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## Chemical and Pharmacological Evaluation of *Hygrophila spinosa* Root

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**Chemical investigation of *Hygrophila spinosa* root exhibited the presence of a greasy mass, lupeol and lupenone in petroleum ether extract. Crude petroleum ether extract, when administered (i.p.) to mice, potentiated the sedative-hypnotic action of chlorpromazine, diazepam, pentobarbitone, chlordiazepoxide and protected against strychnine-induced convulsions.**

*Hygrophila spinosa* (Acanthaceae), a small herb, is found to be distributed throughout India<sup>1</sup>. Its roots, seeds and leaves are reported to be used in different diseases<sup>2,3,4</sup>. Chemical investigations on seeds<sup>5</sup>, leaves<sup>6</sup>, and roots<sup>7</sup> led to the isolation of a sterol, a triterpene alcohol, xylose, and uronic acids. Petroleum ether fraction of the root has been reported to exhibit antitumour activity in mice<sup>8</sup>. During a toxicity study<sup>9</sup>, the petroleum ether extract of *H. spinosa* root was found to exhibit mild passivity and decreased touch response. Hence the extract was investigated for CNS depressant action. The present communication deals with chemical evaluation of *Hygrophila spinosa* root and activity of crude petroleum ether extract on central nervous system.

The roots *Hygrophila spinosa* were shade dried and powdered. It was extracted with petroleum ether (60°-80°)

in a soxhlet and the extract was concentrated under vacuum. Thin layer chromatography of the crude petroleum ether extract (yellowish white in colour) using chloroform as solvent indicated the presence of four components, I, II, III and IV showing R<sub>f</sub> values 0.95, 0.7h, 0.5h and 0.1m respectively. To separate the components, the crude extract was chromatographed over silica gel (60-120 mesh) in a column using petroleum ether:ethyl acetate (90:10) as the eluting system. The first elute after evaporation was found to be a greasy mass (component I). The second fraction was a white mass (component II) which was crystallised from n-hexane (m.p 166°) and analysed spectroscopically. Mass spectra (associated machine model JEOL-JMS AX 500 Spectrometer) of the compound indicated molecular ion peak (M<sup>+</sup>) at 424. Its IR spectra (associated machine model 873 Perkin Elmer Spectrometer) exhibited the presence of a keto carbonyl group (y<sub>max</sub> at 1770 cm<sup>-1</sup>). From the mass, IR and

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Table I - Effect of petroleum ether extract on drug-induced sleeping time in mice

Treatment	Sleeping time (min)		Latent period of sleep (min)		
	Vehicle (5 ml/kg)	Extract (80 mg/kg)	Vehicle	Extract	% increase in sleeping time
Chlorpromazine (10 mg/kg)	71.4±4.03	89.6±2.94*	13.5±1.71	7.0±1.23*	25.5
Diazepam (6 mg/kg)	51.8±2.33	69.8±2.84*	21.8±4.63	11.8±2.98*	34.7
Pentobarbitone (40 mg/kg)	40.8±2.87	58.0±4.65*	6.0±1.47	3.5±0.65*	42.2
Chlordiazepoxide (20 mg/kg)	65.4±3.09	82.4±2.94*	20.3±2.47	9.0±0.71*	26.0

Sleep was induced by chlorpromazine or diazepam or pentobarbitone or chlordiazepoxide in presence either vehicle or extract. All values are mean±SEM of 10 determinations and asterisks denote statistical significance at  $P \leq 0.05$ .

PMR(Bruker 300 MHz Spectrometer) spectral data, the compound was identified as lupenone. Further elution of the column material was done by increasing the polarity of the solvent mixture (petroleum ether:ethyl acetate, 85:15). The eluted solution after evaporation indicated the presence of another compound (component III) which was also a white mass. It was recrystallised from petroleum ether (40°-60°, m.p. 212°) and analysed spectroscopically. IR spectra confirmed the presence of -OH group ( $y_{\max}$  at 3330  $\text{cm}^{-1}$ ). Mass ( $M^+$  426), UV, mixed IR and NMR spectra confirmed the compound as lupeol, which is triterpine alcohol.

Preliminary investigations indicated that the crude extract has low toxicity when tested on mice<sup>9</sup>.  $LD_{50}$  was determined according to the method of Litchfield and Wilcoxon<sup>10</sup> and the value was found to be more than 700 mg/kg, i.p. The crude extract exhibited CNS depressant effect at different i.p. dose levels. From the dose response curve the optimum effect was found to be at the i.p. dose level of 80 mg/kg. Albino Swiss mice of either sex weighing between 20-25 g were used for all experiments. They were fed standard pellet diet and given tap water *ad libitum*. Sedative-hypnotic and anticonvulsant actions of the crude petroleum ether extract were evaluated after 18 h of fasting and injections were given intraperitoneally.

The animals were divided into 11 groups, containing ten mice in each group and were given the following treat-

ments : Group I, saline control (5 ml/kg, 0.9% NaCl w/v); group II, vehicle control (ground nut oil, 5 ml/kg); group III, crude extract (80 mg/kg); group IV, pentobarbitone (40 mg/kg); group v, crude extract (80 mg/kg), 15 minutes prior to administration of pentobarbitone (40 mg/kg); group VI, chlorpromazine (10 mg/kg); group VII, crude extract (80 mg/kg), 15 minutes prior to administration of chlorpromazine(10m/kg); group VIII, diazepam (6 mg/kg); group IX, crude extract (80 mg/kg), 15 minutes prior to administration of diazepam (6 mg/kg); group X, chlordiazepoxide (20 mg/kg) and group XI, crude extract (80 mg/kg, 15 minutes prior to administration of chlordiazepoxide (20 mg/kg). Sleeping time was measured as the interval between the loss and recovery of the righting reflex<sup>11</sup>. The sleeping time and the latent period of sleep were noted.

For evaluating the anticonvulsant effect, another batch of animals were divided into four groups, A,B, C and D each containing ten mice. Strychine alone (2 mg/kg) was given to group A. 0.9%, w/v aqueous NaCl (normal saline, 5 ml/kg); ground nut oil (vehicle, 5 ml/kg) and crude extract (80 mg/kg) were given 30 minutes prior to the administration of strychine (2 mg/kg) to group B, C and D respectively. The per cent mortality and onset of convulsion were noted for each group.

Results were analysed statistically by Students

**Table II - Effect of petroleum ether extract on strychnine-induced convulsion in mice**

Treatment	% mortality	Onset of convulsion (Sec)
Strychnine (2 mg/kg)	100	49.8±2.26
Strychnine (2 mg/kg) + Vehicle (5 ml/kg)	100	54.8±2.52
Strychnine (2 mg/kg) + Pet.et.ext. (80 mg/kg)	50	99.5±5.78*

*Convulsion was induced by strychnine in presence either vehicle or extract. All values are mean±SEM of 10 determinations and asterisks denote statistical significance at  $P \leq 0.05$ .*

unpaired 't' test<sup>12</sup> and statistical significance were considered only when  $P < 0.05$ .

From the experimental data it is confirmed that the root extract alone has no sedative-hypnotic action, but it significantly potentiates the sleeping time induced by standard reference drugs (sodium pentobarbitone-42.2%; diazepam-34.7%; chlorpromazine-25.5% and chlordiazepoxide-26.0% respectively). There are some compounds which bring prolongation of sleeping time of certain sedative-hypnotic compounds, by a vascular mechanism affecting the absorption of the drug from the site of injection, its penetration into the "blood brain barrier" or its break down or excretion<sup>13</sup>. The crude extract may enhance pentobarbitone/chlorpromazine/chlordiazepoxide/diazepam-induced sleeping time by any of the above mentioned effects. Strychnine (2 mg/kg) caused seizures in all the animals. Pretreatment with the extract signifi-

cantly decreased the mortality (50%) caused by strychnine-induced convulsions. Strychnine produces a tonic type extensor convulsion and thought to exert its convulsive activity by antagonizing postsynaptic inhibition<sup>14</sup>. Pretreatment of crude extract inhibited the strychnine-induced convulsions fifty per cent probably by blocking multineuronal pathways in the spinal cord with minimal effects on monosynaptic pathways<sup>15</sup>.

From increase in sleeping time and decrease in myoclonic spasms and seizures it may be concluded that petroleum ether extract appeared to possess CNS depressant activity and the mechanism of which is not clear.

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