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ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITIES OF *HIBISCUS CANNABINUS (L)* SEED EXTRACT

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ABSTRACT

The petroleum ether extract of the seeds of Hibiscus cannabinus was investigated for its antinociceptive and anti-inflammatory activity in respective animal models. Significant antinociceptive was observed at higher dose of extract in writhing, tail immersion and hot plate animal models and anti-inflammatory activity was observed in carrageenan, serotonin and histamine induced paw oedema in rats. The extract exhibit significant decreased paw edema in anti-inflammatory models. Thus, the present study demonstrated that H. cannabinus seed extract has a anti-inflammatory and analgesic action against different phases of inflammation.

KEYWORDS: *Hibiscus cannabinus,* Anti-inflammatory, Analgesic.

INTRODUCTION

Inflammation involves a complex array of enzyme activation mediator release, extravasations of fluid, cell migration, tissue breakdown and repair.[1, 2] Inflammation has become the focus of global scientific research area because of its implication and complications in all human and animal diseases. Recent treatment (NSAID) have various side effects. Thus, the use of NSAID as anti-inflammatory and analgesic agents have not been successful in all cases,[3, 4] Therefore, new herbal anti-inflammatory and analgesic drugs lacking these side effect are being researched as alternative to current therapies.[3, 5] Most of the researchers are being focused on the investigation of the safety and efficacy of plant based drugs used in traditional medicine because they are economical and have less side effects.[3, 5, 6] Many indigenous

drugs have been claimed to have analgesic and anti-inflammatory effect in Ayurvedic system of medicine but they were not properly investigated.

Hibiscus cannabinus L. (Malvaceae) is a fast grooving, woody to herbaceous annual plant (4-5 m in height) producing large cream coloured flowers, popular in the western world as "Kenaf" and widely grown as a fibre crop. It is known by various names in India such as Bimli, Deccan hemp, Gogu, Channa, Ambadi Gongura, Sunkura and Sunbeeja.[7] This plant was traditionally prescribed in traditional folk medicine in Africa and India; reported to contain several active components as tannins, saponins, polyphenolics, alkaloids, lignans, essential oils and steroids. The plant possesses hepatoprotective.[8] haematinic.[9] cholesterol lowering.[10] and antioxidative.[11] activities. The seeds were used externally to treat aches and bruises. In addition, this plant has been reported to be an anodyne, aperitif, aphrodisiac, as well as fattening, purgative and stomachic.[12] Since no detail scientific data is available regarding antinociceptive and anti-inflammatory activity of *Hibiscus cannabinus* (L.), therefore the present study was carried out to provide pharmacological evidence for the folklore medicinal consideration of *Hibiscus cannabinus* (L.) as antinociceptive and anti-inflammatory.

MATERIAL AND METHODS

Collection of plant material

Fresh seeds of *Hibiscus cannabinus* were collected from local area of Jalgoan district, Maharashtra, India in the months of July-October. This plant was identified and authenticated by Dr. A. S. Upadhye, Scientist, Agharkar Research Institute, Pune. Voucher specimens No. (S-156) have been kept in Agharkar Research Institute, Pune, Maharashtra, India.

Animals

Adult male Wistar albino rats, weighing between 180 - 220 g and albino mice (25-30 g) were used and acclimatized to laboratory condition for one week. All animals were housed in well ventilated polypropylene cages at 12 h light/dark schedule with 25±2ºC and 55-65% relative humidity. The rats had fed with commercial pelleted rats chow and water *ad libitum* as a standard diet. Institutional Animal Ethics Committee approved the experimental protocol in accordance with CPCSEA.

Preparation of seed extract

The seeds were collected and dried in shade and ground. Coarsely powdered seeds were used for the study. Coarsely powdered seed material (1000 g) was subjected to successive extraction with petroleum ether (60 – 80^{0}C) in a soxhlet extractor at a temperature of 45-50^{0}C to 45 cycles per batch for 2 batches. The extraction was continued until the solvent in the thimble becomes clear indicating the completion of the extraction. After each extraction the solvent was distilled off and concentrated extract was transferred to previously weighed petri dish and evaporated to dryness at room temperature to obtain dried extracts. After completion of drying the petri dish was weighed again. The yield of extract was calculated by subtracting original weight of empty petri dish. The yield was 6.3 g/100 g.

Preliminary phytochemical studies

Preliminary qualitative phytochemical screening for the identification of the phytoconstituents of the petroleum ether extract of seeds of *Hibiscus cannabinus* has been carried out.[13]

Acute oral toxicity of the extract

Adult Albino mice (25-30 g) were divided into five groups containing ten mice each. The mice were fasted for 6 h and access only water *ad libitum* before experimental study. Group I received only vehicle (distilled water). Group II, III, IV and V animals received with different doses of petroleum ether extract of seeds of *Hibiscus cannabinus* (PEHC) i.e. 1000, 2000, 3000 and 4000 mg/kg respectively. All the doses and vehicle were administered orally. The animals were observed for 72 h for mortality.[14]

Antinociceptive activity

Writhing test

Male Swiss albino mice (25-30 g) were divided into five groups containing six animals each as follows Group I: Vehicle control rats received distilled water (10 ml/kg, p.o.), Group II: Indomethacine (10 mg/kg, p.o.), Group III: PEHC (100 mg/kg, p.o.), Group IV: PEHC (200 mg/kg, p.o.), Group V: PEHC (400 mg/kg, p.o.).[15] All the drug treatments were given 1 hour before i.p. injection of 0.6 % (v/v) acetic acid, at a dose of 10 ml/kg.[16] Writhing is a syndrome characterized by a wave of contraction of the abdominal musculature followed by a wave of contraction of hind limbs. The hind limbs contractions that occurred over a period of 10 min were counted. A reduction in time of writhing initiation and number of writhing as compared to the vehicle treated group was considered as evidence for the analgesia.

Tail immersion test

Swiss Albino Mice (25-30 g) were divided into five groups of six animals each as follows: Group I: Vehicle control mice received distilled water (10 ml/kg, p.o.), Group II: Aspirin (100 mg/kg, p.o.), Group III: PEHC (100 mg/kg, p.o.), Group IV: PEHC (200 mg/kg, p.o.), Group V: PEHC (400 mg/kg, p.o.). The lower 5 cm portion of the tail was immersed in a beaker containing water and temperature maintained at 55 ± 0.5ºC.[17] The time in seconds for tail withdrawal from the water was taken as the reaction time, with a cut-off time of immersion set at 10s. The reaction time was measured 1 h before and 0.5, 1, 2, 3, 4 and 6 h after oral administration of drugs.[18]

Hot Plate Method

Swiss Albino Mice (25-30 g) were divided into five groups of six animals each as follows: Group I: Vehicle control mice received distilled water (10 ml/kg, p.o.), Group II: Pentazocine (10 mg/kg, i.p.), Group III: PEHC (100 mg/kg, p.o.), Group IV: PEHC (200 mg/kg, p.o.), Group V: PEHC (400 mg/kg, p.o.). Mice were placed on a hotplate maintained at a temperature of 55 ± 1ºC for a maximum time of 15 s. The time between placement of animal on the hot plate and occurrence of licking of the fore or hind paws, shaking or jumping off from the surface was recorded as response latency. Mice with basal latencies of more than 10 s were eliminated from the study. The testing of response latencies was measured before distraction (basal) and 30, 60 and 90 min. after treatment. The cut off time for hotplate latencies was set at 15 s.[19]

Anti inflammatory activity

Carrageenan induced rat paw Oedema

The Wistar rats (180-220 g) were starved overnight and divided into five groups of six animals each as follows Group I: Vehicle control rats received distilled water (10 ml/kg, p.o.), Group II: Diclofenac sodium (10 mg/kg, i.p.), Group III: PEHC (100 mg/kg, p.o.), Group IV: PEHC (200 mg/kg, p.o.), Group V: PEHC (400 mg/kg, p.o.). After selection of animals, 0.1 ml of 1% carrageenan solution was injected into the left hind paw. The pretreatment time was 1 h before carrageenan injection. The paw volume was recorded immediately and at 1 h, 2 h, 3 h, 4 h and 6 h by using plethysmometer (UGO Basile 7140). Mean increase in the volume of oedema was measured and percentage inhibition was calculated.[20-21]

Serotonin induced rat paw Oedema

The Wistar rats (180-220 g) were starved overnight and divided into five groups of six animals each as follows Group I: Vehicle control rats received distilled water (10 ml/kg, p.o.), Group II: Diclofenac sodium (10 mg/kg, i.p.), Group III: PEHC (100 mg/kg, p.o.), Group IV: PEHC (200 mg/kg, p.o.), Group V: PEHC (400 mg/kg, p.o.). After selection of animals, 0.05 ml of 1% freshly prepared s**erotonin** solution was injected into the left hind paw. The pretreatment time was 1 h before serotonin injection. The paw volume was recorded immediately and at 1 h, 2 h, 3 h, 4 h and 6 h by using plethysmometer (UGO Basile 7140). Mean increase in the volume of oedema was measured and percentage inhibition was calculated.[22-23]

Histamine induced rat paw Oedema

The Wistar rats (180-220 g) were starved overnight and divided into five groups of six animals each as follows Group I: Vehicle control rats received distilled water (10 ml/kg, p.o.), Group II: Diclofenac sodium (10 mg/kg, i.p.), Group III: PEHC (100 mg/kg, p.o.), Group IV: PEHC (200 mg/kg, p.o.), Group V: PEHC (400 mg/kg, p.o.). After selection of animals, 0.05 ml of 1% freshly prepared histamine solution was injected into the left hind paw. The pretreatment time was 1 h before histamine injection. The paw volume was recorded immediately and at 1 h, 2 h, 3 h, 4 h and 6 h by using plethysmometer (UGO Basile 7140). Mean increase in the volume of oedema was measured and percentage inhibition was calculated.[22-23]

RESULTS AND DISCUSSION

The present study establishes the antinociceptive and anti-inflammatory activity of the petroleum ether extract of the seeds of *H. cannabinus* in the models used.

*H. cannabinus (*L.) is the reservoir for many potentially active chemical compounds which acts as drugs against various diseases and disorders. The petroleum ether extract of *H. cannabinus* (L.) showed the presence of alkaloids, saponins, tannins, sterols and glycosides (Table 1). The PEHC was found to be safe at all doses used and found no mortality up to the dose of 4000 mg/kg when administered orally. In view of this, we have taken 400 mg/kg as the therapeutic dose and made variations by taking 100 mg/kg as lower dose and 400 mg/kg as higher dose.

The study indicated that petroleum ether extract of *H. cannabinus* has both peripheral and central analgesic activity. With respect to the writhing test, the research group of Deraedt et

al.[24] described the quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of rats, obtained after intraperitoneal injection of acetic acid. They found high levels of prostaglandins during the first 30 min after acetic acid injection. Nevertheless, it was found that the intraperitoneal administration of acetic acid induces the liberation not only of prostaglandins, but also of the sympathetic nervous system mediators.[25-27]

H. cannabinus significantly reduced writhings and stretchings induced by acetic acid (nociceptive stimuli). The significant protective effect was dose dependent with 21.99 % (*P<0.001*), 34.94 % (p<0.001) and 63.62 % (p<0.001) reduction observed for 100, 200 and 400 mg/kg respectively. Indomethacine (10 mg/kg) showed 65.74 % ($P < 0.001$) inhibition (Table 2).

The first phase of tail immersion and hotplate test results showed central protective effect of extract. The tail immersion test indicated that the pharmacological actions were mediated by mu (μ) opioid receptors rather than kappa (k) and delta receptors.[28-29] The reaction time of animal showed a significant increase (p<0.001) with increasing latency (time). Oral administration with PEHC (200, 400 mg/kg) showed significant (p<0.001) increased pain latencies at 1, 2, 3, 4 and 6 h as compared to vehicle treated animal. Treatment with PEHC (100 mg/kg, p.o.) did not show significant activity (Table 3). Pretreatment with PEHC (200 and 400 mg/kg, p.o.) increased the pain latency in the hotplate test. The latency response was found to be significantly increased (p<0.001) at 20, 60 and 90 min. The known centrally acting analgesic drug pentazocine also increased the response latencies at 20, 60 and 90 min. The PEHC (100 mg/kg, p.o.) did not show significant effect (Table 4).

Carrageenan induced oedema is a multimediated phenomenon that liberates diversity of mediators like histamine, 5-HT, kinins and prostaglandins at various time intervals. It is believed to be biphasic the first phase (60 min) involves the release of serotonin and histamine while the second phase (over 60 min) is mediated by prostaglandins, the cyclooxygenase products, and the continuing between the two phase is provided by kinins.[30] Development of oedema induced by carrageenan is commonly correlated with early exudative stage of inflammation.[31]

The dose of PEHC (400 mg/kg) showed a maximum 64.97 % inhibition in carrageenan (Table 5), 70.13 % inhibition in serotonin (Table 6) and 68.49 % inhibition in histamine

(Table 7) induced rat paw oedema as compared to vehicle treated group. PEHC (100 and 200 mg/kg, p.o.) did not show significant effect.

Since carrageenan induced inflammation model is a significant test for anti-inflammatory agent acting by the mediators of acute inflammation.[32] The results of this study showed that H. *cannabinus* can be effective in acute inflammatory disorders.

So we can conclude that the present study supports the claims by traditional medicine practitioners about the usefulness of *H. cannabinus* in inflammatory diseases. More detailed phytochemical studies are, however, necessary to identify the active principle(s) and exact mechanism(s) of action.

Table 1: Phytochemical screening of the petroleum ether extract of *Hibiscus cannabinus*

Sr. No.	TEST	Inference
1	Alkaloids	+ve
2	Flavonoids	-ve
3	Saponins	+ve
4	Tannins	+ve
5	Sterols	+ve
6	Carbohydrates	-ve
7	Test for glycosides	+ve

Table 2: Effect of Petroleum ether extract of *Hibiscus cannabinus* on acetic acid induced writhing in mice

Treatment	Dose Mg/kg	No of wriths	% inhibition
Vehicle	10	34.83±1.17	-
Indomethacine	10	12.00±0.58***	65.54
PEHC	100	27.17±0.65***	21.99
PEHC	200	22.00±0.86***	34.94
PEHC	400	12.67±0.61***	63.62

Data was expressed as means ± S.E.M and analysed by one way ANOVA followed by Dunnett's test, n=6, ***p<0.001

Table 3: Effect of Petroleum ether extract of *Hibiscus cannabinus* on latency period (s) in tail immersion method

Treatment	Dose mg/kg	Latency period (S)						
		0 h	0.5 h	1 h	2 h	3 h	4 h	6 h
Vehicle	10	4.46±0.25	4.25±0.20	3.27±0.08	2.76±0.11	2.40±0.09	2.26±0.05	2.19±0.05
Aspirin	100	4.22±0.15	5.01±0.18**	5.98±0.11***	7.26±0.17***	8.43±0.19***	9.08±0.08***	9.71±0.16***
PEHC	100	4.12±0.16	3.57±0.16	3.16±0.07	2.70±0.16	2.52±0.14	2.42±0.08	2.29±0.05
PEHC	200	4.05±0.22	4.43±0.25	5.22±0.24***	5.93±0.05***	6.28±0.04***	6.95±0.05***	7.61±0.11***
PEHC	400	4.13±0.10	4.84±0.11*	5.77±0.17***	6.48±0.14***	7.34±0.14***	7.94±0.12***	8.57±0.11***

Values are means ± S.E.M from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. ***P < 0.001 compared to vehicle animals.

Table 4: Effect of Petroleum ether extract of *Hibiscus cannabinus* on hot plate method in mice

Treatment	Dose Mg/kg	Pain latency (min.)			
		0	20	60	90
Vehicle	10	11.22±0.55	9.32±0.22	8.62±0.15	7.50±0.12
Pentazocine	10	11.23±0.35	16.22±0.77***	15.80±0.66***	16.70±0.43***
PEHC	100	12.02±0.52	11.52±0.29**	10.42±0.12*	9.73±0.24**
PEHC	200	11.22±0.39	12.87±0.55***	15.43±0.27***	17.73±0.31***
PEHC	400	11.15±0.57	13.92±0.36***	16.55±0.45***	18.67±0.36***

Values are means ± S.E.M from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. ***P < 0.001 compared to vehicle animals.

Table 5: Effect of Petroleum ether extract of *Hibiscus cannabinus* on carrageenan induced rat paw oedema

Treatment	Dose Mg/kg	Change in paw vol (h)					
		0	1	2	3	4	6
Vehicle	10	1.09±0.03	1.27±0.03	1.33±0.06	1.33±0.02	1.40±0.05	1.45±0.06
Diclofenac sodium	10	1.10±0.05	1.18±0.05 (51.85)	1.18±0.05 (66.66)	1.17±0.05 (70.83)	1.20±0.04* (67.56)	1.20±0.05** (72.35)
PEHC	100	1.18±0.05	1.35±0.05 (5.55)	1.33±0.05 (37.5)	1.32±0.05 (41.66)	1.35±0.07 (43.78)	1.35±0.07 (51.15)
PEHC	200	1.18±0.05	1.33±0.03 (16.66)	1.31±0.04 (44.44)	1.30±0.04 (50)	1.33±0.04 (51.35)	1.32±0.03 (61.29)
PEHC	400	1.11±0.04	1.25±0.07 (22.22)	1.23±0.06 (50)	1.20±0.06 (62.5)	1.24±0.06 (57.83)	1.24±0.06* (64.97)

Values are means ± S.E.M from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. *P < 0.05, **P<0.01 compared to vehicle animals.

Table 6: Effect of Petroleum ether extract of *Hibiscus cannabinus* on serotonin induced rat paw oedema

Treatment	Dose Mg/kg	Change in paw vol (h)					
		0	1	2	3	4	6

Vehicle	10	1.21±0.03	1.39±0.03	1.45±0.06	1.45±0.03	1.52±0.05	1.58±0.07
Diclofenac sodium	10	1.22±0.05	1.31±0.04 (48.62)	1.30±0.05 (65.27)	1.29±0.05 (69.17)	1.31±0.04* (69.35)	1.32±0.05** (72.39)
PEHC	100	1.30±0.05	1.48±0.05 (2.75)	1.46±0.05 (31.25)	1.46±0.04 (31.50)	1.49±0.07 (36.55)	1.50±0.07 (45.24)
PEHC	200	1.33±0.04	1.46±0.03 (24.77)	1.45±0.03 (47.91)	1.43±0.04 (59.58)	1.46±0.04 (56.45)	1.47±0.03 (62.44)
PEHC	400	1.22±0.05	1.39±0.06 (2.75)	1.38±0.05 (31.25)	1.35±0.06 (46.57)	1.35±0.05 (55.91)	1.33±0.05** (70.13)

Values are means ± S.E.M from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. *P < 0.05, **P<0.01 compared to vehicle animals.

Table 7: Effect of Petroleum ether extract of *Hibiscus cannabinus* on histamine induced rat paw oedema

Treatment	Dose Mg/kg	Change in paw vol (h)					
		0	1	2	3	4	6
Vehicle	10	1.19±0.03	1.37±0.02	1.43±0.06	1.43±0.04	1.49±0.04	1.55±0.05
Diclofenac sodium	10	1.23±0.05	1.33±0.03 (43.63)	1.34±0.03 (54.48)	1.32±0.04 (63.26)	1.32±0.03 (69.39)	1.34±0.03* (68.94)
PEHC	100	1.27±0.05	1.44±0.05 (10)	1.42±0.05 (40)	1.41±0.05 (44.89)	1.44±0.07 (44.80)	1.44±0.07 (52.96)
PEHC	200	1.30±0.03	1.42±0.03 (35.45)	1.45±0.03 (38.62)	1.44±0.04 (42.85)	1.45±0.04 (51.36)	1.46±0.04 (55.25)
PEHC	400	1.21±0.03	1.34±0.07 (30)	1.32±0.06 (55.17)	1.29±0.06 (68.02)	1.33±0.06 (61.20)	1.33±0.06** (68.49)

Values are means ± S.E.M from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. *P < 0.05, **p<0.01 compared to vehicle animals.

REFERENCES

1. Vane JR, Bolting RM. New insights into the made of action of anti-inflammatory drugs. Inflamma Res., 1995; 44: 1-10.
2. Perianayagam JB, Sharma SK, Pillai KK. Anti-inflammatory activity of *Trichodesma indicum* root extract in experimental animals. J Ethanopharmacol., 2006; 104: 410-414.
3. Dharmasiri JR, Jayalcody AC, Galhena G, Liyanage SSP, Ratansooriya WD. Antiinflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*. J Ethanopharmacol., 2003; 87: 199-206.
4. Park JH, Son KH, Kim SW, Chang HW, Bae K, Kang SS, Kim HP. Anti-inflammatory activity of *synurus deltoids*. Phytother Res., 2004; 18: 930-933.

5. Kumara NKVMR. Identification of strategies to improve research on medicinal plants used in Shri Lanka. In: WHO Symposium. University of Ruhuna, Galle. Shri Lanka., 2001: 12-14.
6. Li RW, Myers SP, Leach DN, Lin GD, Leach G. A cross cultural study anti-inflammatory activity of Australian and Chinese plants. J Ethanopharmacol., 2003; 85: 25- 32.
7. The Wealth of India; A dictionary of Indian Raw Materials and Industrial Products. Raw materials, C.S.I.R. New Delhi, 78.
8. Gabrile A Agbor, Julius E Oben, Blaise Nkegoum, Jean Pierre Takala, Jeanne Y Ngogang. Hepatoprotective activity of *Hibiscus cannabinus* (Linn.) against carbon tetrachloride and paracetamol induced liver damage in rats. Pak J Biological Sci., 2005; 13: 397 - 401.
9. Agbor GA, Oben JE, Ngogang JY. Haematinic activity of *Hibiscus cannabinus*. Afr J Biotech., 2005; 4(8): 833 - 337.
10. Tamaki Y, Jinjo K, Uechi S, Hongo F, Sameshima K, Yoga S, Mokuzai Gakkaishi. Cholesterol lowering effect of water soluble polysaccharides from kenaf (*H.cannabinus*) seeds in rats, Fac.Agric University Ryukyusa Okinawa Japan., 2001; 47(2): 159 - 163.
11. Agbor GA, Oben JE, Ngogang JY. Antioxidative activity of *Hibiscus cannabinus* leaf extract. Food Africa., 2003; 01 - 05.
12. Lee YG, Byeon SE, Kim JY, Lee JY, Rhee MH, Hong S, Wu JC, Lee HS, Kim MJ, Cho DH, Cho JY. Immunomodulatory effect of *Hibiscus cannabinus* extract on macrophage functions. *J. Ethnopharmacol.*, **2007;** 113: 62.
13. Harborne JB. *Phytochemical methods*, 3rd edn, Chapman and hall, London; 1998.
14. Ravichandran V, Suresh B, Sathishkumar MN, Elango K, Srinivasan R. Antifertility activity of hydroalcoholic extract of *Ailanthus excels* (Roxb): An ehanomedicines used by tribals of Nilfiris region in Tamilnadu. J Ethanopharmacol., 2007; 112: 189-191.
15. Mate GS, Naikwade NS, Magdum CS, Chowki AA, Patil SB. Evaluation of anti-nociceptive activity of *Cissus quadrangularis* on albino mice. Int J Green Pharm., 2008; 2: 118-121
16. Zakaria MN, Islam MW, Radhakrishnan R. Anti-nociceptive and anti-inflammatory properties of *Caralluma arabica*. J Ethnopharmacol., 2001; 76: 155-158.
17. Janssen PAJ, Niemegeers CJE, Dony JGH. The inhibitory effect of fentanyl and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats. Arzneimittel-Forsch., 1963; 6: 502–507.

18. Vogel GH, Vogel WH. Drug Discovery and Evaluation. Pharmacological Assays. Springer., 1997; 360–418.
19. Eddy NB, Leimback D. Synthetic analgesics. II. Dithienylbutenyl and dithienylbutylamines. J Pharmacol Exp Ther., 1953; 107**:** 385–393.
20. Winter CA, Risley EA, Nuss GW. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. P Soc Exp Biol Med., 1962; 111: 544–547.
21. Young H., Luo Y, Cheng H, Hsieh W, Liao J, Peng W. Analgesic and anti-inflammatory activities of [6]-gingerol. J Ethnopharmacol., 2005; 96: 207–210.
22. Maity TK, Mandal SC, Mukharjee PK, Saha K, Das J, Pal M, Saha BP. Studies on anti inflammatory effect of Cassia tora leaf extract (Fam Leguminosae). PTR. Phytother Res., 1998; 12: 221-223.
23. Mandal SC, T.K. Maity TK, Das J, Saba BP and Pal M, Antiinflammatory evaluation of *Ficus racemosa* Linn. Leaf extract. J Ethnopharmacol., 2000; 72: 87-92.
24. Deraedt R, Jouquey S, Delevallee F, Flahaut M. Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur J Pharmacol., 1980; 61: 17–24.
25. Duarte JDG, Nakamura M, Ferreira SH. Participation of the sympathetic system in acetic acid induced writhing in mice. Braz J Med Biol Res., 1988; 21: 341–343.
26. Hokanson GC, Acetic acid for analgesic screening. J Nat Prod., 1978; 41: 497–498.
27. Neto AG, Costa JMLC, Belati CC, Vinholis AHC, Possebom LS, Da Silva Filho AA, Cunha WR, Carvalho JCT, Bastos JK, e Silva MLA. Analgesic and anti-inflammatory activity of a crude root extract of *Pfaffia glomerata* (Spreng) Pedersen. J Ethnopharmacol., 2005; 96: 87–91.
28. Schmauss C, Yaksh TL. In vivo studies on spinal opiate receptor systems mediating antinociception. II. Pharmacological profiles suggesting a differential association of mu, delta and kappa receptor with visceral chemical and cutaneous thermal stimuli in the rat. J Pharmacol Exp Ther., 1984; 228: 1–12.
29. Aydin S, Demir T, Ozturk Y, Baser KHC. Analgesic activity of *Nepeta italica* L. Phytother Res., 1999; 13: 20–23.
30. Perianayagam JB, Sharma SK, Pillai KK. Anti-inflammatory activity of *Trichodesma indicum* root extract in experimental animals. J Ethanopharmacol., 2006; 104: 410-414.
31. Adedapa A, Sofidiya MO, Inaphosa V, Inaya B, Masika PJ, Afolayan AJ. Antiinflammatory and analgesic activities of the aqueous etract of *Cussonia Paniculata* stem bark, Rec Nat Prod., 2008; 2(2): 46-53.

32. Dagar HS, Chagtitai SA. *Trichosanthes bracteata* (lam) voight (Cucurbetaceae) a promising ethanomedicinal taxon in Andaman and Nicobar Islands. Indian J Appl Pure Biol 1989; 4(2): 131-132.