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Constituents of *Mallotus nepalensis* Muell. Arg.: a Mild CNS Depressant

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Abstract – *Mallotus nepalensis* (Euphorbiaceae) is a small tree found in central and eastern Himalayas, Nepal, Wallich and Sikkim. The 90% ethanolic extract of *Mallotus nepalensis* exhibited mild CNS depressant activity. Four compounds, lupeol (**1**), β-sitosterol (**2**), ursolic acid (**3**) and β-sitosterol- β-D-glucoside (**4**) were isolated from the 90% ethanolic extract of this plant of which 1, 3 and 4 are being reported for the first time from this specie.

Keywords – *Mallotus nepalensis*, Lupeol, Ursolic acid, CNS depressant

Introduction

Mallotus nepalensis Muell. Arg. (Euphorbiaceae) is a small tree found in central and eastern Himalayas, Nepal, Wallich, Sikkim at an altitude of 5000-7000 feet and Khasia mountains at an altitude of 4000-5000 feet. Leaves 3-8 inch in diameter, thinly coriaceous, glaborous above except when young, quite entire, base truncate, rarely subcordate, or acute in young leaves, colour of pubescence very variable, nerves and nervules strong. Male racemes 6-12 inch, bracts lanceolate, flowers 1-4 inch diameter, glaborous in buds, stamens very numerous. Female racemes very stout, especially in fruits, pedicels short, stout, sepals narrow, ovary shaggy with short tomentose processes. Capsules densely crowded, 1-2 inch diameter, crenile, cocci very thin. Seeds 1-6 inch long, sub-hemispheric, black opaque (Hooker, 1885).

Literature survey showed that although chemical constituents of *Mallotus* species have been extensively studied, not much work has carried out on this specie. Just moretenone, moretenol and β-sitosterol are the constituents that have been isolated (Sil *et al.*, 1980). During the course of the present study, the ethanolic extract of *M. nepalensis* was found to exhibit CNS depressant activity as indicated by gross CNS observations and barbiturate sleeping time test in mice. Also, four compounds- lupeol (**1**), β-sitosterol (**2**), ursolic acid (**3**) and β-sitosterol-β-D-glucoside (**4**) were isolated from this plant, of which **1**, **3** and **4** are being reported for the first time from this specie.

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Experimental

Plant material – *Mallotus nepalensis* (aerial parts) was collected from Bomdila, West Kameng, Arunachal Pradesh, India. It was authenticated by matching with the typed specimen present in the Institute's herbarium and the specimen deposited in the departmental herbarium depository for future reference.

General – Mps uncorrected. ^{1}HNMR spectra were taken at 300 MHz with Bruker DRX 300 spectrometer. EIMS was recorded on a Jeol SX-102 spectrometer.

Animals – Male sprague - Drawley rats (160-180 g) were kept in the departmental animal house at 26 ± 2°C and relative humidity 44-55% light and dark cycles of 10 and 14 h respectively for one week before the experiment. Animals were provided with rodent diet (Amruth, India) and water *ad libitum*. All studies were conducted in accordance with the National Institute of Health "Guide for the Care and Use of Laboratory Animals".

Extraction and isolation – The dried and powdered aerial parts of *M. nepalensis* (2 Kg) were extracted with 90% aq. ethanol (3 × 5l). The combined ethanolic extract was concentrated under reduced pressure below 50°C which was then successively fractionated into chloroform (3.2 g), ethyl acetate (3.0 g), *n*-butanol (7.0 g) and water (4.8 g) soluble fractions. The chloroform fraction (3.0 g) of *M. nepalensis* was subjected to column chromatography over silica gel using different solvents. Fractions were collected and checked by TLC. Fr. Nos. 21-25, eluted in benzene (fr. A), 26-35, eluted in benzene:chloroform:: 3:1 and 1:1 (fr. B), 66-68, eluted in benzene:chloroform:: 1:3 (fr. C) and 99-102, eluted in chloroform:ethyl acetate::1:3

(fr. D) contained compounds **1**, **2**, **3** and **4** as the major constituents respectively. Fractions A, B and D on recrystallization with methanol yielded compounds **1**, **2** and **4** respectively. Fr. C was subjected to repeat chromatography over silica gel and fr. Nos. 41-64, eluted in benzene:methanol::99.5:0.5, were washed with hexane and recrystallised from acetone to yield compound **3**.

Compound 1 (Lupeol) – Colourless needles, m.p.: 216°C ; EIMS: *m/z* 426 (M^+), 411 (M-CH_3), 220, 218, 207 and 189 ; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.76 (3H, s, H-24), 0.79 (3H, s, H-28), 0.83 (3H, s, H-25), 0.94 (3H, s, H-27), 0.97 (3H, s, H-23), 1.03 (3H, s, H-26), 1.68 (3H, s, H-30), 3.15 (1H, dd, *J*=5, 10 Hz, H-3), 4.56 (2h, br.d, H-29).

Compound 2 (β-Sitosterol) – Colourless flakes, m.p.: 136°C ; EIMS: *m/z* 414 (M^+),^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.68 (3H,s, H-18), 0.81 (3H, d, *J*=6.9 Hz, H-27), 0.83 (3H, d, *J*=7.3 Hz, H-26), 0.87 (3H, t, *J*=7.6 Hz, H-29), 0.92 (3h, d, *J*=6.6 Hz, H-21), 1.01 (3H, s, H-19), 3.52 (1H, m, H-3), 5.35 (1H, br d, *J*=5.4 Hz, H-6). Compound **2** (5 mg) was subjected to acetylation with acetic anhydride and pyridine (0.5 ml each) by keeping it overnight at room temp. The reaction mixture was evaporated under reduced pressure and co-distilled with toluene (3 × 1 ml) to remove excess reagents. The acetate, **2a**, was crystallized from chloroform and methanol, m.p. 125°C. Its ^{1}H NMR showed signals at (1.94 (3H, s, $\underline{CH_3}CO$), 4.48 (1H, m, H-3) and 5.27 (1H, m, olefinic proton).

Compound 3 (Ursolic acid) – Colourless crystals, m.p.:290°C ; EIMS: *m/z* 456 (M^+), 438 (M^+-H_2O), 423 (M^+-H_2O-CH_3), 248 (rDA fragment), 207, 203, 189 and 133 ; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.79 (3H, d, *J*=7Hz, H-29), 0.91 (3H, s, H-24), 0.93 (3H, d, *J*=7Hz, H-30), 1.00 (6H, s, H-25, H-27), 1.15 (3H, s, H-23), 1.27 (3H, s, H-26), 2.83 (2H, br. d, *J*=15Hz, H-11), 3.22 (1H, dd, *J*=5, 10HZ, H-3), 5.30 (1H, br. s, H-12).

Compound 4 (β-Sitosterol-β-D-glucoside) – Colourless crystals, m.p.:275°C ; positive Fiegel's test for glycosides. Compound **4** (10 mg) was refluxed with 2 N HCl in 80% aq. ethanol (2 ml) for 3 hr. The hydrolysate was diluted with water and concentrated under reduced pressure to remove alcohol and then further refluxed at 100° for 1 hr. It was then cooled and extracted with chloroform. The organic layer was washed with water, dried over anhydrous sodium sulphate and concentrated. The aglycone crystallized from methanol, m.p. 136°. Its EIMS gave M^+ at *m/z* 414. It was identified as β-sitosterol (**2**) by mmp and comparison of its data with that obtained for **2**. The aqueous portion was neutralized with Amberlite 410 (CO_3^-) resin, and was found to contain glucose as the sugar moiety by paper chromatography.

Pentobarbital-induced sleeping time – Thirty minutes after intraperitoneal injection of extract (79 mg/kg), vehicle (control) and DZP (1 mg/kg), all the three groups, with 6 animals per group, received sodium pentobarbital (40 mg/kg) intraperitoneally. The time since the injection up to the loss of the righting reflex is recorded as sleeping latency and the time elapsed between the loss and voluntary recovery of the righting reflex is recorded as sleeping time (Wambebe, 1985; Rolland *et al.*, 1991).

Results and Discussion

The absolute values of the sleeping latency and sleeping time are presented in Table 1 (Fig. 1). The intraperitoneal treatment with extract (79 mg/kg) and DZP (1 mg/kg) resulted in a statistically significant decrease in sleeping latency [extract: 210.5 ± 16.8 min] and increase in sleeping time [extract: 2756.5 ± 147.2 min]. The effects of extract in the central nervous system were evaluated by the potentiation of sodium pentobarbital sleeping time. Decrease in sleeping latency and increase in sleeping time are classically related to central nervous system depressant drugs (Willianson *et al.*, 1996). Our results showed that the ethanolic extract of *M. nepalensis* decreased the sleeping latency time and increased the sleeping time at

Table 1. Effect of ethanolic extract of *M. nepalensis* on pentobarbital induced sleeping time

Groups	Sleeping latency time (min)	Sleeping time (min)
Group1	328.7 ± 24.5	2001.0 ± 185.3
Group2	210.5 ± 16.8[b]	2756.5 ± 147.2[a]
Group3	187.6 ± 9.2[b]	4769.2 ± 340.4[b]

Values are mean ± S.E.M. of 6 rats per group. Group 1: control; Group 2: extract treated; Group 3: DZP treated.
P values are calculated based on paired-t-test.
a: P < 0.05, b: P < 0.001 as compared to control group.

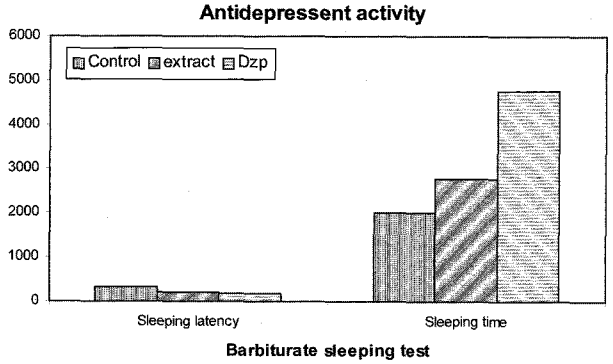
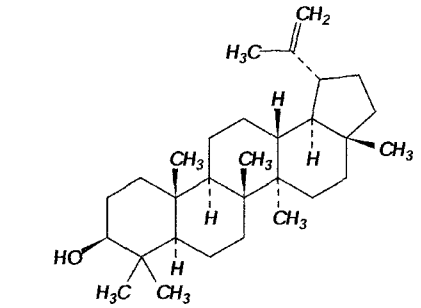


Fig. 1. Effect of ethanolic extract of *Mallotus nepalensis* on sleeping time.

the dose of 79 mg/kg, thereby exhibiting a CNS depressant action. Solvent fractionation and repeated column chromatography of the ethanolic extract of M. nepalensis led to the isolation of compounds **1**, **2**, **3** and **4**.

Compound **1** crystallised from methanol as colourless needles, m.p. 216°. The ^{1}H NMR of substance **1** showed signals for 7 methyl groups in the region δ 0.75-1.70. It also showed a broad doublet at 4.56 for CH_3-C=CH_2 and a double doublet at 3.15 for C<u>H</u>OH. EIMS of 1 gave major fragment ions at *m/z* 426 (M^+), 411, 220, 218, 207 and 189. The identity of compound **1** was confirmed as lupeol on the basis of co-TLC and comparison of its data with that reported in the literature (Yue *et al.*, 2004)

Compound **2** was crystallized from methanol as colourless flakes, m.p. 136°. Its **EIMS** showed M^+ at *m/z* 414. It gave a positive Lieberman Burchard test and



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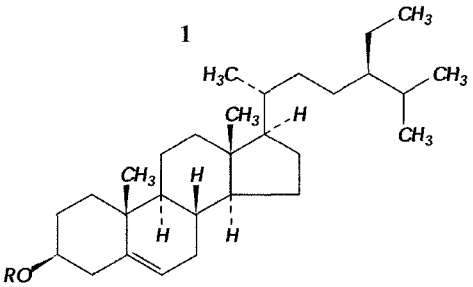
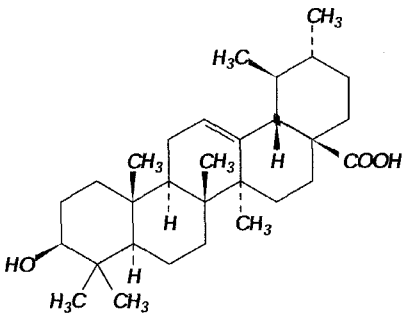


2 R = H
2a R = Acetyl
4 R = Glucosyl



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formed a monoacetate, m.p. 125°C (M^+ 456). It was identified as β-sitosterol by co-TLC, mmp. and comparison of spectral values (Yun-Choi *et al.*, 2003).

Compound **3** was crystallized from acetone as colourless crystals, m.p. 290°C. It gave a positive Lieberman Burchard test for triterpenoids. Its solubility in sodium bicarbonate indicated it to be a triterpenoid acid. The ^{1}H NMR spectrum showed the presence of seven quarterly methyl groups in the region of δ 0.75-1.30. Two of these methyl signals were present as doublets (δ 0.79 and 0.93, *J*=7 Hz each). An olefinic proton signal was observed at δ 5.30. These observations indicated the presence of an ursane skeleton. Its mass spectrum showed major fragment ions at *m/z* 456 (M^+), 248, 233, 207 and 203. Compound **3** was thus finally identified as ursolic acid.

Compound **4** crystallised from methanol as colourless needles, m.p. 275°C. It gave a positive Fiegel's test for glycosides. On acid hydrolysis followed by the usual work up, it gave an aglycone and a sugar which were identified as β-sitosterol and D-glucose respectively. Compound **4** was thus identified as β-sitosterol-β-D-glucoside and confirmed by mmp. and co-TLC with an authentic sample.

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