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Research Article

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

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ABSTRACT

Objective: This study was designed to investigate analgesic and anti-inflammatory activity of the xanthones 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 xanthones. Structures of isolated xanthones were elucidated by spectroscopic 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 xanthones namely Decussatin (**1**), Gentiacaulein (**2**) and Swertianin (**3**) 1, 8-dihydroxy-2, 6-dimethoxyxanthone (**4**) and two new xanthones 8-hydroxy-1, 2, 4, 6-tetramethoxyxanthone (**5**) 1, 2, dihydroxy-6-methoxyxanthone-8-O-β-D-xylopyranosyl (**6**) were isolated. Among the isolated xanthones, 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 antinoceceptive which could be used as drug candidates against inflammation related conditions.

Keywords: Xanthone dixylopyranoside, Anti-inflammatory, Carrageenan-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 xanthonoids, flavonoids, irridoids 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, cholecystitis, pneumonia, dysentery and cancer [9]. Xanthones are naturally occurring polyphenols and structurally similar to flavonoids[10,11]. Xanthones 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 xanthones 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. ^{1}H-NMR, ^{13}C-NMR and 2D NMR (H-H COSY, C-H COSY and HMBC) were recorded on Bruker AV 500. (500 MHz (^{1}H) and 125 MHz (^{13}C) NMR spectrometer using tetramethysilane (TMS) as an internal reference. The chemical shifts are expressed in part per million (PPM). Mass spectra (MS) were recorded on Auto spec EI + 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 Vellingiri 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 (90: 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, ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.87

(s, 3H, C7-OCH_3) 3.93 (s, 3H, C3-OCH_3), 4.00 (s, 3H, C8-OCH_3), 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* = 9.00Hz, H-6) 7.32 (d, 1H, *J* = 9.00 Hz, H-5). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 55.72 (C8-OCH_3), 57.07 (C7-OCH_3), 61.73 (C3-OCH_3), 91.97 (C-4), 96.81 (C-2), 103.98 (C-9a), 112.72 (C-5), 115.68 (C-8a), 120.30 (C-6), 148.76 (C-10a), 149.20 (C-4a), 150.91 (C-7), 157.07 (C-8), 163.77 (C-1), 166.35 (C-3), 181.13 (C=O). EIMS: *m/z* (%) 302 (M^+ 75 %), 287 (100 %), 273 (10 %), 259 (45 %), 201 (12 %), 97 (25 %), 83 (40 %), 69 (48 %), 43 (62 %), 41 (40 %). $C_{16}H_{14}O_6$ (302). Anal. Calcd. C: 63.58 %, H: 4.64 %: Found C: 63.30 %, H: 4.77 %.

Gentiacaulein (2)

Yellow needles, m.p.192–194 °C; IR (KBr) cm^{-1}, 3415-3310 (OH), 1653 (C=O), 1596, 1475, 1276 (O-CH_3), 1323; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.88 (s, 3H, C3-OCH_3) 4.03 (s, 3H, C8-OCH_3), 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), 7.14 (d, 1H, *J* = 9.00 Hz, H-6), 7.37 (d, 1H, *J* = 9.00 Hz, H-5), 13.16 (s, 1H, C1-OH),. ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 55.73 (C8-OCH_3), 62.73 (C3-OCH_3), 91.99 (C-4), 96.31 (C-2), 103.98 (C-9a), 113.01 (C-5), 114.63 (C-8a), 122.46 (C-6), 144.23 (C-10a), 145.44 (C-4a), 150.62 (C-7), 157.16 (C-8), 163.54 (C-1), 166.44 (C-3), 180.52 (C=O). EIMS: *m/z* (%) 288 (M^+, 62 %) 270 (100 %), 259 (12 %), 245 (62 %), 229 (20 %), 214 (40 %), 202 (26 %), 184 (12 %), 111 (30 %), 85 (56 %), 71 (50 %), 69 (58 %), 47 (30 %). $C_{15}H_{12}O_6$ (288). Anal. Calcd. C: 62.50 %, H: 4.16 %: Found C: 62.41 %, H: 4.29 %. Gentiacaulein 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 C_5- and C_6-H. The DEPT-135 spectrum displayed 4-CH signals besides the OCH_3 and C=O, the remaining being quarternary carbons.

Swertianin (3)

Yellow needles; m.p. 224–227 °C; IR (KBr) cm^{-1}: 3388-3442 (OH groups), 1662 (C=O), 1282 (O-CH_3). ^1H NMR (300 $CDCl_3$) δ (ppm): 3.90 (s, 3H, C6-OCH_3) 5.44 (b s, 3H, C2-OH), 6.34 (d, 1H, *J* =2.40 Hz, H-5) 6.41 (d, 1H, *J* = 2.40 Hz, H-7) 6.85 (d, 1H, *J* =9.00 Hz, H-3), 7.30 (d, 1H, *J* =9.00 Hz, H-4), 11.87 (s, 1H C8-OH) 11.94 (s, 1H C1-OH). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 60.69 (C6-OCH_3), 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 %), 246 (50 %), 245 (96 %), 231 (64 %), 216 (13 %), 202 (30 %) 185 (16 %), 171 (14 %), 157 (23 %), 152 (18 %), 137 (34 %), 123 (88 %), 101 (36 %), 98 (30 %), 77 (35 %), 63 (40 %), 51 (62 %). $C_{14}H_{10}O_6$ (274). Anal. Cal Found C: 61.46 %, H: 3.52 %. Swertianin 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 6.85 (*J* = 9.00 Hz, C3-H) and one proton doublet at 7.30 (*J* = 9.00 Hz, C4-H) have connectivity and assigned for C_3-H and C_4-H.

1, 8-dihydroxy-2, 6-dimethoxyxanthone (4)

Yellow needles, m.p.184–187 °C; IR (KBr): υ $_{max}$ 3433 (OH), 1694(C=O), (1604) (Ar). ^1H NMR ($CDCl_3$, 500MHz) δ 3.81 (3H s, OCH_3-6), 3.86 (3H, s, OCH_3-2), 6.23 (1H, d, *J* = 2.00 Hz, H-7), 6.29 (1H, d, *J* = 2.00 Hz, H-5), 6.75 (1H, d, *J*= 9.00 Hz, H-4), 7.17 (1H, d, *J* = 9.00 Hz, H-3), 11.87 (1H, s, OH-8), 12.00 (1H, s, OH-1). ^{13}C NMR ($CDCl_3$) δ 55.90 (OCH_3, C-6), 57.05 (OCH_3, C-2) 92.88 (C-5), 97.18 (C-7), 102.31 (C-8a), 105.56 (C-4), 107.75 (C-9a) 120.38 (C-3), 142.91 (C-2), 149.58 (C-4a), 150.13 (C-1), 158.06 (C-10a) 162.93 (C-8), 167.42 (C-6), 184.99 (C=O, C-9). 1, 8-dihydroxy-2, 6-dimethoxyxanthone was further supported structurally by 2D NMR studies (H-H COSY and HMBC) and EIMS: *m/z* (%) 289.13 [M+H]+ (Calc. for $C_{15}H_{12}O_6$).

8-hydroxy-1, 2, 4, 6-tetramethoxyxanthone (5)

Yellow needles, m.p. 165-167 °C, IR (KBr): υ $_{max}$ 3435, 2922, 2853, 1651, 1577 and 1306. ^1H NMR ($CDCl_3$) δ 3.88 (3H, s, OCH_3-6), 3.94 (3H, s, OCH_3-2), 3.96 (1H, s, OCH_3- 4), 4. 01 (1H, s, OCH_3-1), 6.32 (1H, d, *J* = 2.00 Hz, H-7), 6.46 (1H, d, *J* = 2.00 Hz, H-5), 6.97 (1H, s, H-3). 13.29 (1H, s, OH-8). ^{13}C NMR ($CDCl_3$) δ 55.81 (OCH_3, C-6), 56.88 (OCH_3, C-1), 57.65 (OCH_3,C-4), 61.87 (OCH_3, C-2), 92.03 (C-5), 97.39 (C-7), 104.22 (C-8a) 105.14 (C-3), 116.05 (C-9a), 141.05 (C-2), 141.47 (C-4a), 144.49 (C-1), 148.65 (C-4), 156.94 (C-10a), 163.56 (C-8), 166.42 (C-6), 181.09 (C=O, C-9). 8-hydroxy-1, 2, 4, 6-tetramethoxyxanthone was further supported structurally by 2D NMR studies (H-H COSY and HMBC).

1, 2, dihydroxy-6-methoxyxanthone-8-O-β-D-xylopyranosyl: Swertianin xyloside (6)

Yellow needles; m.p. 245–247 °C; IR (KBr): υ $_{max}$ 3350-3430cm^{-1} (OH groups), 1657cm^{-1} (C=O), 1285cm^{-1} (O-CH_3). ^1H NMR (500, $CDCl_3$) δ (ppm): 2.87-3.88 (m, 15H, sugar protons), 3.90 (3H, s, OCH_3-6) 4.20 (1H, d, *J* = 7.00 Hz, H-1''), 5.29 (1H, d, *J* = 7.00 Hz, H-1'), 5.44 (1H, b s, OH-2), 6.34 (1H, d, *J* = 2.40 Hz, H-5) 6.41 (1H, d, *J* = 2.40 Hz, H-7) 6.85 (1H, d, *J* = 9.00 Hz, H-3), 7.30 (1H, d, *J* = 9.00 Hz, H-4), 11.94 (1H, s, OH-1). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 60.69 (OCH_3, C-6), 65.30 (C-5'), 68.38 (C-5''), 69.50 (C-4'), 69.62 (C-4''), 73.02 (C-2'), 73.69 (C-2''), 75.68 (C-3'), 75.97 (C-3''), 97.33 (C4), 101.84 (C-2), 102.77 (C-1'), 104.03 (C-1''), 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,C-9). EIMS: *m/z* (%) 540 (M^+ absent), 274 (M-265, 100 %) 273 (5 %), 248 (40 %), 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 %). $C_{24}H_{26}O_{14}$ (538). Anal. Calcd. C: 53.53 %, H: 4.87 %: Found C: 53.25 %, 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 20-30g 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±3ºC with dark/light cycle 12/ 12 h; 35-60 humidity). They were fed with standard pellet diet (VRK Nutritional solutions, Sangli, Maharastra) 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 cleared by same before beginning the experiment (688/02/C/CPCSEA).

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 24h. 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 motoractivity, tremors, convulsions, straub reaction, aggressiveness, piloerection, loss of lighting reflex, sedation, muscle relaxation, hypnosis, analgesia, ptosis, lacrimation, diarrhea and skin colour were observed for the first one hour and after 24 h of test drug administration.

Analgesic activity

Hot plate method

The hotplate 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±0.5ºC. 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

treatment) and 30, 60, 120, and 240 min after oral administration of samples and intraperitoneal administration of standard. A latency period of 15s was observed to avoid damage to the paw. The percentage thermal pain stimulus relief or protection was determined by applying the formula:

$$\% \text{ Protection against thermal pain stimulus} = \frac{[(\text{test mean} - \text{control mean})/\text{test mean}] \times 100}{\text{Acetic acid-induced writhing}}$$

The test was carried out using the method of Siegmund et al[26]. This method was used to preferentially evaluate possible peripheral effects of isolated compounds 1-6 as analgesic substance. Fourteen groups swiss albino male mice (n = 6) were fasted overnight prior the start of the experiment, and water *ad libitum*. The peripheral analgesic drug, indomethacin was used as a positive control. Group 1 received the vehicle distilled water (10 ml/kg, p.o.) and group 2 was treated with indomethacin (25 mg/kg, p.o.). Whereas groups 3 to 14 groups animals were orally administered with compounds **1-6** (25 and 50mg/kg, p.o). Thirty minutes after treatment, the mice were injected (i.p.) with 0.1 ml of 1% acetic acid solution to induce the characteristic writhings. After 5 min, the mice were placed in an observation box and the number of writhes in a 10 min period was counted. The response of the extract and indomethacin treated groups were compared with those of animals in the control group.

Anti-inflammatory activity

Carrageenan induced paw edema

For the experiment, the male wistar rats (120–150 g) were divided into fourteen groups (n = 6). The animals were fasted overnight prior to the start of the experiment, and water *ad libitum*. The first group received normal water (10 ml/kg, p.o.), while the second group was treated with indomethacin (25 mg/kg, p.o.). The third to fourteen groups were administered with compounds **1-6** (25 and 50mg/kg, p.o respectively). Acute inflammation was produced by the subplantar administration of 0.1 ml of 1% carrageenan (in 1% CMC w/v) in the right hind paw of the rats. The vehicle, extracts and the standard drugs were administered 60 min prior to the injection of the phlogestic agent. The volumes of edema of the injected and the contralateral paws were measured at 1, 2, 3, 4, and 5 h after the induction of inflammation. The inflammation was measured by using an electronic vernier caliper (CD-6 CSX; Digimatic caliper, Mitutoyo, Japan) and calculates the percentage of paw edema inhibition[27].

Statistical analysis

For anti- inflammatory and analgesic activity of the isolated compounds, the results were recorded as mean ± standard deviation (SD) (n = 6) and subjected to one-way analysis of variance (ANOVA) followed by post hoc Duncan's multiple range test using SPSS (version 9, SPSS Inc., Chicago, USA). $P < 0.05$ was chosen as the criterion for statistical significance.

RESULTS AND DISCUSSION

The known xanthones, decussatin (**1**), gentiacaulein (**2**), swertianin (**3**) and 1,8-dihydroxy-2, 6-dimethoxy xanthone (methylswertianin) (**4**) were identified by comparison of their physical and spectroscopic data (IR, 1D and 2D (^{1}H–^{1}H COSY and HMBC) NMR and MS) with values found in the literature[11, 28-30]. Two new xanthones, 8-hydroxy-1, 2, 4, 6-tetramethoxyxanthone (**5**) and 1, 2, dihydroxy-6-methoxyxanthone-8-O-β-D-xylopyranosyl **(**Swertianin xyloside) (**6**) were isolated first time from *Swertia corymbosa* Figure 1.

Compound **4** was obtained as a yellow amorphous powder. The IR spectrum showed absorption band at υ_{max} 1629 cm^{-1} reminiscent of xanthone carbonyl group. The ^{1}H NMR spectrum showed the presence of two methoxy group at δ: 3.81 (OCH_3-6) and 3.86 (OCH_3-3), four aromatic protons at 6.23 (1H, d, *J* = 2.00Hz, H-7), 6.29 (1H, d, *J* = 2.00Hz, H-5), 6.75 (1H, d, *J* = 9.00Hz, H-4) 7.17 (d, 1H, C3-H, *J* = 9.00 Hz) two hydroxyl groups δ: 12.00 (OH-1), 11.87 (OH-8). The HMBC spectrum showed an interaction of C-6-methoxy proton δ 55.90 with C-6 (δ 167.42) and C-2 methoxy proton (δ 85.05) with C-2 (δ 142.91). The C-7-H (δ 97.18) showed crossed peak with C-8 (δ 162.93) and C-6 (δ 167.42) and also C-5 (δ 92.88) showed cross peak with C-6 (δ 167.42) and C-10a (δ 158.06). The C-4 proton (δ105.56) showed an interaction with C-2 (δ 142.91) and C-4a (δ 149.58). The C-3 (δ 120.38) showed HMBC correlation with C-1 (δ 150.13), C-4a (δ 149.58) and C-2 (δ 142.91). The C-8 –hydroxy proton (δ162.93) having correlation with C-7 (δ 97.18), C-8 (δ162.93) and C-8a (δ102.31).The C-1-hydroxy proton (δ 150.13) showed correlation with C-2 (δ 142.91), C-1 (δ 150.13), and C-9a (δ 107.75) respectively.

Compound **5** was obtained as a yellow crystal. The molecular formula was assigned as $C_{16}H_{14}O_8$. The IR spectrum showed absorption band at υ_{max} 1632 cm^{-1} reminiscent of xanthone carbonyl group. The 1H NMR spectrum showed the presence of four methoxy group at δ 3.88(OCH_3-6), 3.94 (OCH_3-2), 3.96 (OCH_3- 4) and 4.01(OCH_3-1) and one hydroxy group at δ 13.29 (OH-8). In addition, three protons at δ 6.32, 6.46 and 6.97 as assignable to H-7, H-5 and H-3. The ^{13}CNMR spectrum data of compound **5** showed four methoxy group and one carbony group at δ 183.3 indicated a double-chelated carbonyl, meaning there was one hydroxy attached at C-8. The HMBC (Fig.1) spectrum showed the coupling of the C-6 methoxy proton (δ 3.88) with C-6 (δ 166.42), C-2 methoxy proton (δ 3.94) with C-2 (δ141.05), C-4 methoxy proton (δ 3.96) with C-4 (δ148.65) and C-1 methoxy proton (δ 4.01) exhibited HMBC interaction with C-1 (δ144.49). The C-7 -H (δ 6.32) showed crossed peak with C-8 (δ 163.56) and C-5 (δ 92.03) and C8a (δ 104.22). C-5 proton δ 6.46 correlated with C-7 (δ 97.39), C-6 (δ 166.42), C-10a (δ 156.94) and C8a (δ 104.22). The HMBC correlation of C-3 proton (δ 6.97) exhibited the correlation of C-4 (δ 148.65), C-2 (δ 141.05), C-1(δ 144.49) and C-4a (δ 141.47) respectively. Compound **5** is reported for the first time in this plant species.

Compound **6** was obtained as yellow needles. The molecular formula was assigned as $C_{24}H_{26}O_{14}$. It gave a positive response (red colour) in the presence of $MgCl_2$ which is characteristic for a xanthone glucoside [31]. IR spectra showed OH groups at 3350-3430 cm^{-1}, 1657 (C=O) cm^{-1} and 1285 (O-CH_3) groups. The 1H NMR spectrum showed the presence of one methoxy group at δ 3.90 (OCH_3-6) and two hydroxy group δ 5.44 (OH-2) and 11.94 (OH-1). In addition, four protons at δ 6.34 (H-5), 6.41 (H-7), 6.85 (H-3) and 7.30 (H-4) 2.87-3.88 (m, 15H, sugar protons). The presence of two xylose moiety was confirmed by 11 carbohydrate signals (δ_C 65.30, 68.38, 69.50, 69.62, 73.02, 73.69, 75.68, 75.97, 101.84, 102.77 and 104.03 respectively) in the ^{13}C NMR spectrum. Thus, **6** should be a 1, 2, dihydroxy-6-methoxyxanthone-8-O-β-D-xylopyranosyl. The connectivity of the xylose moiety was established with C-8 of xanthone nucleus which was the only possible place for attachment. As there was no further vacancy in the ring for attachment of a second xylose fragment, it was therefore assumed that two xylose units could be bonded to one another through an oxygen atom. These data clearly established that the xylopyranosyl residue in **6** was attached to the C-8 of the xanthone nucleus through an OH atom. The HMBC spectrum showed the coupling of the C-6 methoxy proton (δ 3.90) with C-6 (δ 60.69), H-1″ proton (δ 4.20) with C-4′ (δ 104.03) and H-1′ proton (δ5.29) exhibited HMBC interaction with C-8 (δ102.77). The C-2 -OH (δ 5.44) with C-3 (δ 152.01) and C-5 (δ 6.34) interaction with C6 (δ 97.33). C-7 proton δ 6.41 correlated with C-5 (δ 110.77), C-8 (δ 162.79) and C-8a (δ 128.75). The HMBC correlation of C-3 proton (δ 6.85) exhibited the correlation of C-1 (δ 167.27) and C-4a (δ 152.01). The C-4 proton (δ 7.30) exhibited the correlation of C-2 (δ 101.84) and C-4a (δ 152.01). The correlation between anomeric proton (δ 5.29) of one xylose and C-4 signal (δ 69.62) of the other xylose suggests that two xylosyl units are connected by a 1→4 pattern and second xylose protons C-1 (δ 4.20) correlation with xanthone skeleton at C-8(δ 162.79). The configurations of two xylose residues were deduced to be both β from the *J* values (7.00, 7.00 Hz respectively) of two anomeric protons. According to the above evidence, 1, 2, dihydroxy-6-methoxyxanthone-8-O-β-D-xylopyranosyl-(1→4)-β-D-xylopyranoside, to the best of our knowledge, it has not been encountered before in nature (Fig.1).

In mice, oral administration of isolated xanthone **1-6** at a dose of 2, 000 mg/kg did not produce any signs of toxicity and no animals were died up to 3 days. It showed that isolated xanthone was nontoxic in rats up to an oral dose of 2000 mg/kg bw. Therefore, investigation of inflammatory and antinoceceptive activity was carried out using 25 and 50 mg/kg dose levels.

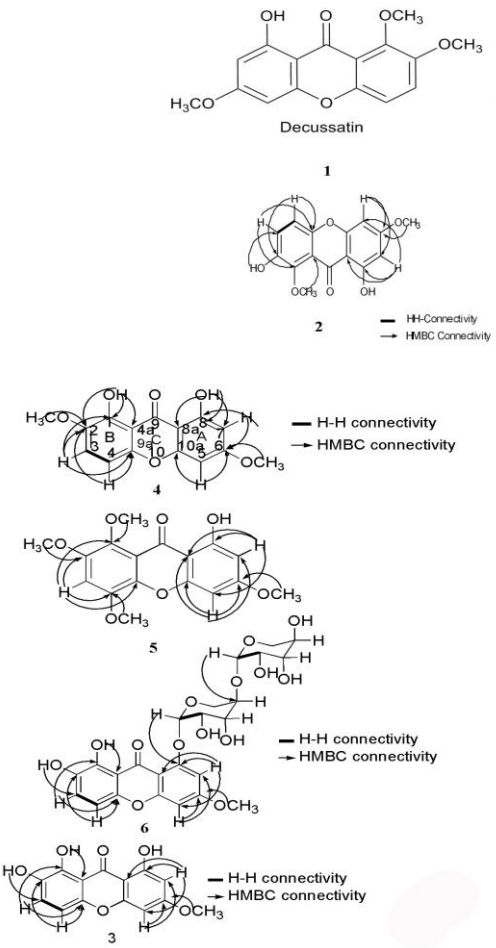

Fig. 1

Table 1: Anti-inflammatory effects of isolated compound 1-6 from *Swertia corymbosa* on carrageenan-induced paw edema in rats.

Treatment group	Dose	Swelling thickness (mm) ±SD (inhibition %)				
	(mg/kg b.w.)	1h	2h	3h	4h	5h
Control	10 ml/ kg b.w.	4.23±0.65	5.78±0.43	6.28±0.45	7.53±0.43	8.69±0.10
Indomethacin	25	3.27±0.15 (22.69%)	3.48±0.76 (39.79%)	3.51±0.45 (44.10%)	3.42±0.43 (54.58%) ***	3.09±0.71 (64.44%) **
Compound 1	25	3.76±0.61 (11.11%)	4.16±0.01 (28.02%)	4.28±0.61 (31.84%)	4.82±0.13 (35.98%)	4.91±0.33 (43.49%)
	50	3.51±0.17 (21.51%)	3.73±0.51 (34.34%)	4.02±0.11 (35.98%)	4.24±0.61 (43.69%)	4.31±0.21 (50.40%)
Compound 2	25	3.43±0.61 (17.01%)	3.76±0.31 (34.94%)	3.97±0.22 (36.78%)	4.12±0.17 (45.28%)	4.22±0.23 (51.43)***
	50	3.32±0.17 (21.51%)	3.47±0.22 (39.96%)	3.58±0.09 (42.99%)	3.92±0.41 (47.94%)	4.12±0.80 (52.58%) ***
Compound 3	25	3.34±0.11 (21.52%)	3.41±0.51 (41.00%)	3.46±0.15 (44.90%)	3.50±0.13 (53.51%) ***	3.69±0.12 (57.53%)**
	50	3.13±0.27 (26.00%)	3.00±0.32 (48.09%)	3.12±0.06 (50.31%)	3.23±0.51 (57.10%) **	3.41±0.98 (60.28%) **
Compound 4	25	3.53±0.15 (15.54%)	3.62±0.15 (37.37%)	3.79±0.22 (37.37%)	3.91±0.37 (48.07%)	4.16±0.62 (52.12%) ***
	50	3.44±0.25 (18.67)	3.56±0.25 (38.40%)	3.59±0.12 (48.83%)	3.64±0.72 (51.66%) ***	3.82±0.83 (56.04%) **
Compound 5	25	4.13±0.65 (02.36%)	4.93±0.65 (14.70%)	5.22±0.39 (16.87)	5.93±0.73 (21.24%)	6.32±0.60 (27.27%)
	50	3.73±0.32 (11.82%)	4.62 ±0.32 (20.06%)	4.75±0.35 (24.36%)	5.41±0.51 (28.15%)	5.78±0.30 (33.48)
Compound 6	25	3.12±0.25 (26.24%)	3.31±0.83 (42.73%)	3.35±0.21 (46.65%)	3.41±0.43 (54.71%) ***	3.39±0.12 (60.98%) **
	50	2.97±0.40 (29.78%)	2.81±0.40 (51.38%)	2.57±0.76 (59.07%) **	2.82±0.28 (62.7.54%) **	2.45±0.82 (71.80%)*

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 5h 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 5h) 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 than 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 54.58% and 64.44 % respectively. The result obtained is clearly indicates that compound **6** is appeared to be more effective than that of indomethacin and crude extracts. The inflammatory response induced by carrageenan is characterized by a biphasic response with marked edema formation resulting from the rapid production of several inflammatory mediators[32-34]. Early phase is attributed to a release of histamine, serotonin and bradykinin, whereas late phaseis due to the over production of prostaglandin and nitric oxide with peak at 3 h, produced by inducible isoforms of COX (COX-2) and nitric oxide synthase (iNOS)[35]. According to Table 1, compound **6** significantly reduced paw edema at 5 and 6 h after carrageenan injection, but was less effect at 1 and 2 h. This evidence allows us to suggest that the compound **6** acted in the second phase of the inflammatory response. The mechanism underlying the anti-inflammatory activity of the compound **6** may therefore involve inhibition of the release of inflammatory mediators, such as prostaglandins and nitric oxide. Previous reports showed that xanthone glucosides and prenylated xanthones are known to possess effective anti-inflammatory activities[36,37]. Banerjee et al[38] investigated the anti-inflammatory efficacy of 1, 5-dihydroxy-3, 8-dimethoxyxanthone from *S. chirata* showed that 57% of inhibition at a dose of 50 mg/kg. In our result, compound **3** and **6** showed a dose of 50 mg/kg inhibited 60.28 % and 71.80 % respectively, similarly compound **4** (1, 8-dihydroxy-2, 6-dimethoxyxanthone) **5** (8-hydroxy-1, 2, 4, 6-tetramethoxyxanthone) showed 56.04 % and 33.48 % respectively. This result indicated that orthohydroxyl groups and number of hydrogen play an important role in reduced formation of paw edema after 6h.

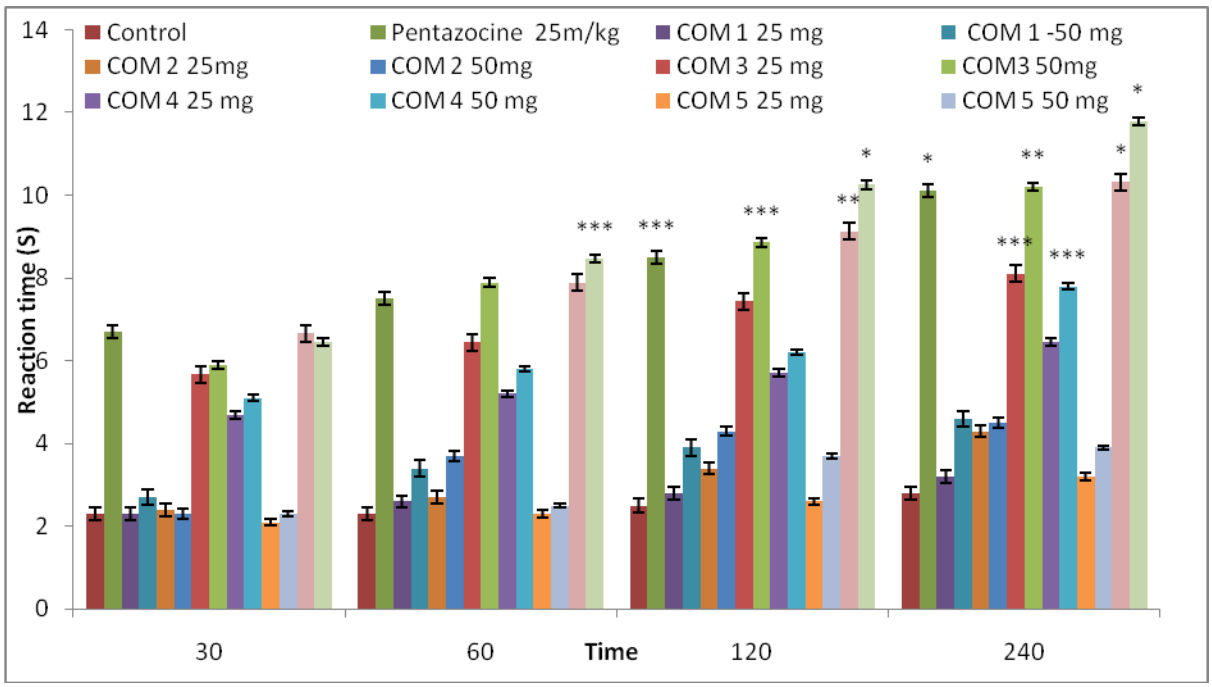

Fig. 2: Antinociceptive effects of isolated compound 1-6 from *Swertia corymbosa* on the hot-plate test.

Values are means of 3 replicate determinations ±SD; ***p<0.05. **p<0.01. *p<0.001 significant compared to control.

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** (10.21± 0.89, 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 opioids[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 level 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 are 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 PGE_1. Our findings also provide evidences 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 2: Antinociceptive effects of isolated compounds from *Swertia corymbosa* on acetic acid-induced writhing test.

Treatment group	Dose (mg/kg b.w.)	Number of writhings ±SD	Inhibitory ratio (%)
control	10 ml/ kg b.w.	83.25± 1.43	-
Indomethacin	25	23.87 ± 4.66	72.32*
Compound 1	25	58.49± 3.21	29.74
	50	51.53± 2.10	38.10
Compound 2	25	52.33± 5.57	37.14
	50	45.10± 2.10	45.83***
Compound 3	25	28.32± 1.29	65.92**
	50	24.47± 1.60	70.60*
Compound 4	25	51.89± 2.37	37.66
	50	46.43± 1.23	44.22***
Compound 5	25	67.49± 3.45	18.93
	50	61.23± 4.98	26.45
Compound 6	25	29.35± 1.78	64.74**
	50	19.22± 1.12	76.85*

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 is 1, 2, dihydroxy-6-methoxyxanthone-8-O-β-D-xylopyranosyl 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.

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