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Original Article

# PHARMACOLOGICAL EVALUATION OF ANTI-INFLAMMATORY, ANALGESIC AND ANTIPYRETIC EFFECTS OF *GYNOCARDIA ODORATA ROXB* IN ANIMAL MODELS

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**ABSTRACT**

**Objective:** The purpose of this investigation was to study the anti-inflammatory, analgesic, and antipyretic properties of methanol extract of *Gynocardia odorata roxb* (MEGO) in rats and mice.

**Methods:** The plant material was extracted with methanol. Dose of 200, 400 mg/kg of each extracts were used in carrageenan-induced paw oedema, cotton-pellet granuloma in rats, writhing nociception in mice, and yeast induced hyperpyrexia in rats.

**Results:** All compounds reduced paw oedema into the control group at 5 h post carrageenan injection. The methanol extract of *G. odorata roxb* were similar to phenylbutazone in reduction of paw oedema and cotton-pellet granuloma. The extract as well as paracetamol induced antinociception in writhing test in comparison to control. Positive results for flavanoids and phenolic compounds were investigated by phytochemical analysis of the extract. The higher antinociception effects of the extract might be due to the presence of flavanoids, and phenol compounds. The methanol extract produced a significant dose dependent inhibition of temperature elevation.

**Conclusion:** These data suggest that the extract of *G. odorata roxb* produce antinociceptive, anti-inflammatory, and anti-pyretic activities that could be due to the effect of one or a combination of the bio active components in each extract.

**Keywords**: *Gynocardia odorata roxb* leaves, Anti-inflammatory, Antinociception, Anti-pyretic activity.

## INTRODUCTION

Traditional medicaments chiefly obtained from plants have played a vital role in sustaining disease free human existence in the planet. Over the centuries, phytopharmaceuticals have been utilized by different communities of the world [1,2]. Pain, inflammation and fever are very common complications in human beings. Several plants and their products are claimed and proved to possess antipyretic property [3]. As a result of adverse effects such as gastric lesions caused by non-steroidal anti-inflammatory drugs (NSAID), tolerance and dependence induced by opiates the use of these drugs as anti-inflammatory and analgesic agents have not been successful in all cases. Attention is being focused on the investigation of the efficacy of plant-based drugs used in the traditional medicine because they are cheap, have few side effects and according to WHO, about 80% of the world population still rely mainly on herbal remedies [4-6]. In recent times, focus on plant research has increased and non-steroidal antiinflammatory drugs (NSAID) constitute one of the most widely used classes of drugs. Herbal drugs are being proved as effective as synthetic drugs with lesser side effects. Herbal medicines are in line with nature, with less hazardous reactions [7].

*Gynocardia odorata roxb* is also known as Chaulmoogra plant belongs to the family of Flacourtiaceae, which is indigenous to parts of India, Malaysia and tropical countries of the world contain fatty acids chaulmoogric acid, hydnocarpic acid. Chaulmoogra oil is an important therapeutic agent in certain medical traditions [8]. The seeds of *G. odorata roxb* are most commonly used. The fruits are hot anthelmintic and used in bronchitis, skin diseases, small tumor's leprosy, and as an analgesic. *G. odorata roxb* is reported to contain antioxidant properties [9]. *G. odorata roxb* may have its antiulcer activity because of its active constituents like flavonoids and especially quercetin [10-11]. It was reported that *G. odorata roxb* could be a natural medication alternative of thrombolytic agents as well as the source of potent bioactive compounds [12].[7] Among several traditional claims, the usefulness of *G. odorata roxb* in fever, inflammation and pain have been emphasized more in literature. Hence it was considered that investigations for these medicinal properties might give scientific authentication to the traditional claims. Thus the aim of this present study was to carry out a pharmacological evaluation of methanol extract of *G. odorata roxb* for its anti-inflammatory, analgesic, and antipyretic properties.

## MATERIALS AND METHODS

### Collection and extraction

The fresh leaves of *Gynocardia odorata roxb* were collected from the Authenticated crude drug supplier in Delhi and authentication of the plant was carried in Botanical Survey of India, Coimbatore, India. A voucher specimen has been deposited in the laboratory for future reference (BSI/SC/7/46/13-14/TECH.785). The leaves of the plant were shade dried and pulverized. The powder was defatted with petroleum ether. Later, it had been subjected to continuous hot extraction with 95% aqueous methanol in a Soxhlet apparatus. The extract (MEGO) was concentrated under vacuum and dried in desiccators (yield 69 gm, 6.9%w/w). The dry extract was kept in vacuum desiccators until use.

### Animals

Adult male Wistar albino rats (150–200 g) and Swiss albino mice (20-25 g) were procured from Venkateshwara Enterprises, Bangalore, Karnataka, India and used throughout the study. All the animals were under the age of 8–12 weeks. They were housed in a very clean polypropylene cage and maintained under standard laboratory conditions (temperature 25 ± 2°C with dark/light cycle 12/12 h). They were fed with standard pellet diet and water *ad libitum*. The animals were acclimatized to laboratory conditions for one week before experiment. Experiments were performed complied with the rulings of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) New Delhi, India under the registration No: 1135/a/07/CPCSEA.

### Phytochemical screening

The preliminary phytochemical investigations were performed for various phytoconstituents such as alkaloids, flavonoids, tannins, terpenoids, saponin, and steroids etc which are probably responsible for the activity of the extract [13].

**Acute toxicity**

Acute toxicity studies were performed as per OECD-423 guidelines [14]. Male Wistar albino rats selected by random sampling technique were utilized during this study. The animals were fasted for 4h with free access to water only. The plant extract was administered orally at a dose of 5mg/kg initially and mortality if any was observed for 3 days. If mortality was ascertained in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was ascertained in only one animal out of three animals then the identical dose was repeated again to confirm the toxic effect. If no mortality was ascertained, then higher (50,300 and 2000 mg/kg) doses of extract were utilized for further toxicity studies.

**Drugs and chemicals**

The drugs and fine chemicals were purchased from Sigma Chemical Co., St. Louis, USA. All other chemicals and solvents were obtained from local firms (India) and were of highest purity and analytical grade.

**Studies on inflammation**

**Acute inflammation study**

**Carrageenan-induced paw oedema in rats**

Pedal inflammation in male Waster rats (150-200 g) was produced according to the method described earlier [15]. An injection (s. c.) was made of 0.1 ml of 1% carrageenan into the right paw of each rat under the sub plantar aponeurosis. The test groups of rats were administered intraperitonially with 200 and 400 mg/kg of the methanol extract of *G. odorata roxb*1 h before carrageenan injection. At the same time, the control group received 5 ml/kg of 5% gum acacia and the reference group received 100 mg/kg phenyl butanone (i. p.). The paw value was measured immediately after carrageenan injection and at 1,2,3,4 and 5 h intervals after the administration of the edematogenic agent using a plethysmograph-apparatus up to the anatomical hairline on lateral malleolus [16], and compared with the control animals, which received only the vehicle. The inhibitory activity was calculated according to the following formula [17].

$$\text{Percent inhibition (\%)} = 100 - \frac{\text{(oedema volume control)}}{\text{(oedema volume in the treated)}} \times 100$$

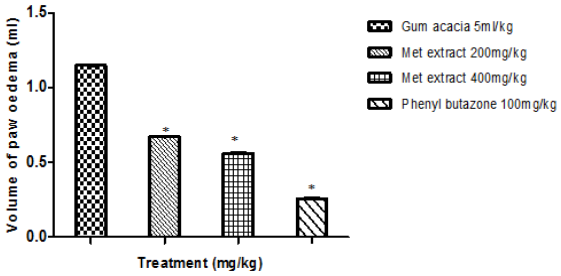


**Fig. 1: Effect of *G. odorata roxb* on Carrageenan induced Paw oedema in rats. *P<0.001 vs control.**

**Sub-acute inflammation study**

**Cotton-pellet granuloma in rats**

This study was carried out by cotton-pellet implantation method in rats [18]. This method used here was adopted from sheth et al., [19], with a slight modification of using only male rats [20]. Under light ether anaesthesia, sterile cotton- pellets (10 mg) were implanted subcutaneously in the axilla and groin regions of the rats. The animals were treated orally with methanol extract at different doses of *G. odorata roxb* (200 and 400 mg/kg) daily for 7 consecutive days. Animals in the control group received either normal saline or the vehicle gum acacia. Phenyl butazone (100 mg/kg, orally.) was given to animals in the reference groups. They were sacrificed on day 8, the cotton-pellet removed, freed from extraneous tissue and dried overnight at 60°c and weighed. The percent inhibition of the dry weight of the granuloma were calculated and compared.

**Antinociceptive activity**

**Effect on acetic acid-induced writhing in mice**

Analgesic activity was evaluated on the acetic acid-induced writhing according to Koster et al., [21]. The writhes were induced by intra-peritoneal injection of 0.6% acetic acid (v/v) (10 ml/kg). Two different doses (200 and 400 mg/kg) of the extract of *G. odorata roxb* were administered orally to different groups of six animals each, 30 min before chemical stimulus. The numbers of writhing movements were counted 10, 20, 30, and 40 min after acetic acid injection. Antinociception (analgesia) expressed as the reduction of the number of abdominal constrictions between control animals (acetic acid treated mice) and mice pre-treated with the extract.

**Antipyretic activity**

**Yeast induced hyperthermia**

Four groups of six rats each were injected subcutaneously with 10 ml/kg body weight. Yeast suspension (15% aqueous suspension) to induce pyrexia, after measuring the basal rectal temperature (0°c) of each animal. Nineteen hours after yeast injection, the rectal temperature was recorded again and animals showing a rise in temperature of <0.6°c were discarded [22]. Thereafter, treatment was carried out as follows:

Group I: Distilled water (10 ml/kg; p. o.),

Group II-Methanol extract of *G. odorata roxb* (200 mg/kg: p. o)

Group III- Methanol extract of *G. odorata roxb* (400 mg/kg: p. o)

Group 1V: Paracetamol (100 mg/kg: p. o.).

Rectal temperatures were then recorded at 20, 21, 22, 23 and 24h (T °c) after yeast injection.

**Statistical analysis**

The results were expressed as mean ± S. E. M. The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's test. The *p*-value < 0.05 was considered to be statistically significant.

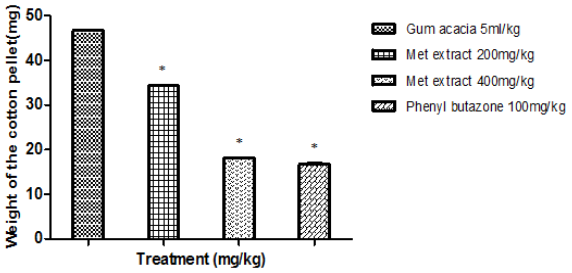


**Fig. 2: Effect of *G. odorata roxb* on Cotton-pellet granuloma in rats. *P<0.001 vs control.**

**RESULTS**

**Phytochemical Screening**

Preliminary phytochemical analysis revealed the presence of flavonoids, alkaloids, and steroids in MEGO Plant material.

**Studies on inflammation**

**Acute inflammation study**

**Carrageenan – induced paw oedema in rats**

Carrageenan – induced rat paw oedema was markedly inhibited by intraperitonial treatment with either the extract (200, 400 mg/kg) or phenyl butazone (100 mg/kg). The extract of *G. odorata roxb* showed highly significant ($p < 0.001$) acute inflammatory effect in a dose related manner, more or fewer equals to the effect were produced by phenyl butazone. The results were shown in Fig. 1.

**Sub- acute inflammation study**

**Cotton Pellet granuloma in rats**

In sub acute studies Methanol extract of *G. odorata roxb* shows highly significant sub acute anti-inflammatory effect **[Fig. 2].**

**Antinociceptive activity**

**Acetic acid induced writhing in mice**

The Methanol extract of *G. odorata roxb* (200-400 mg/kg, i. p.) were significantly reduced ($p < 0.001$) acetic acid-induced abdominal constrictions and stretching of hind limbs in a dose dependent manner **[Fig. 3].**

**Antipyretic activity**

As shown in **Fig. 4** subcutaneous injection of yeast caused elevation of rectal temperature in control rats 19h after administration. Oral administration of the extract produced a significant ($P<0.001$) dose dependent inhibition of temperature elevation. Peak inhibitory effect was observed at 1 h post-therapy, i. e., 20 h post-yeast injection ($p<0.001$).

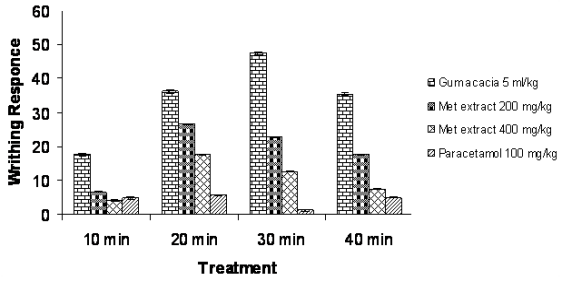


**Fig. 3: Effect of *G. odorata roxb* on Acetic acid induced writhing in mice**

**DISCUSSION**

In the present study, the anti-inflammatory, analgesic and anti-pyretic activity of the methanol extract of *G. odorata roxb* has been established. The test extract at two different doses (200-400 mg/kg) were found to significantly inhibit the carrageenan-induced rat paw oedema, a test, which has significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation [23]. Oedema formation due to carrageenan in the rat paw is the biphasic event [24]. The initial phase is attributed to the release of histamine and serotonin. The second phase of oedema is due to release of prostaglandins, protease and lysosome [25].

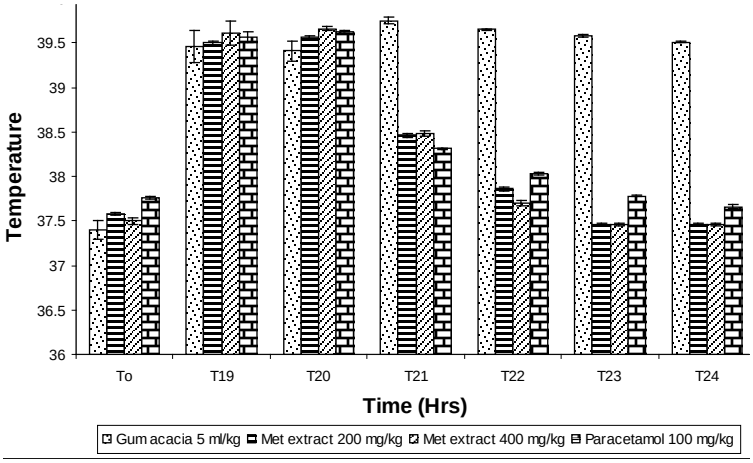


**Fig. 4: Effect of *G. odorata roxb* on yeast induced Hyperpyrexia in rats**

The second phase is sensitive to most clinically effective anti-inflammatory drugs [26]. Besides in the carrageenan-induced rat paw oedema model the production of protanoides has been through the serum expression of COX-2 by a positive feedback mechanism [27]. Therefore, it is suggested that the mechanism of action of test extract may be related to prostaglandin synthesis inhibition, as described for the anti-inflammatory mechanism of nonsteroidal anti-inflammatory drugs in the inhibition of an inflammatory process induced by carrageenan.

Likewise, the granulomatous tissue formation is related to the chronic inflammatory process, which is characterized by several phases [28]. In this regard, the oral treatment with 200, 400 mg/kg of the extract of *G. odorata roxb* and 100 mg/kg of phenyl butazone lead to 54.78 and 68.69% reduction of the granulomatous tissue formation, respectively ($P<0.001$).

In addition, the classification of antinociceptive drugs is usually based on their mechanism of action either on the central nervous system or on the peripheral nervous system [29]. With respect to the writhing test the research group of Deraedt et al., [30], described the quantification of prostaglandins by radio immuno assay in the peritoneal exudates of rats, obtained after intra peritoneal injection of acetic acid. They found high levels of prostaglandins, PGE2 and PGF2 alpha during the first 30 min after acetic acid injection. Nevertheless, it was found that the intra peritoneal administration of acetic acid-induces the liberation not only of prostaglandins, but also of the sympathetic nervous system mediators [31, 32]. Thus, the results obtained for the writhing test using acetic acid are similar to those obtained for the oedematogenic test using carrageenan. Therefore anti-inflammatory substances may also be involved in the peripheral analgesic activity.

The pain induction caused by liberating endogenous substances as well as some other pain mediators such as arachidonic acid via cyclooxygenase, and prostaglandin biosynthesis. This pain paradigm is widely used for the assessment of peripheral analgesic activity

due to its sensitivity and response to the compounds at a dose which is not effective in other methods. The local peritoneal receptor could be the cause of abdominal writhings. Pain sensation in acetic acid induced writhing paradigm is elicited by producing localized inflammatory response due to release of free arachidonic acid from tissue phospholipids via cyclo-oxygenase (COX), and producing prostaglandin specifically PGE2 and PGF2α, the level of lipoxygenase products may also increases in peritoneal fluids [33-34].

Indeed, non-steroidal anti-inflammatory drugs (NSAIDS), like paracetamol, exert their antipyretic action by largely inhibiting prostaglandin (E-type) protection in the hypothalamus [35]. Consequently, elevated plasma prostaglandin level, as observed in fever, is suppressed. Acetyl salicylic acid, another reference anti-pyretic drug (not used in this study), also brings about the same effect by a selective action on a specific cyclo-oxygenase (COX) isoenzyme in the CNS. The Methanol extract of *G. odorata roxb* demonstrated effective anti pyretic activity as evident in the inhibition of the temperature elevation in the yeast model. The antipyretic action of the extract may possibly be through inhibition of prostaglandin production, leading to suppression of elevated plasma level, especially since the extract had been shown to possess analgesic and anti-inflammatory activities.

## CONCLUSION

From these investigations, it may be concluded that the Methanol extract of *G. odorata roxb* showed analgesic, anti-inflammatory and antipyretic effects, similar to those observed for non-steroidal drugs such as, phenyl butazone and paracetamol. It is important to point out that the phytochemical analysis showed the presence of flavonoids and this might be responsible for anti-inflammatory and analgesic activity. Further investigations are under process in our laboratory to isolate and characterize the specific active components of the plant extract which is responsible for observed pharmacological actions.

## CONFLICT OF INTEREST

Authors declare no conflict of interest.

## REFERENCES

1. Sumanta Mondal, Raja S, Suresh P, Kumar GS. Analgesic, anti-inflammatory and antipyretic properties of Acacia suma stem bark. Int J Phytomed 2013;5:302-7.
2. Naveed M, Muhammad S, Haroon K. Antipyretic, analgesic and anti-inflammatory activity of Viola betonicifolia whole plant. BMC Comp Alter Med 2012;12:59-67.
3. Manoj Kumar R, Mahesh CS, Manoj G, Gajendar Kamal S, Somdatt G. Antipyretic activities of ethanolic extract of the whole plant of Fagonia schweinfurthii Hadidi on albino rats-An experimental study. Spatula DD 2012;2:159-64.
4. Dharmasiri JR, Jayakody AC, Galhena G, Liyanage SSP, Ratnasooriya WD. Antiinflammatory and analgesic activities of mature fresh leaves of Vitex negundo. J Ethnopharmacol 2003;87:199-206.
5. Park JH, Son KH, Kim SW, Chang HW, Bae K, Kang SS, *et al*. Antiinflammatory activity of Synurus deltoids. Phytother Res 2004;18:930-3.
6. Kumara NKVMR. Identification of strategies to improve research on medicinal plants used in Sri Lanka. In: WHO Symposium. University of Ruhuna, Galle, Sri Lanka; 2001. p. 12-4.
7. Sahu SK, Das D, Tripathy NK, Sundeep kumar HK, Banerjee M. Anti-inflammatory, analgesic and antipyretic effects of mollugo pentaphylla. Rasayan J Chem 2011;4:533-8.
8. Roxburgh RW, Coromandel P. Plant (introduction) gynocardia odorata. Indian Council Med Res 1820;3:95-299.
9. Jagan Mohan, Deepa L, Ubaidulla U, Ganesh N. *In vitro* antioxidant activity of hydroalcoholic extract of Gynocardia odorata roxb leaf. Int J Res Pharm Nano Sci 2013;2:351-7.
10. Khan H, Gupta N, Mohammed MS, Meetu A, Khan G, Mohan G. Antiulcer activity of seed extracts of Gynocardia odorata roxb on pylorus ligation and indomethacin induced gastric lesions in albino rats. Int J Dev Res 2013;3:49-54.
11. Shrish Kumar S, Hemant kumar, Mrityunjoy A. Phytochemical screening and ulcer protective activity of ethanolic seeds extract of Gynocardia odorata in different ulcer model. Am J Pharm Tech Res 2014;4:446-52.
12. Faisal A, Arshida ZB, Nur A, Muhammad T, Sharmin Reza C, Mohammad AR. Canvassing of thrombolytic, cytotoxic, and erythrocyte membrane-stabilizing attributes in *in vitro* screening of Gynocardia odorata. J Anal Sci Tech 2014;5:36-42.
13. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. New Delhi: Springer (India) Pvt. Ltd; 1998.
14. Ecobichon DJ. The basis of Toxicology testing. 1st Edn. CRC Press, New York, ISBN; 1997.
15. Winter CA, Risley EA, Nuss GW. Carrageenan-induced oedema in hind paws of the rat as an assay for anti-inflammatory drugs. In: Proceedings of the Society. Exp Biol Med 1962;111:544-7.
16. Goldenberg MM, IIse AC. Anti-inflammatory activity in EU-2972. Arch Int Pharmacodyn 1977;228:153-61.
17. Chu D, Kovacs BA. Anti-inflammatory activity in Oakgall extracts. Arch Int Pharmacodyn 1977;230:166-76.
18. Meier R, Schuler DP, Zur Fragedes MDH, Des Binde GC. Weles Wattsluns dur Cortisone. Experientia 1950;6:469-71.
19. Sheth UK, Dadkar NK, Kamat UG. Selected Topics in Experimental Pharmacology. The Kothari Book Depot: Bombay India; 1972. p. 164-5.
20. Lassman HB, Kirby RE, Wilker JC, Mc Fadden AR, Aultz DE, Hoffman D, *et al*. Pharmacology of a new non-steroidal anti-inflammatory agent HP-549. Arch Int Pharmacodyn 1977;227:143-5.
21. Koster R, Anderson M, Beer EJ. Acetic acid for Analgesic screening. Federation Proceeds 1959;18:412-6.
22. Mukherjee K, Saha BP, Mukherjee PK. Evaluation of antipyretic potential of Leucas lavandulaefolia aerial part extract. Phytother Res 2002;16:686-8.
23. Mossa JS, Rafatullah S, Galal AM, Al-Yahya MA. Pharmacological Studies of Rhus retinoeehoea. Int J Pharmacog 1995;33:242-6.
24. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenan oedema in rats. J Pharmacol Exp Therap 1969;166:96-103.
25. Crunkhon P, Meacock SER. Mediators of the inflammation induced in the rat paw by Carrageenan. Br J Pharmacol 1971;42:392-402.
26. Di Rosa M, Giroud JP, Willough DA. Studies of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. Pathol 1971;104:15-29.
27. Nantal F, Denis D, Gordon R, Northey A, Cirino M, Metters KM, *et al*. Distribution and regulation of cyclo-oxygenase-2 carrageenan induced inflammation. Braz J Pharmacol 1999;128:853-9.
28. Swingle KF, Shideman FE. Phases of inflammation response to subcutaneous implantation of Cotton-pellet and other modifications by certain anti-inflammatory agents. J Pharmacol Exp Therap 1972;13:226-34.
29. Planas E, Sanchez S, Rodriguez L, Polo Pug MM. Antinociceptive, anti-edema effects of liposomal morphine during acute inflammation of the rat paw. Pharmacol 2000;60:121-7.
30. 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;61:17-23.
31. Hokansan GC. Acetic acid for analgesic screening. J Nat Prod 1978;41:497-8.
32. Duarte JDG, Nakamura M, Ferria SH. Participation of the Sympathetic system in acetic acid induced Writhing in mice. Braz J Med Biol Res 1988;21:341-3.
33. Khan H, Saeed M, Gilani AUH, Khan MA, Dar A, Khan I. The antinociceptive activity of Polygonatum verticillatum rhizomes in pain models. J Ethnopharmacol 2010;127:521–7.
34. Mbiantcha M, Kamanyi A, Teponno R, Tapondjou A, Watcho P, Nguelefack T. Analgesic and anti-inflammatory properties of extracts from the bulbils of Dioscoreabulbifera L. var sativa (Dioscoreaceae) in mice and rats. eCAM 2011.
35. Rang HP, Dale MM, Ritter JM. Anti-inflammatory and immunosuppressant Drugs. In: Pharmacology. Churchill Livingstone, Edinburgh. 4th Edn; 1999. p. 229-47.