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Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

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To cite this article: Sakshi Bajaj, Sharad Wakode, Avneet Kaur, Shivkanya Fuloria & Neeraj Fuloria (2020): Anti-inflammatory and ulcerogenic activity of newer phytoisolates of *Swertia alata* C.B. Clarke, Natural Product Research, DOI: 10.1080/14786419.2020.1775224

To link to this article: https://doi.org/10.1080/14786419.2020.1775224

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2020.1775224

Anti-inflammatory and ulcerogenic activity of newer phytoisolates of *Swertia alata* C.B. Clarke

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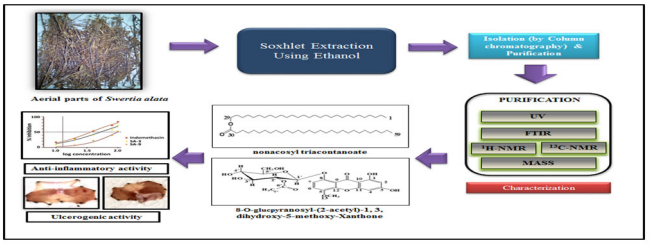
ABSTRACT

The present study was intended to evaluate the *in vitro* (COX-1/COX-2) and *in vivo* anti-inflammatory and ulcerogenic activity of newer phytoconstituents isolated from the aerial parts of *Swertia alata* C.B. Clarke (*Gentianaceae*). For isolation of newer phytoconstituents, the ethanolic extract of aerial parts of *S. alata* was subjected to column chromatography using mixture of petroleum ether and chloroform in various concentrations, which yielded two phytoisolates characterised as nonacosyl triacontanoate **(SA-3)** and 8-*O*-glucpyranosyl-(2-acetyl)-1,3-dihydroxy-5-methoxy-xanthone **(SA-9)**. Identification of compounds was based on melting point, UV, FTIR, ^{1}H-NMR, ^{13}C-NMR and mass spectrometric data. The isolates were screened for *in vitro* COX-1/COX-2 inhibitory activity, *in vivo* anti-inflammatory and ulcerogenic activity. Among the two compounds, **SA-3** was found to be more effective than **SA-9.** The ulcerogenic study revealed significant gastric tolerance of **SA-3** and **SA-9** in comparison to indomethacin.



ARTICLE HISTORY
Received 16 January 2020
Accepted 17 May 2020

KEYWORDS
Phytochemistry; partitioned; chemical methods; chromatographic; phytoconstituents

1. Introduction

Swertia (family *Gentianaceae*) is a large genus of herbs distributed in the mountainous regions of tropical area at an altitude of 1200–3600 m. Genus *Swertia* is well known for its medicinal plant species (Patro et al. 2005; Jiang et al. 2016). *Swertia alata* C.B. Clarke is a

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Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2020.1775224.

branched herbaceous plant with yellowish green flowers (Natrajan and Prasad 1972). It is widely used in the Indian system of medicine (ISM). The *S. alata* plant is generally used as a substitute of *Swertia chirata* in many herbal formulations (Bajaj and Wakode 2017). *S. alata* is known to possess oleanolic acid, swertisin (Pant et al. 2000), swertiamarin (Negi et al. 2011), swertianin (Khetwal et al. 1997), methyl swertianin, methylbellidifolin (Khetwal et al. 1997), bellidifollin (Bajpai et al. 1991), swertiaperennine and decussatin (Karan et al. 2005), but still very limited data is available over aerial parts of *S. alata*.

Facts suggest chronic inflammatory diseases are associated with cardiovascular diseases, inflammatory bowel disease and acceleration of ageing, that are the major cause for morbidity and mortality in the world (MacNee, Rabinovich, and Choudhury 2014; Mason and Libby 2015; Alatab et al. 2020). The NSAIDs are one of the most widely prescribed drugs in the world and are extensively used to alleviate clinical cases especially for pain and inflammation (Brooks et al. 1999). However, these drugs are well-known to induce gastric/intestinal ulceration, anemia, platelet inhibition, delay ulcer healing, and in some cases increase the risk of myocardial infarction (Hippisley-Cox and Coupland 2005; Lanas et al. 2005; Craig et al. 2008).

Steroidal anti-inflammatory drugs prevent or suppress inflammation but do not attack the root cause of the disease, and the prolonged use of these compounds can inhibit the synthesis of the inducible isoform of nitric oxide synthase enzyme and cause pituitary-adrenal suppression, hyperglycemia, glycosuria and an increased susceptibility to infections and peptic ulcers (Ogirala et al. 1991).

Despite recent advances, an adequate remedy for NSAID-induced gastropathy remains elusive. Discovery of potent NSAIDs with very low or no gastrointestinal (GI) side effects is the area of prime interest. Use of most commonly prescribed drug from analgesics such as aspirin nowadays have been limited due to their potential side effects like severe gastric disorders. With the introduction of cyclooxygenase-2 (COX-2) inhibitors problem of GI side effects is overcome up to certain level (Chhabria and Doda 2005; Dutt et al. 2007).

The World Health Organization has stressed over the need for development of drugs from plant origin owing to their inexpensive, accessibility (particularly to rural people of developing countries) and less/no side effects. Hence, development of drug having anti-ulcerogenic property from plant source without compromising the efficacy and safety would be expected to benefit millions of suffering humanity. The Ayurvedic and Yunani system highlights the astringent, stomachic, sedative and anti-inflammatory potential of a large genus called *Swertia* belonging to the *Gentianaceae* family (Negi et al. 2011; Mehta et al. 2017). But, evaluation of anti-inflammatory potential of isolates of *S. alata* is still due. Hence, based on the aforementioned findings, an attempt has been made to isolate and characterise the phytoconstituents from *S. alata* and evaluate against *in vitro* (COX-1/COX-2) and *in vivo* anti-inflammatory and ulcerogenic activity.

2. Results and discussions

2.1. Structure elucidation of compounds

2.1.1. Nonacosyl triacontanoate (SA-3)

SA-3 (Figure 1) was obtained as white crystalline powder from elution of the column with chloroform:petroleum ether (2:3), recrystallised from methanol, 80 mg (0.06%) and

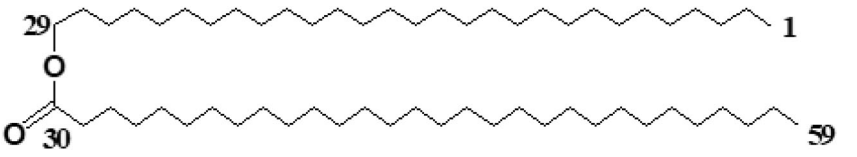

Figure 1. Structure of nonacosyl triacontanoate.

having melting point 78–79 °C. UV (MeOH) max: 257 nm; FTIR: (KBr, ν_{max}, cm^{-1}): 2917, 2848 (C–H stretching), 1735 (C==O stretch) cm^{-1}. ^{1}H-NMR (DMSO-d_6, 300 MHz): 0.85 (t, $J = 6.9$ and 6.9, attributed to 6H of 02 terminal methyl units), 1.16–1.25 (m, attributed to 110H of 55 methylene groups), 1.61 (t, $J = 6.9$, attributed to 2H of methylene unit at position 29), 2.32 (t, $J = 6.9$ and 6.9, attributed to 2H of methylene unit at position 31). ^{13}C-NMR (DMSO-d_6, 300 MHz): 14.21 (C1 and C59), 29.26 (C-29), 32.02 (C-31), 76 (methylene 54 carbon units), 164 (carbonyl carbon). LC-ESI-MS *m/z* (Relative intensity): No parent ion peak at *m/z*: 859; characteristic peaks fragment ion peaks at *m/z*: 423, 395, 367, 283, 255, 227]$^{+}$ (Fragmentation pattern given in Supplementary Material Figure S1).

Chemical Identification: Compound SA-3 (80 mg) was acid hydrolysed using 2 M H_2SO_4 and confirmed the presence of ester group and steryl alcohol.

The Compound **SA-3**, identified as nonacosyl triacontanoate, was obtained as white amorphous powder. It was acid hydrolysed and responded positively for steryl alcohol and ester group. The presence of sterol nucleus was confirmed by acid hydrolysis using 2 M H_2SO_4. The IR spectra of SA-3 exhibited characteristics IR absorption bands at 2917 cm^{-1}, 2848 cm^{-1} (due to C–H stretching) and carbonyl group at 1735 (due to C==O stretch). Its + ve ion ESI mass spectra showed no molecular ion peak, which indicated saturated nature of molecule. The generation of the prominent ion peaks at *m/z* 423 $[C_{29}H_{59}O]^{+}$ signaled the presence of steryloxy group. Signals at 395 $[C_{27}H_{55}O]^{+}$, 367 $[C_{25}H_{51}O]^{+}$, 283 $[C_{19}H_{39}O]^{+}$, 255 $[C_{17}H_{35}O]^{+}$, 227 $[C_{15}H_{31}O]^{+}$ suggested the presence straight chain saturated steryl moiety (Supplementary Material Figure S1). The ^{1}H-NMR spectra of compound SA-3 displayed a 6 proton triplet at δ 0.85 ($J = 6.9$ and 6.9 Hz) assigned to two terminal methyl groups, one triplet at δ 1.61 ($J = 6.9$ Hz) assigned to two hydrogen protons present on H-29 unit, one triplet at δ 2.32 ($J = 6.9$ and 6.9 Hz) assigned to two hydrogen protons present on H-31 unit and remaining protons appeared as broad multiplet in the range of δ 1.16–1.25, assigned to remaining 55 methyl units. The ^{13}C-NMR spectral data of SA-3 exhibited important signals for two methyl carbons at δ 14.21 (C-1 and C-59), signal at δ 29.26 for (C-29), signal at 32.02 for (C-31 as signal at 164 is assigned to carbonyl carbon) and remaining 55 carbon signals at δ 76. The IR data, mass fragmentation pattern, ^{1}H- and ^{13}C-NMR chemical shifts of the fatty acid ester were compared with related compounds viz steryl alcohol and ester group. On the basis of spectral data analysis and acid hydrolysis, chemical identification of compound SA-3 has been formulated as nonacosyl triacontanoate. This is a new ester reported for the first time in *S. alata* species.

2.1.2. 8-O-β-D-Glucopyranosyl-(2-acetyl)-1,3-dihydroxy-5-methoxy-xanthone (SA-9)

SA-9 (Figure 2) was obtained as yellow amorphous powder from elution of the column with chloroform:petroleum ether (8:2), recrystallised from methanol, 88 mg

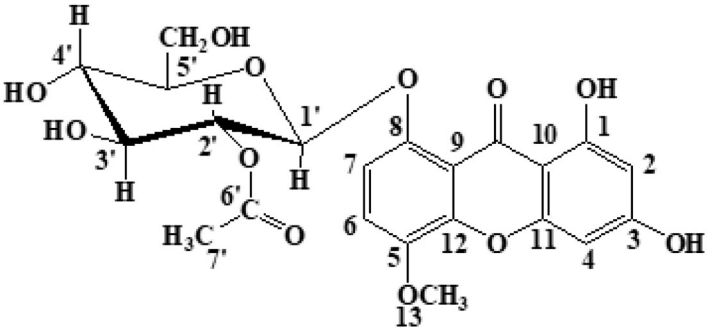

Figure 2. Structure of 8-*O*-β-D-glucopyranosyl-(2-acetyl)-1, 3, dihydroxy-5-methoxy-xanthone.

(0.073%) and having melting point 121 °C. FTIR: (KBr, ν_{max}, cm^{-1}): 3394 (OH), 2919, 2861 (C–H), 1736 (C==O), 1564, 1601 (C==C); ^{1}H-NMR (DMSO-d_6, 300 MHz) (in δ): 2.31 (s, 3H, C==O CH_3), 3.79 (s, 3H, OCH_3), 3.78 (H5′), 3.82 (H4′), 3.95 (CH_3′), 4.02 (H2′), 4.64(s, 1H, H2), 6.68 (s, 1H, H4), 7.04 (d, 1H, $J = 4.5$, H1′), 7.42 (d, 1H, H7, $J = 3.3$), 7.48–7.49 (d, 1H, $J = 3.3$, H7), 8.6 (OH) and ^{13}C-NMR (DMSO-d_6, 500 MHz): 76.60–77.44 (C1′, C2′, C3′, C4′, C5′, C13), 24 (C6′), 105–109 (C2, C4, C7, C6, C9, C10), 155–160 (C1, C3, C11, C5, C12, C8), 165 (C6′), 180 (C==O)**;** LC-ESI-MS *m/z* (Relative intensity): 478 (M^+), 477 (*M* – 1), 257 (Fragmentation pattern given in Supplementary Material Figure S2).

The compound **SA-9** identified as 8-*O*-β-D-glucopyranosyl-(2-acetyl)-1,3-dihydroxy-5-methoxy-xanthone was obtained as yellow amorphous powder. It gave positive tests for carbohydrate. Its IR spectrum displayed characteristic absorption band for hydroxyl groups at 3394 cm^{-1} (OH), 2919 cm^{-1}, 2861 cm^{-1} for C–H stretching, 1736 cm^{-1} for C==O stretching and 1564, 1601 and 1146 cm^{-1} for C==C stretching. Its + ve ion FAB mass spectra showed molecular ion peak at *m/z* 478 $[C_{22}H_{22}O_{12}]^+$ corresponds to $M + 1$, and 477 corresponds to *M* – 1. The generation of the prominent ion peaks at *m/z* 299 $[C_{15}H_9O_7]^+$, 257 $[C_{14}H_9O_5]^+$ showed the presence of xanthone nucleus. Fragment ion peak at 179 $[C_7H_{14}\ O_5]^+$ suggested the presence of glucopyranosyl moiety. The peak at 165 $[C_6H_{11}O_5]^+$ and other fragmented peaks suggested the presence of gucopyranosyl substituted xanthone (Supplementary Material Figure S2).

The ^{1}H-NMR spectra of compound **SA-9** displayed a singlet at δ 2.31 assigned to three protons of methyl group (H-7′). Another singlet at δ 3.79 was assigned to proton of methoxy protons, hydroxy protons showed a broad signal at δ 8.6. The aromatic protons displayed their signals from δ 6.68 to 7.49. The protons of glucose moiety showed their signals at δ 3.78, 3.82, 3.95, 4.02 and 7.04 glucopyranosyl protons. The ^{13}C-NMR spectral data of **SA-9** exhibited important signals for carbonyl group at δ 165 and 180 for carbonyl of xanthone and ester group, respectively. Remaining carbons signaled between δ 76.6 to 77.44, 105 to 109 and 155 to 160. The ^{1}H- and ^{13}C-NMR chemical shifts of the fatty acid ester were compared with related compounds viz steryl alcohol and ester group. On the basis of spectral data analysis and acid hydrolysis, chemical identification of compound **SA-9** has been formulated as 8-*O*-β-D-glucopyranosyl-(2-acetyl)-1,3-dihydroxy-5-methoxy-xanthone. This is a new ester reported for the first time in *S. alata* species. The spectral characterisation of molecular structure

of isolated compounds **SA-3** and **SA-9** was also confirmed and supported by standard literature (Tapondjou et al. 2003; Brahmachari et al. 2004).

Based on phytochemical tests and characterisation of spectral data, the presence of a newer phytoisolates nonacosyl triacontanoate (**SA-3**) and 8-*O*-glucopyranosyl-(2-acetyl)-1,3-dihydroxy-5-methoxy-xanthone (SA-9) was revealed in aerial parts of *S. alata*. This study has enhanced the knowledge base related to phytochemical composition of *S. alata*.

2.2. In vitro COX-1 and COX-2 inhibitory assay

The enzyme that catalyses the formation of prostaglandin GS_2 (PGG_2) from arachidonic acid followed by its conversion into PGH_2 is designated as COX, prostaglandin-endoperoxide synthase, or prostaglandin H synthase. There are two mammalian isozymes encoded by different genes: the constitutive COX-1 and the inducible COX-2. Both enzymes are the major pharmacologic targets of NSAIDs (Taketo 1998). Facts suggest that COX-1 and COX-2 catalyse the biosynthesis of PGH_2 from arachidonic acid substrate. Inhibition of COX-1 results in some undesirable side-effects, whereas COX-2 inhibition provides therapeutic effects in pain, inflammation, cancer, glaucoma, Alzheimer's and Parkinson's disease (Blobaum and Lawrence 2007). The COX-2 is an inducible enzyme, whereas COX-1 is constitutive that is present even in the absence of inflammatory conditions. In addition to pro-inflammatory prostaglandins, COX-1 is responsible for the synthesis of those prostaglandins that are necessary to maintain the integrity of gastrointestinal mucosa. A higher inhibition of COX-1 increases the tendency of a drug to induce gastric ulcers and related complications (Yang et al. 2017).

The inhibitory activity of isolated compounds (**SA-3** and **SA-9**) was evaluated against ovine COX-1 and human recombinant COX-2 using enzyme immunoassay kit (Supplementary Material Figure S3) and IC_{50} (μM) values were determined (Supplementary Material Table S1). The property of indomethacin for nonselective inhibition of COX-1 and COX-2 enzyme justifies its suitability as a positive control for the present study (Takeuchi et al. 2010; Guo et al. 2013). The IC50 values of indomethacin for COX-1 and COX-2 were observed as 53.00 μM and 36.56 μM, respectively. The results of the *in vitro* COX-1 and COX 2 inhibitory studies revealed that the isolated compounds (**SA-3** and **SA-9**) potentially inhibit COX-2 (IC_{50} = 98.54–52.36 μM) over COX-1 (120.3–79.89 μM). Whereas, isolated compound **SA-3** (COX-1/COX-2 = 79.89/52.36) were found to be potent inhibitor of COX-2 than **SA-9** (COX-1/COX-2 = 120.3/98.54) (Supplementary Material Figure S4). To understand the inhibitory activity, the isolated compounds were further evaluated for their *in vivo* anti-inflammatory activity.

2.3. In vivo activity

2.3.1. Anti-inflammatory activity

Anti-inflammatory activity of the phytocompounds (**SA-3** and **SA-9)** was evaluated in Wistar albino rat by carrageenan-induced rat paw edema method (Winter et al. 1962). Carrageenan-induced paw edema is a standard assay for acute inflammation that is effectively employed to evaluate the phytoisolate against anti-inflammatory activity

(Crunkhorn and Meacock 1971). The biological data of the selected compounds is shown in Supplementary Material Table S2, which clearly implies that the isolated compounds exhibit varying degree of anti-inflammatory activity ranging from 37.27% to 59.54%. The results were compared with standard anti-inflammatory drug such as indomethacin (8 mg/kg) which showed effective inhibition (65.90%) at 3 h. The findings of the present study revealed that the phytoisolates **SA-3** and **SA-9** show dose-dependent anti-inflammatory activity by suppressing the rat paw edema.

It was observed that at a higher dose of 8 mg/kg, **SA-3** showed significant decrease in edema volume after 3 h. Edema is produced by a sequential release of inflammatory mediators such as histamine, serotonin, kinnins, prostaglandins and bradykinins which leads to fluid accumulation (Sedgwick and Willoughby 1989). Edema is characteristic of acute inflammatory response (Brattsand et al. 1982). The release of histamine or serotonin occurs in the first phase (up to 1 h) and the second phase (over 1 h) is associated with the production of bradykinins (Wallace 2002). Statistical analysis revealed that **SA-3** (2, 4, 8 mg/kg) significantly inhibited paw edema at 3 h after treatment ($p < 0.05$). These results support the possible role of **SA-3** in inhibition of lysosomal enzyme secretion and arachidonic acid release from membranes by inhibiting lipoxygenase cyclooxygenase, and phospholipase A2. Such arachidonic acid inhibition by inflamed cells could reduce endoperoxides, prostaglandins, prostacyclin and thromboxanes from the lipoxygenase pathway as well as hydroperoxy and hydroxy eicosatetraenoic acids and leukotrienes from the cyclooxygenase pathway (Gabor 1986). Indeed, previous studies have shown that this compound plays a pharmacological role in inflammation (Laavola et al. 2016).

2.3.2. Ulcerogenic activity

It has been reported that NSAIDs are inadequately dissolvable in gastric acid and stay in contact with the stomach wall for a more extended period, thus produces a highly dangerous local concentration. This manifests in local irritation of the stomach wall after ulceration (Kumar and Mishra 2006). Both compounds **SA-3** and **SA-9** possessing *in vivo* anti-inflammatory activity when screened for their ulcerogenic activity, exhibited significant ulcerogenic potential compared to the control treated group at a dose of 12 and 24 mg/kg. A strong correlation between potency of NSAID's as inhibitor of prostaglandin synthesis and ulcerogenic activity has been observed (Boyle et al. 1982). The resultant data (Supplementary Material Table S3 and Figure S5) revealed that tested compounds **SA-3** and **SA-9** showed better GI safety profile with the ulcer score of 2.0 ± 0.35 and 2.44 ± 0.56, respectively, in comparison to standard drug indomethacin 2.7 ± 0.27. Further, **SA-3** possesses significantly less ulcerogenic potential as compared with **SA-9** at a dose of 16 mg/kg. Most potent compound **SA-3** showed severity index less than the standard drug indomethacin. Hence, this compound may ascertain to have better safety margin on gastric mucosa than indomethacin (Singh et al. 1992).

3. Experimental

3.1. General experimental procedure

The melting points of isolated compounds were uncorrected and determined on Visual Melting Range apparatus. Ultraviolet spectra were recorded in methanol (MeOH)

on a Shimadzu UV-160A UV Visible Recording Spectrophotometer (Shimadzu Scientific Instruments, Kyoto, Japan). IR spectra were recorded as KBr pellets on Nicolet FTIR spectrophotometer. MS were recorded by effecting electron impact ionisation at 70 eV on an ESIMS analyst QS TOF (Canada) mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were scanned on Bruker DRX-300,500 NMR (300 MHz) instrument in $CDCl_3$ and D_2O using tetramethyl silane (TMS) as the internal standard and coupling constants (*J* values) are expressed in Hertz (Hz). Silica gel G (Qualigen, 60-120 mesh) was used for column chromatography. TLC was performed on plates coated with silica gel G (E. Merck, Germany).

3.2. Plant material and extraction

The dried plant material was supplied by Almas Pharmaceutical Ltd., Uttar Pradesh and identified by Dr. H.B Singh, Scientist F and Head, Raw Material Herbarium and Museum, NISCAIR (National Institute of Science Communication and Information Resources) Pusa Gate, New Delhi. The voucher specimen (NISCAIR/RHMD/2013/2185/190) of the test drug has been deposited in the herbarium of NISCAIR for future reference. In the present study, the aerial parts were carefully collected and air dried under shade. The air dried materials were reduced to coarse powder. The coarse powdered material (1.2 kg) was subjected to exhaustively extraction with 95% ethanol in a Soxhlet apparatus (50 h). The extract was concentrated in rotary evaporator to obtain dried greenish brown colored 52.63 g (4.385%) residue.

3.3. Isolation and purification

The residue was chromatographed on a silica gel-based column chromatography. The residue was dissolved in the minimum amount of ethanol and absorbed on column grade silica gel (60–120 mesh, stationary phase) to obtain slurry, dried in air to free flow and subjected to silica gel-based column loaded in petroleum ether. The column was eluted successively using solvent system of petroleum ether and chloroform and offered two phytoconstituents **SA-3** and **SA-9**. The structures of the compounds were established by spectroscopic method and by comparison with the previous reported works. All spectroscopic data are available in Supplementary Material Figures S6–S13.

3.4. Animals

The animals (Wistar albino rats) used in the study were procured from Animal House Center and were divided and housed in different cages at 25–28 °C, under well maintained hygienic and environmental conditions with relative humidity of 50%–65%, under 12-h light and dark cycles. All animals were acclimatised for a week before use. All experimental work was conducted after receiving the approval from Institutional Animal Ethics Committee (IAEC) via protocol no. IAEC/2015-I/Prot no. 09, 10 and IAEC/2016-I/Prot no. 10, Delhi Institute of Pharmaceutical Sciences and Research, New Delhi.

3.5. In vitro activity

3.5.1. In vitro COX-1 and COX-2 inhibitory assay

The isolated compounds were screened for their *in vitro* COX-1 and COX-2 enzymatic activity using enzyme immunoassay kit (catalog No. 560131, Cayman Chemicals Inc., Ann Arbor, MI, USA). Enzymatic assay was performed as per the manufacturer's assay instructions and literature procedure (Herrera-Salgado et al. 2005). The efficiencies of the test compounds that causes 50% inhibition of COX-2 was calculated as IC_{50} from the concentration response curve. The compounds were further tested for their *in vivo* anti-inflammatory activity.

3.6. In vivo activity

3.6.1. Anti-inflammatory activity

The anti-inflammatory activity of isolated compound was evaluated on Wistar albino rat by carrageenan-induced rat paw edema method as described earlier (Winter et al. 1962; Turner and Hebborn 1971). The animals were divided into six groups, each with five animals. They were starved overnight with water prior to the day of experiment. The acute hind paw edema was produced by injecting 0.1 mL of freshly prepared 1% (wt/vol) carrageenan solution in normal saline locally into the plantar region of the left hind paw of rats of each group. Isolated compounds were administered orally at the dose of 2, 4 and 8 mg/kg body weight (b. wt) while the two other groups received standard drug indomethacin (8 mg/kg, b. wt) and normal saline. The compounds with three dose levels, indomethacin and normal saline were administered orally 1 h prior to injection of carrageenan. The rat paw volume up to the ankle joint was measured using Plethysmometer (Ugo-Basyl, Italy) at the interval of 3 h (0, 1, 2 and 3 h) after injection of carrageenan and the percentage inhibition of edema in the various treated groups was calculated using the following formula:

$$\% \text{ Inhibition} = 1 - \left(\frac{v_s}{v_c} \right) \times 100$$

where,

v_s = paw volume in sample treated group

v_c = paw volume in control group.

3.6.2. Acute ulcerogenic activity

The ulcerogenic activity of the isolated compound was performed according to previous method (Cioli et al. 1979). Each study group consisted of five Wistar albino rats. The animals were fasted for 18 h before the administration of the test compound, while water was given continuously. The dose quantity was made three times (6, 12, 24 mg/kg) of the administered dose for anti-inflammatory studies (2, 4, 8 mg/kg). The control group received only normal saline. After 6 h of the drug administration the rats were sacrificed, stomach was removed and opened around the greater curvature. Inner lining was washed properly with distilled water followed by normal saline. The mucosal damage was examined, and number of ulcers and severity index was

calculated on a scale of 0–3, where: 0 = no lesions; 0.5 = redness; 1.0 = spot ulcers; 1.5 = hemorrhagic streaks; 2.0 = ulcers > 3 but ≤5; 3.0 = ulcers > 5.

3.7. Statistical analysis

Experimental data are expressed as mean. Statistical difference between the treated and control group was evaluated by one-way analysis of variance (ANOVA) followed by Turkey's test as a post ANOVA (Graph Pad Prism 5, San Diego, CA, USA) to determine the statistical significance. The significance level was $\#p < 0.05$, $\#\#p < 0.01$ and $\#\#\#p < 0.001$, when compared to control and $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$, when compared to control.

4. Conclusion

In view of phytochemical tests and interpretation of spectral data, the present study concludes the presence of phytoconstituents **SA-3** and **SA-9** in aerial parts of *S. alata*. This is the first report of isolation of these compounds from this species. Ayurvedic knowledge bolstered by modern science is necessary to isolate, characterise and standardise the dynamic constituents from herbal sources for anti-inflammatory and anti-ulcer activity.

Acknowledgement

The corresponding author is thankful to DIPSAR, AIRF JNU, IIT Delhi, India and AIMST University, Malaysia for providing necessary facilities for successful completion of the study.

Disclosure statement

The authors declare that they have no conflict of interest.

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