EVALUATION OF ANTI-INFLAMMATORY AND ANTAGONIST ACTIVITY OF XANTHONES FROM SWERTIA CORYMBOSA (GRISEB.) WIGHT EX C.B. CLARKE

G. MAHENDRAN1*, M. MANOJ2, K.J. RAJENDRA PRASAD2, V. NARMATHA BAI1
1Department of Botany, School of Life sciences, 2Department of Chemistry, School of Chemical sciences, Bharathiar University, Coimbatore 641046, Tamilnadu, India. Email: mahendran0007@gmail.com.

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ABSTRACT

Objective: This study was designed to investigate analgesic and anti-inflammatory activity of the xanthis isolated from Swertia corymbosa.

MATERIALS AND METHODS: Aerial part of Swertia corymbosa was extracted with petroleum ether and ethyl acetate, further subjected to chromatographic separation for isolation of xanthis. Structures of isolated xanthis were elucidated by spectrocopic methods. Anti-inflammatory (carrageenan-induced paw edema in rat), analgesic action was estimated in mice using the acetic acid-induced writhing test and the hot-plate method and the acute oral toxicity study in mice.

Results: Four known xanthis namely Decussatin (1), Gentianalequin (2) and Swertianin (3) 1, 8-dihydroxy-2, 6-dimethoxyxanthone (4) and two new xanthis 8-hydroxy-1, 2, 4, 6-tetramethoxyxanthone (5) 1, 2, dihydroxy-6-methoxyxanthone-8-O-β-D-xylopyranosyl (6) were isolated. Among the isolated xanthis, compound 3 and 6 showed stronger suppression on carrageenan-induced rat paw edema (60.28%, 71.80 %) and increase in hot plate reaction time (9.88, 11.78 sec), while reduced the number of writhing (70.60, 76.85 %) in acetic acid test.

Conclusion: Based on the results of the present study, it is concluded that compound 3 and 6 had potential anti-inflammatory and antinociceptive which could be used as drug candidates against inflammation related conditions.

Keywords: Xanthonic dicycloxypranoiside, Antinflammatory, Carrageenin-induced paw edema, Swertia corymbosa.

INTRODUCTION

Inflammation is fundamentally a protective response, ultimate goal of which is to get rid of the noxious things, but sometimes it may be potentially harmful and needs pharmacological treatment to control its symptoms[1]. Inflammation is the body’s way of dealing with infections, maintaining a subtle balance between the beneficial effects of inflammation cascades to restrict the infection and potential for long-term tissue destruction[2,3] and involves a complex array of enzyme activation, mediator release, fluid extra vacations, cell migration, tissue breakdown and repair[4]. If not controlled, inflammation can lead to development of diseases such as chronic asthma, rheumatoid arthritis and rheumatoid bowel disease, etc[5,6,7]. Till date a very few anti-inflammatory drugs from herbal origin have been found and a number of plants from ethno-medicinal databases are under laboratory investigations across the world[8].

Swertia, an important genus of family Gentianaceae, are rich sources of xanthones, flavonoids, iridoids and terpenoids[9]. The herbs of this genus are extensively used as bitter tonic and febrifuge in the Ayurvedic system of medicine[9]. The extracts of a number of species have long been used in folk medicine for the treatment of hepatitis, cholercystitis, pneumonia, dysentery and cancer [9]. Xanthis are naturally occurring polyphenols and structurally similar to flavonoids[10,11]. Xanthis exhibit various pharmacological properties such as antioxidant[12,13], anti-inflammatory activities [14], inhibition of a variety of tumor cell lines growth[15,16] and inhibiting α-glucosidase [17, 18].

Swertia corymbosa (Griseb.) Wight ex C.B. Clarke commonly known as Shirattakuchi by Irulars tribe. This plant has a long history of being used by Irulars and Paliyan ethnic medical practitioners have been used for medicinally as diarrhea, fever, jaundice, diabetic, inflammation, anxiety, promote sleep, antiepileptic, nervous disorders, antidote and as a stomach wash in cattle [19,20]. The antimicrobial and phytochemical screening[21], antioxidant activity[22], anti-inflammatory activity[23] and isolation of 1, 5, 8-trihydroxy-3-methoxyxanthone[24]. Till date, no other articles devoted to pharmacological or phytochemical properties of this plant have been published. On the basis of the previously references of S. corymbosa, the present study focuses on the isolation, structure elucidation and investigates the anti-inflammatory, analgesic activities of the xanthis from S. corymbosa.

MATERIALS AND METHODS

General experimental procedures

Melting points (m.p) were determined on Mettler FP 51 apparatus (Mettler Instruments, Switzerland) and are uncorrected. They are expressed in degree centigrade (°C). IR spectra were recorded on Shimadzu FTIR-8201 PC Spectrophotometer (Shimadzu-Japan) using KBr disc. 1H-NMR, 13C-NMR and 2D NMR (H-H COSY, C-H COSY and HMBC) were recorded on Bruker AV 500. (500 MHz (H) and 125 MHz (13C) NMR spectrometer using tetramethylsilane (TMS) as an internal reference. The chemical shifts are expressed in part per million (ppm). Mass spectra (MS) were recorded on Auto spec E1 + Shimadzu QP 2010 PLUS GC-MS. Micro analyses were performed on a vario EL III model CHNS analyser (Vario, Germany) at the department of Chemistry, Bharathiar University. The purity of the product was tested by TLC with plates coated with silica gel-G.

Plant material

The plant material was collected from Vellungiri hills, Coimbatore (Tamilnadu) at an altitude of 1850 MSL. The plant was authenticated by Botanical Survey of India and the herbarium was deposited in the Department of Botany, Bharathiar University, Coimbatore. (Accession Number: BUH6144).

Extraction, isolation and identification

The air-dried aerial parts of S. corymbosa (3 kg) were extracted with petroleum ether and ethyl acetate using soxhlet apparatus. The resulting crude extract was subjected to TLC which showed three major spots and they were separated by column chromatography using silica gel with petroleum ether and chloroform and chloroform and ethyl acetate as gradient solvents. Compound 1, 2 and 3 were separated using petroleum ether and chloroform in the ratio of (98: 2), (95: 5) and (98: 10) respectively. Compound 4, 5 and 6 were separated using chloroform and ethyl acetate in the ratio of (95: 5), (92: 7) and (85: 15) respectively.

Physical properties and spectral data

Decussatin (1)

Yellow needles; m.p 152–155 °C; IR (KBr) cm−1 3321(OH), 1654(C=O), 1600, 1483, 1282, 1356, 1H NMR (300 MHz, CDCl3) δ (ppm): 3.87.
(3, 3H, 7-OCH3) 3.93 (s, 3H, C3-OCH3), 4.00 (s, 3H, C8-OCH3), 6.30 (d, 1H, J = 2.40 Hz, H-4) 6.32 (d, 1H, J = 2.40 Hz, H-2) 7.15 (d, 1H, J = 9.00 Hz, H-5) 3.72 (d, 1H, J = 9.00 Hz, H-5). 13C NMR (75 MHz, CDCl3) δ (ppm): 55.72 (C8-OCH3), 57.07 (C7-OCH3), 61.37 (C3-OCH3), 91.97 (C-4'), 96.81 (C-2'), 103.98 (C-9a), 112.75 (C-5), 115.68 (C-8a), 120.30 (C-6), 140.90 (C-10a), 149.20 (C-10a), 159.91 (C-7), 157.07 (C-8), 163.77 (C-1), 166.35 (C-13), 181.13 (C-O), EIMS: m/z (%) 302 (M+ 75%), 287 (100%), 273 (10%), 259 (45%), 201 (12%), 97 (25%). 84 (40%), 69 (48%), 43 (62%), 41 (40%). Calcd.: C = 63.58 %, H = 4.64 %. Found: C = 63.30 %, H = 4.77 %.

Gentianic acid (2)

Yellow needles, mp 192–194 °C; IR (KBr) cm−1: 3413-5301 (OH groups), 1653 (C=O), 1596, 1475, 1276 (O-CH3), 1323 (H NMR (300 MHz, CDCl3) δ (ppm): 3.88 (s, 3H, C3-OCH3) 4.03 (s, 3H, C8-OCH3), 6.02 (b s, 3H, C7-OH), 6.32 (d, 1H, J = 2.40 Hz, H-2) 6.35 (d, 1H, J = 2.40 Hz, H-4) 1/4 (d, 1H, J = 9.00 Hz, H-5), 1.73 (t, 1H, J = 9.00 Hz, H-5), 2.14 (s, 3H, C1-CH3). 13C NMR (75 MHz, CDCl3) δ (ppm): 55.73 (C8-OCH3), 57.07 (C7-OCH3), 91.69 (C-4'), 96.31 (C-2'), 103.98 (C-9a), 113.01 (C-5). 114.63 (C-3a), 122.46 (C-6), 144.23 (C-20a), 144.44 (C-4a), 150.62 (C-7), 151.76 (C-15), 163.54 (C-16), 180.52 (C=O). EIMS: m/z (%) 302 (M+ 100%), 270 (12%), 245 (62%), 229 (20%), 214 (40%), 202 (26%), 184 (12%), 111 (30%), 85 (56%), 57 (30%), 47 (30%). FAB: m/z (%) 262.50 (H2O+ 2H), 17.20 (H2O+ H), 41.20 (H2O+ H2). Gentianic acid was further supported structurally by 2D NMR studies (H-H COSY and HMBC) and HMBC connectivity diagram is shown Figure 1. In the H-H COSY one proton doublet at 7.14 (J = 9.00 Hz) and one proton doublet at 7.37 (J = 9.00 Hz) have connectivity and assigned for C3-OH and C8-OH, the remaining being quartenary carbons.

Swertianin (3)

Yellow needles, mp: 224–227 °C; IR (KBr) cm−1: 3388-3442 (OH groups), 1662 (C=O), 1282 (O-CH3). 1H NMR (300 CDCl3) δ (ppm): 3.90 (s, 3H, C6-OCH3) 5.44 (b s, 3H, C2-OH), 3.63 (d, 1H, J = 2.40 Hz, H-5) 6.41 (d, 1H, J = 2.40 Hz, H-7) 6.75 (d, 1H, J = 0.90 Hz, H-5), 7.30 (d, 1H, J = 9.00 Hz, H-4), 1.18 (s, 3H, C8-OH) 11.94 (s, 1H, C1-CH3). 13C NMR (75 MHz, CDCl3) δ (ppm): 60.69 (C5-OCH3), 97.33 (C-4'), 101.84 (C-2), 106.89 (C-9a), 110.77 (C-5), 112.30 (C-8a), 128.75 (C-6), 145.23 (C-10a), 152.01 (C-4a), 153.28 (C-7), 162.79 (C-8), 167.27 (C-1), 171.92 (C-3'), 189.51 (C=O). EIMS: m/z (%) 274 (M+ 100 %), 260 (5 %), 259, 258 (3 %), 247 (2%), 245 (25%), 231 (10%), 216 (13%), 203 (35%) 190 (14 %), 175 (10 %), 147 (5 %), 123 (10 %), 101 (36 %), 95 (10 %), 81 (5 %). 69 (10 %), 43 (12 %). Calcd.: C = 53.25 %, H = 4.70 %. Found: C = 53.53 %, H = 4.70 %. 1, 2-dihydroxy-6-methoxyxanthone-8-O-β-D-xylopyranosyl was further supported structurally by 2D NMR studies (H-H COSY and HMBC)

Animals

Swiss albino mice weighing 20-25 g and Wistar albino rats of 200-250 g were used for the pharmacological studies. They were housed in a clean polypropylene cage and maintained under standard laboratory conditions (temperature 25±2°C with dark/light cycle 12/12 h; 35-60 humidity). They were fed with standard pellet diet (VRK Nutritional solutions, Sangli, Maharashtra) and water ad libitum. The studies were carried out at Nandha College of Pharmacy and Research Institute, Perundurai, Tamil Nadu, India. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee, and was clarified by the same before experiment (68B/02/C/PCPSEA).

Acute toxicity

Acute oral toxicity study was performed according to acute toxic class method. Swiss albino mice (n=6) of either sex selected by random sampling technique were used for acute toxicity study. The animals were kept fasting for overnight providing only water. Isolated compounds 1–6 (suspended in 0.5% carboxy methyl cellulose) were administered orally at a dose of 5, initially to separate groups of mice and mortality was observed for 24 h. 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, 1000 and 2000 mg/kg. The general behaviors such as motactivity, tremors, convulsions, strab reaction, aggressiveness, pilorection, loss of lighting reflex, sedation, muscle relaxation, hypnotis, analgesia, ptosis, lacrimation, diarrhoea and skin colour were observed for the first one hour and after 24 h of test drug administration.

Analytic activity

Hot plate method

The hot plate method was used to measure response latencies according to the method described by MacDonald et al [25]. For the experiments, fourteen groups (n = 6) of swiss albino mice (20–25 g) were placed on a plate maintained at room temperature for 15 min. Group 1: normal control fed with water (vehicle) 10 ml/kg p.o., and Group 2: treated with pentazocine (25mg/kg, i. p.) whereas Groups 3 to 14 groups animals received compounds 1-6 (25 and 50mg/kg, p.o.). The animals were positioned on Eddy’s hot plate kept at a temperature of 55±0.5°C. The time taken by the animals to lick the fore or hind paw or jump out of the place was taken as the reaction time. The latency was recorded at the time of 0 (just before any
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Table 1: Anti-inflammatory effects of isolated compound 1-6 from *Swertia corymbosa* on carrageenan-induced paw edema in rats.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose (mg/kg b.w.)</th>
<th>Swelling thickness (mm) ±SD (inhibition %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1h</td>
<td>2h</td>
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<tr>
<td>Control</td>
<td>10 ml/kg b.w.</td>
<td>4.32±0.65 (5.87±0.43)</td>
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<tr>
<td>Indomethacin</td>
<td>25 (22.69%)</td>
<td>3.27±0.15 (3.48±0.76)</td>
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<tr>
<td>Compound 1</td>
<td>25 (1.11%)</td>
<td>3.76±0.61 (4.16±0.01)</td>
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<td>50 (21.51%)</td>
<td>3.51±0.17 (3.72±0.51)</td>
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<td>Compound 2</td>
<td>25 (1.70%)</td>
<td>3.43±0.61 (3.76±0.31)</td>
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<td>50 (2.152%)</td>
<td>3.32±0.17 (3.47±0.22)</td>
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<tr>
<td>Compound 3</td>
<td>25 (21.51%)</td>
<td>3.34±0.11 (3.41±0.51)</td>
</tr>
<tr>
<td></td>
<td>50 (21.52%)</td>
<td>3.13±0.27 (3.09±0.32)</td>
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<tr>
<td>Compound 4</td>
<td>25 (15.54%)</td>
<td>3.53±0.15 (3.62±0.15)</td>
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<td>50 (16.87%)</td>
<td>3.13±0.27 (3.09±0.32)</td>
</tr>
<tr>
<td>Compound 5</td>
<td>25 (18.67%)</td>
<td>4.13±0.66 (4.93±0.65)</td>
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<tr>
<td></td>
<td>50 (20.36%)</td>
<td>3.73±0.32 (4.62±0.32)</td>
</tr>
<tr>
<td>Compound 6</td>
<td>25 (21.82%)</td>
<td>3.12±0.25 (3.31±0.83)</td>
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<tr>
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<td>50 (2.62%)</td>
<td>2.97±0.40 (2.81±0.40)</td>
</tr>
</tbody>
</table>
| Control (vehicle) – normal water, The data represent the mean ± SD (n = 6). Values given in parentheses represent percentage of inhibition; ***P<0.05. **P<0.01. *P<0.001 compared to control.
The anti-inflammatory activity of isolated compounds (1-6) against acute paw edema (induced by carrageenan) is shown in Table-15 and the results are comparable to that of the standard drug indomethacin, a potent inhibitor of the prostaglandins. Carrageenan induced paw edema remained even 5 h after its injection into the sub plantar region of rat paw. Indomethacin inhibited the edema formation due to carrageenan to an extent of (3.09±0.71) (at 5 h) at the dose of 25 mg/kg. Compound 6 showed dose-dependent suppression of paw edema formation at 25 and 50 mg/kg with the percentage inhibition of 60.91 % (P < 0.01) and 71.80 % (P < 0.001) respectively. Further, the highest dose of compound 3 and 4 (50 mg/kg) also significantly suppressed the edema formation with the percentage inhibition 56.04 % (P < 0.05) and 60.28 % (P < 0.01) (Table-1). No significant action of compounds to reduce carrageenan-induced paw edema was observed at first two hour. Compared to indomethacin at a dose of 25 mg/kg showed significant reduction of hind paw edema at 4 and 5 h with the percentage inhibition of 54.58% and 64.44 % respectively. The result obtained is clearly indicates that compound 6 is appeared to be more effective that of indomethacin.

No significant action of compounds to reduce carrageenan-induced paw edema was observed at first two hour (P > 0.05 for all doses tested). Compared to the control group, a known anti-inflammatory agent indomethacin at a dose of 25 mg/kg showed significant reduction of hind paw edema at 4 and 5 h with the percentage inhibition of 54.58% and 64.44 % respectively. The result obtained is clearly indicates that compound 6 is appeared to be more effective that of indomethacin.

The analgesic activity of isolated compounds (1-6) was assessed using hot plate test in Swiss albino mice is illustrated in Figure-2. In this analgesic testing model, pentazocine (25 mg /kg, b.w) and compounds 3, 4 and 6 (25 and 50 mg/kg, b.w) significantly prolonged the reaction time of animals with relatively extended duration of stimulation confirming centrally mediated activity. Compound 3 and 6 showed significant analgesic activity at dose levels of 25 and 50mg/kg, b.w. Analgesic activity of the later dose was often comparable that of the standard drug pentazocine at the dose level 50 mg/kg b.w. and time 240 minutes reaction lime, the analgesic activity of compound 3 and 6 (10.2±1.89, 11.78±0.64 sec respectively) of the later dose was often comparable that of the standard drug pentazocine (10.11±0.21Sec). In the present study, compounds 1, 2 and 5 (25 and 50 mg/kg, p.o) did not show any significant increase in the baseline. The hot-plate test is an established and reliable test of nociception. Thermal stimulus-induced hyperalgesia is specific for centrally-mediated nociception[39] and is thought to involve opioid receptors[40]. The effect of compound 6 was found to be better than that of pentazocine (25 mg /kg s.c). Hence, compound 6 may possess central antinociceptive actions involving opioid-like receptor mediation.

Peripheral analgesic activity was assessed by acetic acid induced writhing test, the compound 3 and 6 showed significant (p< 0.001) suppression of writhes in the experimental rats. Pretreatment with compound 3 and 6 at doses of 25 and 50 mg/ kg b.w reduced the
number of writhing in a dose dependent manner and it in the later dose registered higher levels of analgesic activity than the standard drug indomethacin. This inflammatory pain model has been used to assess the central and peripheral antinociceptive or anti-inflammatory activity of new agents[41]. Intraperitoneal administration of acetic acid causes an increase in cyclooxygenase (COX), lipoxygenase (LOX), prostaglandins (PGs), histamine, serotonin, bradykinin, substance P, IL-1β, IL-8 and TNF-α in the peripheral tissue fluid. Increased levels of these mediators causes the excitation of primary afferent nociceptors entering dorsal horn of the central nervous system[42]. These mechanisms are associated with the development of inflammatory pain and abdominal constriction[43]. The potent antinociceptive effect of compound 3 and 6 in the acetic acid-induced model suggests that compound 3 and 6 may be involved in the inhibition of COX, LOX, bradykinin and other inflammatory mediators resulting interrupted signal transduction in primary afferent nociceptors. Xanthone derivatives like mangostin, isomangostin and mangostin triacetate is known to possess significant anti-inflammatory activities[38]. Islam et al[44] xanthones isolated from Swertia chirata has already been reported to inhibit the inflammatory mediators such as 5-HT, Carrageenin, Bradykinin, Dextran, and PGE2. Our findings also provide evidence supporting the use of S. corymbosa in such painful and inflammatory conditions.

The evidence from the hot plate test and acetic acid induced-writing test experiment that compound 3 and 6 isolated from S. corymbosa posses both central and peripheral analgesic activities. Much evidence has shown that the production of free radicals at the site of inflammation contributes to tissue damage and stimulation of pain. Painful stimulation increases the production of free radicals and it increases lipidperoxidation. The application of antioxidants, in the present case compound 3 and 6 increase the anti-oxidation capacity and thus enhances the protection against the consequences of pain. Antioxidants are known to protect CNS against free radical and also decreased sensation of pain[45] .

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose (mg/kg b.w.)</th>
<th>Number of writhings ±SD</th>
<th>Inhibitory ratio (%)</th>
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<tbody>
<tr>
<td>control</td>
<td>10 ml/kg b.w.</td>
<td>83.25± 1.43</td>
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<tr>
<td>Indomethacin</td>
<td>25</td>
<td>23.87± 4.66</td>
<td>72.32*</td>
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<tr>
<td>Compound 1</td>
<td>25</td>
<td>38.49± 3.21</td>
<td>29.74</td>
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<tr>
<td>Compound 2</td>
<td>50</td>
<td>51.53± 2.10</td>
<td>38.10</td>
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<tr>
<td>Compound 3</td>
<td>25</td>
<td>52.33± 5.57</td>
<td>37.14</td>
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<td>Compound 4</td>
<td>50</td>
<td>45.10± 2.10</td>
<td>45.83***</td>
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<td>Compound 5</td>
<td>25</td>
<td>28.32± 1.29</td>
<td>65.92**</td>
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<td>Compound 6</td>
<td>50</td>
<td>24.47± 1.60</td>
<td>70.60*</td>
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<td>Compound 7</td>
<td>25</td>
<td>51.99± 2.37</td>
<td>37.66</td>
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<td>Compound 8</td>
<td>50</td>
<td>46.42± 1.23</td>
<td>44.22***</td>
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<td>Compound 9</td>
<td>25</td>
<td>67.49± 3.45</td>
<td>18.93</td>
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<td>Compound 10</td>
<td>50</td>
<td>61.23± 4.98</td>
<td>26.45</td>
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<td>Compound 11</td>
<td>25</td>
<td>29.35± 1.78</td>
<td>64.74**</td>
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<td>Compound 12</td>
<td>50</td>
<td>19.22± 1.12</td>
<td>76.85*</td>
</tr>
</tbody>
</table>

Control (vehicle) – normal water. The data represent the mean ± SD (n = 6). Values given in parentheses represent percentage of inhibition; ***P<0.05. **P<0.01. *P<0.001 compared to control

CONCLUSION

The present study has established the traditional utilization of Swertia corymbosa in inflammatory disorders. This study showed that it is 1, 2, dihydroxy-6-methoxyxanthone-8-O-β-D-xylpyranosyl one of the active components in Swertia corymbosa responsible for the anti-inflammatory and antinociceptive. This compound could be a good candidate for further development of a new phytotherapeutic agent.

REFERENCES


