* on carbohydrate metabolizing enzymes in N-nitrosodiethylamine induced hepatocellular carcinoma in rats. The methanolic extract of *H. auriculata* (200 mg/kg) produced significant decrease in hexokinase, phosphogluco-isomerase, aldolase, while increased glucose-6-phosphatase in the plasma and liver of carcinoma bearing rats at the end of the treatment period (28 days). However, the changes in the above parameters were comparable with control. Thus, methanolic extract of *H. auriculata* reverted the altered carbohydrate metabolizing enzymes which is associated with biochemical changes of hepatomas to near normal in HCC bearing rats due to the presence of polyphenols and flavonoids [19].

**Antioxidant activity**

- Sunilkumar and Klausmuller (1999) screened 28 different plant species of Nepalese medicinal plants including seeds of *A. longifolia* used traditionally in indigenous system of medicine to treat inflammatory diseases for the inhibitory
effect on lipid peroxidation and reported that the plant inhibited lipid peroxidation with an IC$_{50}$ value of 20 ug/mL [20].

**Antibacterial activity**

- Boily and Vampuyvelde (1986) screened anti-microbial property of ethanolic extract of leaves, stem, fruits and root of *H. auriculata* against *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Escherachia coli, Candida albicans* and *Mycobacterium smegmatis* and reported that the leaves exhibited active anti-microbial activity against *S. aureus, B. subtilis, C. albicans and M. smegmatis* [21].

- Vlientick *et al.*, (1995) screened anti-microbial property of ethanolic extract of leaves, stem, fruits and roots of *H. auricalata* against *Staphylococcus aureus, Pseudomonas aeroginosa, Echterachia coli, Candida albicans, Tricophyton mentagrophytes* and *Mycobacterium canis* and reported that the leaves exhibited active anti-microbial activity against *S. aureus, C. albicans, M. canis and T. mentagrophytes*, stem exhibited activity against *C. albicans, M. canis and T. mentagrophytes* [22].

- The antibacterial activity of petroleum ether, chloroform, alcoholic and aqueous extracts of leaves of *H. spinosa* against *Escherachia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeroginosa* evaluated by disc-diffusion methods. The chloroform and alcoholic extract exhibited significant antibacterial activity, whereas the aqueous extract has moderate effect and petroleum ether extract was showed least action against the microorganismS [23].

**Anthelmintic activity**

- The anthelmintic activity of petroleum ether, chloroform, alcoholic and aqueous extracts of leaves of *H. spinosa* against *Pherithima posthuma* as a test worms in various concentration (10-100 mg/ml) were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms. The alcoholic extract showed the significant anthelmintic activity at higher concentration 100 mg/ml, whereas chloroform and aqueous extract showed moderate activity and petroleum ether extract exhibited least anthelmintic activity [23].
Anti-nociceptive activity

- Shanmugasundram et al., (2005) found that the aqueous extracts at the aerial parts and roots of *H. auriculata* at the dose of 200mg/kg p.o exhibited potent antinociceptive activity in animal model of thermally induced analgesia in mice [24].

- Petroleum ether, choloroform, alcoholic and aqueous extracts of the leaves of *H. spinosa* (Acanthaceae) were screened for analgesic activity. The choloroform, alcoholic and aqueous extracts at dose of 200 and 400 mg/kg of body weight significantly inhibited the abdominal constriction produced by acetic acid and also increased the pain threshold of mice towards the thermal source in a dose dependent manner comparable to the standard drug aspirin (100 mg/kg of body weight) [25].

Anti-inflammatory and antipyretic activities

- Patra et al., (2009) carried out the anti-inflammatory and antipyretic activities of the petroleum ether, choloroform, alcoholic and aqueous extracts of the leaves of the *H. spinosa*. The anti-inflammatory activity of the various extracts was studied based on their effects on carrageenan-induced paw oedema in rats while antipyretic activity was evaluated on the basis of their effect on Brewer’s yeast-induced pyrexia in rats. Chloroform and alcoholic extracts of leaves of *H. spinosa* exhibited significant anti-inflammatory and antipyretic activities in a dose-dependent manner. On the other hand, petroleum ether and aqueous extracts did not show anti-inflammatory and antipyretic effect. The maximum anti-inflammatory activities produced by the chloroform and alcoholic extract at a dose of 400 mg/kg body weight [26].

- Patra et al., (2009) reported the antipyretic activity of the alcoholic extract of leaves and roots of *H. spinosa* using Brewer’s yeast-induced pyrexia in rats. Both the extracts at a dose of 200 and 400 mg/kg body weight significantly reduced the increased rectal temperature and exhibited potent antipyretic action in animal model [26].
Mohapatra et al., (2011) reported the chloroform extract of aerial part of *H. auriculata* was screened for different study like acute toxicity study, analgesic activity by thermal (hot plate) and chemical methods (acetic acid induced writhing) and anti-inflammatory activity was evaluated on the basis of effect on formalin induced arthritis (paw edema) in rats and measured by slide calipers method. In thermal method the maximum analgesic activity of the extract at dose 250 mg/kg, 350 mg/kg (p.o) increased pain threshold of mice towards thermal source and in chemical method the maximum analgesic activity of the extract at dose 250 mg/kg, 400 mg/kg (p.o) inhibited the abdominal constriction induced by acetic acid. The maximum anti-inflammatory activity of chloroform extract at dose 200 mg/kg, 300 mg/kg reduce formalin induced arthritis (paw edema). From the result it was concluded that the chloroform extract exhibited analgesic and anti-inflammatory effects by central and peripheral mechanism [27].

**Antimotility activity**

- Antimotility activity was studied by charcoal meal feeding method and atropine sulphate at a dose of 0.1 mg/kg (i.p.) was used as the standard drug. The alcoholic extracts of leaves of *H. spinosa* at a dose of 400mg/kg body weight significantly decreased the distance travelled by charcoal meal through the gastrointestinal tract. The results suggested that the extracts exhibited antimotility activity [28].

**CNS activity**

- Mazumdar et al. (1999) carried out chemical investigation of the petroleum ether extract of root of *H. spinosa* and reported for the presence of active constituents like lupeol and lupenone. They also reported that the i.p. administration of crude petroleum ether extract in mice potentiated the sedative-hypnotic action of chlorpromazine, diazepam, phenobarbitone, chlordiazepoxide and protected against strychnine induced convulsions [29].

**Diuretic activity**

- The diuretic potential of aqueous, alcoholic extract and different fraction of alcoholic extract of whole plant of *H. auriculata* was evaluated by using methods
described by Lipschitz et al., (1943). The diuretic effect was examined by treating different groups of Wistar Albino rats with single (200 mg/kg) oral doses of alcoholic extract/fractions. Furosemide (10 mg/kg) was used as positive control in the study. Out of the different fractions and extract, the n-butanol fraction (200 mg/kg) significantly and markedly increased the urine output. The pattern of diuresis induced by the n-butanol fraction was shown almost similar to that produced by the furosemide [30].

- Preethi et al., (2012) investigated the diuretic properties of the seeds of H. auriculata (Acanthaceae) in normal Wistar Albino rats. The alcoholic extract of seeds of H. auriculata (300 mg/kg and 500 mg/kg p.o.) were investigated for diuretic activity by measuring the total urine output over 24hrs and electrolytes (sodium, potassium and chloride) estimation in Wistar rats (n=6). Frusemide (20 mg/kg, p.o), a high ceiling diuretic served as positive control and normal saline (25 ml/kg, p.o) as placebo control. The alcoholic extract of H. auriculata showed significant diuretic properties. At both the doses employed there was a significant increase in electrolyte excretions, however increase in urine volume was significant only at 500mg/kg [31].

**Erythropoietic activity**

- An administration of the ethanolic extract of A. longifolia at the doses of 100 mg/kg and 200 mg/kg body weight, i.p., demonstrated a significant (P<0.05) increase in erythrocyte count, haemoglobin count, serum iron and serum protein etc. This effect may be due to the presence of iron (622 µg/50 mg) in extract estimated by spectrophotometric method. An ethanolic extract of A. longifolia effectively restored the hematological parameters, serum iron and serum protein and normalized the microcytic (smaller in size), anisocytosis (disturbed shape) and hypochromic RBCs [32].

**Free radical scavenging activity**

- Free radical scavenging potential of aqueous, alcoholic and different fraction of whole plant of H. auriculata was evaluated by using 1,1’-diphenyl-2-picryl-
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hydrazyl (DPPH), deoxyribose degradation against OH•, nitric oxide and lipid peroxidation radical assays. Vitamin E was used as a standard for the study. The results of study revealed the n-butanol fraction exhibited the potent free radical scavenging activity in dose dependant manner and comparable to the standards vitamin E [33].

- The alcoholic extract of seeds of H. auriculata exhibited strong scavenging effect on 2, 2-diphenyl-2-picryl hydrazyl (DPPH) free radical, superoxide, nitric oxide radical and ABTS radical scavenging assay. The free radical scavenging effect of H. auriculata extract was comparable with that of the reference antioxidants. The obtained data of the study suggests that the extract of H. auriculata seed have potent antioxidant activity against free radicals, prevent oxidative damage to major biomolecules and afford significant protection against oxidative damage [34].

**Hepatoprotective Activity**

- A methanolic extract of the seeds of H. auriculata at the dose of 200 mg/kg/p.o exhibited potent hepatoprotective activity against paracetamol and thioacetamide induced liver damage in rats [35].

- Shanmugasundram et al., (2005) found that aqueous extract of the roots of H. auriculata at the dose of 150 mg/kg/p.o exhibited potent hepatoprotective activity against carbon tetrachloride induced liver damage in rats [36].

- Hewawasam et al., (2003) to reported that the aqueous extract of A. longifolia was tested for hepatoprotective activity against carbon tetra chloride and paracetamol induced acute hepatotoxicity in mice. The plant exhibited significant hepatoprotective activity by reducing carbon-tetra chloride and paracetamol induced changes in biochemical parameters that were evident by enzymatic examination. The plant extract may interfere with free radical formation, which may conclude in hepatoprotective action. A. longifolia elicited significant hepatoprotective activity against carbon tetra chloride and paracetamol, comparable with standard drugs [37].
• The *A. longifolia* whole plant slurry was tested against carbon tetra chloride induced liver dysfunction in rats. The plant exhibited significant hepatoprotective activity by reducing carbon-tetra chloride induced changes in biochemical parameters that were evident by enzymatic examination. The whole plant slurry of *A. longifolia* showed significant hepatoprotective efficacy against carbon tetra chloride, comparable with a known hepatoprotectant, silymarin [38].

• Solomon *et al.*, (2011) reported the antihepatotoxic activity of the ethanolic extract of *H. spinosa* roots was carried out using CCl₄ induced antihepatotoxic in albino rats. Silymarin at the dose of 25 mg/kg, p.o. was used as reference standard drug and it exhibited significant protection [39].

• Usha *et al.*, (2007) aqueous extract of the roots of *H. spinosa* at the dose of 200 mg/kg/p.o exhibited potent hepatoprotective activity against carbon tetrachloride induced liver damage in rats [40].

- Hypoglycemic activity

• Fernando *et al.*, (1991) carried out preliminary investigations on hypoglycaemic activity of aqueous extracts of whole plant of *A. longifolia* and started that the extract significantly lowers the fasting blood glucose level and markedly improves the glucose tolerance of rats at a therapeutic dose equivalent to 5 g/kg of starting material [41].

• Ethanolic extract of the aerial parts of *H. auriculata* at the dose of 100 and 250 mg/kg body weight for 3 weeks displayed significant reduction in blood glucose, thiobarbituric acid reactive substances (TBARS) and hydroperoxide in both liver and kidney against streptozotocin induced diabetic rats. The treatment with ethanolic extract of aerial parts of *H. auriculata* significantly increased the glutathione (GSH), glutathione peroxidase (GPx), glutathione S-transferase (GST) and catalase (CAT) in the drug-treated group, which is comparable to the control group. The ethanolic extract of *H. auriculata* and glibenclamide-treated rats also showed decreased lipid peroxidation that is associated with increased activity of superoxide dismutase (SOD) and catalase. The result of this study indicated that ethanolic extract of aerial parts of *H. auriculata* possessed significant anti-
diabetic activity along with potent antioxidant potential in diabetic conditions [42].

- The chloroform, ethyl acetate and alcoholic roots extracts of *H. spinosa* tested for their antidiabetic activity in alloxan-induced diabetic rats. The ethyl acetate and alcoholic extracts have shown significant anti-diabetic activity at a dose of 200 mg/kg, p.o. [43].

- **Haematinic effect**

  The ethanolic extract of aerial parts of *H. spinosa* at the dose of 100 and 200 mg/kg/p.o significantly increased the haemoglobin, haematocrit and RBC in anaemic male rats indicating the haematinic effect of the extract [44].

- **Nephrotoxicity activity**

  The ethanolic extract of whole plant of *H. spinosa* at the dose of 50 and 250 mg/kg/p.o significantly showed gentamicin induced nephrotoxicity activity and free radical scavenging activities to confirm the therapeutic activity of this plant extract [45].
3.4. EXPERIMENTAL

❖ MATERIALS AND METHODS

➢ Phytochemical Investigation

• Collection of plant material

The fresh plants of *Hygrophila auriculata* (K. Schum) Heine were collected from the field area of Baryahi, Saharsa District, Bihar, India, in January 2009. The plant specimen was authenticated by Prof (Dr.) Anjani Kumar Sinha, Principal, M L T Saharsa College Saharsa, Saharsa, Bihar. A voucher specimen no SHC 55/01/2009 has been deposited in the herbarium, Department of Botany, M L T Saharsa College Saharsa- 852201.

• Preparation of extract

*H. auriculata* (4.6 kg) was shade dried, coarsely powdered and extracted exhaustively with methanol in a Soxhlet apparatus. The methanolic extracts of the plant was then concentrated on steam bath and dried under reduced pressure to get 167.0 g of dark brown mass.

• Preparation of slurry

The concentrated extract (80 g) of the drug was dissolved in minimum amount of methanol with constant stirring, till desired consistency was obtained. A weighed quantity of silica gel for column chromatography (60-120 mesh) was then added slowly with continuous mixing until the whole methanolic solution of plant extract adsorbed on the silica gel particles. It was dried in the air; the larger lumps were broken and finally passed through a sieve (No. 8) to get uniform particle size.

• Packing of column & Isolation of phytoconstituents

A column of 3.0 feet, height and 16 mm internal diameter was taken, cleaned properly and dried. The lower end of the column was plugged with non-absorbent cotton wool. The column was clamped and fitted in a vertical position on a stand. The column was half filled with petroleum ether (b.p. 60-80 °C). Silica gel (for column, 60-120 mesh) was then
poured in small portions and allowed to settle down and the dried plant extract slurry was loaded over the column. The developments and elution of the column were carried out with successive series of different solvents in various combinations, such as petroleum ether (100), petroleum ether: chloroform (3:1, 1:1, 1:3), chloroform (100), chloroform: methanol (99:1, 98:2, 97:3, 19:1, 9:1, 4:1) and methanol to isolate the compounds.

- **Homogeneity of the fractions**

  The fractions collected were subjected to thin layer chromatography (TLC) to check homogeneity of various fractions. Chromatographically identical fractions (having same $R_f$ values) were combined together and concentrated. They were then crystallized with suitable solvent system.

### 3.4.2 Observation

Following compounds have been isolated from the *H. auriculata*:

- **n-Hexacos-21-one-1-ol (HA-01)**

  Elution of the column with chloroform: methanol (19:1) gave pale yellow crystalline mass of **HA-01**, recrystallized from acetone-methanol (1:1), 115 mg (0.21 % yield).

  *$R_f$ 0.54 (CHCl$_3$-MeOH, 19:1)*

  *m.p.: 105-106°C;*

  *UV $\lambda_{max}$ (MeOH): 208 nm.*

  *IR $\nu_{max}$ (KBr): 3415, 2925, 2853, 1710, 1655, 1458, 1377, 1244, 1073 cm$^{-1}$.*

  *$^1$H NMR (MeOD): $\delta$ 3.79 (2H, t, J= 7.2 Hz, H$_2$-1), 2.33 (2H, t, J= 7.5 Hz, H$_2$-20), 2.03 (2H, t, J= 6.3 Hz, H$_2$-22), 1.58 (2H, m, CH$_2$), 1.37 (4H, brs, 2xCH$_2$), 1.33 (8H, brs, 4xCH$_2$), 1.29 (20H, brs, 10xCH$_2$), 1.26 (8H, brs, 4xCH$_2$), 0.87 (3H, t, J= 6.9 Hz, Me-26).*

  *$^{13}$C NMR (MeOD): $\delta$ 203.13 (C-21), 63.16 (C-1), 35.05 (CH$_2$), 33.22 (CH$_2$), 30.90 (CH$_2$), 30.24 (CH$_2$), 29.94 (CH$_2$), 29.91 (CH$_2$), 29.16 (CH$_2$), 23.88 (CH$_2$), 14.61 (Me-26).*

  *$^{+}$ve ESI MS $m/z$ (rel. int.): 397 [M+H]$^+$ (C$_{26}$H$_{55}$O$_2$) (9.8), 99 (73.6).*
trans- Tetracos-7-en-1-ol (HA-02)

Further elution of the column with chloroform: methanol (19:1) gave afforded yellow crystalline mass of HA-02, recrystallised from chloroform methanol (1:1), 139 mg (0.221% yield).

Rf 0.57 (CHCl₃-MeOH, 19:1)

m.p.: 112-113°C;

UV λmax (CHCl₃): 274 nm.

IR νmax (KBr): 3429, 2922, 2853, 1645, 1461, 1335, 1026, 722 cm⁻¹.

1H NMR (CDCl₃): δ 5.37 (1H, m), 5.07 (1H, m), 2.28 (2H, m), 1.95 (2H, m), 1.59 (4H, m), 2.29 (8H, brs, 4×CH₂), 1.25 (56H, brs, 28×CH₂), 0.85 (3H, t, J=6.9 Hz, Me-40).

13C NMR (CDCl₃): 123.45 (C-7), 120.89 (C-8), 67.27 (C-1), 33.84 (CH₂), 31.72 (CH₂), 29.45 (32×CH₂), 22.61 (CH₂), 14.22 (Me-40).

+ve ESI MS m/z (rel. int.): 577 [M+H]⁺ (C₄₀H₈₁O) (19.8), 475 (15.2).

n-Octacosan-8-one (HA-03)

Elution of the column with chloroform: methanol (93:7) gave greenish crystalline mass of HA-03, recrystallised from acetone: methanol (1:1), 89 mg (0.0143% yield).

Rf 0.67 (CHCl₃-MeOH, 95:5)

m.p.: 107-108°C;

UV λmax (CHCl₃): 204 nm.

IR νmax (KBr): 2924, 2855, 1709, 1461, 1403, 1169, 725 cm⁻¹.

1H NMR (CDCl₃): δ 2.34 (2H, t, J=7.5 Hz, H₂-7), 2.05 (2H, t, J=5.7 Hz, H₂-9), 1.65 (2H, m,CH₂), 1.63 (2H, m, CH₂), 1.61 (2H, m, CH₂), 1.29 (6H, brs, 3×CH₂), 1.25 (34H, brs, 17× CH₂), 0.88 (3H, t, J=6.0 Hz, Me-1), 0.83 (3H, t, J=6.9 Hz, Me-28).
13C NMR (CDCl3): δ 203.5 (C-8), 33.90 (CH2), 32.15 (CH2), 29.91 (20×CH2), 29.58 (CH2), 29.43(CH2), 22.91 (CH2), 14.33 (Me-1, Me-28).

+ve ESI MS m/z (rel. int.): 409 [M+H]+ (C28H57O) (34.7), 309 (18.4), 281(58.5).

**n-Octatriacontan-3-one -19,31,-diol (HA-04)**

Further elution of the column with chloroform: methanol (9:7) gave colourless crystalline mass of HA-04, recrystallised from acetone: methanol (1:1), 125 mg (0.133% yield).

Rf 0.49 (CHCl3-MeOH, 19:1)

M.P.: 136-137°C

UV λmax (MeOH): 209 nm.

IR νmax (KBr): 3420, 3370, 2927, 2857, 1707, 1459, 1249, 1070, 720 cm⁻¹.

1H NMR (MeOD): 3.88 (1H, brm, w1/2=7.8 Hz, H-19β), 3.69 (1H, brm, w1/2=10.2 Hz, H-31β), 2.31 (2H, t, J=7.5, H2-4), 2.03 (2H, m, H2-2), 1.57 (4H, m, 2×CH2), 1.41 (4H, m, 2×CH2), 1.37 (4H, brs, 2×CH2), 1.33 (6H, brs, 3×CH3), 1.29 (34H, brs, 17×CH2), 1.26 (10H, brs, 5×CH2), 0.96 (3H, t, J=7.5 Hz, Me-1), 0.87 (3H, t, J=7.2 Hz, Me-38).

13C NMR (MeOD): δ 208.26 (C-3), 76.41 (C-19), 68.90 (C-31), 33.22 (CH2), 32.30 (CH2), 30.88 (25×CH2), 30.26 (CH2), 30.08 (CH2), 30.01 (CH2), 29.83 (CH2), 29.77 (CH2), 23.87 (CH2), 14.57 (Me-1, Me-38).

+ve ESI MS m/z (rel. int.): 580 [M]+ (C38H76O3) (26.1), 551 (59.8), 523 (11.2), 313 (78.4), 283 (8.7), 129 (16.1).

**n-Heptacosan-11α-ol (HA-05)**

Elution of the column with chloroform: methanol (9:1) gave colourless crystalline mass of HA-05, recrystallized from acetone: methanol (1:1), 115 mg (0.103% yield).

Rf 0.52 (CHCl3-MeOH, 9:1)
m.p.: 125-126°C

UV $\lambda_{\text{max}}$ (CHCl$_3$): 306 nm.

IR $\nu_{\text{max}}$ (KBr): 3423, 2920, 2852, 1643, 1454, 1376, 1164, 1024, 722 cm$^{-1}$.

$^1$H NMR (CDCl$_3$): 3.35 (1H, brm, $\omega_{1/2}$ 9.1 Hz, H-11β), 2.02 (4H, m, 2×CH$_2$), 1.73 (14H, brs, 7×CH$_2$), 1.51 (2H, m, CH$_2$), 1.41 (2H, m, CH$_2$), 1.33 (2H, m, CH$_2$), 1.25(24 H, brs, 12×CH$_2$), 0.88 (3H, t, J=6.1 Hz, Me-1), 0.85 (3 H, t, J=7.2 Hz, Me-27).

$^{13}$CNMR (CDCl$_3$): $\delta$ 64.13 (C-11), 33.45 (CH$_2$), 31.81 (CH$_2$), 29.92 (20×CH$_2$), 25.36 (CH$_2$), 22.50(CH$_2$), 14.33 (Me-1), 14.30 (Me-27).

$^+$ve ESI MS $m/z$ (rel. int.): 397 [M+H]$^+$ (C$_{27}$H$_{57}$O) (100), 255 (18.7), 171(6.1).

3.5. RESULTS AND DISCUSSION

Compound HA-01, an aliphatic keto alcohol, was obtained as a pale yellow crystalline mass from chloroform: methanol (19:1) eluants. Its IR spectrum showed characteristic absorption bands for hydroxyl groups (3415 cm$^{-1}$) and carbonyl function (1710 cm$^{-1}$). Its mass spectrum had a molecular ion peak at $m/z$ 397 [M+H]$^+$ consistent to the molecular formula of the keto alcohol C$_{26}$H$_{53}$O$_2$. The prominent ion peak arising at $m/z$ 99 [CH$_3$(CH$_2$)$_4$CO]$^+$ suggested the existence of the keto group at C-21. The $^1$HNMR spectrum of HA-01 one displayed three two-proton triplets at $\delta$ 3.79 (J= 7.2 Hz) and 2.33 (J=7.5 Hz) and 2.03 (J=6.3 Hz) assigned to hydroxymethylene H$_2$-1 and methylene H$_2$-20 and H$_2$-22 protons respectively adjacent to the C-21 keto group. The other methylene proton appeared as broad singlet’s from $\delta$ 1.37 to 1.26. A three-proton triplet at $\delta$ 0.87 (J=6.9 Hz) was accounted to terminal C-26 primary methyl protons. The $^{13}$C NMR spectrum of 1 exhibited signal for carbonyl carbon at $\delta$ 203.13 (C-21) hydroxyl methylene carbon at $\delta$ 63.16 (C-1), other methylene carbons from $\delta$ 35.05 to 23.88 and methyl carbon at $\delta$ 14.61 (C-26). On the basis of spectral data analysis the structure of HA-01 has been identified as n-hexacos-21-one-1-ol.
**Compound HA-02** an unsaturated aliphatic alcohol was obtained as a yellow crystalline mass from chloroform: methanol (19:1) eluants. Its IR spectrum showed absorption bands for hydroxyl groups (3429 cm\(^{-1}\)), unsaturation (1645 cm\(^{-1}\)) and long aliphatic chain (722 cm\(^{-1}\)) and had a molecular ion peak in the mass spectrum at \(m/z\) 577 [M+H]+ corresponding to the molecular formula of vinylic alcohol C\(_{40}\)H\(_{81}\)O. The ion peak generating at \(m/z\) 475 [CH\(_3\)(CH\(_2\))\(_{31}\) CH=CH]+ supported the presence of vinylic linkage at C-7. The \(^1\)H NMR spectrum of HA-02 displayed two one-proton multiplets at \(\delta\) 5.37 and 5.07 with half-width of 16.6 Hz each assigned to trans oriented vinylic H-7 and H-8 protons respectively. A two-proton triplet at \(\delta\) 3.59 (J=11.2 Hz) was ascribed to hydroxymethylene H\(_2\)-1 protons. The other methylene protons signals appeared between \(\delta\) 2.28 - 1.26. A three-proton triplet at \(\delta\) 0.85 J= 6.9 Hz was accounted to terminal C-40 primary methyl protons. The \(^{13}\)C NMR spectrum of HA-02 displayed signals for vinylic carbons at \(\delta\) 123.45 (C-7) and 120.89 (C-8), hydroxymethylene carbon at \(\delta\) 67.27 (C-1), other methylene carbons between \(\delta\) 33.84-22.61 and methyl carbon at \(\delta\) 14.22 (C-40). On the basis of the above discussion the structure of HA-02 has been characterized as t-Tetracont-7-en-1-ol.

**Compound HA-03** an aliphatic ketone, was obtain as a pale yellow crystalline mass product from chloroform: methanol (93:7) eluants. Its IR spectrum showed characteristic absorption bands for keto group (1709 cm\(^{-1}\)) and long aliphatic chain (725 cm\(^{-1}\)). Its mass spectrum displayed a molecular ion peak at \(m/z\) 409 [M+H]+ corresponding to the molecular formula of a saturated ketone, C\(_{28}\)H\(_{57}\)O. The ion peaks arising at \(m/z\) 281 [CH\(_3\)(CH\(_2\))\(_{19}\)]+ and 309 [CH\(_3\)(CH\(_2\))\(_{19}\)CO]+ supported the location of the keto group at C-8. The \(^1\)H NMR spectrum of HA-03 exhibited two triplets at \(\delta\) 2.34 (J=7.5 Hz) and 2.05 (J=5.7 Hz), integrating for two protons each, assigned to methylene H\(_2\)-7 and H\(_2\)-9, respectively, nearby to the C-8 keto group. The other methylene protons appeared from \(\delta\) 1.65 to 1.25. Two three-proton triplets at \(\delta\) 0.88 (J=6.0 Hz) and 0.83 (J=6.9 Hz) were accounted to terminal C-1 and C-28 primary methylene protons, respectively. The \(^{13}\)C NMR spectrum of HA-03 displayed signals for keto carbon at \(\delta\) 203.51 (C-3), methylene carbons between at \(\delta\) 33.90 - 22.91 and methyl carbons at \(\delta\)
14.33 (C-1, C-28). Based on these evidences, the structure of **HA-03** has been elucidated on n-Octacosan-8-one.

**Compound HA-04**, an aliphatic keto diol, was obtained as a colourless crystalline mass from chloroform: methanol (93:7) eluants. Its IR spectrum showed absorption band for hydroxyl groups (3420, 3370, cm\(^{-1}\)), keto group (1707 cm\(^{-1}\)) and longs aliphatic chain (720 cm\(^{-1}\)). On the basis of mass and \(^{13}\)C NMR spectra, the molecular ion peak of **HA-04** was determined at \(m/z\) 580 consistent to the molecular formula of a keto diol C\(_{38}\)H\(_{76}\)O\(_3\). The ion fragments generating at \(m/z\) 129 \([\text{CH}_3(\text{CH}_2)_{16}\text{CHOH}]+\), 283 \([\text{CH}_3(\text{CH}_2)_{16}\text{CHOH}-(\text{CH}_2)_{11}]+\) and 313 \([\text{CH}_3(\text{CH}_2)_{16}\text{CHOH}-(\text{CH}_2)_{11}\text{CHOH}]+\) indicated the existence of the hydroxyl groups at C-19 and C-31 positions. The ion peaks arising at \(m/z\) 523 \([\text{CH}_3(\text{CH}_2)_{16}\text{CHOH}(\text{CH}_2)_{11}\text{CHOH}-(\text{CH}_2)_{15}]+\) and 551 \([\text{M-C}_2\text{H}_5]+\) supported the location of the carbonyl group at C-3 (Scheme-3.4).

The \(^1\)H NMR spectrum of **HA-04** displayed two one–proton multiplets at \(\delta\) 3.88 (\(\text{w}1/2=7.8\text{Hz}\)) and 3.69 (\(\text{w}1/2=10.2\text{Hz}\)) assigned to \(\beta\)-oriented carbinol H-19 and H-31 protons, respectively. A two-proton triplet at \(\delta\) 2.31 (J=7.5 Hz) and a two-proton multiplet at \(\delta\) 2.03 were ascribed to methylene H\(_2-4\) and H\(_2-2\) protons, respectively, nearby the carbonyl function. The other methylene protons resonated from \(\delta\) 1.57 to 1.26. Two three-proton triplets at \(\delta\) 0.96 (J=7.5 Hz) and 0.87 (J=7.2 Hz) were accounted to terminal C-1 and C-38 primary methyl protons, respectively. The \(^{13}\)C NMR spectrum of **HA-04** exhibited signals for keto carbon at \(\delta\) 208.26 (C-3), carbinol carbons at \(\delta\) 76.41 (C-19) and 68.90 (C-31), methylene carbons in the range of \(\delta\) 33.22 - 23.87 and methyl carbons at \(\delta\) 14.57 (C-1, C-38). The absence of any signal beyond \(\delta\) 3.88 in the \(^1\)H NMR spectrum and between \(\delta\) 208.26 - 76.41 in the \(^{13}\)C NMR spectrum supported saturated nature of the compound.

On the basis of evidences, the structure of **HA-04** has been established as n-Octatriacontan-3-one-19\(\alpha\), 31\(\alpha\)-diol. This is a new aliphatic keto diol.

**Compound HA-05**, an aliphatic alcohol, was obtained as a colourless crystalline mass product from chloroform: methanol (90:10) eluants. Its IR spectrum showed absorption bands for hydroxyl group (3433 cm\(^{-1}\)) and long aliphatic chain (722 cm\(^{-1}\)). It had a molecular ion peak at \(m/z\) 397 \([\text{M+H]}^+\) in the mass spectrum corresponding to the molecular formula of an aliphatic alcohol C\(_{27}\)H\(_{57}\)O. The ion peaks arising at \(m/z\)
255[CH₃(CH₂)₁₅CHOH]⁺ and 171[CH₃(CH₂)₉CHOH]⁺ indicated the location of the hydroxyl group at C-11 (Scheme-3.5). The ¹H NMR spectrum of HA-05 displayed a one- proton broad multiplet at δ 3.35 with half width of 9.1 assigned to β-oriented carbinol H-11 proton. The methylene protons appeared between δ 2.02 -1.25. Two three –proton triplets at δ 0.88 (J=6.1 Hz) and 0.85 (J=7.2 Hz) were associated with terminal C-1 and C-27 methyl protons, respectively. The ¹³C NMR spectrum of HA-05 exhibited signals for carbinol corbon at δ 64.13 (C-11), methylene carbons between δ 33.45 - 22.50 and methyl corbons at δ 14.33 (Me-1) and 14.30 (Me-27). The absence of any signal beyond δ 3.35 in the ¹H NMR spectrum and δ 64.13 in the ¹³C NMR spectrum suggested saturated nature of the molecule. On the basis of the foregoing account, the structure of HA-05 has been determined as n-heptacosan-11α-ol.

Table 3.1: Phytoconstituents isolated from H. auriculata

<table>
<thead>
<tr>
<th>Code</th>
<th>Compound name</th>
<th>Eluants</th>
<th>M.wt.</th>
<th>m.p.(°C)</th>
<th>% Yield</th>
<th>Nomenclature</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA-01</td>
<td>Hexacosanol</td>
<td>C: M (19:1)</td>
<td>396.69</td>
<td>105-106 °C</td>
<td>0.21 %</td>
<td>n-Hexacos-21-one-1-ol</td>
</tr>
<tr>
<td>HA-02</td>
<td>Tetracontenol</td>
<td>C: M (19:1)</td>
<td>577.06</td>
<td>112-113 °C</td>
<td>0.24 %</td>
<td>trans-Tetracont-7-en-1-ol</td>
</tr>
<tr>
<td>HA-03</td>
<td>Octacosanone</td>
<td>C: M (93:7)</td>
<td>408.74</td>
<td>107-108 °C</td>
<td>0.0143%</td>
<td>n–Octacosan-8-one</td>
</tr>
<tr>
<td>HA-04</td>
<td>Octatriacontanondiol</td>
<td>C: M (93:7)</td>
<td>580</td>
<td>106-107 °C</td>
<td>0.133%</td>
<td>n-Octatriacontan-3-one-19α, 31α-diol (New)</td>
</tr>
<tr>
<td>HA-05</td>
<td>Heptacosanol</td>
<td>C: M (9:1)</td>
<td>396.73</td>
<td>125-126 °C</td>
<td>0.103%</td>
<td>n-Heptacosan-11α-ol</td>
</tr>
</tbody>
</table>

C= Chloroform, M= Methanol
Structure of isolated compound from *H. auriculata*

n-Hexacos-21-one-1-ol (HA-01)

trans- Tetracont-7-en-1-ol (HA-02)

n-Octacosan-8-one (HA-03)

n-Octatriacontan-3-one -19,31,-diol (HA-04)

n-Heptacosan-11α-ol (HA-05)
Mass fragmentation pattern of isolated compounds

HA-01

\[
\text{CH}_3\overbrace{\text{(CH}_2\text{)}_4\text{C-CH}_2\text{-}\overbrace{\text{(CH}_2\text{)}_{18}\text{-CH}_2\text{-OH}}}^{99} + \text{CH}_3\text{(CH}_2\text{)}_4\text{C}^{396}
\]

**Scheme 3.1:** Mass fragmentation pattern of n-Hexacos-21-one-1-ol (HA-01)

HA-03

\[
\text{CH}_3\overbrace{\text{(CH}_2\text{)}_{18}\text{-CH}_2\text{-C-CH}_2\text{-}\overbrace{\text{(CH}_2\text{)}_5\text{-CH}_3}}^{281} + \text{CH}_3\text{(CH}_2\text{)}_{18}\text{-CH}_2\text{-C}^{309}
\]

**Scheme 3.2:** Mass fragmentation pattern of n–Octacosan-8-one (HA-03)
Scheme 3.3: Mass fragmentation pattern of n-Octa-triacontan-3-one-19α, 31α-diol
HA-05

Scheme 3.4: Mass fragmentation pattern of n-Heptacosan-11α-ol (HA-05)
Spectra of isolated compounds

**Spectrum 3.1.** $^1$H NMR spectrum of n-Hexacos-21-one-1-ol (HA-01)

**Spectrum 3.2.** $^{13}$C NMR spectrum of n-Hexacos-21-one-1-ol (HA-01)
Spectrum 3.3. Mass spectrum of n-Hexacos-21-one-1-ol (HA-01)

Spectrum 3.4. $^1$H MNR spectrum of trans- Tetraccont-7-en-1-ol (HA-02)
Spectrum 3.5. $^{13}$C NMR spectrum of trans- Tetracont-7-en-1-ol (HA-02)

Spectrum 3.6. Mass spectrum of trans-Tetracont-7-en-1-ol (HA-02)
Spectrum 3.7. $^1$H NMR spectrum of n-Octacosan-8-one (HA-03)

Spectrum 3.8. $^{13}$C NMR spectrum of n-Octacosan-8-one (HA-03)
Spectrum 3.9. Mass spectrum of n-Octacosan-8-one (HA-03)

Spectrum 3.10 $^1$H NMR spectrum of n-Octatriacontan-3-one -19,31,-diol (HA-04)
Spectrum 3.11. $^{13}$C NMR spectrum of n-Octatriacontan-3-one-19,31,-diol (HA-04)

Spectrum 3.12. Mass spectrum of n-Octatriacontan-3-one-19,31,-diol (HA-04)
**Spectrum 3.13.** $^1$H NMR spectrum of n-Heptacosan-11α-ol (HA-05)

**Spectrum 3.14.** $^{13}$C NMR spectrum of n-Heptacosan-11α-ol (HA-05)
Spectrum 3.15. Mass spectrum of n-Heptacosan-11α-ol (HA-05)
Chapter 3

Hygrophila auriculata (K. Schum) Heine

3.6. References


