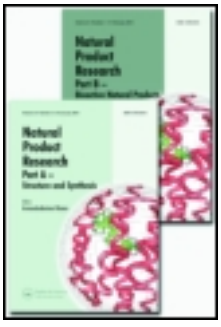

Natural Product Research: Formerly Natural Product Letters
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gnpl20

A novel lupene-type triterpenic glucoside from the leaves of Clerodendrum inerme

Mehtab Parveen [a], Zakia Khanam [a], Mohammad Ali [b] & Syed Ziaur Rahman [c]

[a] Department of Chemistry, Aligarh Muslim University, Aligarh - 202002, UP, India

[b] Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi - 110062, India

[c] Department of Pharmacology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh - 202002, UP, India

Published online: 13 Jan 2010.

To cite this article: Mehtab Parveen , Zakia Khanam , Mohammad Ali & Syed Ziaur Rahman (2010): A novel lupene-type triterpenic glucoside from the leaves of Clerodendrum inerme , Natural Product Research: Formerly Natural Product Letters, 24:2, 167-176

To link to this article: http://dx.doi.org/10.1080/14786410902975566

Natural Product Research
Vol. 24, No. 2, 20 January 2010, 167–176

A novel lupene-type triterpenic glucoside from the leaves of *Clerodendrum inerme*

Mehtab Parveen[a]*, Zakia Khanam[a], Mohammad Ali[b] and Syed Ziaur Rahman[c]

[a]*Department of Chemistry, Aligarh Muslim University, Aligarh – 202002, UP, India;* [b]*Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi – 110062, India;* [c]*Department of Pharmacology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh – 202002, UP, India*

(*Received 13 January 2009; final version received 10 April 2009*)

A new triterpenic glucoside, lup-1,5,20(29)-trien-3-*O*-*β*-D-glucopyranoside (**4**), along with three known phytoconstituents: *n*-octacosane, friedelin and *β*-amyrin, has been isolated from the leaves of *Clerodendrum inerme* (L.) Gaertn. (Verbenaceae). Structure elucidation was carried out on the basis of chemical and physical evidence (IR, ^{1}H-NMR, ^{13}C-NMR, DEPT and MS spectra). The alcoholic and aqueous extracts of the leaves of *C. inerme* showed significant antinociceptive activity in analgaesiometer tests.

Keywords: *Clerodendrum inerme*; triterpenic glucoside; lup-1,5,20(29)-trien-3-O-*β*-D-glucopyranoside; *n*-octacosane; friedelin; *β*-amyrin; antinociceptive activity

1. Introduction

Clerodendrum inerme (L.) Gaertn. (Verbenaceae) is a perennial shrub commonly known as lanjai or garden quinine. The plant is generally grown as a hedge to stabilise sand dunes in deserts and to check erosion in water sheds (Anonymous, 2001). For many years, *Clerodendrum* spp. has been used in Indian and Chinese traditional medicine. In Siddha medicine, *C. inerme* is utilised under the names of 'chankan kuppi' and 'pechagnan' (Sasikala, Usman, & Kundu, 1995). The plant leaves are prescribed to cure skin ailments, elephantiasis, asthma, oedema, tetanus, atopic rhinitis, epilepsy, topical burns, umbilical cord infections, to aid cleaning of the uterus, and to treat coughs and rheumatism (Anonymous, 2001; M.B. Reddy, K. Reddy, & M.N. Reddy, 1988, 1989; Sudarsanam, Reddy, & Nagaraju, 1995). The shrub acts as a substitute for quinine (Kirtikar & Basu, 1991). Recently, *C. inerme* has been demonstrated to possess growth inhibitory and antifeedant activities against *Earias vitella*, *Spondoptera litura*, *Bemesia tabaci*, *Musca domestica* and *Culex quinquefasciatus* (Kumari, Balachandran, Arvind, & Ganesh, 2003; Pereira & Gurudutt, 1990; Rao & Kumar, 2006), as well as persistent toxicity against *Culex fatigans*, *Tribolium castaneum*, *Castor semilooper* and *Achaea janata* (Basappa & Lingappa, 2002; Rizvi, Ahmad, Azmi, Ahmad, & Akhtar, 2001a; Rizvi, Azmi, Akhtar, & Ahmad, 2001b). The plant extracts exhibited hepatoprotective, antiviral and antifungal activities (Anitha & Kannan, 2006; Gopal & Sengottuvelu, 2008; Prasad, Shankar, Kumar, Shetty, &

*Corresponding author. Email: mehtab_organic@rediffmail.com

ISSN 1478–6419 print/ISSN 1029–2349 online

DOI: 10.1080/14786410902975566
http://www.informaworld.com

Prakash, 2007) and this therefore justified the therapeutic use of this plant in tribal medicine. Extensive chemical studies of the phytoconstituents of *C. inerme* have been reported due to its medicinal properties. Numerous compounds such as flavonoids, iridoids, diterpenes, triterpenes and sterols have been isolated from various parts of the plant (El-Shamy, El-Shabrawy, & El-Fiki, 1996; Kanchanapoom, Kasai, Chumsri, Hinraga, & Yamasaki, 2001; Nan, Wu, Yin, & Zhang, 2006; Pandey, Verma, & Gupta, 2005; Pandey, Verma, Singh, & Gupta, 2003). As a part of studies to investigate the active principles of *C. inerme*, a new triterpenic glucoside characterised as lup-1,5,20(29)-trien-3-*O*-β-D-glucopyranoside (**4**) has been isolated, together with *n*-octacosane (**1**), friedelin (**2**) and β-amyrin (**3**) from the leaves of the plant. Its alcoholic and aqueous extracts have also been screened for antinociceptive activity.

2. Results and discussion

Fresh leaves of *C. inerme* (2 kg) were procured from the Botanical Garden of Aligarh Muslim University, Aligarh, India and identified by Dr Athar Ali, Taxonomist, Department of Botany, Aligarh Muslim University, Aligarh. After being defatted with light petroleum ether (60–80°C), the dried leaves of *C. inerme* were exhaustively extracted with 95% ethanol in a soxhlet apparatus. The ethanolic extract was concentrated under reduced pressure and the residue was extracted successively with benzene, chloroform, ethyl acetate, acetone and methanol. The benzene and chloroform extracts showed similar behaviour on TLC examination, and hence were mixed together. The mixed concentrate was chromatographed over silica gel column. Elution of the column in increasing polarity of different solvent systems (light petroleum ether, light petroleum ether-benzene (9 : 1–1 : 1) mixtures, benzene, benzene–ethyl acetate (9 : 1–6 : 4) mixtures) afforded four compounds marked as **1**, **2**, **3** and **4**.

Compound **1** was obtained as white granular crystals from light petroleum ether : benzene (9 : 1) eluate. The FABMS of **1** displayed a quasi molecular ion peak at m/z 395 $[M+1]^+$ consistent with a molecular formula of $C_{28}H_{58}$, which was also supported by its ^{13}C-NMR spectrum. The presence of C-1 and C-28 terminal methyl protons was indicated by a signal δ 0.82, which corresponded to a signal at δ_C 21.48 in the ^{13}C-NMR spectrum. The signals between δ 0.98–2.02 and δ_C 27.53–45.35 were associated with a methylene group in ^{1}H- and ^{13}C-NMR spectra, respectively. On the basis of the above spectral data, the structure of compound **1** has been established as *n*-octacosane (Lei et al., 2003).

Compounds **2** and **3** have been identified as friedelin (Budzikiewecz, Wilson, & Djerassi, 1963; Crawford, Hanson, & Kokar, 1975; Gunatilaka, De Silva, Sotheeswaran, Balasabramaniam, & Wazeer, 1984) and β-amyrin (Inoue et al., 1978) by comparison of TLC, Co-TLC, m.p., co-m.p. and spectral data with those of authentic samples.

The compound **4**, a lupeol diene glycoside, was obtained as a colourless crystalline mass from benzene : ethyl acetate (6 : 4) eluate. It responded positively to Liebermann–Burchard and Molisch tests for triterpenic glycosides. Its IR spectrum showed characteristic absorption bands for hydroxyl groups at 3510 and 3416 cm^{-1} and unsaturation at 1638 cm^{-1}. The elemental analysis and ESIMS of **4** exhibited a quasi molecular ion peak at m/z 585 compatible with triterpenic glycoside, $C_{36}H_{56}O_6$. It indicated nine double bond equivalents; five of them were adjusted in the pentacyclic carbon framework of the triterpenic molecule, three in the vinylic linkages and the remaining one in the glycoside

moiety. The mass spectrum showed important ion fragments (Figure 1) at *m/z* 543 $[M–C_3H_5]^+$, 528 $[543–Me]^+$, 513 $[528–Me]^+$, 498 $[513–Me]^+$, 569 $[M–Me]^+$, 554 $[569–Me]^+$, 539 $[554–Me]^+$, 524 $[539–Me]^+$, 421 $[M–C_6H_{11}O_5]^+$, 391 $[421–2Me]^+$, 376 $[391–Me]^+$, 389 $[M–C_6H_{12}O_6–Me]^+$, 374 $[389–Me]^+$ and 329 $[344–Me]^+$, indicating the attachment of a C_6 monosaccharide to the triterpenic aglycone. The ion peaks arising at *m/z* 312, 272 $[C_{6,7}–C_{9,10}$ fission$]^+$, 366, 218 $[C_{8,14}–C_{9,11}$ fission$]^+$, 380, 204 $[C_{8,14}–C_{11,12}$ fission$]^+$, 394 $[C_{8,14}–C_{12,13}$ fission$]^+$, 379 $[394–Me]^+$, 364 $[379–Me]^+$ suggested the existence of vinylic linkages in ring A and ring B, the saturated nature of ring C and the presence of a carbinol carbon in ring A which was placed at C-3 on the basis of biogenetic considerations. The ion peaks formed at *m/z* 434 $[C_{14,15}–C_{13,18}$ fission$]^+$, 271 $[434–C_6H_{11}O_5]^+$, 419 $[434–Me]^+$, 254 $[434–C_6H_{12}O_6]^+$ and 448 $[C_{15,16}–C_{13,18}$ fission$]^+$

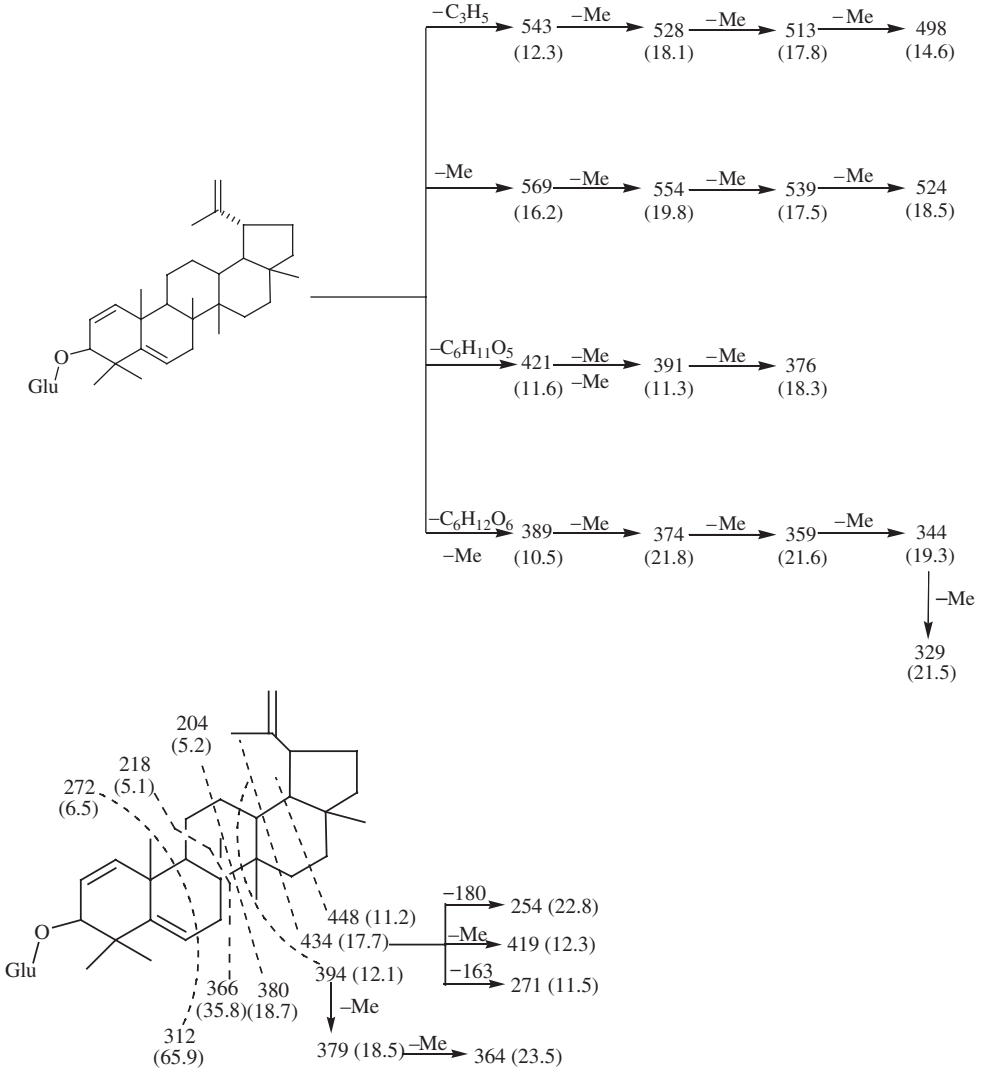

Figure 1. Mass fragmentation pattern of compound **4**.

supported the saturated nature of the ring D and the presence of an isopropenyl group in the lupene-type triterpene.

The ^{1}H-NMR spectrum (Table 1) of **4** displayed two one-proton multiplets at δ 5.53 and 5.29 assigned to vinylic H-2 and H-6 protons, respectively. The two one-proton doublets at δ 5.26 ($J = 5.7$ Hz) and 5.05 ($J = 7.2$ Hz) were ascribed to vinylic H-1 and anomeric H-1′ protons, respectively. A two-proton broad signal at δ 4.83 was accounted to unsaturated methylene protons H_2-29. Then 3 one-proton multiplets at δ 4.52, 3.99 and 3.95, a one-proton double doublet at δ 4.28 ($J = 7.2$, 6.8 Hz) and a two-protons broad signal at δ 3.92 were associated with the sugar protons. A one-proton

Table 1. ^{1}H-NMR and ^{13}C-NMR spectral data of lup-1,5,20(29)-trien-3-*O*-β-D-glucopyranoside (**4**).

Position	^{1}H-NMR	^{13}C-NMR
1	5.26 d (5.7)	135.70
2	5.53 m	137.69
3	4.24 d (5.5)	80.86
4	–	40.00
5	–	140.0
6	5.29 m	121.78
7	–	34.52
8	–	42.96
9	–	54.35
10	–	37.43
11	–	22.68
12	–	23.38
13	–	32.61
14	–	46.61
15	–	32.35
16	–	34.41
17	–	57.58
18	–	52.02
19	–	39.30
20	–	150.71
21	–	30.95
22	–	33.00
23	1.36 br s	25.53
24	0.85 br s	15.53
25	1.02 br s	14.77
26	0.89 br s	20.36
27	0.61 br s	14.51
28	0.98 br s	14.51
29	4.83 br s	110.05
30	1.68 br s	21.08
3-O-Glc		
1′	5.05 d (7.2)	102.43
2′	4.28 dd (7.2, 6.8)	78.32
3′	3.99 m	75.20
4′	3.95 m	71.54
5′	4.52 m	77.69
6′	3.92 br s	62.69

Note: Spectrum recorded in $CDCl_3$ at 500 MHz, using TMS as the internal standard; coupling constants in hertz are given in parentheses.

doublet at δ4.24 with coupling interaction of 5.5 Hz was accounted to a carbinol H-3 proton in α-orientation. A three-proton broad signal at δ1.68 was due to C-30 methyl protons attached to a C-20 vinylic carbon. Six three-proton broad signals at δ1.36, 1.02, 0.98, 0.89, 0.85 and 0.61 were accommodated correspondingly to tertiary C-23, C-25, C-28, C-26, C-24 and C-27 methyl protons. The remaining multiplets between δ1.80 and 2.50 were associated with 9 methylene and 13 methine protons. The ^{13}C-NMR spectrum (Table 1) of **4** showed 36 carbon signals, and the important signals appeared for vinylic carbons at δ 135.70 (C-1), 137.69 (C-2), 140.00 (C-5), 121.78 (C-6), 150.71 (C-20) and 110.05 (C-29), the carbinol carbon at δ80.86 (C-3), the anomeric carbon at δ102.43 (C-1′) and the other sugar carbons between δ78.32 and 62.69. The ^{13}C-NMR values of the triterpenic carbons were compared with the reported lupene-type δ_C values (Ali, 2001; Mohato & Kundu, 1994). The DEPT spectrum showed the presence of seven methyl, seven quaternary carbons, nine methylene and 13 methine carbons. The ^{1}H–^{1}H cosy spectrum of **4** showed correlations of H-3 with H-2, H-1 and H-1′; H-6 with H_2-7 and H_3-23; and H_2-29 with H-19 and H_3-30.

Acid hydrolysis of **4** yielded luptrienol and D-glucose. On the account of the spectral evidence, the structure of **4** has been established as lup-1,5,20(29)-trien-3-*O*-β-D-glucopyranoside (Figure 2).

2.1. *Pharmacological studies*

Oral administration to Charles Foster rats of ethanolic and aqueous extracts of the leaves of *C. inerme* at doses of 25 and 100 mg kg^{-1} p.o. showed significant antinociceptive activity in analgaesiometer tests (Figure 3). After 2 h of drug administration, the aqueous extract at doses of 25 and 100 mg kg^{-1} in rats caused a gradual increase in reaction time, which reached its maximum values of 7.50 ± 0.10 and 7.19 ± 0.17 in 100 and 40 min, respectively (Table 2). The effect gradually declined within the next 160 min for both doses. The administration of the ethanolic extract in doses of 25 and 100 mg kg^{-1} p.o. resulted in a gradual increase to its peak values of 6.94 ± 0.16 and 7.50 ± 0.12 in 200 and 180 min after drug administration. The effect gradually decreased within the next 80 and 160 min, respectively (Table 2). The maximum response (duration 240 min) was noted with 25 mg kg^{-1} of aqueous extract, while with crude ethanolic extract maximum response (duration 200 min) was observed at 100 mg kg^{-1}. The ethanolic extract showed analgaesic effect in a dose dependent manner. The peak effect (40 min) with aqueous extract (100 mg kg^{-1}) was observed earlier in comparison with ethanolic extracts. The effect of

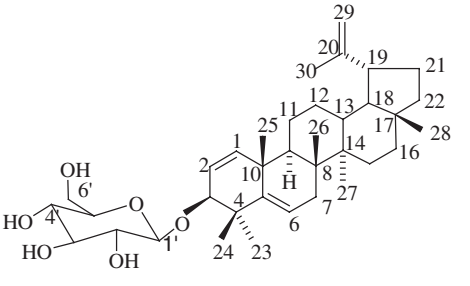

Figure 2. Structure of compound **4**.

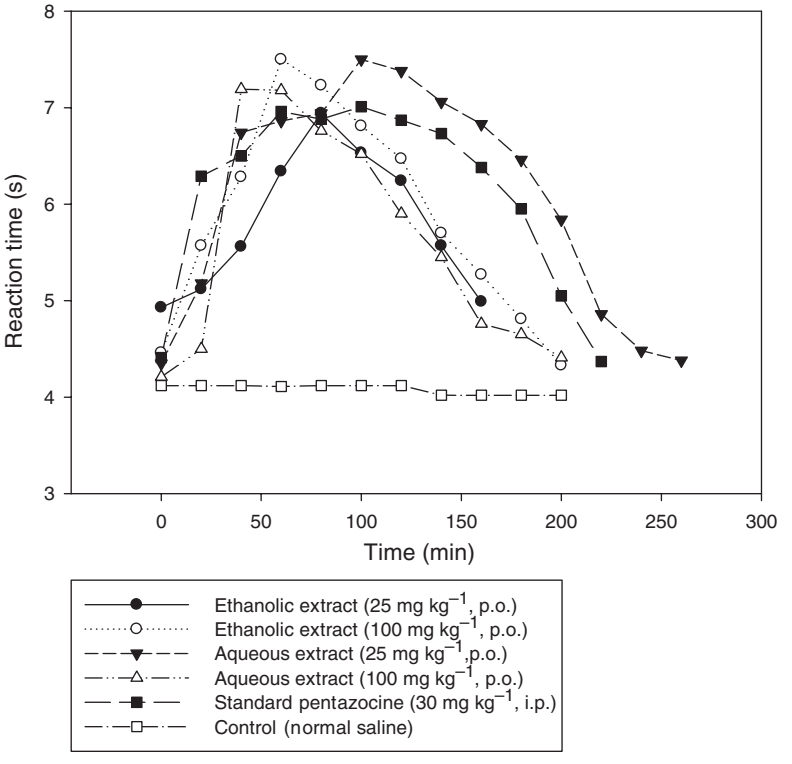

Figure 3. Time response curve of aggregate mean of reaction time of *C. inerme* extracts and pentazocine in analgaesiometer.

Table 2. Antinociceptive activity of ethanolic and aqueous extracts of the leaves of *C. inerme*.

Group (*n*)	Treatment	Onset	Peak	Recovery	Duration
Control (8)	N. saline	4.12 ± 0.21	4.23 ± 0.11	4.02 ± 0.22	0 min
Standard (8)	Pentazocine (30mg kg^{-1})	6.29 ± 0.58● (20 min)	7.01 ± 0.17* (100 min)	4.37 ± 0.04▲ (220 min)	200 min
Test (8)	*C. inerme* (25 mg kg^{-1}) Ethanolic extract	5.12 ± 0.14● (20 min)	6.94 ± 0.16* (80 min)	4.99 ± 0.18● (160 min)	140 min
	C. inerme (100 mg kg^{-1}) Ethanolic extract	5.57±.019* (20 min)	7.50 ± 0.12* (60 min)	4.33 ± 0.08■ (220 min)	200 min
	C. inerme (25 mg kg^{-1}) Aqueous extract	5.18 ± 0.21● (20 min)	7.50 ± 0.10* (100 min)	4.38 ± 0.22■ (260 min)	240 min
	C. inerme (100 mg kg^{-1}) Aqueous extract	4.50 ± 0.14♦ (20 min)	7.19 ± 0.17* (40 min)	4.41 ± 0.13▲ (200 min)	180 min

Note: *$p < 0.001$, ●$p < 0.01$, ♦$p < 0.1$, ▲$p < 0.2$, ■$p < 0.3$.

C. inerme extracts was compared with the standard drug pentazocine, which was given in a dose of 30 mg kg^{-1} i.p. and was found almost equivalent in efficacy. The significant increase in reaction time suggested that *C. inerme* could be one of the analgaesic alternative herbal drugs.

3. Experimental

3.1. *General experimental procedure*

The melting points were taken on a Kofler block and are uncorrected. ^{1}H-NMR and ^{13}C-NMR were recorded on Bruker Advance 500 MHz spectrometers with TMS as an internal standard. IR spectra were taken on a Shimadzu IR-408 Perkin Elmer 1800 (FTIR). The MS were recorded on a JEOL MSD-300 spectrometer. Elemental analysis was performed on an Elementar Vario EL-III. The silica gel used for different chromatographic purposes was obtained from E. Merck (India) and SRL (India). Reagents and solvents used were mostly of LR grade.

3.2. *Plant material*

The leaves of *C. inerme* were collected from the Botanical Garden, Aligarh Muslim University, Aligarh, India and were identified by Dr Athar Ali, Taxonomist, Department of Botany, Aligarh Muslim University, Aligarh, India.

3.3. *Extraction, isolation and identification*

The fresh leaves of *C. inerme* (2 kg) were dried under shade and crushed to a powder. The powdered leaves were defatted with light petroleum (60–80°C) and then extracted thoroughly with 95% ethanol in a soxhlet apparatus. The EtOH extract was evaporated to dryness under reduced pressure. The dark green viscous mass (290 g) left behind was extracted successively with benzene, chloroform, ethyl acetate, acetone and methanol successively. TLC examination of benzene and chloroform extracts revealed similar behaviour on the TLC plates and hence they were mixed together. Further, TLC examination of the combined extract in different solvent systems (light petroleum ether : diethyl ether (9 : 1), light petroleum ether : benzene (1 : 1), benzene : chloroform (1 : 1) and chloroform : methanol (9 : 1)) showed there to be an intricate mixture of a number of compounds. Therefore, it was chromatographed over silica gel (60–120 mesh) column. The column was eluted successively with light petroleum ether, light petroleum ether–benzene (9 : 1–1 : 1) mixtures, benzene and benzene–ethyl acetate (9 : 1–6 : 4) mixtures. Fractions showing similar behaviour on TLC plates and the same IR spectra were pooled together. Repeated column chromatography of the fractions followed by fractional crystallization afforded four compounds, **1**–**4**.

n-Octacosane (**1**): The fraction obtained by the elution of the column with light petroleum ether : benzene (9 : 1) mixtures was crystallised with chloroform–acetone as white granular crystals (75 mg) m.p. 60–61°C; IR ν_{max}^{KBr} cm^{-1}: 2926, 2853, 1451, 1369, 1160, 1068, 1019 and 889; FABMS *m*/*z* 395 $[M+1]^+$ ($C_{28}H_{58}$) (38.6); ^{1}H-NMR ($CDCl_3$) δ; 2.02 (4H, br s, 2 × CH_2), 1.56 (2 H, br s, CH_2), 1.40 (2H, br s, CH_2), 1.23 (42 H, br s, 21 × CH_2), 0.98 (2H, br s, CH_2), 0.82 (6H, br s, Me–1, Me–28); ^{13}C-NMR ($CDCl_3$) δ; 45.35 (CH_2), 30.01 (CH_2), 29.65 (23 × CH_2), 27.53 (CH_2), 21.48 (Me–1, Me–28). It was characterised as *n*-octacosane.

Friedelin (**2**): This was obtained from the column with petroleum ether–benzene eluate and crystallised from chloroform–methanol as a white solid (35 mg); m.p. 262–264°C. It was identified as friedelin by comparison with TLC, co-TLC, m.p., m.m.p. and spectral data of authentic samples.

β-Amyrin (**3**): This was obtained from the column with benzene eluate and crystallised from chloroform–methanol as a white solid (45 mg); m.p. 198–199°C. It was identified as *β*-amyrin by comparison with TLC, co-TLC, m.p., m.m.p. and spectral data of authentic samples.

Lup-1,5,20(29)-trien-3-*O*-*β*-D-glucopyranoside (**4**): This was obtained from the column with benzene : ethyl acetate (9 : 1) eluate and crystallised from chloroform–methanol as colourless crystals (80 mg); m.p. 238–240°C. IR ν_{max}^{KBr} cm^{-1} 3510, 3416, 2930, 2851, 1638, 1460, 1370, 1167, 1073, 1024 and 889; ESIMS *m/z* 585 $[M+1]^+$ ($C_{36}H_{56}O_6$) (5.8), 569 (16.2), 554 (19.8), 543 (12.3), 539 (17.5), 528 (18.1), 524 (18.5), 513 (17.8), 498 (14.6), 448 (11.2), 434 (17.7), 419 (12.3), 421 (11.6), 394 (12.1), 391 (11.3), 389 (10.5), 380 (18.7), 379 (18.5), 373 (18.3), 374 (21.8), 366 (35.8), 364 (23.5), 359 (21.6), 344 (19.3), 329 (21.5), 312 (65.9), 272 (6.5), 271 (11.5), 260 (6.5), 254 (22.8), 218 (5.1) and 204 (5.2); ^{1}H-NMR and ^{13}C-NMR values are given in Table 1.

3.3.1. *Acid hydrolysis of **4***

Compound **4** (25 mg) was dissolved in ethanol (5 mL), dilute HCl (2 mL) was added and the reaction mixture was refluxed for 2 h on a steam water bath. The solution was dried under reduced pressure and the residue was dissolved in $CHCl_3$ (5 mL). The organic phase was washed with water (2 × 5mL), dried over anhydrous Na_2SO_4 and concentrated to get luptrienol, IR ν_{max}^{KBr} 3450 cm^{-1}. EIMS *m/z* 422 $[M]^{+\bullet}$ ($C_{30}H_{46}O$). The residue after separation of the triterpene was dissolved in a minimum amount of water and chromatographed on paper with a standard sample of D-glucose using *n*-butanol : water : -acetic acid (4 : 1 : 5) as a developing solvent; the R_f value was comparable with that of an authentic sample of D-glucose.

3.4. *Pharmacological method*

3.4.1. *Antinociceptive activity*

The analgaesic activity tested in this study was the tail flick response in which change in the latency of the tail flick escape from noxious heating of the tail skin was used to assess the antinociceptive effect. The normal reaction time of rats on analgaesiometer ranges from 4 to 5 s. The cut-off time was obtained after determination of the reaction time of each untreated rat at 0, 20 and 40 min. Rats were selected by preliminary screening. Those showing variation of more than 1 s between two reaction times at 20 min intervals or more than 3 s from the group mean were discarded. Charles Foster rats weighing 150–200 g of either sex were placed in a restraining holder so that the tail between the hole and tail tip or single point 3–5 cm from the tip of tail are directly kept over a heated nichrome wire. The reaction due to thermal stimulus in the form of a tail flick in normal, untreated rats was adjusted within 4–5 s. Since the stimulus capable of producing tissue damage was stated to be about twice that required to produce pain, the system was able to automatically cut off at 7–8 s (average multiplied by 1.5) (Turner, 1965). The rats were housed in standard breeding cages with access to a solid diet (Gold Mohar, Lipton, India, Ltd) and tap water, except during the experiment. The animal observation room was controlled so that the light dark cycle (light period 6:00 am to 6:00 pm, 12 h) and temperature (22 ± 2°C) were constant (Yamauchi, Fujita, Obara, & Ueda, 1981). Six groups of eight animals each were taken and grouped as: Group 1: control (double distilled water); Group 2: 25 mg kg^{-1},

ethanolic extract; Group 3: 100 mg kg^{-1} ethanolic extract; Group 4: 25 mg kg^{-1} aqueous extract; Group 5: 100 mg kg^{-1} aqueous extract; and Group 6: 30 mg kg^{-1} standard drug (pentazocine). Initially, the basal reaction time to heat was observed by placing the tip of the tail directly over the nichrome wire of the analgaesiometer. After administration of drugs, the reaction time was noted 2 h later in Groups 2, 3, 4 and 5, while 20 min later in Groups 1 and 6. All readings were taken in an interval of 20 min each.

References

Ali, M. (2001). *Techniques in terpenoid identification* (p. 284). Delhi: Birla Publication.

Anitha, A., & Kannan, P. (2006). Antifungal activity of *Clerodendrum inerme* (L). and *Clerodendrum phlomidis* (L). *Turkish Journal of Biology, 30*, 139–142.

Anonymous. (2001). *Wealth of India* (Vol. 2, pp. 67–68). New Delhi: National Institute of Science Communication and Information Resources (NISCAIR) and Council of Scientific and Industrial Research (CSIR).

Basappa, H., & Lingappa, S. (2002). Persistent toxicity of botanical insecticides against *Castor semilooper, Achaea janata* Linn. (Lepidoptera: Noctuidae). *Indian Journal of Plant Protection, 30*, 187–190.

Budzikiewecz, H., Wilson, J.M., & Djerassi, C. (1963). Mass spectrometry in structural and stereochemical problems. XXXII. Pentacyclic triterpene. *Journal of the American Chemical Society, 85*, 3688–3699.

Crawford, M., Hanson, S.W., & Kokar, M.E.S. (1975). The structure of cymbopogon, a novel triterpenoid from lemon grass. *Tetrahedron Letters, 16*, 3099–3102.

El-Shamy, A.M., El-Shabrawy, A.R.O., & El-Fiki, N. (1996). Phytochemical study of *Clerodendrum inerme* L. growing in Egypt. *Zagazig Journal of Pharmaceutical Science, 5*, 49–53.

Gunatilaka, A.A.L., De Silva, A.M.Y.J., Sotheeswaran, S., Balasabramaniam, S., & Wazeer, M.I.M. (1984). Triterpenoid and biflavonoid constituents of *Calophyllum calba* and *Garcinia spicata* from Sri Lanka. *Phytochemistry, 23*, 323–328.

Gopal, N., & Sengottuvelu, S. (2008). Hepatoprotective activity of *Clerodendrum inerme* against CCl_4 induced hepatic injury in rats. *Fitoterapia, 79*, 24–26.

Inoue, T., Ishidata, Y., Fugita, M., Kubo, M., Fukushina, M., & Nagai, M. (1978). Studies on the constituents of Aceraceae plants. I. Constituents in the leaves and stem bark of *Acer nikoense* Maxim. *Yakugaku Zasshi, 98*, 41–46.

Kanchanapoom, T., Kasai, R., Chumsri, P., Hinraga, Y., & Yamasaki, K. (2001). Megastigmane and iridoid glucosides from *Clerodendrum inerme*. *Phytochemistry, 58*, 333–336.

Kirtikar, K.R., & Basu, B.D. (1991). *Indian medicinal plants* (2nd ed., Vol. 3, pp. 1945–1947). Dehradun: B. Singh and M.P. Singh Publications.

Kumari, G.N.K., Balachandran, J., Arvind, S., & Ganesh, M.R. (2003). Antifeedant and growth inhibitory effects of some *neo*-clerodane diterpenoids isolated from *Clerodendrum* species (Verbenaceae) on *Earis vitella* and *Spodoptera litura*. *Journal of Agricultural and Food Chemistry, 51*, 1555–1559.

Lei, Y., Nan, P., Tsering, T., Bai, Z., Tian, C., & Zhong, Y. (2003). Chemical composition of the essential oils of two *Rhodiola* species from Tibet. *Zeitschrift für Naturforschung, 58c*, 161–164.

Mohato, S.B., & Kundu, A.P. (1994). ^{13}C NMR spectra of pentacyclic triterpenoids – a compilation and some salient features. *Phytochemistry, 37*, 1517–1575.

Nan, H., Wu, J., Yin, H., & Zhang, C. (2006). Terpene compounds in *Clerodendrum inerme* (L.) Gaertn. *Zhongcaoyao, 37*, 508–509.

Pandey, R., Verma, R.K., & Gupta, M.M. (2005). *Neo*-clerodane diterpenoids from *Clerodendrum inerme*. *Phytochemistry, 66*, 643–648.

Pandey, R., Verma, R.K., Singh, S.C., & Gupta, M.M. (2003). 4α-Methyl-24β-ethyl-5α-cholesta-14,25-dien-3β-ol and 24β-ethylcholesta-5, 9(11), 22e-trien-3β-ol, sterols from *Clerodendrum inerme*. *Phytochemistry, 63*, 415–420.

Pereira, J., & Gurudutt, K.N. (1990). Growth inhibition of *Musca domestica* L. and *Culex quinquefasciatus* by (-)-3-epicaryoptin isolated from leaves of *Clerodendrum inerme* (Gaertn.) (Verbenaceae). *Journal of Chemical Ecology, 16*, 2297–2306.

Prasad, H.P., Shankar, U.A.C., Kumar, B.H., Shetty, S.H., & Prakash, H.S. (2007). Management of bean common mosaic virus strain blackeye cowpea mosaic (BMCV-BLCM) in cowpea using plant extracts. *Archives of Phytopathology and Plant Protection, 40*, 139–147.

Rao, P.N., & Kumar, K.P. (2006). White fly management in cotton agro-ecosystem with botanical peticides: An ecofriendly approach. *Journal of Ecophysiology and Occupational Health, 6*, 57–60.

Reddy, M.B., Reddy, K.R., & Reddy, M.N. (1988). A survey of medicinal plants of Chenchu tribes of Andhra Pradesh, India. *International Journal of Crude Drug Research, 26*, 189–196.

Reddy, M.B., Reddy, K.R., & Reddy, M.N. (1989). A survey of plant crude drugs of Anantapur district, Andhra Pradesh, India. *International Journal of Crude Drug Research, 27*, 145–155.

Rizvi, S.A., Ahmad, A., Azmi, M.A., Ahmad, I., & Akhtar, K. (2001a). Determination of toxicity of *Clerodendrum inerme* and cypermethrin against *Tribolium castaneum* and their effects on acid phosphatase and cholinesterase enzyme. *Proceedings of Pakistan Congress of Zoology, 21*, 175–180.

Rizvi, S.A., Azmi, M.A., Akhtar, K., & Ahmad, I. (2001b). Toxicological studies on *Clerodendrum inerme* and DDVP in *Culex fatigans* (wild strain) and their effects on cholinesterase and acid phosphatase activity. *Proceedings of Pakistan Congress of Zoology, 21*, 275–280.

Sasikala, E., Usman, A.S. & Kundu, A.B. (1995). On the pharmacognosy of *Clerodendrum inerme* (L) Gaertner leaves. Seminar on Research in Ayurveda and Siddha (Vol. 90, pp. 20–22). New Delhi: CCRAS.

Sudarsanam, G., Reddy, M.B., & Nagaraju, N. (1995). Veterinary crude drugs in Rayalaseema, Andhra Pradesh, India. *International Journal of Pharmacognosy, 33*, 52–60.

Turner, R.A. (1965). *Screening methods in pharmacology* (p. 99). New York: Academic Press.

Yamauchi, C., Fujita, S., Obara, T., & Ueda, T. (1981). Effects of room temperature on reproduction, body and organ weights, food and water intake, and hematology of rats. *Laboratory Animal Science, 31*(3), 251–258.