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Antinociceptive activity of selected Cadaba species

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ABSTRACT

The present study was designed to investigate the antinociceptive activity of two species of Cadaba leaf extract using central and peripheral pain model viz., tail flick, tail immersion, hot plate method and acetic acid-induced writhing method (thermal and chemical stimuli). Alcohol and aqueous extract of Cadaba fruticosa and Cadaba trifoliata was administered orally at doses of 100 and 300 mg/kg to Swiss albino mice and Wistar rats. It was evident that 100mg and 300mg of aqueous and alcohol extract of C.trifoliata exhibited statistically significant antinociceptive activity against thermal stimuli. The effect was more profound in the heat-induced pain model than chemical-induced. This finding substantiates the traditional claim mentioned in the classical texts.

Keywords— *Analgesic, Antinociceptive action, Cadaba trifoliata, Cadaba fruticosa, Hot plate and tail clip method*

1. INTRODUCTION

Cadaba fruticosa and C.trifoliata belonging to the family Capparaceae found in Peninsular India, Africa and Australia, Saudi Arabia. They are erect, divaricately branched herb frequently growing in semi aquatic environment in the rice field and near the seacoast. About 3 species of *Cadaba* have been reported (Gamble, 1987), out of which two species are represented in South India (Hooker 1872). They are *Cadaba fruticosa* (L.) Druce and *Cadaba trifoliata* (Roxb). Wt. & Arn. These two are commonly known weeds. *Cadaba fruticosa* or Viluthi is one of the ingredients in Siddha formulations namely Mandoorathi adaikudineer, Veezhi ennai, Karpavyathikku ennai and Peenisankatku thailam (Anonymous 2009) also Maraviluthi (*Cadaba trifoliata*) is used as its substitute. These plants have been selected since their uses are reported in the literature for number of ailments. The scientific evaluation is not available for both plants. The medicinal attributes of *Cadaba trifoliata* are well established (Yoganarasimman 1996). Traditionally *Cadaba species* are used as anthelmintic, antipyretic, antiphlogistic and in rheumatism (Mitra, 1985; Iyengar 1976). So far, no scientific reports are available to substantiate these uses. Hence Antinociceptive activity of Cadaba species is evaluated in the present study.

2. MATERIALS AND METHODS

The leaves of *Cadaba fruticosa* and *Cadaba trifoliata* were collected from the local areas of Tamil Nadu, India and specimens were identified and authenticated with the help of available literature and confirmed by a botanist. The voucher specimens are deposited in the Department of Pharmacognosy, K.K. College of Pharmacy, Chennai (KKCP013 and 014). The collected material was shade dried, finely powdered (sieve no.40) and 100g of powder exhaustively extracted with alcohol in separate soxhlet apparatus. The extract was evaporated to dryness under reduced pressure (40° c). The aqueous extract was prepared by macerating 100 g of powder with 300 ml of water and 2% chloroform for 3 days. It was then evaporated to dryness. Albino rats of Wistar strain weighing between 150-180g and Swiss albino mice weighing between 18-25g of either sex were used and maintained at 25 ± 2°c. They were kept in a well-ventilated animal house under the natural photoperiodic condition in large polypropylene cages and were fed with standard rodent diet and water *ad libitum*. The animal experiment was approved by institutional Animal Ethical Committee.

2.1 Tail flick test

The tail flick test was used to calculate analgesic activity by the method defined by D'amour and Smith 1941, with minor alterations. The tail flick method was utilized to study the antinociceptive activity in mice. A radiant heat automatic tail flick analgesiometer was applied to measure reaction latencies. The basal reaction time of animals to radiant heat was recorded by locating the tip (last 1-2 cm) of the tail on radiant heat source. The tail removal from the radiant warmth was taken as end point. The cutoff time of 15 seconds was used to avoid tail injury by heat. Mice were divided into 10 groups of 6 each. Alcohol and aqueous extract of *C.fruticosa* and *C. trifoliata* were administered 100 and 300 mg/kg body weight perorally in groups 2 to 9. Group 1 served as vehicle control. Group 10 treated with morphine (10 mg/kg) intraperitonealy. The latent period of the tail-flick response was determined at 30, 45, 60, and 75 minutes after the administration of drugs.

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2.2 Tail immersion method

Wistar Rats (six per group) were randomly divided in to ten groups. Group, I animals received only 1% v/v Tween 80 solution (10 ml/kg, p.o.). Group II & IX animals received aqueous and alcohol extract of *C.fruticosa* and *C.trifoliata* 100 and 300 mg/kg, p.o., in 1% v/v Tween 80 emulsion respectively. Group X animals received Morphine (5 mg/kg, i.p.). The animals were screened for the sensitivity test by immersing 3 cm of the tail of the rat gently in hot water maintained at 55 ± 0.5 °C. Within a few secs, the rats reacted by withdrawing the tail. The reaction time was recorded with a stopwatch. Each animal served as its own control and two readings were obtained for the control at 0 and 10 min interval. The average of the two values was taken as the initial reaction time. After 30 min, tail withdrawal time of each group animals was noted and the % protection of analgesia was calculated by using the formula:

$$C\text{-}T \div C \times 100$$

Where 'C' represents the tail withdrawal (in a sec) of control and 'T' to that of treated groups.

2.3 Hot plate method

Albino rats were divided into 10 groups each consisting of six animals. One group served as vehicle control, second to ninth group received 100 and 300 mg/kg of alcohol and aqueous extract of *C. fruticosa* and *C. trifoliate* orally. The tenth group treated with 10mg/kg b.w. of Morphine as standard drug. After 30 min. of treatment rats were placed on a hot plate (55°c) and the time interval between the placement of the animals and the occurrence of licking or shaking the hind paws was recorded as reaction time. The cut off time was set as 20 seconds (Eddy NB, 1953).

2.4 Acetic Acid Writhing Test

The acetic acid writhing test of *C.fruticosa* and *C.trifoliata* was carried out as per the method described by Koster, 1959. In this method, Mice were used in groups of 6 per dose of plant extracts, standard drug paracetamol or DMSO. They were placed singly in a transparent perspex mouse cage and allowed to acclimatize to their environment for a 30 min period prior to the commencement of the experiment. In the control experiment, the animals were pretreated with 0.25mL of physiological saline (i.p.) for 15 min and then given intraperitoneal injection of 0.20mL of 3% acetic acid solution, an irritant, used to induce writhing (pain). The mice were then left for 5min, and the writhes were counted for the next 20 min. Writhe is defined as contraction of the abdominal muscles accompanied by elongation of the body and the hind limbs.

In the test experiment, a group of 6 mice were pretreated for 15min with either the plant extract orally or the standard analgesic drug paracetamol (i.p.), after which they were injected with 0.20mL of the 3% acetic acid intraperitoneally, allowed to wait for 5 min and then the number of writhes counted for 20 min as for the control experiment. The experiment was repeated with another group of 6 mice pretreated with 0.25mL of DMSO solution (i.p.) for 15 min, after which they were injected with 0.20mL of the 3% acetic acid intraperitoneally, allowed to wait for 5 min, and then the number of writhes counted for 20min. All experiments were performed in a quite laboratory with an ambient temperature of 22 ± 3°C. The ability of the plant extract to prevent or significantly reduce the number of acetic acid-induced writhes was an indication of an antinociceptive activity.

3. STATISTICAL ANALYSIS

Data were subjected to statistical analyis using ANOVA and statistical comparison was done using Tukey Kramer multiple comparison test. Values of $p<0.01$ were considered statistically significant.

4. RESULTS AND DISCUSSION

Analgesic effect of alcohol and aqueous extracts of ***C. fruticosa*** and ***C. trifoliata*** on mice was performed by tail flick, tail immersion, hot plate, and acetic acid induced writhing methods and the results are presented in Table-1 – 4 respectively.

Table 1: Analgesic effect by tail flick method

Groups	Time in seconds @30min	Time in seconds @45min	Time in seconds @60min	Time in seconds @90min
Control	**1.83 ± 0.30**	**1.93 ± 0.10**	**1.63 ± 0.21**	**2.03 ± 0.20**
100mg alc. *C.trifoliata*	**7.03 ± 0.49 [b]**	**8.33 ± 0.49 [b]**	**8.93 ± 0.49 [b]**	**9.33 ± 0.49 [b]**
300mg alc. *C.trifoliata*	**7 ± 0.68 [b]**	**7.4 ± 0.28 [b]**	**8.5 ± 0.48 [b]**	**9.3 ± 0.68 [b]**
100mg aq. *C.trifoliata*	**7.1 ± 0.70 [b]**	**7.7 ± 0.20 [b]**	**8.1 ± 0.20 [b]**	**9.1 ± 0.33 [b]**
300mg aq. *C.trifoliata*	**5.6 ± 0.49 [a]**	**6.6 ± 0.39 [a]**	**7.6 ± 0.42 [a]**	**9.6 ± 0.46 [a]**
100mg alc. *C.fruticosa*	**5.5 ± 0.7**	**6.5 ± 0.7**	**6.9 ± 0.17a**	**7.2 ± 0.1**
300mg alc. *C.fruticosa*	**6.3 ± 0.4 [b]**	**6.8 ± 0.74**	**7.3 ± 0.4 [b]**	**7.93 ± 0.54**
100mg aq. *C.fruticosa*	**5 ± 0.5**	**5.5 ± 0.52**	**6.5 ± 0.5**	**6.0 ± 0.35**
300mg *a*lc. *C.fruticosa*	**3.5 ± 0.7**	**3.5 ± 0.7**	**3.5 ± 0.7**	**3.5 ± 0.7**
Morphine 10mg/kg by i.p	**8.5 ± 0.22**	**9.5 ± 0.82**	**9.85 ± 0.32**	**9.85 ± 0.12**

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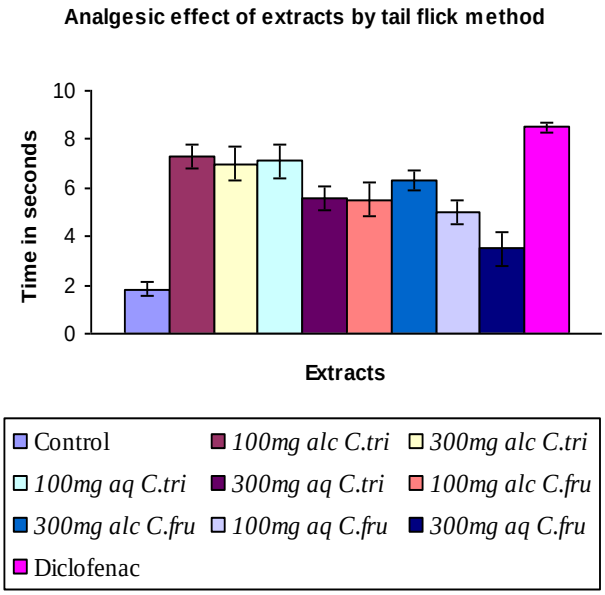

Fig. 1: Analegsic effect of extracts by tail flick method

Values are mean ± SEM for 6 repetitive experiments. p values are expressed as [a] p > 0.05 and [b] p > 0.01 compared with control group.

Alcohol extract (100 and 300 mg/kg), 100 mg aqueous extract of ***C. trifoliata*** and 300 mg of alcohol extract of ***C. fruticosa*** exhibited significant analgesic effect by tail clip method when compared to that of standard diclofenac. In general, alcohol and aqueous extracts of test plants treated animals responded for the thermal stimuli.

Table 2: Tail immersion

Groups	Time in seconds
Control	1.83 ± 0.30
100mg alc. ***C.trifoliata***	7.33 ± 0.49 [b]
300mg alc. ***C.trifoliata***	7 ± 0.68 [b]
100mg aq. ***C.trifoliata***	7.1 ± 0.70 [b]
300mg aq. ***C.trifoliata***	5.6 ± 0.49 [a]
100mg alc. ***C.fruticosa***	5.5 ± 0.7 a
300mg alc. ***C.fruticosa***	6.3 ± 0.4 [b]
100mg aq. ***C.fruticosa***	5 ± 0.5 [a]
300mg alc. ***C.fruticosa***	3.5 ± 0.7
Diclofenac	8.5 ± 0.22

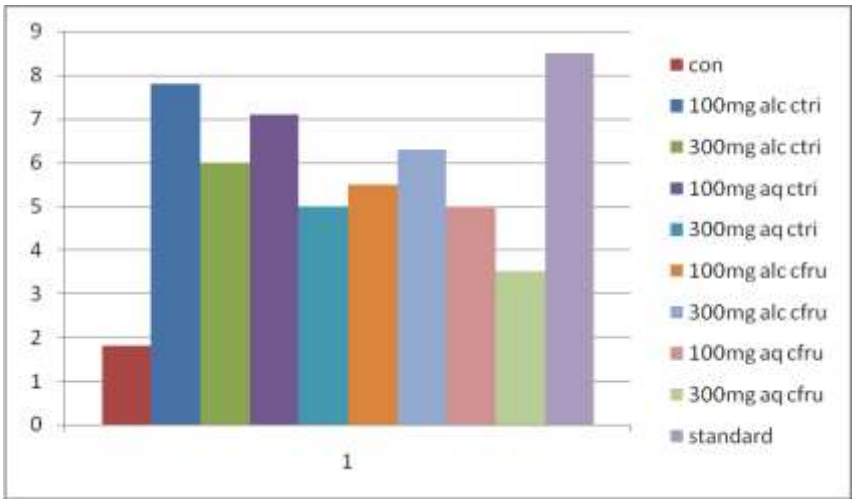

Fig. 2: Analgesic effect of extracts by tail immersion method

Values are mean ± SEM for 6 repetitive experiments. p values are expressed as [a] p > 0.05 and [b] p > 0.01 compared with control group.

Alcohol extracts of C.trifoliata at 100mg and 300mg dose levels, as well as 300mg aqueous extract, exhibited significant analgesic effect by tail immersion method. The produced analgesic effect was comparable with that of the standard Diclofenac.

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Table 3: Hot plate

Groups	Latency Time in seconds
Control	6.1 ± 0.4
100mg alc. ***C.trifoliata***	5.0 ± 0.5
300mg alc. ***C.trifoliata***	7.8 ± 0.4[b]
100mg aq. ***C.trifoliata***	3.5 ± 0.7
300mg aq. ***C.trifoliata***	5.8 ± 1.1
100mg alc. ***C.fruticosa***	6.6 ± 0.8
300mg alc. ***C.fruticosa***	8.5 ± 0.4[b]
100mg aq. ***C.fruticosa***	7.1 ± 0.4[a]
300mg aq. ***C.fruticosa***	8.3 ± 0.6[b]
Aspirin	11 ± 1.3

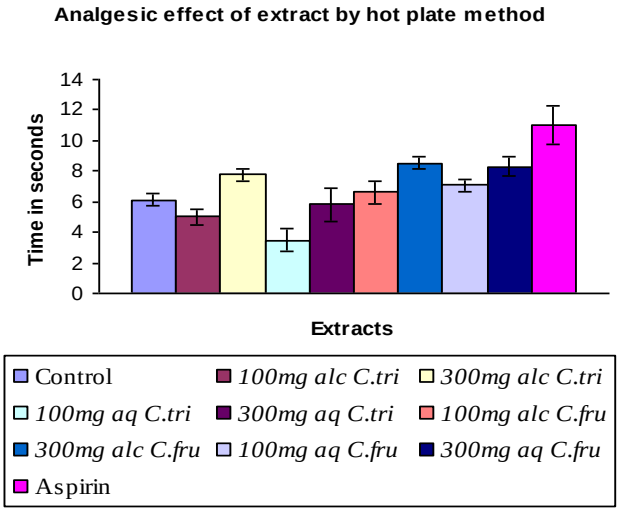

Fig. 3: Analgesic effect by hot plate method

Values are mean ± SEM for 6 repetitive experiments. p values are expressed as [a] p > 0.05 and [b] p > 0.01 compared with control group.

Alcohol extract of ***C. trifoliata***, aqueous and alcohol extract of ***C. fruticosa*** showed the significant analgesic effect at 300 mg/kg body weight that was comparable to the reference standard aspirin. Alcohol and aqueous extracts of both plants exhibited dose dependent analgesic effect by hot plate method.

Table 4: Acetic acid induced writhing method

Groups	No. of writhes	% Inhibition
Control	46.1 ± 0.4	-
100mg alc. ***C.trifoliata***	25.0 ± 0.5 [b]	46
300mg alc. ***C.trifoliata***	17.8 ± 0.4[a]	61
100mg aq. ***C.trifoliata***	33.5 ± 0.7	27
300mg aq. ***C.trifoliata***	25.8 ± 1.1 [b]	44
100mg alc. ***C.fruticosa***	26.6 ± 0.8	42
300mg alc. ***C.fruticosa***	28.5 ± 0.4[b]	39
100mg aq. ***C.fruticosa***	37.1 ± 0.4	19
300mg aq. ***C.fruticosa***	28.3 ± 0.6	39
Aspirin	11 ± 1.3	76

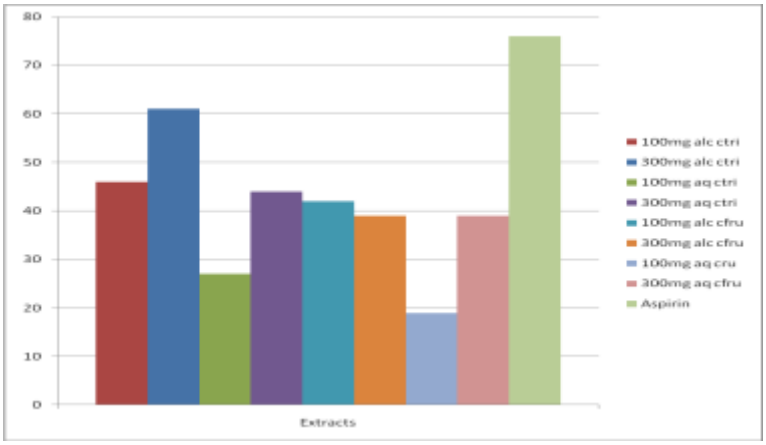

Fig. 4: Acetic acid induced writhing method

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Alcohol extract of *C.trifoliata* at 300 mg/kg produced a reduction in the no. of writhes that was comparable with the standard aspirin. Also 300 mg of aqueous extract of *C.trifoliata*, 100 mg of *C.fruticosa*, 300 mg of alcohol and aqueous extracts of *C.fruticosa* produced feeble activity in acetic acid induced writhing test.

Analgesics are drugs acting either peripherally or centrally to alleviate the pain. Peripheral acting analgesics act by inhibiting the generation of impulses at chemoreceptor site of pain (Tripathi, 2004). Centrally acting analgesics act by raising the threshold for pain and alter the physiological response to pain (Sridhar *et al.,* 2009, Wigdor & Wilcox 1987). In the present investigation, thermal and chemical stimuli are applied and the methods adopted were Tail flick, Tail immersion, Eddy's hot plate for thermal stimuli and acetic acid induced writhing for chemical stimuli. It was evident that 100mg and 300mg of aqueous and alcohol extract of *C.trifoliata* exhibited statistically significant antinociceptive activity against thermal stimuli. The effect was more profound in heat-induced pain models. It is believed that hot plate method demonstrates the supraspinal reflex mediated by μ1 and μ2 opioid receptors whereas the tail immersion test monitors the spinal reflex involving μ2 and δ opioid receptors (Arslan and Bektas, 2010). These methods are, therefore, useful for screening molecules effective in spinal and supraspinal region. The increase in latency in these two tests by alcohol extract (100mg and 300mg) of *C.trifoliata* and 300 mg of *C.fruticosa* indicates the modulation of central nervous system pain signaling. This hypothesis is further supported by the antinociception of *C.trifoliata* and *C.fruticosa* at tested doses by tail flick method. Upon chemical stimulation (acetic acid induced writhing test) method, animals treated with 300 mg/kg of alcohol extract of *C.trifoliata* reduced the number of writhes that indicate the plant extracts respond for the chemical stimuli also.

The major bioactive components of most medicinal plants include flavonoids, phenolic compounds, tannins, alkaloids, and saponins. The aqueous extract of the leaves of *C. fruticosa* had shown to contain terpenoids, flavonoids, proteins, and furals while the alcoholic extract has shown to contain terpenoids, flavonoids, steroids, phenols, alkaloids, gums, sugars and saponins (Gopalaiah *et al.,* 2001). Preliminary studies showed that the presence of Tannins, Steroids, Alkaloids, Glycosides, Flavonoids and Phenolic compounds (Velmurugan *et al*., 2010). The main important compound phytol (C20H40O) and a diterpene compound contain activity such as anti-cancer, anti-diabetic, anti-inflammatory, anti-oxidant activity and antimicrobial.

These findings indicate that the antinociceptive activity of *C.trifoliata* and *C.fruticosa* is mediated through the central and peripheral mechanisms. The presence of common phytoconstituents such as alkaloids, flavonoids, glycosides, tannins, and phenolic compounds may be responsible for the observed antinociceptic activity. It may be further postulated that the observed activity is due to either single phytoconstituent or synergistic effect of multiple constituents.

5. CONCLUSION

It is concluded that the alcohol extract of *C.trifoliata* and alcohol extract of *C.fruticosa* exhibited antinociceptive activity on thermal and chemical stimuli induced animals. The probable mechanism of action of the extracts may be spinal and supraspinal reflux mediated by μ1, μ2 and δ opioid receptors. This action is contributed due to the presence of either one of the phytoconstituent or synergistic effect of many of the phytoconstituents present in the extracts.

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