5.1 PLANT DESCRIPTION

5.1.1 Family: Asteraceae

5.1.2 Synonyms: *Vernonia anthelmintica* Willd

5.1.3 Vernacular Name: English-Purple fleebane; Hindi-Kaliyira, Bakri; Kannada-Kadijira; Malayalam-Krisatru, Kattirarakam, Sanskrit-Aranyajiraka, Brhapali; Tamil-Kattuccirakam, Cittila; Telugu-Adavji lakarra.

5.1.4 Morphology: *Centrtherum anthelminticum* is an annual robust, erect, leafy; stems 60-90 cm high, branched, pubescent. Leaves 5-9 by 2.5-3.2 cm, lanceolate or elliptic-lanceolate, acute, coarsely serrate, more or less pubescent on both sides, base tapering into the petiole. Heads 1.3-2 cm in diameter, subcorymbos, many (about 40) flowered, with a linear bract near the tops of the peduncle. Outer involucral bracts linear, hairy, herbaceous, shorter than those of the inner rows; intermediate bracts with herbaceous hairy tips, linear, acute or subobtuse, often constricted at the base of the herbaceous part, equaling or shorter (rarely longer) than the innermost; innermost bracts usually the longest, linear, subacute, scarious, often tipped with purple. Pappus reddish, the exterior row very short, subpaleaceous, persistent, the inner hairs somewhat flattened, deciduous, much shorter than the glabrous corollas. Achenes 4.5-6 mm, long, oblong-cylindrical in shape, 10 ribbed, pubescent.

5.1.5 Medicinal Uses: The seeds have a sharp taste; acid, astringent to the bowels, anthelmintic; cure ulcers ‘vata’ and ‘kapha’; used in skin diseases leucoderma and fever according to the Ayurvedic literature and in Unani
literature these are described as having sharp bitter taste; anthelmintic, purgative; used for asthma, kidney troubles, hiccup; applied in inflammatory swellings; remove blood from liver; good for sores and itching of the eyes; a depilatory (Kirtikar & Basu, 2000).

5.2 CHEMICAL CONSTITUENTS
Several flavonoids including 2', 3, 4, 4'-tetrahydroxychalcone, 5, 6, 7, 4'-tetrahydroxyflavone and butin, were separated from the seeds of *V. anthelmintica* by high-speed counter-current chromatography using a two-step operation. Two different types of solvent systems were used: chloroform-dichloromethane-methanol-water (2:2:3:2, v/v) and 1,2 dichloroethane-methanol-acetonitrile-water (4:1.1:0.25:2, v/v). From 1 kg of seeds of *V. anthelmintica* the method yielded about 45 mg of 2', 3, 4, 4'-tetrahydroxychalcone, 40 mg of 5, 6, 7, 4'-tetrahydroxyflavone, and 55 mg of butin. Each isolated component showed 95-97% purity as determined by high-performance liquid chromatography analysis. These purified compounds were characterized by MS and NMR (Tian et al., 2004).

A novel 4α-methylsterol isolated from the seeds of *V. anthelmintica* was shown to have the structure 4α-methyl-5α-stigmasta-8, 14, 24(24')-Z-trien-3β-ol (4α-methylvermisterol) based on spectroscopic methods. The 4-demethylsterol and 4,4-dimethylsterol fractions from the seed material were also investigated. The 4-demethylsterol fraction contained 5α-stigmasta-8, 14, 24(24')-Z-trien-3β-ol (vernisterol) and 5α-stigmasta-7, 24(24')-Z-dien-3β-ol (avenasterol) as the dominant sterols. 4α-Methylvermisterol is the possible intermediate in the biosynthesis of vernisterol in *V. anthelmintica* seeds (Akihisa et al., 1992).

Asaka et al., in 1977 have reported a new elemanoide lactone, crystalline vernodalol by repeated chromatography on silica gel of ether extracts of the dried seeds of *V. anthelmintica*.

A new glycosylated triterpene has been isolated from the seeds of *C. anthelminticum*. The structural analysis of its acetylated derivative was performed by 1H, 13C NMR, 1H-1H COSY, HMQC, HMBC and DEPT spectroscopy. The saponin was shown to contain hederagenin and six sugar residues forming two glycosyl chains. The complete structure of the saponin was established as 3-O-[β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl]-28-O-[β-D-glucuronopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl]-hederagenin (Mehta et al., 2004).

The isolation and structure elucidation of six new and one known compound, from the extract of *C. anthelminticum* seeds was described. The extract was fractionated and collected in bulk and monitored by TLC. Repeated chromatography afforded 7 compounds in pure form. The seven compounds were identified as hexatetracontan-16-ol, 6,9-cicosadiene, butyl-11-hydroxy octadecanoate, hexyl 3-hydroxynonanoate, hexyl 9-hydroxyheptatriacontanoate, heptadecyl nonadecanoate and stigmasterol (Verma et al., 2004).
In another study by The composition of oil and seed meal of *C. anthelminticum* was determined. The oil is a greenish semi viscous substance with 0.945 sp. gr., insoluble in 70% alcohol. Vernolic acid (70.28%) is the major component of the oil, while saturated palmitic acid (2.30%) and stearic acid (0.42%) were lower than essential linoleic (15.84%) and oleic acids (5.65%). The defatted seed meal contained 5.2% ash and 28.4% protein (Singh and Kaul, 1999).

The seeds contains amino acids, linoleic, myristic, oleic, palmitic, stearic and vernolic acids, brassicasterol, stigmasterol an elemanoUde lactone, vernodalol (Prajapati et al., 2003).

Two non-glaucolide, germacolide sesquiterpene lactones, 8α-methacryloyloxyxystrophanthidin-1(10), 4(5)-diepoxide and 1-oxo-10α-OH-8α-methacryloyloxyxystrophanthidin (not fully characterized), were isolated from *V. jonesii* and the previously described glauconolide B was identified from *V. pookae*. This is one of the few reports of non-glaucolide type germacrolides in a New World species of *Vernonia*, although members of this class of sesquiterpene lactones are common constituents of old World species of the genus. The chemical evidence supported the suggestion that *V. jonesii* may be part of a relictual, isolated group of the New World *Vernonia*, closely related to some of the Old World taxa (Gershenzon et al., 1984).

From the roots of *V. cinerea* a new natural sterol and a new aliphatic acid characterized as stigmast-5, 17(20)-dien-3β-ol and 26-methylheptacosanoic acid, respectively, have been isolated together with stigmasterol and sitosterol (Misra et al., 1984).

Ohno et al., in 1979 has reported a germacerane namely centratherin from *C. punctatum* leaves and stem parts.

Isocentratherin- another germacerane with cytotoxic activity has also been reported from *C. punctatum* aerial parts (Bevelle et al., 1981).

The investigation of three South African *Vernonia* species afforded minute amounts of five new glauconolides, two monoepoxides and three diepoxides. The structures were elucidated by 1H NMR spectroscopy. The roots of *V. sutherlandii* contain, in addition to vernonataloide, bergamotene and santalene, minute amounts of the corresponding acetoxy derivatives (Bohlmann et al., 1984).

In another investigation of four South African *Vernonia* species afforded, in addition to known compounds, six ketohirsutinolides with different oxygen fun (Bohlmann et al., 1983). Investigation of six spices, all belonging to the veronieae, afforded guaianolides. The structures were elucidated by spectroscopic methods (Bohlmann et al., 1980).

The investigation of *V. compactiflora* afforded in addition to known compounds a hirsutinolide and a glauconolide; the latter is most probably the direct precursor of the former. The aerial parts of *V. monocephala* contain the same hirsutinolide as
well as the corresponding acetate, while those of \textit{V. chalybata} afforded glaucolide B. A reinvestigation of the aerial parts of \textit{V. cotoneaster} gave in addition to the allenic lactone isolated before two further known ones as well as a new glaucolide. The structures of the lactones were elucidated by spectroscopic methods and some chemical transformations. The erroneously assigned stereochemistry at C-10 in the hirsutinolides has been revised. The biogenetic relationship of the \textit{Vernonia} lactones is discussed briefly (Bohlmann et al., 1982).

A re-investigation of \textit{V. arkamsana} afforded several new sesquiterpene lactones, three bourbonenolides, obviously closely related to those isolated from this plant previously, a glaucolide and a methoxy derivative most probably formed by fragmentation of the corresponding bourbonenolide from \textit{V. profuga} in addition to known lactones a new guaianolide was isolated. The biogenetic relationships of the \textit{Vernonia} sesquiterpene lactones are discussed briefly (Bohlmann et al., 1981).

5.3 BIOLOGICAL ACTIVITIES

Compositions comprising an extract from the seeds of the plant \textit{C. anthelminticum} are suitable for use in the treatment of skin disorders, such as acne and impetigo, and fungal infections of the skin and nails. The composition is especially useful for the treatment of acne which results from bacterial infection by \textit{Staphylococcus} sp., \textit{Pitrosporum} sp. and/or \textit{Propionaebacterium} sp. The composition may also include an extract from the leaves, bark or roots of \textit{Melia azadirachta} and/or an extract from the seeds of the plant \textit{Cassia tora}. A number of other plant extracts may be present in the composition also. The compositions are preferably formulated in powder form which may then be made up as a potable decoction, i.e., a drink; a mouthwash or as drops for nasal application. Alternatively, the composition may be dispersed in a suitable carrier for topical application, such as Ghee. Compositions comprising \textit{C. tora} and one or more of a variety of plant extracts including \textit{M. azadirachta} or compositions comprising \textit{M. azadirachta} and one or more of a variety of plant extracts including \textit{C. anthelminticum} are also suitable for use in the treatment of skin disorders and fungal infections of the skin and nails (Shah, 2001).

In another study related to anthelmintic activity forty-eight helminth-free lambs were divided into eight groups (A-H) of six animals. Groups A-G were infected artificially with 10,000 third stage larvae of \textit{Haemonchus contortus} and 20,000 third stage larvae of \textit{Trichostrongylus colubriformis}, whereas group H remained uninfected. Thirty days post-infection the lambs were treated orally with a single dosage of one of the following products: group A with 3 mg/kg body weight of an aqueous ethanol extract (70%, v/v) of the seeds of \textit{Azadirachta indica} (Meliaceae); group B with 1 g/kg body weight of a raw powder of the leaves of \textit{Ananas comosus} (Bromeliaceae); group C with 0.3 mg/kg body weight of an aqueous ethanol extract of a 1:1 mixture (g/g) of \textit{V. anthelmintica} (Asteraceae) seeds and \textit{Embelia ribes} (Myrsinaceae) fruits; group D with 183 mg/kg body weight of an aqueous ethanol extract of the whole plants of \textit{Fumaria parviflora} Lam. (Fumariaceae); group E with 28 mg/kg body weight of an aqueous ethanol extract of the seeds of \textit{Caesalpinia crista} (Caesalpiniaceae); group F with 25 mg/kg body weight of pyrantel tartrate and group G with 50% ethanol. Group H remained untreated.
Only the ethanol extract of *F. parviflora* caused a strong reduction of the fecal egg counts (100%) and a 78.2 and 88.8% reduction of adult *H. contortus* and *T. colubriformis* on day 13 post-treatment. The extract was as effective as the reference compound pyrantel tartrate. Therefore, the ethanol extract itself or single constituents of *F. parviflora* could be a promising alternative source of anthelmintic for the treatment of gastrointestinal trichostrongyliids in small ruminants (Hordegen et al., 2003).

A study was undertaken to evaluate and compare the efficacy of a proprietary herbal anthelmintic (Kriminth) with piperazine citrate against economically important toxocara vitulorum in cow and buffalo calves. Each 10 mL of the herbal anthelmintic contains: *Embelia ribes* 0.5; *Punica granatum* 0.55; *Caesalpinia cristata* 0.5; *Artomisia maritima* 0.45; *Cuminum cyminum* 0.5; *Gardenia gymnfolia* 0.4; *C. anthelminticum* 0.4; *Butea monosperma* 0.8, *Cassia angustifolia* 0.5 and *Acorns calamus* 0.4 (all in g). Based on the findings of the present study it was concluded that efficacy of piperazine citrate was superior to the herbal anthelmintic. It was recommended that the herbal drug may be used as an alternative next to piperazine citrate (Devi et al., 2000).

Effect of aqueous and alcoholic extract of *C. anthelminticum* was studied on the spontaneous movements of the whole worm and nerve-muscle preparation of *Setari cervi*. Ethyl acetate, acetone and methanol extract showed similar effect, of causing inhibition of spontaneous motility of the nerve-muscle preparation of *S. cervi* characterized by decreased amplitude and frequency of contractions. The inhibitory effect on the motility was reversible. Further, the extracts did not involve the blockade of cholinergic receptors as evidenced by the presence of unaltered stimulant response of acetylcholine in the presence of drug in bathing fluid (Singhal et al., 1992).

The in vitro antimicrobial efficacy of different extracts of seeds of *C. anthelminticum* was studied by the filter paper disk method against several human pathogenic bacteria and fungi. Some of the extracts showed significant effect over tested bacteria and fungi (Sharma et al., 1991).

**Compounds Reported From C. anthelminticum**

![Chemical structure of C. anthelminticum compounds](attachment:image.png)
Chapter 5

*C. anthelminticum*

7. (Z) 24(28)-stigmastadienol

8, 14(Z)24(28)-stigmastatrienol acetate

Hexatetracontan-16-ol

Vernodalol
Chapter 5

C. anthelminticum

5.4 EXPERIMENTAL

5.4.1 Materials and Methods

5.4.1.1 General: Mps were uncorrected; Perfit melting point apparatus; IR: Bio-Rad FTIR Spectrophotometer KBr; UV: Lambda Bio 20 Spectrophotometer, MeOH; ¹H-NMR (300 MHz); DPX 300, Bruker Spectrospin, CDCl₃ and DMSO-d₆; ¹³C-NMR (300 MHz); DPX 300, Bruker Spectrospin, CDCl₃ and DMSO-d₆ with TMS as an internal standard; MS: ESIMS JEOL-JMS-DX 303; CC: Silica gel (Qualigens), 60-120 mesh; TLC: Silica gel G (Qualigens). Spots were visualized by exposure to iodine vapours, UV radiation and by spraying with ceric ammonium sulphate and perchloric acid.

5.4.1.2 Collection of material: C. anthelminticum seeds were procured from the Khari Baoli market of Delhi and was identified by Dr. M.P. Sharma, Department of Botany, Jamia Hamdard, New Delhi. A voucher specimen is deposited in the herbarium of the Phytochemical Research Laboratory, Faculty of Pharmacy.

5.4.1.3 Extraction and Isolation: The dried drug (2 Kg) was coarsely powdered, defatted with petroleum ether and then exhaustively extracted with ethanol (95%). The combined extracts were then concentrated on a water bath and dried under reduced pressure to get 85 g (4.25% yield) of dark brown mass. The viscous dark brown mass was dissolved in a small quantity of methanol and adsorbed on silica gel (60-120 mesh) for the preparation of slurry. It was dried, packed and chromatographed over silica gel column packed in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol successively in the order of increasing polarity to isolate following compounds:

Centratherum olein (CA-1)

Elution of column with petroleum ether-CHCl₃ (4:1) (fraction No. 5-86) afforded orange sticky mass of CA-1, recrystallised from MeOH, 125 mg (0.00625% yield). Rᵧ 0.70 (petroleum ether).

UV λₘₚₓ (MeOH): 277 nm (log ε 5.3).

IR νₘₚₓ (KBr): 3500, 3350, 2950, 2845, 1725, 1650, 1560, 1540, 1430, 1260, 890 cm⁻¹.

¹H NMR (CDCl₃): δ 7.55 (1H, dd, J=8.4, 1.8 Hz, H-3'''), 7.52 (1H, dd, J=8.4, 1.8 Hz, H-7'''), 7.35 (1H, brs, H-5''), 7.14 (1H, m, H-4'''), 7.11 (1H, m, H-6'''), 5.36 (1H, m, H-8'), 5.01 (1H, m, H-9'), 4.90 (2H, brs, H2-1'''), 4.32 (1H, d, J=6.6 Hz, H2-1a), 4.28 (1H, d, J=6.6 Hz, H2-1b), 4.06 (1H, m, H-2), 3.66 (1H, d, J=8.7 Hz,
**H$_2$-3a**, 3.61 (1H, d, $J$=8.7 Hz, H$_2$-3b), 2.89 (1H, d, $J$=7.5 Hz, H$_2$-2'a), 2.84 (1H, d, $J$=7.5 Hz, H$_2$-2'b), 2.59 (2H, m, H$_2$-7'), 2.26 (2H, m, H$_2$-10'), 2.05 (2H, m, H$_2$-3'), 1.42 (2H, m, H$_2$-6), 1.33 (4H, brs, 2xCH$_2$), 1.28 (6H, brs, 3xCH$_3$), 1.25 (8H, brs, 4xCH$_2$), 0.85 (3H, t, $J$=6.5 Hz, Me-17').

**$^{13}$C NMR (CDCl$_3$):** δ 172.98 (C-1'), 146.65 (C-2''), 138.02(C-5''), 130.46 (C-3''), 128.41 (C-7''), 124.33 (C-4''), 124.03 (C-6''), 123.55 (C-8''), 118.69 (C-9'), 72.62 (C-1''), 67.73 (C-2) 65.13 (C-1), 39.08 (C-2''), 36.11 (C-7''), 34.44 (CH$_2$), 33.35 (CH$_2$), 31.02 (CH$_2$), 29.28 (3 x CH$_2$), 28.95 (3xCH$_3$), 28.21 (2xCH$_2$), 22.27 (CH$_2$), 13.69 (Me-17').

**+ve ESI MS** $m/z$: 465 [M]+ C$_{27}$H$_{45}$O$_6$.

**Centratherum ricinolein (CA-2)**

Elution of column with CHCl$_3$-MeOH (99:1) (fraction. No. 160-166) afforded cream-coloured semisolid mass of CA-2, recrystallised from CHCl$_3$-acetone (8:2), 350 mg (0.0175% yield).

**UV** (MeOH): 246 nm ($\log$ e 4.3).

**IR** $\nu_{max}$ (KBf): 3450, 2926, 2855, 1725, 1640, 1360, 1180 cm$^{-1}$.

**$^1$H NMR (DMSO-$d_6$):** δ 5.51 (1H, m, H-10'), 5.43 (1H, m, H-10''), 5.35 (1H, m, H-9'), 5.27 (1H, m, H-9''), 4.32 (1H, d, $J$=4.2 Hz, H$_2$-1a), 4.28 (1H, d, $J$=4.2 Hz, H$_2$-1b), 4.15 (1H, t, $J_{t/t}=8.85$ Hz, H-2), 3.94 (1H, m, H-12'), 3.65 (1H, d, $J$=9.1 Hz, H$_2$-3a), 3.61 (1H, d, $J=9.1$ Hz, H$_2$-3b), 2.33 (1H, d, $J=7.5$ Hz, H$_2$-2'a), 2.31 (1H, d, $J=7.5$ Hz, H$_2$-2'b), 2.07 (1H, d, $J=7.3$ Hz, H$_2$-2''a), 2.03 (1H, d, $J=7.3$ Hz, H$_2$-2''b), 1.82 (1H, m, H$_2$-11'a), 1.80 (1H, m, H$_2$-11''), 1.77 (1H, m, H$_2$-8'), 1.74 (1H, m, H$_2$-8''), 1.74 (1H, m, H$_2$-8''), 1.65 (1H, m, H$_2$-11''a), 1.62 (1H, m, H$_2$-11''b), 1.59 (1H, m, H$_2$-8'a), 1.56 (1H, m, H$_2$-8'b), 1.52 (2H, m, CH$_2$), 1.48 (2H, m, CH$_2$), 1.42 (2H, m, CH$_2$), 1.30 (26H, brs, 13xCH$_2$), 1.25 (6H, brs, 3xCH$_3$), 0.91 (3H, t, $J=6.1$ Hz, CH$_3$-18'), 0.88 (3H, t, $J=6.3$ Hz, CH$_3$-18').

**$^{13}$C NMR (DMSO-$d_6$):** δ 67.57 (C-1), 68.75 (C-2), 61.94 (C-3), 173.08 (C-1'), 34.72 (C-2'), 32.52 (C-3'), 29.34 (C-4'), 28.92 (C-5'), 28.92 (C-6'), 31.73 (C-7'), 31.73 (C-8'), 124.12 (C-9'), 133.13 (C-10'), 73.41 (C-12'), 33.12 (C-13'), 28.92 (C-14'), 27.54 (C-15'), 26.08 (C-16'), 25.18 (C-17'), 13.85 (C-18'), 172.67 (C-1''), 34.48 (C-2''), 31.96 (C-3''), 29.34 (C-4''), 28.92 (C-5''), 28.92 (C-6''), 31.56 (C-7''), 31.56 (C-8''), 121.82 (C-9''), 133.6 (C-10''), 33.45 (C-11''), 73.64 (C-12''), 32.73 (C-13''), 28.92 (C-14''), 27.23 (C-15''), 26.26 (C-16''), 26.65 (C-17'), 13.85 (C-18'').

**+ve ESI MS** $m/z$: 652 [M]+ C$_{39}$H$_{62}$O$_7$. 

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**Chapter 5**

*C. anthelminticum*

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Centratherum ricinolpalmitein (CA-3)

Elution of column with CHCl₃-MeOH (49:1) (fraction No. 175) afforded colourless semisolid mass of CA-3, recrystallised from MeOH, 475 mg (0.02375% yield).

Rf 0.60 (Tolune:ethyl acetate: acetic acid; 5:4:5:0.5).

UV $\lambda_{max}$ (MeOH): 247 nm (log $\varepsilon$ 4.6).

IR $\nu_{max}$ (KBrs): 3455, 2928, 2856, 1743, 1379, 1240, 1160, 1098, 794, 724 cm⁻¹.

$^1$H NMR (CDCl₃): $\delta$ 5.44 (1H, m, H-9”), 5.35 (1H, m, H-10”), 5.29 (1H, m, H-9”), 5.22 (1H, m, H-10”), 4.27 (1H, m, H-1”), 4.11 (1H, d, $J=6.0$ Hz, H-2”), 4.07 (1H, d, $J=6.0$ Hz, H-2”), 3.89 (1H, m, H-12”), 3.42 (1H, d, $J=13.56$ Hz, 3a”), 3.37 (1H, d, $J=13.56$ Hz, H-2”), 2.23 (1H, d, $J=7.2$ Hz, H-2”), 2.03 (1H, d, $J=7.5$ Hz, H-2”), 1.99 (1H, d, $J=7.5$ Hz, H-2”), 1.75 (2H, m, H-11”), 1.59 (2H, m, H-8”), 1.57 (2H, m, H-8”), 1.55 (2H, m, H-11”), 1.26 (30H, brs, 15 x CH₂), 1.21 (8H, brs, 4 x CH₂), 0.86 (3H, t, $J=6.1$ Hz, Me-18’), 0.83 (3H, t, $J=6.2$ Hz, Me-16’).

$^{13}$C NMR (CDCl₃): $\delta$ 173.15 (C-1”), 172.73 (C-1’), 132.06 (C-10”), 124.80 (C-9’), 124.59 (C-10’), 124.06 (C-9”), 73.68 (C-12”), 67.48 (C-1), 61.94 (C-3), 34.69 (C-11), 34.34 (C-2”), 33.81 (C-2’), 33.35 (C-8”), 32.68 (C-11”), 32.45 (C-8’), 31.69 (CH₂), 31.47 (CH₂), 31.11 (CH₂), 29.30 (CH₂), 28.88 (9 x CH₂), 27.19 (CH₂), 26.23 (CH₂), 25.15 (CH₂), 24.87 (CH₂), 24.62 (CH₂), 22.38 (CH₂), 13.84 (Me-18’, Me-16’)

+ve ESI MS m/z: 608 [M]+ C₃₇H₅₈O₆.

Centratherum naphthylpentol (CA-4)

Elution of column with CHCl₃-MeOH (97:3) (fraction No. 183-186) afforded green amorphous mass of CA-5, recrystallised from CHCl₃, 220 mg (0.011% yield).

Rf 0.60 (CHCl₃).

UV $\lambda_{max}$ (MeOH): 243 nm (log $\varepsilon$ 5.2).

IR $\nu_{max}$ (KBrs): 3410, 3350, 3310, 2955, 2845, 1640, 1560, 1175, 1162, 1042, 950, 860 cm⁻¹.

$^1$H NMR (DMSO-d₆): $\delta$ 5.78 (1H, brs, H-1), 5.69 (1H, brs, H-4), 5.16 (1H, d, $J=11.1$ Hz, H-6), 5.11 (1H, d, $J=11.1$ Hz, H-7), 4.76 (1H, d, $J=11.8$ Hz, H-14’), 4.36 (1H, dd, $J=11.8$, 11.4 Hz, H-15), 4.01 (1H, d, brs, H-2”), 3.95 (1H, d, H-11”), 3.79 (1H, m, H-20”), 2.81 (1H dd, $J=11.8$, 10.8 Hz, H-13”), 2.56 (1H, m, H-12’), 2.50 (1H, m, H-16”), 1.89 (1H, m, H-17”), 1.59 (2H, m, H-19”), 1.23 (2H, m, H-18’).

$^{13}$C NMR (DMSO-d₆): $\delta$ 140.69 (C-1), 164.45 (C-2), 163.75 (C-3), 138.38 (C-4), 132.77 (C-5), 123.55 (C-6), 115.06 (C-7), 141.88 (C-8), 166.73 (C-9), 127.74 (C-10), 59.31 (C-11), 52.14 (C-12”), 51.76 (C-13’), 70.46 (C-14”), 70.03 (C-15”), 50.24 (C-16”), 50.24 (C-17’), 37.46 (C-18”), 37.46 (C-19”), 68.68 (C-20’).

+ve ESI MS m/z: 358 [M]+ C₃₀H₄₂O₆.
Centratherumnaphthyl hexol (CA-5)

Elution of column with CHCl₃-MeOH (97:3) (fraction No. 183-186) afforded white amorphous mass of CA-4, recrystallised from CHCl₃, 725 mg (0.0362% yield)

Rᵣ 0.80 (CHCl₃).

UV λₒₘₙₐₓ (MeOH): 242.6 nm (log ε 4.9).

IR νₘₐₓ (KBr): 3410, 3350, 3355, 2960, 2855, 1650, 1587, 1481, 1046, 795 cm⁻¹.

¹H NMR (DMSO-d₆): δ 5.78 (IH, brs, H-1), 5.69 (IH, brs, H-4), 5.16 (IH, d, J=11.7 Hz, H-6), 5.12 (1H, d, J=11.7 Hz, H-7), 4.76 (1H, d, J=11.4 Hz, H-14), 4.36 (1H, dd, J=11.4, 11.8 Hz, H-15), 4.01 (2H, brs, H₂-11), 3.81 (1H, m, H-20), 3.67 (1H, brs, H-19), 2.81 (1H, dd, J=9.0, 10.5 Hz, H-13), 2.56 (1H, m, H-12), 2.50 (1H, m, H-16), 1.90 (1H, m, H-17), 1.59 (1H, m, H-18a), 1.51 (1H, m, H₂-18b).

¹³C NMR (DMSO-d₆): δ 140.67 (C-1), 164.44 (C-2), 163.75 (C-3), 138.3 (C-4), 132.78 (C-5), 123.55, 115.06 (C-7), 141.87 (C-8), 166.74 (C-9), 127.74 (C-10), 59.30 (C-11), 52.11 (C-12), 51.76 (C-13), 70.46 (C-14), 70.01 (C-15), 50.22 (C-16), 50.22 (C-17), 37.44 (C-18), 68.67 (C-19), 79.18 (C-20).

+ve ESI MS m/z: 374 [M]+ C₂₀H₂₂O₇.

5.5 RESULTS AND DISCUSSION

Compound CA-1 designated as centratherum olein, was obtained as orange sticky mass from chloroform-methanol (4:1) eluents. It responded positively to tetranitromethane (TNM) and bromine water test for unsaturation. Its IR showed characteristics absorption for hydroxyl group (3550, 3350 cm⁻¹), ester group (1725 cm⁻¹) and unsaturation (1650 cm⁻¹) and aromatic ring (1540, 890 cm⁻¹). The ¹H NMR spectrum of CA-1 exhibited two down field double doublets, one-proton each at δ 7.55 (J=8.4, 1.8 Hz) and 7.52 (J=8.4, 1.8 Hz) assignable to ortho-meta coupled H-3" and H-7" aromatic protons. Other aromatic protons appeared as a broad signal at δ 7.35 (H-5") and two one-proton multiplets at δ 7.14 (H-4") and 7.11 (H-6"), respectively. Two one-proton multiplets at δ 5.36 and 5.01 were attributed to H-8' and H-9' vinylic protons whereas another two protons broad signals at δ 4.90 were attributed to H₂-1" protons. Four one-proton doublets at 4.32 (J=6.6 Hz), 4.28 (J=6.6 Hz), 3.66 (J=8.7 Hz) and 3.61 (J=8.7 Hz) were assigned correspondingly to H₂-1a, H₂-1b, H₂-3a and H₂-3b oxygenated methylene protons. The oxygenated methine proton H-2 appeared as a multiplet at δ 4.06. Another set of two one-proton doublets at δ 2.89 (J=7.5 Hz) and 2.84 (J=7.5 Hz), were assigned to methylene protons adjacent to the ester group H₂-2'.

The remaining methylene protons resonated between δ 2.59-1.25. The primary methyl protons Me-17' appeared as a triplet at δ 0.85 (J=6.5 Hz) integrating for three protons. The ¹³C NMR spectrum provided significant evidence in support of the structure of CA-1. It exhibited important signal for ester carbons C-1' at δ 172.98 and six aromatic carbons between δ 124.03-146.65. The vinylic carbons C-8' and C-9' appeared correspondingly at δ 123.55 and 118.69. The carbinol carbon C-3 and oxygenated carbon C-2 and C-1 resonated at δ 64.20, 67.73 and 65.13, respectively. The primary methyl carbon C-17' appeared at δ 13.69.
whereas rest of the methylene resonated between $\delta$ 36.11-22.27. On the basis of foregoing discussions the structure of CA-1 has been elucidated as glyceryl-1-heptadec-8-enoate-2-phosphobenzoate.

Compound CA-2 designated as centrathermic ricinolein, was obtained as a cream-coloured semisolid mass from chloroform-methanol (99:1) eluents. It responded positively to TNM and bromine water test for unsaturation. Its IR spectrum exhibited absorption bands for hydroxyl group (3450 cm$^{-1}$), ester group (1725 cm$^{-1}$) and unsaturation (1640 cm$^{-1}$). Its ESI MS showed a molecular ion peak at $m/z$ 652 corresponding to molecular formula $\text{C}_{39}\text{H}_{72}\text{O}_{7}$. Other important peaks at $m/z$ 371 [CO-O fission]$^+$ and 129, 523 [C$_{10}$-C$_{11}$ fission] and 508 [523-Me] supported the CA-2 to be a diglyceride of C$_{9}$-C$_{10}$ unsaturated fatty acids. The $^1$H NMR spectrum of CA-2 exhibited four one-proton multiplet signals at $\delta$ 5.51, 5.43, 5.35 and 5.27 correspondingly attributed to vinylic protons H-10', H-10'', H-9' and H-9''. Two one-proton doublets at $\delta$ 4.32 ($J$=4.2 Hz) and 4.28 ($J$=4.2 Hz) were assigned to oxygenated methylene protons CH$_2$-I whereas the carbinol protons at C-3 appeared as two one-proton doublets at $\delta$ 3.65 ($J$=9.1 Hz) and 3.61 ($J$=9.1 Hz). The oxygenated methine proton H-2 appeared as a multiplet at $\delta$ 4.15. The methylene protons adjacent to the ether groups at C-2' and C-2'' appeared as four one-proton doublets at 2.33 ($J$=7.5 Hz), 2.31 ($J$=7.5 Hz), 2.07 ($J$=7.3 Hz) and 2.03 ($J$=7.3 Hz), respectively. The remaining of the methylene protons resonated between $\delta$ 1.82-1.25. Two primary methyl protons appeared as two triplets at $\delta$ 0.91 ($J$=6.1 Hz) and 0.88 ($J$=6.3 Hz) assigned to Me-18' and C-18'', respectively. Two one-proton multiplets at $\delta$ 3.94 and 3.91 were ascribed to carbinol H-12' and H-12''. The $^{13}$C NMR of CA-2 displayed important signals for ester carbons C-1' and C-1'' at $\delta$ 173.08 and 172.67, respectively. The vinylic carbons C-9', C-10', C-9'' and C-10'' appeared correspondingly at $\delta$ 133.13, 121.82 and 133.6. The carbinol carbons signals at $\delta$ 73.41 and 73.64 were assigned to C-12' and C-12'', respectively. Primary methyl carbons C-18' and C-18'' appeared at $\delta$ 13.85. On the basis of foregoing discussions the structure of CA-2 has been elucidated as glyceryl-1, 2-bis-12-hydroxyoctadec-9-enoate.

Compound CA-3 designated as centrathermic ricinolpalmitein was obtained as colourless semisolid mass from chloroform-methanol (49:1) eluents. It responded positively to TNM and bromine water test for unsaturation. The IR spectrum of CA-3 displayed absorption bands for hydroxyl group (3455 cm$^{-1}$), ester group (1743 cm$^{-1}$), unsaturation (1640 cm$^{-1}$) and long chain (1098, 794, 724 cm$^{-1}$). Its ESI MS exhibited a molecular ion peak at $m/z$ 608 corresponding to molecular formula $\text{C}_{37}\text{H}_{66}\text{O}_{6}$. The $^1$H NMR spectrum of CA-3 displayed four one-proton multiplet signals at $\delta$ 5.44, 5.35, 5.29 and 5.22 correspondingly ascribed to vinylic protons H-9'', H-10'', H-9' and H-10'. Oxygenated methyl protons H$_2$-1 appeared as two one-proton doublets at $\delta$ 4.11 ($J$=6.0 Hz) and 4.07 ($J$=6.0 Hz) whereas the oxygenated methine protons H-2 appeared as a multiplet at $\delta$ 4.27. Two one-proton doublets at $\delta$ 3.42 ($J$=13.56 Hz) and 3.37 ($J$=13.56 Hz) were attributed to H$_2$-3 oxygenated methylene protons. Four doublets, one-proton each, at $\delta$ 2.29 ($J$=7.2 Hz), 2.25 ($J$=7.2 Hz), 2.03 ($J$=7.5 Hz) and 1.99 ($J$=7.5 Hz) were attributed
to four methylene protons adjacent to ester group, \(i.e., \ H_2-2' \) and \( H_2-2'' \), respectively. A one-proton multiplet at \( \delta \ 3.89 \) was ascribed to the \( H-12' \) carbinol protons. The remaining methylene protons resonated between \( \delta \ 1.75-1.21 \). Two primary methyl protons appeared as two triplets at \( \delta \ 0.86 \ (J=6.1 \ Hz) \) and 0.83 \( (J=6.2 \ Hz) \) assigned to \( Me-18' \) and \( Me-16'' \), respectively. The \(^{13}\)C NMR of CA-3 displayed important signals for ester carbons \( C-1' \) and \( C-1'' \) at \( \delta \ 173.15 \) and 172.73, respectively. The vinylic carbons \( C-10', C-9', C-10'' \) and \( C-9'' \) appeared correspondingly at \( \delta \ 132.06, 124.80, 124.859 \) and 124.06. The carbinol carbons \( C-3 \) and \( C-12' \) appeared at \( \delta \ 61.94 \) and 73.68, respectively whereas primary methyl carbons \( C-18' \) and \( C-16'' \) appeared at \( \delta \ 13.84 \). On the basis of foregoing discussions the structure of CA-3 has been elucidated as glyceryl-1-(12-hydroxyoctadec-9-enoate)-2-octadec-9-enoate.

Compound CA-4 designated as centratherumnaphthyl pentol, was obtained as green coloured amorphous mass from CHCl\(_3\)-MeOH (97:3) eluents. It responded positively to FeCl\(_3\) test for phenols. Its IR spectrum showed absorption bands for hydroxyl groups (3410, 3350, 3310 cm\(^{-1}\)), unsaturation (1640 cm\(^{-1}\)) and aromatic nucleus (1560, 1042, 950 cm\(^{-1}\)) that also supported by its UV absorption maxima at \( \lambda \ 243 \) nm. The ESI MS of CA-4 displayed molecular ion peak at \( m/\chi 358 \) corresponding to molecular formula \( C_{20}H_{22}O_6 \). The \(^{1}H\) NMR spectrum of CA-4 exhibited two one-proton broad signals at \( \delta \ 5.78 \) and 5.69 correspondingly attributable to \( H-1 \) and \( H-4 \) vinylic protons. Two \( ortho-ortho \) coupled \( H-6 \) and \( H-7 \) vinylic protons appeared as two doublets, one-protons each, at \( \delta \ 5.16 \ (J=11.1 \ Hz) \) and 5.11 \( (J=11.1 \ Hz) \), respectively. Three carbinol proton signals, one-proton each, appeared as a doublet at \( \delta \ 4.76 \ (J=11.8, 11.4 \ Hz) \), a double doublet at \( \delta \ 4.36 \ (J=11.8, 11.4 \ Hz) \) and a multiplet at \( \delta 3.79 \) correspondingly ascribed to \( H-14, H-15 \) and \( H-20 \) protons. Two downfield one-proton broad signals at \( \delta \ 4.01 \) and 3.95 were attributed to oxygenated methylene protons \( H_2-11a \) and \( H_2-11b \), respectively. The remaining methylene and methine protons resonated between \( \delta \ 2.81-1.23 \) suggesting their saturated nature. The \(^{13}\)C NMR data of CA-4 provided evidences in support of the proposed structure. It exhibited signals for twenty carbons in the molecule. The oxygenated carbons \( C-9 \) and \( C-11 \) appeared at \( \delta \ 166.73 \) and 59.31, respectively while as the aromatic carbons resonated between \( \delta \ 115.06-141.88 \). The saturated carbinol carbon \( C-14, C-15 \) and \( C-20 \) appeared at \( \delta \ 70.46, 70.03 \) and 68.68, respectively whereas their aromatic phenolic carbons \( C-2 \) and \( C-3 \) appeared at \( \delta 164.45 \) and 163.75. On the basis of foregoing discussions the structure of CA-4 has been elucidated as \([a, b]-2,3-Dihydroxynaphthyl-[c,d]-14,15,20-trihydroxy [16,17]-cyclopentanocyclohexyl tetrahydropyran.\n
Compound CA-5 designated as centratherumnaphthyl hexol, was obtained as white crystalline powder from CHCl\(_3\)-MeOH (97:3) eluents. It responded positively to FeCl\(_3\) test for phenols. Its IR spectrum showed characteristic absorption bands for hydroxyl group (3410, 3355, 3350 cm\(^{-1}\)), unsaturation (1650 cm\(^{-1}\)) and aromatic moiety (1587, 1046 cm\(^{-1}\)). Its UV absorption maxima at \( \lambda \ 242.6 \) nm indicate a highly conjugated system in the molecule. The ESI MS of
CA-5 displayed a molecular ion peak at $m/z$ 374 corresponding to molecular formula $C_{20}H_{22}O_7$. The $^1$H NMR spectrum of CA-5 is consistent with the proposed molecular formula as it exhibited two one-proton broad signals at $\delta$ 5.78 and 5.69 corresponding to H-1 and H-4 vinylic protons, respectively. Two ortho-ortho coupled vinylic protons H-6 and H-7 appeared as two doublets, one proton each, at $\delta$ 5.16 ($J$=11.7 Hz) and 5.12 ($J$=11.7 Hz), respectively. Four carbinol proton signals, one proton each, appeared as a doublet at $\delta$ 4.76 ($J$=11.4 Hz), a double doublet at $\delta$ 4.36 ($J$=11.4, 11.8 Hz), a multiplet at $\delta$ 3.84 and a broad singlet at $\delta$ 3.67 correspondingly attributed to H-14, H-15, H-20 and H-19. Another down-field signal at $\delta$ 4.01 was ascribed to oxygenated methylene protons $\text{CH}_2$-11. Rest of the methylene and methine protons resonated between $\delta$ 2.81-1.51. The $^{13}$C NMR of CA-5 provided further evidence in support of the proposed structure. It exhibited signal for 20 carbon atoms in the molecule. The aromatic carbons resonated between $\delta$ 115.06-141.87. Oxygenated carbons C-9 and C-11 appeared at $\delta$ 166.74 and 59.30, respectively. The carbinol carbons C-14, C-15, C-19 and C-20 appeared at $\delta$ 70.46, 70.01, 68.67 and 79.18, respectively supporting the saturated nature of these carbons in contrast to two other phenolic carbon signals at $\delta$ 164.4 and 163.75 for C-2 and C-3, respectively. On the basis of foregoing discussions the structure of CA-5 has been elucidated as $[a, b]$-2, 3-Dihydroxynaphthyl- $[c, d]$-14, 15, 19, 20-tetrahydroxy [16, 17]-cyclopentanocyclohexyl tetrahydropyran.
# Table 5.1. Compounds Isolated From *C. anthelminticum*

<table>
<thead>
<tr>
<th>Code No.</th>
<th>Common Name</th>
<th>m.p. (°C)</th>
<th>Mol. Formula</th>
<th>IUPAC Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA-1</td>
<td>Centratherum olein</td>
<td>-</td>
<td>C27H45O6</td>
<td>glyceryl-1-heptadec-8-enoso-2-phosphobenzoylate</td>
</tr>
<tr>
<td>CA-2</td>
<td>Centratherum ricinolein</td>
<td>-</td>
<td>C39H72O7</td>
<td>glyceryl-1, 2-bis-12-hydroxyoctadec-9-enoate</td>
</tr>
<tr>
<td>CA-3</td>
<td>Centratherum ricinolpalmitein</td>
<td>-</td>
<td>C57H68O6</td>
<td>glyceryl-1-(12-hydroxyoctadec-9-enoate)-2-ocadec-9-enoate</td>
</tr>
<tr>
<td>CA-3</td>
<td>Centratherum napht hyl pentol</td>
<td>124-26</td>
<td>C26H22O6</td>
<td>[a, b]-2,3-Dihydroxynaphthyl-[c,d]-14,15,20-trihydroxy [16,17]-cyclopentanocyclohexyl tetrahydropyran</td>
</tr>
<tr>
<td>CA-3</td>
<td>Centratherum napht hyl hexol</td>
<td>130-32</td>
<td>C26H22O7</td>
<td>[a, b]-2, 3-Dihydroxynaphthyl- [c, d]-14, 15, 19, 20 - tetrahydroxy [16, 17]-cyclopentanocyclohexyl tetrahydropyran</td>
</tr>
</tbody>
</table>

**CA-1**

![Image of CA-1 molecule]

**CA-2**

![Image of CA-2 molecule]

**CA-3**

![Image of CA-3 molecule]

**CA-4**

![Image of CA-4 molecule]

**CA-5**

![Image of CA-5 molecule]
Chapter 5

C. anthelminticum

$^1$H NMR of CA-2

$^{13}$C NMR of CA-2
Chapter 5

C. anthelminticum

\textsuperscript{1}H NMR of CA-3

\textsuperscript{13}C NMR of CA-3
Chapter 5

*C. anthelminticum*

Mass Spectrum of CA-3

IR Spectrum of CA-3
Chapter 5

C. anthelminticum

Mass Spectrum of CA-4

IR Spectrum of CA-4
Chapter 5

C. anthelminticum

$^1$H NMR of CA-5

$^{13}$C NMR of CA-5
Chapter 5

C. anthelminticum

Mass Spectrum of CA-5

IR Spectrum of CA-5
5.6 REFERENCES


Chapter 5

C. anthelminticum


