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Research Article

EVALUATION OF ANTI INFLAMMATORY AND ANALGESIC ACTIVITY OF *TRIDAX PROCUMBENS LINN* AGAINST FORMALIN, ACETIC ACID AND CFA INDUCED PAIN MODELS

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ABSTRACT

This study was designed to evaluate the analgesic activity of *Tridax procumbens Linn.* Phytochemical screening is conducted for both aqueous and ethanolic extracts of the plant using conventional protocol, The analgesic activity is evaluated by two analgesic and one inflammatory in-vivo pain models, male C57 BL6/J mice (25-30g) and male Sprague-Dawley rats (150-230g) was selected for this study. In the formalin test, late phase of moderate pain, which starts about 20 min after formalin injection and lasts about 40 min to 60 min, appears to be caused by tissue and functional changes in the dorsal horn of the spinal cord, Administration of extract demonstrated significant inhibition in late phase Similarly, In the acetic acid-induced abdominal constriction test, T.P extract dose- dependently and significantly reduced the abdominal writhing. In CFA Induced Hyper analgesia Oral administration of T.P extract significantly reduced mechanical hyper analgesia in CFA injected rats. So, it has been observed that *Tridax procumbens* has marked beneficial effects against centrally, peripherally and inflammatory pain models. This protective action may be attributed towards the presence of flavanoid and sterol indicates that the extract of *Tridax procumbens* may be used as an effective analgesic.

Keywords: Biphasic Pain, Flavonoids, Inflammatory Pain, Peripheral Pain, Tridax procumbens.

INTRODUCTION

The clinically useful drugs against pain and inflammation exhibit many adverse effects; this leads to considerable interest in search of safer drug for these conditions. Pain is the human awareness of nociception and has been defined as "an unpleasant sensory and emotional experience associated with tissue damage or described in terms of such damage." Pain is often classified as acute or chronic. Acute pain is frequently associated with anxiety and hyperactivity of the sympathetic nervous system (e.g. tachycardia, increased respiratory rate and BP, diaphoresis, dilated pupils). Chronic pain does not involve sympathetic hyperactivity but may be associated with vegetative signs (e.g., fatigue, loss of libido, and loss of appetite) and depressed mood. People vary considerably in their tolerance for pain [1-3].

Need for a new and potent pain therapy

Though the current therapy to the relieve pain are having some limitation such as, Non steroidal Anti-inflammatory drugs are having GI irritation. Opioids are having the dependency problems. Novel therapies for pain treatment are essential to overcome the adverse effects of existing therapies for pain treatment. It is indeed a need of hour to treat the pain of specific pathologic origin such as cancer pain, neuropathy pain etc. The thirsts for new therapies to treat such painful conditions are alarming. Hence global scenario for new drug discovery is to develop new therapies to treat pain. The study of plants that have been traditionally used as pain killers should still be seen as a fruitful and logical research strategy, in the search for new analgesic drugs [4, 5]. The different pharmacological activities of *Tridax procumbens* are reported in last Few years such as radical scavenging, Wound healing activity, Anti-diabetic activity and blood pressure lowering effect, although *Tridax procumbens* has a reported use as a folklore medicine but has not established through any scientific report present study is an attempt to prove the pharmacological evaluation for analgesic activity of *Tridax procumbens* leaves in rodents. This makes me to evaluate the analgesic activity of the *Tridax procumbens* leaves [6- 9].

MATERIALS AND METHODS

Plant Material

The leaves of *Tridax procumbens* Linn. (Fig.1) was collected from Mannargudi, Tiruvarur District, Tamil Nadu, India and the authentification was done by Prof. Dr. P.Jayraman. Director of National Institute of Herbal Science, Plant Anatomy Research Center, Chennai-45. Plant Profile: *Tridax procumbens* Linn. Belongs to the family of compositae is a small herb having short, hairy blade like leaves. It is a very common weed found in open places and it has yellow corolla. It is widely distributed throughout Indo-Pak region. It is commonly known as 'Ghamra' and 'coat button' in English [10, 11].

Fig. 1: *Tridax procumbens* Linn

Animals

The male C57 BL6/J mice (25-30g) and male Sprague-Dawley rats (150-230g) was selected for this study, and the study was approved by our Industrial Animal Ethical Committee (Registration No.1026/C/2007/CPCSEA and Study approval No.IAEC/JDC-/2008/05), This research was conducted in accordance with the internationally accepted principles for laboratory animal use and care. The animals were allowed to acclimatize to experimental conditions by housing them for 8-10 days prior to the experiments. The mice's were housed in clean polypropylene cages having 6 mice's per cage and maintained under temperature controlled room (27±2° C) with photoperiod of 12h light and 12h dark cycle and good hygienic conditions. The animals were fed with commercially available standard food pellet diet and drinking water *ad libitum*.

Preparation of Plant Extracts

Fresh leaves of *Tridax procumbens* was cleaned and wiped dry. The 700 g leaves were ground in a mixer without adding water or any other vehicle. From this 600 ml leaves juice was obtained after straining extract from muslin cloth. Then 300 ml filtrate was centrifuged for 15 min. at 1000 rpm (Eppendorff Centrifuge). From this 225 ml supernatant was obtained. This supernatant solution was frozen with dry ice and acetone for 15-20 min. Then the frozen compound kept in freeze dryer for Lyophilization at -47 ^{0} C and vacuum for 24 hrs [14, 15]. After completely drying near about 5 g water soluble powder of leaves extract was obtained. The powder

compound obtained from extract of *Tridax procumbens* leaves was administered orally at different doses by dissolving it in Normal saline. Tramadol Hydrochloride was administered as a suspension in 0.5% (w/v) methyl cellulose and two drops of Tween 80. Diclofenac Sodium was administered as a solution in normal saline [16, 17].

Phytochemical screening

Phytochemical screening of both *Tridax procumbens* Linn extracts were carried out using conventional protocol for detecting the presence of different Phytochemical active constitutes in the plant respectively [12, 13] and the result is shown in (Table 1).

Table 1: Preliminary phytochemical screening of aqueous and ethanol extracts of *Tridax procumbens* Linn.

Sr. No.	Active constituents	Aqueous extract	Ethanol extract
1.	Alkaloids	-	-
2.	Amino acid	-	-
3.	Carbohydrates	-	-
4.	Flavonoids	+	+
5.	Proteins	-	-
6.	Phytosterols	+	+
7.	Saponins	-	-
8.	Tannins	-	-

(Where, +ve: Presence of compounds, -ve: Absence of compounds)

Detection of Quercetin by High Performance Thin layer Liquid Chromatography (HPTLC)

As per the pharmcognostical databases, Quercetin is a Flavonoid has both analgesic and antidiabetic activity So, we did the detection of Quercetin in our *Tridax procumbens* leaves extract (TPLE) by HPTLC method and the spectrum shows identification of quercetine and its detection in the TPLE compared with standard Quercetin.

a) Preparation of Quercetin standard solution: Stock solution of standard Quercetin (20 µg/ml) was prepared by transferring 2 mg of Quercetin, accurately weight, into a 100 ml volumetric flask, dissolving in 50 ml methanol. It was then sonicated for 10 min. and the final volume of the solutions was made up to 100 ml. with methanol to get stock solutions containing 20 µg/ml.

b) Preparation of sample solution: Accurately weighed 250 mg of dried leaves extract of *Tridax procumbens* was transferred to a 100 ml volumetric flask dissolving in 80 ml of methanol. It was then sonicated for 10 min. and the contents of the flask were filtered through Whatman No.1filter paper. The final volume of the solution was made up to 100 ml. with methanol to get stock solution containing 2.50 mg/ml.

c) Instrumentation and chromatographic condition: HPTLC was performed on 20 cm × 10 cm aluminum backed plates coated with silica gel 60 F 254. Standard solution of Quercetin and sample solution were applied to the plates as bands 8.0 mm wide, 30 mm apart and 10.0 mm from the bottom edge of the same chromatography plate by use of a Camag (Muttenz, Switzeraland) Linomat V sample applicator equipped with a 100 µl Hamilton syringe. Ascending development to a distance of 80 mm was performed at room temp.(28 ± 2 º C) with toluene : ethyl acetate : formic acid, 5:4:0.2 (v/v/v) as mobile phase, in a Camag glass twin – through chamber previously saturated with mobile phase vapour for 20 min. After development, the plates were dried with a hair dryer and then scanned at 380 nm with a Camag TLC scanner using the Deuterium lamp [18, 19].

Evaluation of analgesic activity

The Analgesic activity of the Tridax procumbens is evaluated by following in vivo methods i) Formalin induced persistent pain (Biphasic pain) ii) Acetic acid induced Writhing test (Peripheral pain) iii) CFA induced hyper analgesia in rat (Inflammatory pain).

I. Formalin induced persistent pain (Biphasic pain)

Sprague-Dawley rats (150-230g) were acclimated after arrival for 1-2 weeks in a temperature controlled room with a 12-h light/dark cycle and allowed free access to standard laboratory chow and water. On day of experiment non fasted animals were weighed and dosed as per randomization; test compounds were dosed orally one hr. before formalin challenge. Formalin was injected into the dorsal lateral surface of the left hind paw and the time spent for licking and biting in seconds by each animal at 5 min interval is Recorded for 40 min immediately after formalin injection in the following order of time intervals: 0-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-35, 35-40. The first phase score is the sum of time spent for licking and biting in seconds from 0-5min, the second phase score is the sum of time spent licking and biting in seconds from 16-40 min, Data was presented as mean ±SEM. % reversal was calculated by following formula

%reversal =100 – (AVG response of test drug/ AVG response of vehicle*100) [20- 22].

II. Acetic acid induced writhing test (Peripheral pain)

C57/BL6J male Mice (25-30g) were acclimated after arrival for 1-2 weeks in a temperature controlled room with a 12-h light/dark cycle and allowed free access to standard laboratory chow and water, On day of experiment non fasted animals were weighed and dosed as per randomization, test compounds were dosed orally before one hour of the acetic acid injection. Acetic acid was injected by intraperitoneal route. Recorded no. of writhes after 5 min interval of acetic acid injection for 15 min in the following order of time intervals: 5-10, 11-15, 16-20, Data is presented as mean ±SEM. % reversal was calculated by following formula, % reversal = 100 – (AVG response of test drug/ AVG response of vehicle*100) [23, 24].

III. CFA induced hyper analgesia in rat (Inflammatory pain)

Sprague-Dawley rats (150-230g) was acclimated after arrival for 1-2 weeks in a temperature controlled room with a 12-h light/dark cycle and allowed free access to standard laboratory chow and water. On the day of experiment, the basal mechanical hyper analgesia was measured with the help of analgesio meter (Randall–Selitto). 0.1 mL of complete Freund's adjuvant (CFA) was injected by interplanetary route into the left hind paw of rat and animals were returned into their home cages. 24 hrs following CFA injection basal mechanical hyper analgesia was measured using analgesio meter (Randall–Selitto). The animals were treated with the test compound i.e. *Tridax procumbens* and standard compound i.e. Diclofenac Sodium. The measurement of mechanical hyperalgesia was done at 30, 60 and 120 min. after the administration of test and standard compound. The cut off time was taken as 150 gm. Data was presented as mean ±SEM. % inhibition was calculated by following formula % inhibition = (Post dose-basal)/ (cut off latency-basal)*100 [25, 26].

RESULTS

I. Formalin induced persistent pain (Biphasic pain)

Analgesic potential of *Tridax Procumbens* by Formalin induced persistent pain method in rats is determined by Total Paw licking and biting events is given in the (Tab.2) and (Fig.2 to 4)

Table 2: Effect of *Tridax Procumbens* on formalin induced persistent pain method in rats

Groups	Pre-treatment time	Vehicle	Dose Volume	1st Phase	2nd Phase
Control	60 min	Normal Saline	Kg/10mL	57±08	194±12
Tramadol-40mpk	60 min	Tween80+0.5%M.C.	Kg/4mL	33±08	44±18
Tridax procumbens-100mpk	60 min	Normal Saline	Kg/10mL	35±06	155±18
Tridax procumbens-200mpk	60 min	Normal Saline	Kg/10mL	35±08	113±19
Tridax procumbens-400mpk	60 min	Normal Saline	Kg/10mL	34±06	95±09

Data was expressed by using two-way ANOVA followed by Bonferroni post test. $p<0.05$was considered as statistical significant, n = 6 in each group.

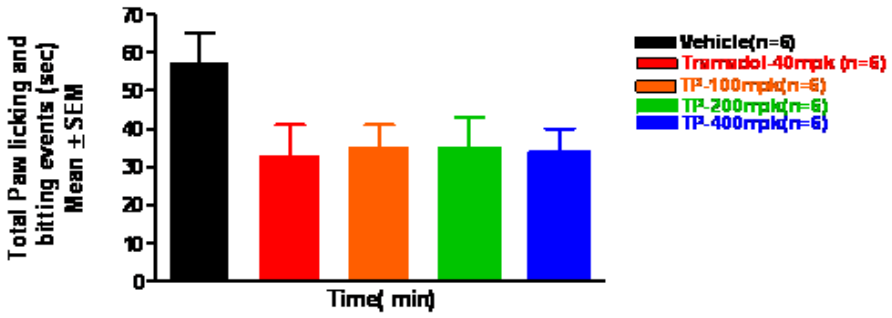

Fig. 2: Total Paw licking and biting events (sec) as Mean ± SEM in 1st phase

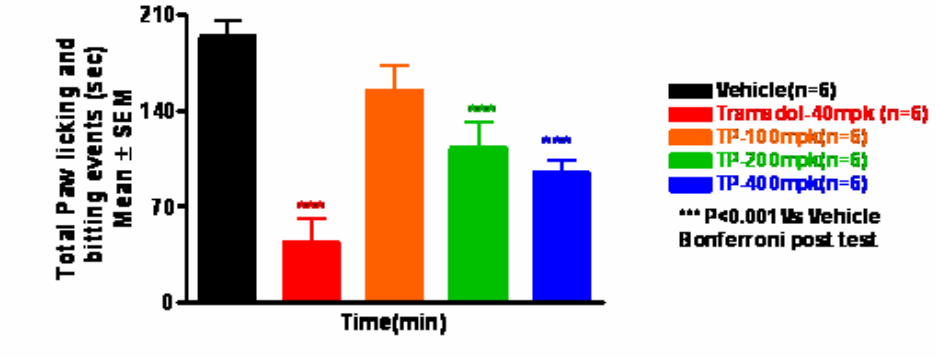

Fig. 3: Total Paw licking and biting events (sec) as Mean ± SEM in 2nd phase

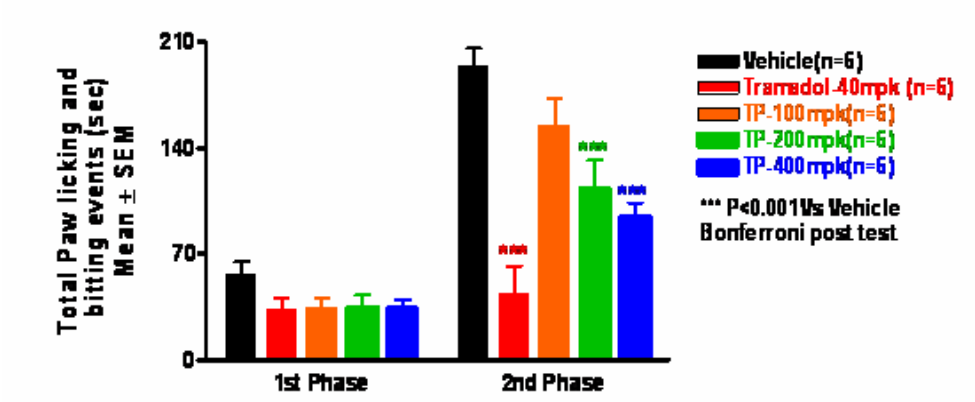

Fig. 4: Total Paw licking and biting events (sec) as Mean ± SEM in both phase

Table 3: Effect of *Tridax Procumbens* on acetic acid induced writhing response on peripheral pain method in mice

Groups	Pre-treatment time	Vehicle	Dose Volume	Total Writhing events	% Reversal
Vehicle	60 min	Normal Saline	Kg/10mL	22±01	16±06
Diclofenac-10mpk	60 min	Tween80+0.5%M.C.	Kg/4mL	08±02	65±10
Tridax procumbens-100mpk	60 min	Normal Saline	Kg/10mL	17±02	22±08
Tridax procumbens-200mpk	60 min	Normal Saline	Kg/10mL	09±01	59±06
Tridax procumbens-400mpk	60 min	Normal Saline	Kg/10mL	05±02	78±07

Data was expressed by using one-way ANOVA followed by Dunnett's post-test. $p<0.05$was considered as statistical significant, n = 9 in each group.

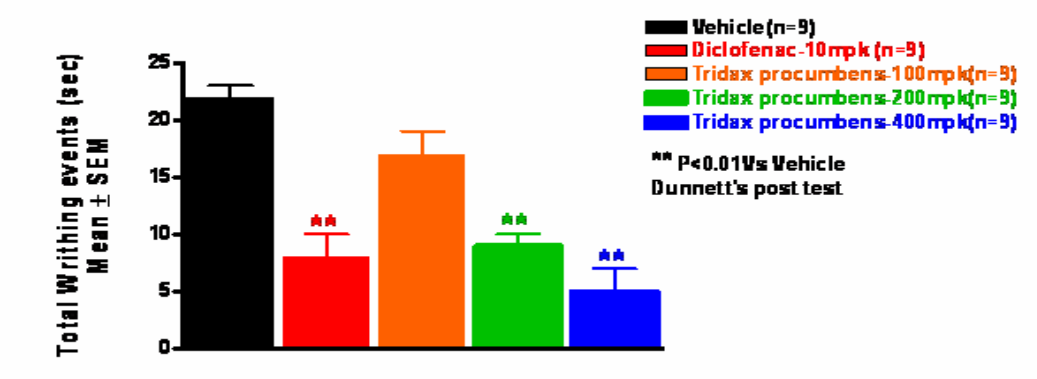

Fig. 5: Total no. of writhing events Mean ± SEM

Table 4: Effect of *Tridax Procumbens* on CFA induced hyper analgesia on inflammatory pain in rats

Groups	Vehicle	Dose Volume	% Pain Inhibition		
			30 min	60 min	120 min
Control	Normal Saline	Kg/10mL	-13±7	-17±12	-16±12
TP100 mpk	Normal Saline	Kg/10mL	45±14	53±15	46±14
TP200 mpk	Normal Saline	Kg/10mL	90±7	95±5	53±10
TP400 mpk	Normal Saline	Kg/10mL	67±11	51±7	37±7
Diclofenac10 mpk	Normal Saline	Kg/10mL	34±13	59±12	27±8

Data was expressed by using two-way ANOVA followed by Bonferroni post test. p<0.05was considered as statistical significant, n = 6 in each group.

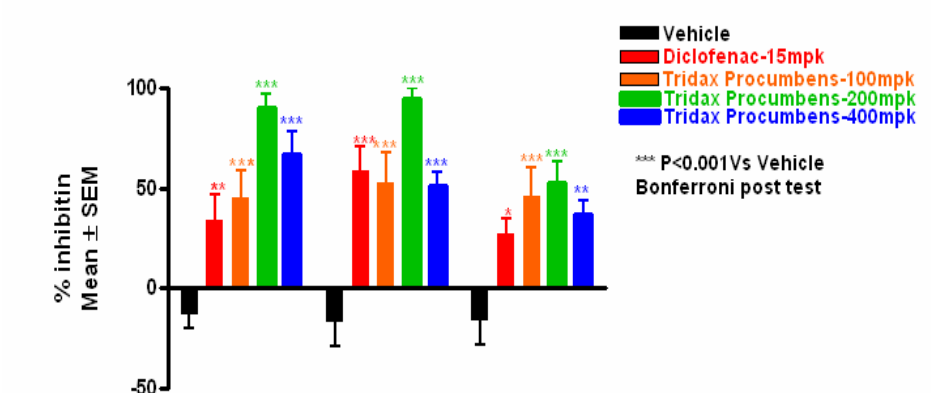

Fig. 6: CFA induced basal mechanical hyper analgesia events as Mean ± SEM.

II. Acetic acid induced writhing test (Peripheral pain)

Writhing assay was done for observing the effect of *Tridax Procumbens* on peripheral Pain and the result is given in (Tab.3) and (Fig.5)

III. CFA induced hyper analgesia in rat (Inflammatory pain)

CFA induced hyper analgesia in rat was done for observing the effect of *Tridax*

Procumbens on inflammatory pain and the observed result is given in (Tab.4) and (Fig.6)

DISCUSSION

The results of the present study demonstrated that the extract of *Tridax procumbens* possessed analgesic activity evident in the two analgesic models and inflammatory pain model, which is suggestive of the presence of both centrally and peripherally mediated mechanisms. In the acetic acid-induced abdominal constriction test, extract of Tridax procumbens dose- dependently and significantly reduced the abdominal writhing. Acetic acid is believed to act indirectly by inducing the release of prostaglandins as well as lipooxygenase products into the peritoneum which stimulate the nociceptive neurons sensitive to the non-steroidal anti inflammatory drugs hence the test is useful for the evaluation of mild analgesic non steroidal anti-inflammatory compounds. Therefore, the result of the acetic acid-induced writhing strongly suggests that the mechanism of this action may be linked partly to inhibition of lipooxygenase and/or cyclooxygenase in the peripheral tissues, thereby reducing prostaglandin synthesis and interfering with the mechanism of transduction in primary afferent nociceptors.

In the formalin test which is sensitive for various classes of analgesic drugs. Formalin injection into the rat hind paw initiates triphasic spontaneous nociceptive behaviors consisting of flinching, and licking and/or biting of the injected paw. The response to formalin is typically biphasic. The early phase of intense pain, which starts immediately after formalin injection, seems to be caused predominantly by activation of C-fibres subsequent to peripheral stimulation. Then, there is a period (about 10 min) of reduced nociceptive activity. The late phase of moderate pain, which starts about 20 min after formalin injection and lasts about 40 min to 60 mins, appears to be caused by tissue and functional changes in the dorsal horn of the spinal cord Administration of extract of Tridax

procumbens demonstrated significant inhibition in late phase Similarly, Tramadol produced marked inhibition of late phases. The centrally acting drugs, such as narcotics, inhibit both phases equally. Inhibition of late phases of pain as observed with the *Tridax procumbens* in this study suggests that they contain active analgesic principles acting centrally. Peripheral action (not significant) in the formalin test is supported by the results recorded in the acetic acid induced writhing test.

Inflammatory pain is one of the most common types of pathological pain in clinical practice. Patients mainly suffer from the ongoing pain (spontaneous pain), evoked pain and hyper analgesia. Injection of complete Freund's adjuvant (CFA) into a rat's hind-paw provides a very good model in order to study the mechanism of chronic inflammatory pain and to screen for anti-inflammatory hyper analgesic drugs. It is well known that mechanical hyper analgesia occurs within several days after CFA injection, i.e. in the acute phase of inflammatory pain. In recent years, some researchers found that there was a mechanical hyper analgesia in acute inflammatory pain rats. In the present study, we observed that mechanical hyper analgesia in rats. For acute inflammatory pain, literature reveals that TRPV1 expression has been shown to be increased in hind paw skin, sciatic nerve and DRG 24hrs, following induction of inflammatory pain with complete Freund's adjuvant (CFA) injection. Oral administration of extract of *Tridax procumbens* significantly reduced mechanical hyper analgesia in CFA injected rats.

As this anti nociceptive property of the extract may be attributed to the presence of flavonoids and phytosterol which are present in the plant However, the isolated flavanoid such as procumbentin and quercetin and sterols such as β sitosterol may show more pronounced analgesic activity compared to the extract, particularly in the formalin – induced pain model, acetic acid induced writhing and in the inflammatory pain model. Previous phytochemical investigations of *Tridax procumbens* described the isolation and structural determination of flavonoids, procumbentin and quercetin and also the presence of the β sitosterol in the plant. In animal study, beta-sitosterol, quercetin and procumbentin has been shown to be anti-inflammatory and analgesic activity. The activity of these flavonoids and sterols could be related to its anti-nociceptive effect. This indicates that the extract of *Tridax procumbens* may be used as an effective analgesic.

CONCLUSION

To summarize the present work, we would like to state that lyophilized extract of *Tridax procumbens* was found to be potent analgesic. In accordance to the present study, it has been observed that *Tridax procumbens* has marked beneficial effects against centrally, peripherally and inflammatory pain models. This protective action may be attributed towards the presence of flavanoid and sterols. We would like to conclude that it is worthwhile to think, to use *Tridax procumbens* as drugs and further studies should be initiated to establish exact mechanism of action and elaborative phytochemical investigations to find out which active constituents responsible for analgesic activity. These reports may serve as a foot step in the research of potent analgesic drug.

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