

## **Analgesic and CNS depressant activities of extracts of *Annona reticulata* Linn. bark**

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**Received:** 2<sup>nd</sup> October, 2011, **Revised:** 7 October 2011, **Accepted:** 7 October 2011

### **Abstract**

The study was designed to evaluate possible effects of various extracts of *Annona reticulata* bark on CNS. Petroleum ether, ethyl acetate and methanol extracts of the bark of *Annona reticulata* L. (Annonaceae) were evaluated for analgesic and CNS depressant activities in different animal models. All the extracts exhibited significant central analgesic activity in the hot plate method in mice. All the extract showed statistically significant mild to moderate central nervous system depressant activity assessed by locomotor activity assay and pentobarbitone sleeping time test.

**Key words:** *Annona reticulata* Linn; bark; analgesic activity; CNS depressant activity; locomotor activity; pentobarbitone sleeping time test

### **Introduction**

*Annona reticulata* L. is a small tree, 4-6 m in height. It occurs throughout India, native to tropical America, particularly the West Indies, completely naturalized and cultivated in some part of India (Kirtikar & Basu, 1987). The bark of the plant *Annona reticulata* L. locally known as Ramphal, is a powerful astringent and is stated to be given as tonic. The West Indies and in Central and South America the fruit is much used as an antidysenteric and anthelmintic (Anonymous, 1994). The plant has been used as anti-inflammatory, in wound healing, anti-anxiety, and anti-stress, anti-mutagenic and spasmolytic. Leaf and stem extract showed inotropic, positive chronotropic and spasmolytic activities (Rastogi & Mehotra, 1993).

The seed of this plant is reported to contain acetogenins mainly cis and transisomurisolenin (Chang *et al.*, 1998), annoreticuin, bullatacin, squamosine and rolliniastatin (Maeda *et al.*, 1993). Leaf and roots contain Sesquiterpenes mainly spathenelol, muurolene, copaene, e-

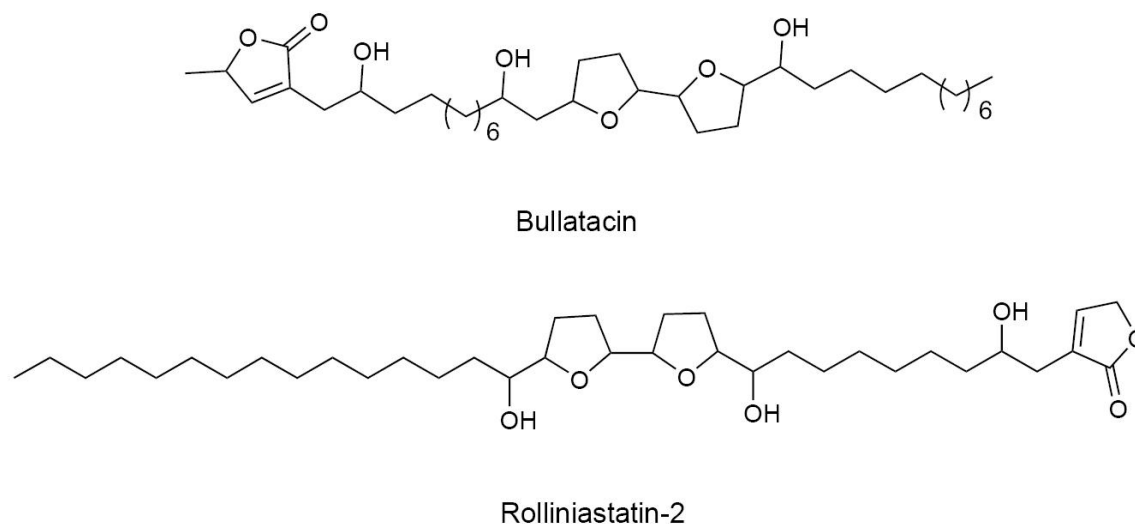


Figure 1. Chemical structures of acetogenins isolated from *Annona reticulata*.

desmol (Chang *et al.*, 1993). Reticullacinone, rolliniastatin-2, molvizarin and kaur-16-ene-19-oic acid are reported in stem bark (Hisham *et al.*, 1994; Chavan *et al.*, 2011). The representative chemical structures of some acetogenins are shown in figure 1. The aim of this work is to evaluate the analgesic activities and Central Nervous System (CNS) effects of various extracts of *A. reticulata* in order to provide a basis for the traditional use of the plant.

## Material and methods

### *Plant material*

Bark of *A. reticulata* L. was collected from Ahmednagar district, Maharashtra. A voucher specimen has deposited in the herbarium of the Botanical Survey of India, Pune Under reference F. No.11629.

### *Preparation of extract*

Shade dried plant material weighing 1 kg was treated with a mechanical pulverized for size reduction. The powder collected was successively extracted with petroleum ether (40-60°C) (1 L), ethyl acetate (1 L) and Methanol (1 L) using soxhlet apparatus for 72 h. The extracts were concentrated under reduced pressure using a rotary vacuum evaporator and dried at room temperature. The percentage yields of extracts were calculated as petroleum ether 2.3% w/w, ethyl acetate 5.58%w/w and methanolic 13.13% w/w. For pharmacological studies extracts were dissolved in 2% dimethyl for-mamide in water for injection.

### *Phytochemical tests*

The freshly prepared extracts were subjected to standard phytochemical screening tests for various constituents (Trease & Evance, 1983). The extracts were screened for the presence of alkaloid, glycoside, flavonoids, tannins, using conventional protocols.

### ***Animals***

Swiss albino mice of either sex weighing 20-25 g were used. The animals were housed in a group of six under standard light/dark cycle in polypropylene cages with standard pellet chow and water *ad libitum*. In all experimental sets six mice were used.

### ***Analgesic activity***

The activity was studied by the hot-plate method (Eddy & Leimbach, 1953) Petroleum ether, ethyl acetate and methanol extract (100 mg/kg) or pentazocin lactate injection (20 mg/kg) was administered intra peritoneal. The first group served as control and received only vehicle (2% dimethyl formamide in water for injection). Mice were placed individually on the Hot plate (Medicraft Eddy's hot plate, mark III) maintained at  $55\pm 0.5^{\circ}\text{C}$  and the latency to lick paws was noted. The basal reaction time was noted before and 30, 60, 90, 120, 150, 180 min. after the administration of treatment.

### ***Locomotor activity***

The locomotor activity was studied in petroleum ether, ethyl acetate and methanol extracts at a dose of (100 mg/kg) and evaluated by using photoactometer. The first group of animals served as control and received only vehicle. The second group was administered standard drug diazepam (2 mg/kg, i.p.). The animals of third to fifth group were administered the extracts. Mice were placed individually in photoactometer. The basal reaction time was noted before and 30 min after the administration of treatment (Kulkarni, 1999).

### ***Pentobarbitone sleeping time***

Group of mice were injected with pentobarbitone sodium (40 mg/kg) fifteen minutes after intraperitoneal administration of both vehicle and bark extracts (100 mg/kg), and time interval between losing and regaining of righting reflex was measured as sleeping time (Dandya & Collumbine, 1953).

### ***Statistical analysis***

All the results were statistically analyzed by student's t-test and expressed as the mean  $\pm$  S.E. Results were regarded as significant when  $P < 0.05$ .

### **Results**

The phytochemical analysis revealed the presence of terpenes and steroids in petroleum ether extract, alkaloids and flavonoids in ethyl acetate extract while methanol extract showed the presence of tannins, flavonoids and glycosides. The extracts of *Annona reticulata* bark showed significant analgesic activity at a dose of 100 mg/kg. Among all the extract, petroleum extract showed highest increase in reaction time. Potency increases from ethyl acetate, methanol and petroleum ether extracts (Table 1). The analgesia induced by the petroleum ether extract was highest between 60 to 120 min. In the animals treated with petro-

Table 1. Effect of various extracts of *Annona reticulata* Linn. bark on thermic stimulus-induced pain in mice (Hot plate test) in mice.

Treatment	Latency to lick the paws (Sec) $\pm$ S.E.M.						
	Pre drug reaction time	30 min	60 min	90 min	120 min	150 min	180min
Control	12.45 $\pm$ 0.5	13.8 $\pm$ 0.88	14.0 $\pm$ 0.44	13.8 $\pm$ 0.37	12.8 $\pm$ 0.20	11.57 $\pm$ 0.58	11.78 $\pm$ 0.6
Pentazocin (20 mg/kg)	10.96 $\pm$ 0.34	9.58 $\pm$ 1.07*	17.23 $\pm$ 0.34*	16.64 $\pm$ 0.58*	16.96 $\pm$ 0.78*	10.71 $\pm$ 0.32	8.57 $\pm$ 0.43
PE (100 mg/kg)	8.35 $\pm$ 1.02	16.26 $\pm$ 0.99	20 $\pm$ 0.18*	20 $\pm$ 0.94*	20 $\pm$ 0.26*	13.67 $\pm$ 0.40*	10.74 $\pm$ 0.20
EA (100 mg/kg)	8.92 $\pm$ 1.2	19.42 $\pm$ 0.95*	18.54 $\pm$ 0.95*	15.78 $\pm$ 0.24*	15.5 $\pm$ 0.73*	14.84 $\pm$ 0.24*	11.55 $\pm$ 0.06
ME (100 mg/kg)	9.45 $\pm$ 0.94	19.04 $\pm$ 0.5*	20 $\pm$ 0.29*	18.36 $\pm$ 0.35*	15.86 $\pm$ 0.44*	11.79 $\pm$ 0.16*	8.19 $\pm$ 0.28

Values are mean  $\pm$  S.E.M., n = 6, P<0.05, significant\* compared to control.

PE – Petroleum ether extract. EA – Ethyl acetate extract. ME – Methanol extract.

leum ether extract, reduction in the locomotor activity scores was more significant than that of standard diazepam and other extracts at a dose of 100 mg/kg (Table 2). Results of effect on pentobarbitone sodium induced narcosis showed that petroleum ether extract potentiated pentobarbitone sodium induced sleeping time up to 215.34% when compared with control, while the ethyl acetate (144.18%) and methanol (56.58%) showed less potentiated pentobarbitone sodium induced sleeping (Table 3).

## Discussion

In vivo methods using intact animals are considered to be the best method for investigating the action of drug on the CNS. In hot plate test, the reaction time of test groups increased to a significant level. In petroleum ether extract peak effect was observed at 60 mi-

Table 2. Effect of various extracts of bark of *Annona reticulata* Linn. on locomotor activity in mice.

Treatment	Locomotor readings $\pm$ S.E.M					
	Pre drug reaction time	30min	60min	90min	120min	180min
Control	122.75 $\pm$ 0.98	119.76 $\pm$ 0.38	121.67 $\pm$ 0.63	118 $\pm$ 0.61	123.76 $\pm$ 0.4	120 $\pm$ 0.54
Diazepam (2 mg/kg)	102 $\pm$ 0.56*	95.75 $\pm$ 0.52*	90.35 $\pm$ 0.73*	85.64 $\pm$ 0.23*	84.5 $\pm$ 0.48*	99.67 $\pm$ 0.51*
PE (100 mg/kg)	105 $\pm$ 0.83*	73 $\pm$ 0.49*	60.53 $\pm$ 0.39*	45.26 $\pm$ 0.72*	63.5 $\pm$ 0.72*	93.34 $\pm$ 0.29*
EA (100 mg/kg)	107.25 $\pm$ 0.48*	81.75 $\pm$ 0.57*	74.57 $\pm$ 0.48*	55.25 $\pm$ 0.74*	68.92 $\pm$ 0.49*	101.87 $\pm$ 0.32*
ME (100 mg/kg)	96.5 $\pm$ 0.72*	80 $\pm$ 0.94*	65.23 $\pm$ 0.62*	55.83 $\pm$ 0.34*	71.59 $\pm$ 0.31*	92.45 $\pm$ 0.45*

Values are mean  $\pm$  S.E.M., n = 6, P<0.05, significant\* compared to control.

PE – Petroleum ether extract. EA – Ethyl acetate extract. ME – Methanol extract

Table 3. Effect of various extracts of bark of *Annona reticulata* Linn. on pentobarbitone sleeping time.

Treatment	Sleeping Time (min)	Onset of action	% effect	% potentiation in sleep
Pentobarbitone sodium (40 mg/kg)	43±1.756	14±0.63	100	100
PE (100 mg/kg)	92.6±0.654**	9.6±0.94*	68.57	215.34
EA (100 mg/kg)	62± 0.674**	9±1.25*	64.29	144.18
ME (100 mg/kg)	24.33±1.064**	37±0.98**	264.28	56.58

Values are mean ± S.E.M., n = 6, P<0.05, significant\*

PE – Petroleum ether extract. EA – Ethyl acetate extract. ME – Methanol extract.

minutes intervals (20±0.18). Ethyl acetate extract induces maximum analgesia at 30 minutes (19.42±0.95), while methanol extract showed increased reaction time at 60 minutes (19.04±0.5) the effect of all the extracts persisted up to 120 minutes. Result of hot plate test is suggestive of strong analgesic effect in all extracts most probably of the opioid type as the positive effect against the thermal nociceptive stimuli are indicative of opioid type analgesic effect (Turner, 1965).

Decrease in sleeping latency and increase in sleeping time are classically related to central nervous system depressant drugs (Williamson *et al.*, 1996). Like many other centrally acting drugs, barbiturates work on the cerebral cortex and thus produced their actions (Bowman & Rand, 1980). Pentobarbital, a barbiturates class hypnotic drug by an allosteric modification of GABA<sub>A</sub> receptor increases the chloride conductance and potentiates GABA<sub>A</sub> mediated postsynaptic inhibitors (Katzung, 2004). The results of pentobarbital sleeping time test showed petroleum ether, ethyl acetate and methanol extracts significantly increases the sleeping time when compared with the control while petroleum ether extract significantly decreased the locomotor activity as shown by the results of locomotor activity assay. The locomotor activity lowering effect was evident at 30 min and continued up to 180 min. The significant CNS depressant activity of all the extracts is probably due to increase in concentration of GABA in brain.

Central depressant activity along with strong analgesic effect may complement to each other and thus, may be used in variety of painful and excitatory conditions. From the above studies, it is quit apparent that all the extracts possess significant analgesic and CNS depressant activity. The study justifies its use in painful conditions in the traditional medicine.

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