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Research Article

ANALGESIC AND ANTI-INFLAMMATORY EFFECTS OF *MERREMIA TRIDENTATA* (L.) HALLIER F.

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ABSTRACT

The study was aimed to investigate the root extracts of *Merremia tridentata* for the *in vivo* analgesic and anti-inflammatory activity. The phenolics and Flavonoids contents were analyzed by using HPTLC in successively acetone extracted root sample. Analgesic and anti-inflammatory activities of these extracts were assessed in rats with hot plate test, writhing test in mice, carrageenan induced paw oedema and histamine induced paw edema in rats. The HPTLC results shows six phenolic compounds and two flavonoid compounds. In acute toxicity study showed the extract did not alter the general behavior and failed produce any mortality even at the highest dose of 1000 mg/kg, p.o. after 3 days and found to be safe. The study highlighted the analgesic and anti-inflammatory of *M.tridentata* root. The present study on extract of *M. tridentata* has demonstrated that this plant has significant analgesic and anti-inflammatory properties, and it justifies the traditional use of this plant in the treatment of various types of pains and inflammation.

Keywords: *Merremia tridentate,* Analgesic, Anti-inflammatory, HPTLC.

INTRODUCTION

Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses [1]. Although it is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases [2]. Currently used anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary.

Merremia tridentate (L.) Hallier f., belonging to the family Convolvulaceae, grows naturally as a perennial, spreading herb with thick root stock, and is distributed throughout India on hedges and open waste lands [3]. Traditionally, the plant is used in piles, swellings, rheumatic affections, stiffness of the joints, hemiplegia, urinary infections, and general debility apart from being a good laxative and astringent [4]. The previous studies conducted on *M. tridentata* have strong wound healing, anti-inflammatory and anti-arthritic activities [5, 6]. *M. tridentata* is also used as a supplementary feed to the grass Panicum maximum for young West African Dwarf Sheep [7]. The aerial parts of the *M. tridentata* contain flavonoids, diosmetin, luteolin, and their 7-O-β-D-glucosides [4]. The acetone extract of root possess high phenolic contents and rich potential of antioxidant activity [8]. In order to understand the highly acclaimed properties of *M. tridentata* and its usage in the traditional systems of medicine, we have attempted to evaluate its analgesic and anti-inflammatory potential.

MATERIAL AND METHODS

Preparation of extracts

Merremia tridentata as collected from Coimbatore, Tamil Nadu State, India during the month of November, 2008, authenticated and deposited in the Botany Herbarium, Bharathiar University with voucher number BUBH-2895. The freshly collected plant roots were washed thoroughly in tap water, shade dried at room temperature (25°C) powdered, and used for solvent extraction. The root samples were successively extracted with petroleum ether (for disposing lipid and pigments) chloroform, acetone, and methanol using Soxhlet apparatus and the air dried residue by the method of maceration for 24 hr. Each time before extracting with the next solvent, the material was dried in hot air oven at 40ºC. The solvents were evaporated using a rotary vacuum-evaporator (RE300; Yamato, Tokyo, Japan) at 50ºC and the remaining water was removed by lyophilization (4KBTXL-75; VirTis Benchtop K, New York, NY, USA). The extract recovery in different solvents was expressed as percent of the root sample dry matter. The freeze-dried extracts thus obtained were dissolved in the respective solvents at the concentration of 1 mg/mL and used for assessment of analgesic and anti-inflammatory through various animal model assays.

Detection of phenolic and flavonoid compounds by using High performance thin layer chromatography (HPTLC) (Egonstahl, 1990) [9]

The given extract sample (50 mg) was dissolved in 1ml acetone and centrifuged. The supernatant was used as test solution for HPTLC analysis to identify phenolic compounds and flavonoids. 3µl of the above solution were loaded in the 5 x 10 Silica gel $60F_{254}$ TLC plate using (Hamilton syringe and LINOMAT 5 instrument) to load the sample. The sample loaded TLC plate was kept in TLC twin trough developing chamber with respective mobile phase for phenolic compounds and flavonoids for chamber saturation up to 20min. The plate was developed in the chamber using respective mobile phase up to 80mm distance. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber and the images were captured at white light, UV 254nm and UV366nm. The plate was sprayed with respective spray reagents for phenolic compounds and flavonoids and dried at 120°C in a hot air oven for 5min. The plate was photo-documented at white light for phenolic compounds and UV 366nm for flavonoids using Photo-documentation chamber. Finally, the plate was fixed in scanner stage and scanning was done at 500nm for phenolic compound and 366nm for flavonoids. The Peak table and densitogram were noted. Mobile phase (For phenolic compounds and flavonoids) Ethyl acetate-Methanol-Water (10: 1.65: 1.35). Spray reagents for phenolic compounds Applied 20% aqueous sodium carbonate solution over the plate followed with 25% aqueous Folin cio-calteu reagent after brisk dry. The plate was dried at 120 C for 5min. For flavonoids Applied 1% ethanolic aluminium chloride solution over the plate and dried at 120 C for 5min. Detection of compounds **f**or phenolic compounds **b**lue colored zone was present in the track at white light in the chromatogram after derivatization, which indicates that phenolic compounds may be present in the given sample extract. For flavonoids **y**ellow fluorescence zone was present in the track at UV366nm in the chromatogram after derivatization, which indicates that flavonoid class compounds may be present in the given samples extract.

Ethics

The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee, and was cleared by same before beginning the experiment (No. KMCRET/M.Sc./1/2009-10)

Animals

Swiss albino male mice weighing 20 – 25 g were used for the study. Animals were fed with standard pellet (Pranav Agro Industries Ltd.,

Sangli) and water ad libitum and maintained at 24 – 28°C temperature, 60 – 70% relative humidity and 12 h day and night cycle. Animals described as fasted were deprived of food for 4 days, but had free access to water.

Acute toxicity

Acute oral toxicity studies were performed Ecobichon[10] according to OECD (organization for economic co-operation and department). Swiss albino male mice (n=6/each dose) selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only. Acetone extract of *M. tridentata* (suspended in 0.5% carboxy methyl cellulose were administered orally at a dose of 5mg/kg initially to separate groups of mice and mortality was observed for 3 days. If mortality was observed in 4/6 or 6/6 animals, then the dose administered was considered as toxic dose.

However, if the mortality was observed in only one mouse out of six animals, then the same dose was repeated with higher doses such as 50, 300 and 1000 mg/kg. The general behaviors such as motor activity, tremors, convulsions, straub reaction, aggressiveness, piloerection, loss of lighting reflex, sedation, muscle relaxation, hypnosis, analgesia, ptosis, lacrimation, diarrhea and skin colour were observed for the first one hour and after 24 h of test drug administration.

Analgesic effect in the hot plate test

The hot plate assay method was employed for the purpose of preferential assessment of possible centrally mediated analgesic effects of acetone extract of *M. tridentata* roots by the method of MacDonald *et al*.[11]. This was adapted for mice. For the experiments, four groups (n=6) of swiss albino mice (20–25g) were placed on a plate maintained at room temperature for 15 min. Food was withdrawn on the preceding night of the experiment.

The experimental model included the following groups. Group 1: normal control fed with 0.5% carboxyl methyl cellulose (vehicle)10 ml/kg p. o., and Group 2: treated with pentazocine (30mg/kg, i. p.) whereas Groups 3 and 4 animals received acetone extract of *M. tridentata* (100 and 200 mg/kg, p. o.). Each animal was then individually placed gently on Eddy's hot plate at 55°C. Latency to exhibit antinociceptive responses, such as licking paws or jumping off the hot plate, was determined 15, 30, 45, 60, and 90 min after administration of the test substances or vehicle[11].

Writhing test in mice

To study the analgesic effect of acetone extract of *M. tridentata* roots against acetic acid-induced writhing in mice by Seigmund *et al.* [12]. Swiss mice weighing 20-25g were divided into three groups each consisting of six animals. Administer 1mL / 100 g body weight of acetic acid solution to the first group (which serves as control) place them individually under glass jar for observation. Note the onset on wriths.

Record the number of abdominal contraction, trunk twist response and extension of hind limb as well as the number of animals showing such response during a period of 10 min. To the second group of animals inject acetyl salicyclic acid (10mg/kg) and to the third group inject plant extract (100mg/kg) 15 minute later administer acetic acid solution to these animals. Note the onset and severity of writhing response. Calculate the mean writhing score in control, acetyl salicyclic acid and plant extract treated groups. Note the inhibition of pain response by acetyl salicyclic acid and plant extract.

Anti-inflammatory activity on carrageenan-induced paw oedema

For the experiments, the male rats (120-150g) were divided into four groups (n = 6). Acute inflammation was produced by the sub plantar administration of 0.1 ml of 1% Carrageenin (in 0.5 % CMC w/v) in the right hind paw of the rats[13]. The paw volume was measured at 0 min, 30 min, 60 min, 120 min and 240 min after Carrageenin injection. The first group received only 0.5% CMC (10ml/ kg p.o.), while the second group received indomethacin (8mg/kg p.o). The third and the fourth groups were treated with the acetone extract of *M. tridentata* (100 and 200 mg/ kg p.o. respectively). The animals were pretreated with the said drugs 1 hour before the administration of Carrageenin.

Histamine induced acute paw edema in rats

To study the anti-inflammatory property of acetone extract of *M. tridentata* roots against histamine induced acute paw edema in rats[14, 15]. Male albino rats (125-150g) were housed in three groups. They were fasted overnight but had free access to water. Make a mark on both the hind paws just beyond tibiotarsal junction, so that every time the paw is dipped in the mercury column up to the fixed mark to ensure constant paw volume. Note the initial paw volume of each rat by mercury displacement method.

The animals are divided into three groups each comprising six rats namely:

Group I: Injection of saline

Group II: Injection of Indomethacin intraperitoneally

Group III: Injection of Plant extracts intraperitoneally

After 30 minutes, inject 0.1 ml of 1% (w/v) histamine in the planter region of the left paw of control as well as plant extract treated group. The right paw will serve as reference non-inflamed paw for comparison. Note the paw volume of both legs of control and plant extract treated rats at 15, 30, 60 and 120 min after histamine injection. Note the reduction in the paw volume.

Statistical analysis

Values were expressed as mean ± S.E.M. Statistical significance for analgesic activity was calculated using a one-way analysis of variance (ANOVA). Significant differences between means were determined by Duncan's multiple-range tests. Values of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

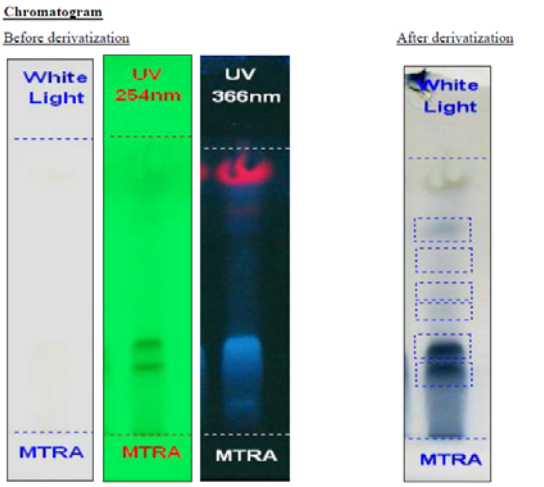
HPTLC

Crude acetone extract of *M. tridentata* roots may contains thousands of chemical compounds. To establish a better characterization of acetone extract, HPTLC was carried out. HPTLC analysis revealed six phenolic compounds present in the acetone extract.

(Showed maximum antioxidant potential) of root Fig1 and 2. Furthermore, two flavonoids were also detected Fig 3 and 4. The beneficial effects of flavonoids and phenols on human health are principally related to their antioxidant activity, which protects the human body from free radicals and retards the progress of many chronic diseases [16]. Moreover, many biological functions such as antimutagenicity, anti carcinogenicity and anti aging, among others, originate from this property [17]. Therefore, the higher phenolic content of acetone extract of *M. tridentata* roots might be responsible for the enhanced antioxidant activities compared with the other extracts. These findings may lead to an increasing demand for *M. tridentata* extract for use as a dietary supplement or as a functional ingredient in nutraceutical and pharmaceutical products.

Acute toxicity

The acetone extract of roots of *M. tridentata* was evaluated for its acute toxicity in mice. The extract did not alter the general behavior and failed produce any mortality even at the highest dose of 1000 mg/kg, p.o. after 3 days and found to be safe.



Peak table

Track	Peak	Rf	Height	Area	Assigned substance
MTRA	1	0.26	316.2	16845.3	Phenolic compound 1
MTRA	2	0.32	357.7	15785.5	Phenolic compound 2
MTRA	3	0.46	30.3	701.6	Phenolic compound 3
MTRA	4	0.5	23.7	734.3	Phenolic compound 4
MTRA	5	0.62	16.8	671.7	Phenolic compound 5
MTRA	6	0.73	96.2	3974.9	Phenolic compound 6

Fig. 1: HPTLC analysis of acetone extract of *M. tridentata* roots showing phenolic compound profile

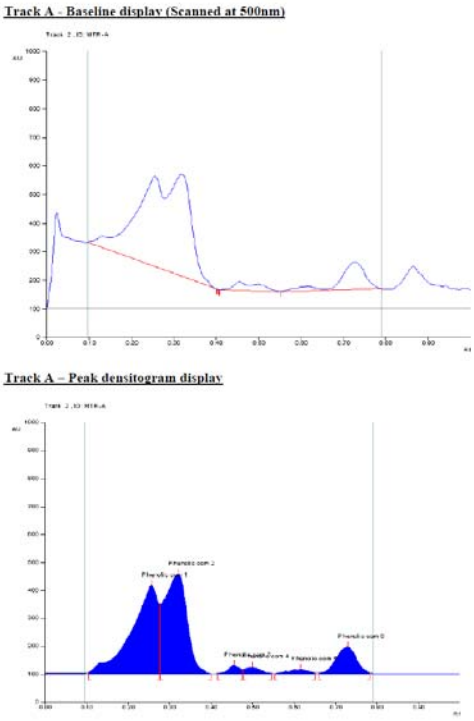
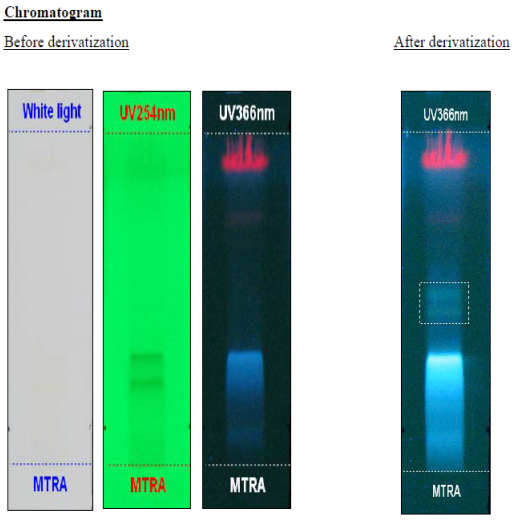


Fig. 2: Densitogram of phenolic compounds of acetone extract of *M. tridentata* roots.



Peak table

Track	Peak	Rf	Height	Area	Assigned substance
MTRA	1	0.47	16.2	618.4	Flavonoid 1
MTRA	2	0.52	24.4	1356.1	Flavonoid 2

Fig. 3: HPTLC analysis of acetone extract of *M. tridentata* roots showing flavonoid profile

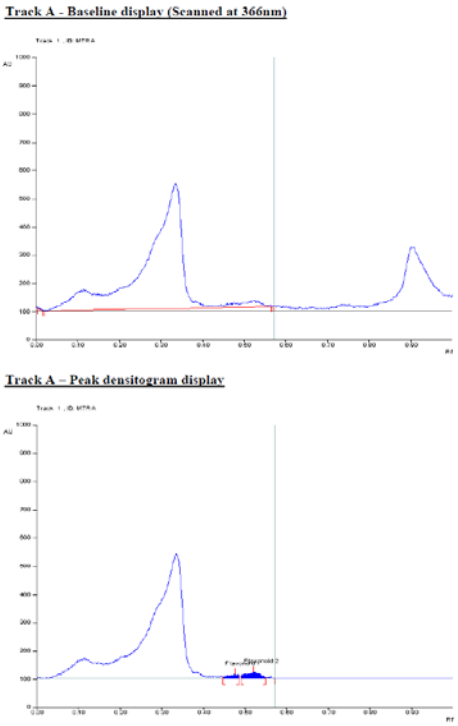


Fig. 4: Densitogram of flavonoid compounds of extract of *M. tridentata* roots

Analgesic activity

Hot plate test in mice

The analgesic effect of acetone extract of *M. tridentata* using hot plate test in mice is presented in Fig 5. The results indicate that oral administration of the extract (200 mg/kg) significantly attenuated the hot-plate thermal stimulation. Analgesic activity of the extract was comparable with the standard drug pentazocine (30 mg/kg). Among the two doses tested, 200mg/kg they showed highest analgesic activity at reaction time 60min (8.0 ± 0.3) which was higher than even the standard drug pentazocine (6.3 ± 0.8). This effect started 15min after treatment and persisted throughout the 90min duration of the experiment. Hot plate test is normally used to study the central analgesic effects of drugs.

Therefore, it is probable that *M. tridentata* could be producing its effects centrally. Non-steroidal anti-inflammatory drugs (NSAIDs) act in a manner that the sensitization of pain receptors by prostaglandin at the inflammation site is inhibited [18]. Several flavonoids isolated from medicinal plants have been discovered to possess significant antinociceptive and/or anti-inflammatory effects [19]. It is, therefore, possible that the analgesic effects observed with this extract may be attributable to its phenolic component, as indicated by the phytochemical analysis Table 2.

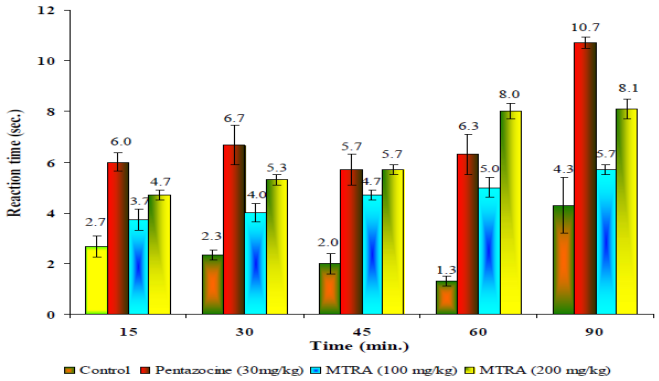


Fig. 5: Analgesic activity of acetone extract of *M. tridentata* roots on the hot plate reaction time

Writhing test in mice

The analgesic effect of acetone extract of *M.tridentata* root using writhing test in mice was presented in Table1. The results indicated that oral administration of the extract (100mg/kg) showed highest analgesic activity (43.8 ± 2.1min) with a reaction time of 20min. Analgesic activites were found in plants possessing some strerols on the models of pain induced by acetyl salicylic acid. The significant analgesic activites were also found in plants possessing some organic acids and flavonoids [20,21].

Table 1: Analgesic activity of *M. tridentata* roots by writhing test in mice

Treatment	Dose (mg/kg, p.o)	No. of writhing in 20 min
Control	-	90.33 ± 3.38
Acetyl salicylic acid	10	44.50 ± 2.20*
MTRA	100	43.83 ± 2.12*

MTRA- *Merremia tridentate* root acetone extract, Degrees of freedom 2, 15, Values are given in mean ± SEM (n=6), *P<0.01 of ANOVA followed by Dunnett test compared with control

Table 2: Anti-inflammatory effect of acetone extract of *Merremia tridentata* roots on carrageenin – induced paw edema in rats

Treatment	Dose (mg/kgb.w)	Before Inflammatiom (mm)	After treatment in inflamed rats (mm)				
			0min	30min	60min	120min	240min
Control (0.5 % vehicle)(10ml/kg)	10	3.92	5.07	5.44	5.27	5.26	5.18
Indomethacin (8 mg/kg)	8	3.60	4.60	4.48	4.30	4.17	4.12
MTRA (100mg/kg)	100	3.95	5.20	5.10	4.90	4.67	4.84
MTRA (200mg/kg)	200	3.87	5.02	4.85	4.71	4.61	4.55

MTRA- *Merremia tridentate* root acetone extract, Degrees of freedom 2, 15, Values are given in mean ± SEM (n=6), *P<0.01 of ANOVA followed by Dunnett test compared with control

Table 3: Anti-inflammatory activity of *Merremia tridentata* roots on histamine induced paw edema in rats

	Dose (mg/kg, p.o)	Rat paw edema volume at different time interval (in ml)		
		0 h	1 h	3 h
Control	-	1.330±0.056	1.485±0.060	1.819±0.060
Indomethacin	10	1.314±0.032	1.483±0.030^{ns}	1.576± 0.055**
MTRA	100	1.258±0.041	1.430±0.026^{ns}	1.523±0.052**

MTRA- *Merremia tridentate* root acetone extract, Degrees of freedom 2, 15, Values are given in mean ± SEM (n=6), **P<0.01, *P<0.05, ^{ns} – not significant of ANOVA followed by Dunnett test compared with control .

Anti-inflammatory activity

Carrageenin induced paw edema model was used for the evaluation of anti-inflammatory activity of the acetone extract of *M. tridentata* roots. There was a dose-dependent, significant reduction in carrageenin-induced rat paw edema at 100 and 200 mg/kg of extract and at 8 mg/kg indomethacin over a period of 240 min as shown in Table 2. Carrageenin induced edema is commonly used as an experimental animal model for acute inflammation and is believed to be biphasic, of which the first phase is mediated by the release of histamine and kinins and then prostaglandin in the later phase [22]. So, the effect of the acetone extract against inflammations produced by these individual mediators was studied. Flavonoids and other phenolics compounds of plant origin have been reported as antioxidants and as scavengers of free radicals [23, 24, 25]. Considering that antioxidants can also exert anti-inflammatory effects[26]. The extract effectively suppressed the inflammation produced by histamine, bradykinin, prostagladins and serotonin Table 2.

Histamine induced acute paw edema in rat

The acetone extract of *M. tridentata* root demonstrated a significant anti-inflammatory effect against Histamine-induced inflammation at a dose of 100mg/kg. The anti-inflammatory effect of the extract was significant (1.523±0.052ml) but was less than that of indomethacin (1.576± 0.055ml). The results were shown in the Table 3. The extract effectively suppressed the inflammation produced by histamine and serotonin. So it may be suggested that its anti-inflammatory activity is possibly backed by its anti-inflammatory activity is possibly backed by its anti-serotonin activity which is responsible for the same. The extract also reduced the edema produced by dextran which is known to be mediated both by histamine and serotonin [27].

CONCLUSION

The present study on extract of *M. tridentata* has demonstrated that this plant has significant analgesic and anti-inflammatory properties, and it justifies the traditional use of this plant in the treatment of various types of pains and inflammation.

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