NATURAL DRUGS

EVALUATION OF ANTIOXIDANT, ANALGESIC AND ANTI-INFLAMMATORY PROPERTIES OF *SEDUM ADENOTRICHUM*

DIL NAZ^{1,2}, ALI MUHAMMAD¹ and ALAM ZEB^{2,*}

¹Department of Zoology, Islamia College University, Peshawar, Khyber Pakhtunkhwa, 25000, Pakistan.

²Department of Biochemistry, University of Malakand, Chakdara, Khyber Pakhtunkhwa, 18800, Pakistan

Abstract: *Sedum adenotrichum* crude extract have been evaluated for *in vitro* antioxidant activities and *in vivo* analgesic and anti-inflammatory potentials. Results showed strong ABTS, H₂O₂ and DPPH free radical scavenging activities. The extract caused a significant decline of acetic acid-induced abdominal constrictions whereas, significantly inhibited both phases of formalin-induced paw licking responses. In tail immersion assay a significant analgesic response of 58.65% was observed at 75 min at a dose of 125 mg and 60.67% at 90 min at a dose of 250 mg. The morphine exhibited dominant activity at 60 min after treatment of 84.86%. Animals treated with naloxone produced a significant reduction in analgesic potentials of morphine and extract. In the carrageenan-induced model for anti-inflammatory activity, edema formation was inhibited by the extract in the 3rd hour at a dose of 100 and 200 mg, by 50.75 and 52.49%, respectively in a dose-dependent manner.

Keywords: *Sedum adenotrichum*, analgesic, antioxidant, anti-inflammatory

Antioxidants are used for the protection of living cells from the attacks of free radicals. These free radicals are molecules or atoms and are very reactive because of the unpaired electrons. The living body produces traces of oxidants normally during metabolism that are beneficial to the body while destroying micro-organisms. However, oxidants in huge quantities are injurious to the body (1). These reactive oxygen species (ROS) have been concerned with more than 100 ailments, including heart diseases, cancer, arteriosclerosis, acquired immune-deficiency syndrome, malaria, stroke, and diabetes with significant involvement in pain generation and tissue damage or inflammation (2).

Pain is an emotional or unpleasant sensory experience leading to damage of tissues. It is typically considered as a warning sign which elicits impulse and conscious avoidance reaction in protecting the body from potential harms (3). There is a complex perception of pain and is under the control of many variables. The primary role of the sensory system is guarding and keeping up pain homeostasis. It is achieved via identification, localization, and

recognition of the damaging processes in the tissue. Although pain is primarily a defensive mechanism that is produced while the tissue is being damaged and made an individual demonstrate response and eradicate the causative stimulant, however, during many circumstances it disturbs social functioning and decreases life quality (4). On the other hand, inflammation is a biological complex response of vascular tissues to harmful stimuli. It is a protective immune vascular response that involves molecular mediators, blood vessels, and immune cells. The typical signs of acute inflammation are pain, heat, redness, swelling, and loss of function (5). In many cases like osteoarthritis, rheumatoid arthritis and atherosclerosis have no resolution and chronic inflammation develops lasting for the life of the individual (6).

Drugs of natural origin are an important source for the treatment of many diseases. Nowadays, the use of alternative medicine and particularly botanicals have been increasing rapidly worldwide, mostly because of their minimal side effects as compared to chemical therapeutic agents (7). Though plants

* Corresponding author: e-mail: azebuom@gmail.com

have been used as a source of analgesic and anti-inflammatory agents since ancient times, the discovery of chemical agents has led to their neglect for a long time, but fortunately, for various reasons, many people are reverting to herbal medicines.

The genus *Sedum* is the most species-rich and taxonomically complex member of the family Crassulaceae. Its members are widely distributed in the world (African region) (8) including Pakistan. Most species of this genus are used in the treatment of infected wounds, ulcers, as hypotensive, anti-inflammatory, antioxidant, anti-nociceptive, antitumor, and hepatoprotective in traditional medicine (9). Several species in this genus possess high economic values as medicines, food, and ornamentals. Recently, *Sedum adenotrichum* has *in vitro* and *in vivo* anti-diabetic properties (10). Some are toxic or allergenic which can adversely affect humans and animals (11). Apart from other traditional uses, *Sedum adenotrichum* is known for its use in rashes, skin eruptions, blisters, burns, constipation, and dyspepsia (12). However, there is a lack of literature regarding its phytochemical composition and medicinal properties. In our recent study (10), we have shown the phenolic profile of the plant. The present study was aimed to explore the scientific basis of the traditional use of the species for possible *in vitro* antioxidant and *in vivo* antinociceptive and anti-inflammatory potentials. Keeping in view the importance of the genus *Sedum* and its phytochemicals, *Sedum adenotrichum* also known as *Rosularia adenotrichum* is selected for the present study.

EXPERIMENTAL

Plant collection

Sedum adenotrichum whole plant was collected from Malakand, and shade dried at room temperature. The plant was identified by Prof. Dr. Jahandar Shah, Taxonomist, University of Malakand. A reference specimen No. SA-11-2015 was deposited to the herbarium, University of Malakand. The dried material (600 g) was finally grounded and subjected to maceration in methanol (6 L) for three weeks at room temperature ($25 \pm 2^\circ\text{C}$) followed by solvent evaporation. The solvent was completely evaporated using an evaporator (Heidolph, Germany) and the gummy extract was poured into separate bottles and labeled. The phenolic composition of the extract was studied using HPLC-DAD and the results were recently reported (10).

Chemicals and drugs

Acetic acid, carrageenan (Sigma-Aldrich, Germany) formalin, and Tween-80 (Merck,

Germany) were used. Standard drugs, diclofenac sodium, indomethacin, morphine, and naloxone were obtained from the local market.

Animals

Swiss Albino mice (18-23 g) and Wister Albino rats (150-170 g) were obtained from the National Institute of Health, Islamabad. They were kept according to standard conditions with open access to water and standard diet at the University of Malakand's animal house. The animals were kept fasting for 12-18 h before starting the experiment. The protocols of the experiment were approved by the Ethical Committee of Pharmacy Department via No: E-SA-11-2009 and ensured its accomplishment with provisions of the "Animal Bye-Laws 2008, Scientific Procedures Issue-I of the University of Malakand". The studies were finally approved by Advanced Studies and Research Board (ASRB), of the Islamia College University, Peshawar, in its 20th meeting.

Acute toxicity

The plant extract was suspended in tween-80 (2%) to determine acute toxicity in mice. Different doses of extract (10, 100, and 1000 mg/kg) were given by i.p route to six groups of mice ($n = 6$) in 1st phase and 1500 and 2000 mg/kg body weight in 2nd phase and observed for 30 min and then six-hourly examination for 24 h. The animals were kept for 14 days for any behavioral changes and the manifestation of the extract toxicity (13).

Antioxidant activity

DPPH radical scavenging assay

The plant samples were tested for antioxidant activity via its ability to scavenge DPPH free radicals. The crude extracts at the concentration of 40, 60, 80, 100, and 200 mg/mL were mixed with 1 mL (90 μM) solution of DPPH respectively and made 4 mL final volume with methanol. The absorbances of blank and test solutions were noted at room temperature at 512 nm using a UV-visible spectrophotometer. Ascorbic acid was used as a positive control in the concentration range of 62.5 to 1000 $\mu\text{g/mol}$. The IC_{50} values, representing test sample concentration that caused 50% DPPH radical neutralization was calculated from the plot of percentage inhibition vs concentrations (14).

ABTS assay

The plant samples were tested for antioxidant potentials using its ability to scavenge ABTS free radical cations which resulted in a decline in

absorbance at 734 nm (14). Briefly, a 7 mM solution of ABTS was mixed with a 2.45 mM solution of $K_2S_2O_8$ and incubated for 14 h in dark at room temperature, which gave a solution of dark color having ABTS cation radical. For adjusting the absorbance at 734 nm, 0.01 M phosphate buffer (pH 7.4) was added to the ABTS cation radical solution. The ABTS solution of 3 mL was added to 300 μ L test samples to find out its radical scavenging ability. The decrease in absorbance after one minute of mixing the solutions was measured using a spectrophotometer. Ascorbic acid in different concentrations was used as a positive control. All the readings were taken in triplicate. Calculated the % scavenging as:

$$\text{ABTS Inhibition (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample} \times 100}{\text{Absorbance of Control}}$$

H₂O₂ scavenging assay

The plant samples were tested for antioxidant potentials via its ability to scavenge hydrogen peroxide radicals by the method described recently (14). Hydrogen peroxide (40 mM) solution was prepared in phosphate buffer (pH 7.4). A solution of hydrogen peroxide was added to the extract in a concentration range of 62.5-1000 μ g/mL and incubated for 10 min. The absorbance of the mixture was measured at 560 nm through a UV-visible spectrophotometer against the blank having phosphate buffer with no hydrogen peroxide. The experiment was repeated thrice. The % scavenging activity was calculated.

Writhing tests

Anti-nociceptive effects of the 125 and 250 mg crude extract were assessed using mice animal models. The different doses (125 and 250 mg/kg) suspended in tween-80 (2%) were dispensed orally and diclofenac sodium (10 mg) was given intraperitoneally to respective groups of mice ($n = 6$). Tween-80 (2%) in normal saline was dispensed to the control group. Acetic acid (1% v/v, 0.1 mL) was administered i.p after 30 min. After 3 minutes of acetic acid injection, the numbers of turning of trunk, abdominal constriction, and hind legs extension for 30 minutes test period were noted (15).

Formalin tests

Formalin induced paw licking reaction of mice was assessed in this test. Formalin (20 μ L) was administered (1% in 0.9% normal saline, s.c) in the hind paw dorsal region and observed licking response while placing mice in the transparent box. The reaction time (sec) duration of paw biting or licking was noted in the 1st phase (0-5 min) and the

second phase (15-30 min). The crude extracts at doses of 125 and 250 mg/kg were given subcutaneously and 10 mg/kg of indomethacin (i.p) were also given to three groups, before 30 min of formalin injection. The control group received 0.1 mL/10 g of the vehicle. The reaction time (sec) of each group was noted with control and demonstrated as percent inhibition (16).

Tail immersion tests

The crude extract was tested for central anti-nociceptive activity in Albino mice. Animals were divided into various groups among which two received 125 and 250 mg of crude extract orally. One group received vehicle (tween-80, 2%) i.p 30 minutes before testing and morphine (5 mg) and naloxone at 2 mg (i.p), 15 minutes before testing. The latency time of mice tail deflection was recorded by immersing 1-2 cm tail in warm water ($53 \pm 1^\circ\text{C}$) (17).

Anti-inflammatory activity

Wister albino rats model was used for the carrageenan-induced paw edema model as per reported protocols (18). The crude extract was given orally to overnight fasted animals ($n = 6$) at doses of 50, 100, and 200 mg/kg. Indomethacin (10 mg/kg) was given to one group, which served as standard. After 30 min, all groups were given carrageenan (1%, 50 μ L, s.c) solution in the left hind paw (planter region). The mean volume of paw after every 1 h for 5 h was measured using a digital plethysmometer and treated and untreated control groups were compared. Percent of paw edema inhibition was noted.

Statistical analysis

Expressed all the data as mean with standard deviation (SD). Analysis of variance and with post hoc, Dunnett's test was performed with GraphPad Prism 5 software.

RESULTS

Polyphenolic composition of the extract

The methanolic extract of Sedum contains phenolic compounds at the lower concentration of below 100 μ g/g of quinic acid, p-hydroxybenzoic acid, gallic acid, salicylic acid, catechin, 3-O-coumaroylquinic acid, and naringenin. Kaempferol-3-O-sophoroside, quercetin-3,4-diglucoside-3-(6-feruloyl glucoside), quercetin-3-(p-coumaroyl glucoside)-7-glucoside, quercetin-3-(p-coumaroyl-diglucoside)-7-glucoside, diosmetin-7-rutinoside, triferuloyl-diglucoside, kaempferol-3-(p-coumar-

oyl-glucoside)-7-glucoside, kaempferol-3,7-diglucoside, kaempferol-3-(p-coumaroyl-diglucoside)-7-glucoside and 4-caffeoyl-5-coumaroylquinic acid were major compounds as reported in our recent work (10). Three phenolic compounds namely kaempferol-3-*O*-sophoroside (3.36 mg/g), vitexin-6-malonyl-2-xyloside (2.1 mg/g), quercetin-3,4-diglucoside-3-(6-feruloyl glucoside) (1.8 mg/g) and diosmetin-7-rutinoside (1.6 mg/g) were present in significantly higher amounts.

***In vivo* acute toxicity**

In vivo acute toxicity test of the extract showed no gross, physical, and behavioral changes for 24 h and no mortality occurred in the next 14 days. Since the extract at a dose of 2000 mg/kg didn't show any mortality in mice so it was considered to be comparatively safe, during the study it was observed that the systemic administration of the compounds didn't produce any sedation, alteration in locomotor activity or motor dysfunction in animals.

Antioxidant activities

It is clear from the results of DPPH free radical scavenging activity, the extract exhibited an excel-

lent antioxidant activity in a dose-dependent way. It showed an activity of 35.0% at the lowest dose of 62.5 and 68.1% at 1000 mg/kg dose, reaching nearly to that of the standard ascorbic acid. The IC₅₀ values of DPPH inhibition were higher than standard ascorbic acid. The extract possesses antioxidant activity in a dose-dependent way (Table 1).

The ABTS free radical scavenging activity of the extract showed good scavenging activity of 52.6% at the highest concentration of 1000 µg/mL. Similarly, in H₂O₂ scavenging activity, the sample showed an activity starting from 21.06 at 62.5 µg/mL and reached 47.9 at the highest dose of 1000 µg/mL as shown in Table 1. The IC₅₀ values of ABTS inhibition were lower (239.8 µg/mL) than standard ascorbic acid (312.3 µg/mL). Similarly, the results of the H₂O₂ inhibition (%) showed lower inhibition as compared to the corresponding ascorbic acid doses. The IC₅₀ values of the extract were lower than 229.1 µg/mL than ascorbic acid standard.

Analgesic activity

The plant extract caused significant inhibition of analgesic response induced via acetic acid with a maximum effect of 57.4% ($p < 0.01$) and 64.7% ($p < 0.001$)

Table 1. Antioxidant activities of the *Sedum adenotrichum* extract.

Dose (µg/mL)	DPPH inhibition (%)		ABTS inhibition (%)		H ₂ O ₂ inhibition (%)	
	Extract	A. acid	Extract	A. acid	Extract	A. acid
62.5	35.0 ± 2.1	61.1 ± 0.7	28.2 ± 1.9	66.2 ± 0.2	21.0 ± 2.2	69.4 ± 0.8
125	36.6 ± 3.2	64.27 ± 0.9	34.6 ± 1.5	69.2 ± 0.4	29.2 ± 2.1	72.2 ± 0.6
250	42.1 ± 1.9*	69.61 ± 0.5	40.9 ± 2.2*	74.1 ± 0.7	34.1 ± 1.9*	77.3 ± 0.3
500	58.4 ± 2.3**	76.20 ± 1.5	46.9 ± 2.2**	81.1 ± 0.9	43.8 ± 2.2**	84.1 ± 0.7
1000	68.1 ± 1.9**	82.33 ± 0.8	52.6 ± 1.8**	87.2 ± 1.5	47.9 ± 2.7**	89.4 ± 0.4
IC ₅₀	374.4	303.8	239.8	312.3	229.1	301.0

DPPH, diphenyl picrylhydrazyl radical; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; A. acid, ascorbic acid; IC₅₀, the concentration of an inhibitor where the response is reduced by half. All values are expressed as mean with standard deviation (SD) for each group. *Significant difference compared with the acetaminophen group ($p < 0.05$). **Significant difference compared with the acetaminophen group ($p < 0.01$).

Table 2. Acetic acid-induced analgesic activity data of *Sedum adenotrichum* extract.

Treatment (dose)	Writhes	% inhibition
Control	78.6 ± 7.6	---
Extract (125 mg)	33.3 ± 1.2**	57.4
Extract (250 mg)	27.8 ± 1.1***	64.7
Diclofenac sodium (10 mg)	9.8 ± 0.3***	87.4

Values expressed as mean with standard deviation (SD) of $n = 6$. ** $p < 0.01$, *** $p < 0.001$ as compared with control.

Table 3. Analgesic activity (Tail flick method) of *Sedum adenotrichum* extract.

Treatment/ Dose	Tail Flick (sec) / Response (%)					
	15 min	30 min	45 min	60 min	75 min	90 min
Control	1.17 ± 0.9	1.32 ± 0.5	1.47 ± 1.2	1.39 ± 1.7	1.29 ± 1.7	1.38 ± 1.5
Extract 125 mg	1.54 ± 1.1* 24.0%	1.92 ± 0.8* 31.2%	2.44 ± 0.3** 39.7%	2.91 ± 0.8** 52.2%	3.12 ± 0.5** 58.6%	3.31 ± 0.9*** 58.3%
Extract 250 mg	1.51 ± 1.4* 22.5%	1.95 ± 0.2* 32.3%	2.76 ± 1.2** 46.7%	3.09 ± 0.4*** 55.0%	3.28 ± 1.3*** 60.6%	3.41 ± 0.9*** 59.5%
Standard Morphine 5 mg	2.31 ± 1.0** 49.3%	3.16 ± 0.2** 58.2%	6.78 ± 0.9*** 78.3%	9.18 ± 0.6*** 84.8%	7.47 ± 1.5*** 82.7%	7.11 ± 0.7*** 80.5%
Extract 125 mg + Naloxone 2 mg	0.76 ± 0.9	0.97 ± 0.6	1.25 ± 0.1	1.43 ± 1.7	1.58 ± 0.5	1.68 ± 0.9
Extract 250 mg + Naloxone 2 mg	0.79 ± 1.7	0.92 ± 1.1	1.41 ± 0.2	1.53 ± 2.5	1.67 ± 0.6	1.8 ± 1.5
Morphine 5 mg + Naloxone 2 mg	1.21 ± 1.4	1.24 ± 1.1	1.39 ± 1.2	1.27 ± 1.6	1.33 ± 0.9	1.35 ± 1.4

Data are mean with SD (n = 6). *p < 0.05, **p < 0.01 and ***p < 0.001 upon comparing to control; one way ANOVA followed by Dunnett's test: compared all vs control test.

Table 4. Formalin-induced paw-licking response of the extract of *Sedum adenotrichum*.

Treatment (Dose)	Licking time Sec		Inhibition %	
	1 st Phase	2 nd Phase	1 st Phase	2 nd Phase
Control	48.6 ± 2.2	72.3 ± 1.9	----	----
Extract (125 mg)	26.6 ± 2.0**	27.6 ± 2.7***	45.1	61.7
Extract (250 mg)	22.7 ± 0.9***	21.7 ± 0.8***	53.2	69.9
Indomethacin (10 mg)	38.5 ± 1.3	17.3 ± 0.9***	20.8	76.0

Data are mean with SD (n = 6). *p < 0.05, **p < 0.01 and ***p < 0.001 upon comparing to control, one way ANOVA followed by Dunnett's test compared all vs control test.

at 125 and 250 mg/kg respectively (Table 2). The results were comparable to diclofenac sodium (standard, 10 mg/kg) that displayed 87.4% at p < 0.001.

The analgesic activity in tail immersion assay of the extract at 125 and 250 mg/kg was significant. A maximum analgesic response of 125 mg extract was noted at 75 min, latency increased 58.6%, at p < 0.01. Similarly, the 250 mg extract showed the maximum response of 60.7%, (p < 0.001) at 90 min. whereas central analgesic, morphine demonstrated powerful activity at 60 min after treatment 84.8% at p < 0.001. Animals treated with naloxone produced a significant reduction in the analgesic activity of morphine and plant extract (125 and 250 mg/kg) as shown in Table 3. The results from antagonistic assay reveal the possible participation of other receptors apart from opioid receptors.

Formalin-induced paw licking

The plant extract significantly inhibited both phases to 45.1% and, 61.75% (p < 0.001) of for-

malin-induced paw licking response respectively at 125 mg/kg. Similarly, inhibition response of 250 mg/kg was observed as 53.2% (p < 0.001) and 69.9% (p < 0.001) as shown in Table 4. Animals pretreated with 10 mg/kg indomethacin caused a marked decline in the paw-licking time to 76.0% (p < 0.001) in the second phase while the mild decline in licking time (20.8%) in phase first was observed.

Anti-inflammatory activity

In the carrageenan-induced model for anti-inflammatory assay, the test sample subdued formation of edema in a 3rd hour at 100 and 200 mg, by 50.7% (p < 0.05) and 52.4 (p < 0.05) respectively in a dose-dependent way. This effect was a bit maintained to the next hour followed by a decrease in the 5th hour of the experiment. At the lowest dose (50 mg) used, it showed 39.0% activity at 3rd h of the experiment as shown in Table 5.

DISCUSSION

The present research work aims to find out antioxidant, analgesic, and anti-inflammatory potentials of *S. adenotrichum*. Important phenolic compounds were present in the extract as reported in our recent work (10). It was shown previously that gallic acid was present in the lyophilized extracts of *Sedum telephium* (19). Another study showed quantification of total glycosides of quercetin and kaempferol, but could not show the exact compounds (20). In a similar study, Oh et al. (21) also identified glycosides of quercetin and kaempferol in *Sedum sarmentosum*. These results suggest that different species of sedum are a good source of important phenolic compounds, especially flavonoids.

The rise of the ROS level contributes to tissue injury and is potentially related to several diseases like cancer, aging, inflammation, and pain (22). In general, the creation, as well as biological reactivity of ROS, are under the control of catalase, glutathione peroxidase, and superoxide dismutase. However, if the creation of these free radicals is increased or the defense via antioxidant is decreased, it leads to oxidative stress (23), which can damage DNA, proteins, tissue, or lipids (24). Many studies have shown that many plant species are useful antioxidants. In this research study, the extract of the root showed excellent antioxidant activity in the highest used dose in DPPH and ABTS free radical scavenging activity. However, in the H₂O₂ free radical scavenging activity, it did not achieve the IC₅₀ even at the highest dose used in the study.

Acetic acid-induced abdominal constriction method is thought to be a sensitive method and a weak analgesic can detect the results. The peripheral anal-

gesic reaction is determined through this method. Acetic acid increases prostaglandin particularly PGE₂ level in mice peritoneal fluid (25). Prostaglandins activate abdominal constriction through activation and sensitization of peripheral chemo-sensitive nociceptors (26), mainly related to inflammatory pain (27). Concerning this model of abdominal constriction, a significant analgesic response was observed from the results at both doses. This analgesic response may be due to its anti-inflammatory response because, in a visceral model of pain, arachidonic acid is released through cyclooxygenase and prostaglandin biosynthesis having a key role in nociception (28). So there may be involved an anti-inflammatory constituent in peripheral analgesic response which leads to inhibition of pain development.

Formalin-induced nociception is applied to determine the activity of the sample for relieving moderate persistent pain produced because of injury to the tissue (29). Acute and chronic phases of formalin-induced nociception demonstrate neurogenic and inflammatory pain respectively. The direct chemical motivation of afferent nociceptive fibers is linked with the acute phase which can be subdued by opioid analgesics such as morphine (30). The release of bradykinin, histamine, serotonin, and prostaglandins in peripheral tissues and functional changes in the dorsal horn of the spinal cord are linked with the 2nd phase (31). The significant inhibition of both phases of formalin-induced nociception indicates that the inhibitory mechanism involves an effect on afferent fibers as well as on inflammatory mediators.

The tail immersion assay is employed for acute pain determination. This method involves spinal pathways in pain response regulation. Raised thermal noci-

Table 5. Effect of crude extract of *Sedum adenotrichum* on carrageenan-induced rats paws edema.

Treatment	Dose	1st h	2nd h	3rd h	4th h	5th h
Saline	10 mL	0.273 ± 0.2	0.261 ± 0.02	0.272 ± 0.02	0.256 ± 0.02	0.261 ± 0.03
Carrageenan	50 µL	0.371 ± 0.1***	0.423 ± 0.03***	0.461 ± 0.02***	0.472 ± 0.02***	0.469 ± 0.02***
Indomethacin	10 mg	0.209 ± 0.04** (43.6)	0.172 ± 0.03* (59.3)	0.161 ± 0.03*** (65.0%)	0.189 ± 0.01*** (59.9%)	0.223 ± 0.02** (52.4%)
Extract	50	0.276 ± 0.03* (25.6%)	0.266 ± 0.02* (37.1%)	0.281 ± 0.02** (39.0%)	0.311 ± 0.02* (34.1%)	0.326 ± 0.03* (30.4%)
	100	0.250 ± 0.02* (32.6%)	0.238 ± 0.01** (43.7%)	0.227 ± 0.02** (50.7%)	0.254 ± 0.03*** (46.1%)	0.291 ± 0.03* (37.95%)
	200	0.239 ± 0.02** (35.6%)	0.227 ± 0.02** (46.3%)	0.219 ± 0.02*** (52.4%)	0.243 ± 0.03*** (48.5%)	0.276 ± 0.02** (41.1%)

Values as mean with SD (n = 6). *p < 0.05, **p < 0.01 and ***p < 0.001 upon comparing with control, one way ANOVA followed by Dunnett's: compared all vs control test.

ception is used in this procedure and samples with good thermal antinociceptive results are considered as potent analgesics (32). The hot plate and tail-flick procedures are thought to be specific tests for central pain evaluation at the spinal and supraspinal levels (33) respectively. In this research work, there was a significant increase at both doses of the extract in the reaction. This increase in time of reaction because of the test sample suggests an analgesic response.

Carrageenan-induced edema involves the synthesis or release of mediators at the site of injury. Serotonin, bradykinins, histamine, and leukotrienes are these mediators, causing pain and fever also (34). Inhibitions of pharmacological effects or reaching of these mediators to the site of injury will normally improve inflammation and/ or symptoms. This work demonstrates that the tested sample possessed a significant antioedematogenic activity on carrageenan-induced paw edema. This is an appropriate *in vivo* experimental representation for anti-oedematous activity evaluation and is biphasic. During the first phase (1 h) histamine and serotonin are released and during the second phase (over 1 h) the release of prostaglandins, products of cyclooxygenase, and kinins occur to provide continuity between the two phases (35).

Phenolic compounds and carotenoids are biologically active compounds that are presently under severe research study for exploring safe constituents of therapeutic value (36). For exploring therapeutically potent herbs and their constituents, this work was designed to explore the biological activities of the crude extract of *S. adenotrichum*. Preliminary, *S. adenotrichum* has tested positive for phenolic compounds and flavonoids presence. The analgesic and anti-inflammatory actions of *S. adenotrichum* may be attributed to these phenolic compounds and flavonoids identified using HPLC. Indomethacin reduces pain receptors sensitization caused by prostaglandins at the inflammatory site (37). The resulted analgesic and anti-inflammatory potentials of the test sample may be due to the overall actions of *S. adenotrichum* components affecting parallel to NSAID, in a complimentary way. Phenolic compounds present in plants possess significant antioxidant activity (38). Flavonoids such as kaempferol and quercetin showed promising anti-inflammatory activity (39, 40). The presence of these phenolic compounds in *S. adenotrichum* may thus be responsible for antioxidant and analgesic activities.

CONCLUSION

This research work shows that *S. adenotrichum* exhibits significant analgesic and anti-inflammatory

effects, justifying its traditional use as a therapeutic agent. It is recommended to perform long-term studies to identify and isolate the active phytoconstituents involved in analgesic and anti-inflammatory effects and to reveal the real mechanism that is involved in analgesic and anti-inflammatory effects of the plant.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial or non-financial relationships that could be construed as a potential conflict of interest.

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