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Anti-inflammatory Activity of the Phenolic-rich Extract of *Schotia brachypetalea* Sond. Fam. Fabaceae, Cultivated in Egypt

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The current study evaluates the anti-inflammatory activity of the methanolic leaf extract of *Schotia brachypetalea*, isolates and identifies the phenolic content and standardizes the crude leaf extract using HPLC.
Place and Duration of study: This study was conducted in the Departments of Pharmacognosy and Pharmacology, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt during the period between July 2013 and December 2014.
Methodology: The anti-inflammatory activity was assessed using 2 models *viz.* carrageenan induced rat paw edema and croton oil induced ear edema. Column and paper chromatography

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were used to fractionate and isolate the phenolic compounds. The isolated compounds were analyzed using UV and ^{1}H-NMR spectroscopy. The methanolic leaf extract was standardized using gallic acid as a reference by HPLC analysis. Total phenolic and flavonoidal contents were determined using Folin-Ciocalteu and Aluminium chloride colorimetric assays, respectively.

Results: Significant dose dependent edema inhibition was observed in the carrageenan model at a dose 200 mg/kg (79.31%, 2hrs) and (85.19%, 3hrs). Also, levels of PGE_2 had decreased significantly at this dose at the corresponding time intervals. In the croton oil model, the methanolic leaf extract of *S. brachypetalea* (50 mg/kg) decreased the edema to about 39.56% while 100 and 200 mg/kg doses decreased the edema to about 23 and 15.8%, respectively. Myloperoxidase (MPO) levels were reduced by 38, 45 and 63% in doses 50, 100 and 200 mg/kg, respectively. TNF-α level was reduced similarly. The anti-inflammatory activity was confirmed by the histopathological examination of the ear tissue revealing that the 200 mg/kg dose of the methanolic leaf extract of *S. brachypetalea* had normal dermal structure with no histopathological changes. The total phenolic content of *S. brachypetala* leaf extract was calculated as 376 mg of caffeic acid equivalents (CAE) per one gram extract while the total flavonoid content was 67.87 mg in one gram extract calculated as quercetin equivalent (QE). Three phenolic constituents were isolated, namely 1 Gallic acid, 2 Myrecitin-3-*O*-*α*-L-$^1C_4$-rhamnoside and 3 Quercetin-3-*O*-*α*-L-$^1C_4$-rhamnoside.

Conclusion: The study showed the potent anti-inflammatory activity of the methanolic leaf extract of *S. brachypetalea;* this might be attributed to its phenolic content.

Keywords: Schotia brachypetalea; fabaceae; anti-inflammatory; HPLC; phenolic; PGE_2; TNF; MPO.

1. INTRODUCTION

Inflammation is a complex biological response of the vascular tissues to the harmful stimuli, such as pathogens, damaged cells or irritants. It is a protective attempt to remove injurious stimuli as well as initiate the healing process for the tissues [1].

Prostaglandin E_2 (PGE_2) is generated from arachidonic acid by the enzyme cyclo-oxygenase (COX) at sites of inflammation in substantial amounts and mediate many of the pathologic features of inflammation. One of the early cellular events of inflammation is the migration of leukocytes, primarily neutrophils. This response can be measured by using the neutrophil specific enzyme myeloperoxidase (MPO), an indicator of the neutrophil accumulation [2].

Although steroidal anti-inflammatory drugs and NSAIDS are currently used to treat inflammation, they show significant side effects even with newer classes [2]. Therefore, there is an urgent need to find safer anti-inflammatory compounds from natural origin [3]. *Schotia brachypetala* (*S. brachypetalea*) Sond. (Fabaceae) is commonly named weeping boer-bean and huilboerbean (Afrikaans), belongs to genus *Schotia*. It is a tree indigenous to Southern Africa. Traditionally, the bark decoction was used to strengthen the body, treat dysentery and diarrhea, nervous and heart conditions, flu symptoms and an emetic in excessive beer drinking. Its roots were also used to treat diarrhea and heartburn. The seeds are edible after roasting [4].

Many biological activities viz. antibacterial [4-5], anti-diarrheal [6], anti-oxidant and anti-malarial[4] had been reported from genus *Schotia*. The stalks showed activity against Alzheimer's disease, which was correlated to their anti-oxidant properties [7]. Several classes of secondary metabolites were isolated and identified from various organs of *Schotia* namely: pentahydroxystilbene [8]; δ-linolenic acid; methyl-5,11,14,17-eicosatetraenoate [5]; catechin and epicatechin [9] and flavonolacyl glucosides [4]. Also, procyanidin isomers; procyanidin dimer gallate; quercetin-3-*O*-rhamnoside; quercetin-hexose-gallic acid;, quercetin-hexose-protocatechuic acid; quercetin-3-*O*-rhamnoside and ellagic acid were also identified [7].To the best of our knowledge, there was little information concerning *S. brachypetalea* cultivated in Egypt. Thus, the present work was aimed to evaluate the anti-inflammatory activity of the leaf extract of *S. brachypetala*; isolate and identify its phenolic constituents in order to provide a scientific explanation to justify its use.

2. MATERIALS AND METHODS

2.1 Plant Material

During spring season (April-May 2012), *S. brachypetalea* leaves were collected from private garden, Alexandia desert road. They were kindly authenticated by Prof. Dr. Mohamed El-Gabaly, Professor of Taxonomy, National Research Center (NRC), Egypt. A voucher specimen (Sbf1513) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University.

2.2 Extraction and Isolation

The powdered plant material (3kg) was boiled in distilled water for 2 hours and filtered while hot. The filtrate was completely evaporated *in vacuo* at ≈ 70ºC to obtain a residue which was then extracted with methanol. The methanol extract was evaporated *in vacuo* till dryness. The residue obtained (120 g) was applied on a column packed with polyamide (Sigma-Aldrich, Germany). Elution was done with water then the polarity was decreased by increasing the percentage of methanol. Similar fractions were combined together according to their paper chromatography (PC) properties using 6% acetic acid (6:94; AcOH:H_2O) and BAW (4:1:5; butanol: AcOH : H_2O; upper layer) as eluents to give 4 main fractions. Fraction II (eluted with water) showed only one major spot to afford compound 1. Fraction III (eluted with 10% - 60%MeOH) was applied on a column packed with Sephadex and eluted with butanol saturated with water. Further purification was done using preparative paper chromatography (PPC) 3MM (Whatmann Ltd., Maidstone, Kent, England) and 6% acetic acid to afford compounds 2 and 3. The isolated compounds were identified using the conventional methods of spectroscopy *viz.* UV (JASCO, V-630) and ^{1}H-NMR (Bruker 400 MHz NMR spectrometer).

2.3 Solvents and Chemicals

Methanol, water and acetic acid were of HPLC grade. All other laboratory chemicals used in current study were of analytical grade and were purchased from Sigma-Aldrich Chemicals.

2.4 Material for Biological Study

2.4.1 Experimental animals

Adult male Sprague–Dawley rats weighing 180–200 g were used. They were obtained from the animal facility, Misr University for Science and Technology (MUST), Egypt. The study was conducted according to ethical guidelines (Ain Shams University, Cairo, Egypt). Rats were housed at a temperature of 23±2ºC with free access to water and standard food pellets. Animals were acclimatized for at least one week prior to the experiment. They were then equally divided into six groups (I-VI), six animals per each group. All animal tests were performed under laboratory conditions.

2.4.2 Measurement of paw volume and PGE_2 in carrageenan-induced rat edema model [10]

Animals were fasted, with free access to water, 12 hours before the experiment. Using an intra-gastric tube, groups I and II were given the vehicle (0.5% CMC-Na). Animals in Group III received Indomethacin (10 mg/kg) as a standard anti-inflammatory drug suspended in aqueous CMC-Na (0.5% w/v), whereas the remaining groups were treated with methanolic leaf extract of *S. brachypetalea* at 3 dose levels (50, 100 and 200 mg/kg, respectively). The dosing volume were kept constant (10 ml/kg) for all the orally treated groups. One hour after oral treatment, group I received 0.05ml saline, whereas groups (II-VI) received 0.05 ml carrageenan (1% solution in saline) subcutaneously on the plantar surface of the right hind paw. The right hind paw volume was measured immediately after carrageenan injection by water displacement using a plethysmometer (model 7140, Ugo Basile, Comerio, Italy). The paw volume was re-measured 1, 2, 3 and 4 hours after carrageenan injection. Right hind paws were removed. A volume of 0.1 ml saline containing 10 µM indomethacin was injected to aid removal of the eicosanoid-containing fluid and to stop further production of PGE_2. Paws were incised with a scalpel and suspended off the bottom of polypropylene tubes with Eppendorf pipette tips to facilitate drainage of the inflammatory exudates. For the purpose of the removal of the inflammatory exudates, paws were centrifuged at 1,800 g for 15 minutes. PGE_2 was quantified using enzyme-linked immunosorbent assay kit obtained from R&D Systems Inc. (Minneapolis, MN, USA), according to the manufacturer instructions. This assay was based on the forward sequential competitive binding technique in which PGE_2 in a sample competes with horseradish peroxidase–labeled PGE_2 for a limited number of binding sites on monoclonal antibodies. PGE_2 in the sample is allowed to bind to the antibody in the first incubation. During the

second incubation, horseradish peroxidase–labeled PGE_2 binds to the remaining antibody sites. Following a wash to remove unbound materials, a substrate solution is added to the wells to determine the bound enzyme activity. The color development is stopped, and the absorbance is read at 450 nm. The intensity of the color is inversely proportional to the concentration of PGE_2 in the sample.

2.4.3 Assessment of ear edema, myloperoxidase (MPO), and histopathology in croton oil–induced ear edema model in rats [11]

An irritant solution was prepared by dissolving four parts croton oil (the irritant) in a solvent mixture of 10 parts ethanol, 20 parts pyridine, and 66 parts ethyl ether. The irritant solution was applied in a volume of 20 µl topically on both sides of the right ear; the left ear was kept untreated to serve as a control. Group I served as the negative control and hence received only the irritant-free solvent mixture, whereas group II received the croton oil solution. Animals in group III received indomethacin (10 mg/kg) as a standard anti-inflammatory drug suspended in aqueous CMC-Na (0.5% w/v), whereas remaining groups (IV-VI) were treated with the methanolic leaf extract of *S. brachypetalea* at 3 dose levels (50, 100 and 200 mg/kg, respectively). One hour later, the solvent mixtures were re-administered to the groups. After 4 hours, animals were sacrificed. 8-mm cork borer was used to punch out discs from both the treated and the control ears. The two punches were weighed immediately after decapitation, and the difference in weight was used to assess the inflammatory response.

The entire tissue of the right ear was homogenized for 10 minutes in an ice bath (10% w/v) in 50 mM phosphate buffer (pH 6.0) containing 0.5% hexa-decyl-tri-methylammonium bromide with a IKA homogenizer (Staufen, Germany). Tissue suspensions were centrifuged at 40,000 g for 15 minutes. The supernatants were collected. An aliquot of 0.1 ml of the supernatant was added to 2.9 ml of 50 mM phosphate buffer (pH 6.0) containing 0.167 mg/ml *O*-di-anisidine dihydrochloride, serving as the MPO substrate, and 0.0005% hydrogen peroxide. The change in absorbance was measured at 460 nm with a Shimadzu UV-1601 spectrophotometer at 25ºC. MPO activity was quantified kinetically; change in absorbance was measured over a period of 2 minutes, sampled at intervals of 15 seconds. The maximal change in absorbency per minute was used to calculate the units of MPO activity based on the molar absorbency index of oxidized *O*-di-anisidinedi hydrochloride, which equals $1.13X10^4$ M^{-1} cm^{-1}. One unit of MPO is defined as that degrading 1 µmol of peroxide/minute at 25ºC. Results were expressed as units of activity per milligram of protein.

A representative ear tissue from each group was fixed in 10% formol saline for 72 hours. Well washing was done in tap water, and then serial dilutions of alcohol (methyl, ethyl, and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56ºC in a hot air oven for 24 hours. Paraffin beeswax tissue blocks were prepared for sectioning at 4 mm thickness with a sledge microtome. The tissue sections obtained were collected on glass slides, deparaffinized, and stained by hematoxylin and eosin then examination was done using the light microscope.

2.4.4 Statistical analysis

Data are presented as mean±S.D. Comparisons were carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer's test for post hoc analysis. Statistical significance was acceptable to a level of $p< 0.05$. All statistical analyses were performed using GraphPad In Stat software, version 3.05 (GraphPad Software, Inc. La Jolla, CA, USA). Graphs were plotted using GraphPad Prism software, version 5.00 (GraphPad Software, Inc. La Jolla, CA, USA).

2.5 HPLC Standardization

The methanolic leaf extract of *S. brachypetala* was standardized using an Agilent 1200 series HPLC equipped with an Agilent quaternary pump connected to a photodiode array detector with variable wavelengths. Separation was carried out on RP C18 column with dimensions (150 mm, 4.6 mm, 5 µm). Gallic acid was used as a reference standard. A stock solution of gallic acid was prepared in a dilution 0.1296 g/10ml methanol from which serial dilutions were prepared to afford different concentrations ranging from 0.02592 to 0.1036mg/ml. All samples were filtered through PTFE 0.5 µm syringe filter and injected in triplicates into the HPLC column using 4% acetic acid in water (solvent A) and methanol (solvent B) in gradient

elution. The gradient program was begun with 100% B and was held at this concentration for the first 4 minutes. This was followed by 50% eluent A for the next 6 minutes after which concentration of A was increased to 80% for the next 10 minutes and then reduced to 50% again for the following 2 minutes. Total run time was 22 minutes. The flow rate was 0.6 ml/min while the injection volume 20 *µ*l. Measurement was done at wavelength 280 nm [12]. A standard calibration curve was designed by plotting the different concentrations of gallic acid against peak area. Regression equation and co-efficient of correlation (R^2) were derived. The concentrations were calculated based on the regression equation. The assay was repeated five times in the same day for intraday precision by the used method. Accuracy was measured by performing a recovery study of gallic acid reference by adding (0.0732g) to the extract solution (0.0324g) and analyzed by the same method.

2.6 Determination of Total Phenolic Content and Total Flavonoid Content

The Folin-Ciocalteu method was used for the determination of the total phenolic content using caffeic acid as a reference standard [13]. Total flavonoid content was determined by the Aluminum chloride colorimetric method using quercetin as the reference standard [14].

3. RESULTS AND DISCUSSION

3.1 Identification of the Isolated Compounds

Three phenolic compounds; one phenolic acid 1Gallic acid and two flavonoid glycosides, 2Myrecitin-3-*O*-*α*-L-$^1C_4$-rhamnoside and 3Quercetin-3-*O*-*α*-L-$^1C_4$-rhamnoside were isolated and identified from leaf extract of *S. brachypetalea*.

Compound 1:

showed one major violet spot on PC under UV and turned blue upon spraying with $FeCl_3$. Thus, it was co-chromatographed with gallic acid standard. The spot showed the same color under UV and the same R_f value.

Compound 2:

was obtained as yellow crystalline powder. It appeared on the chromatograms as dark purple spot on PC under short UV light. $R_f$values: 24.5 (BAW; 4:1:5; butanol: AcOH: H_2O; upper layer) and 13.5 (6% AcOH). It gave dirty green colour with $FeCl_3$, spray reagent specific for phenolics [15]. Also, it exhibited UV spectrum which showed two bands at λ_{max}MeOH (350 nm band I and 258 nm band II) which indicated the flavonol nucleus [16]. Upon addition of sodium methoxide, bathochromic shift (11 nm) occurred in band II and (50 nm) shift then decomposes in band I. While upon using sodium acetate, bathochromic shift (14 nm) was observed in band II and (20 nm) in band I, indicating free OH at positions 3', 4', 5' and 7. A bathochromic shift occurred upon addition of boric acid (61 nm) and (22 nm) in bands II and I, respectively. The ^{1}H-NMR spectra showed the presence of 2 aromatic proton meta coupled, signals appear at δ=6.15ppm (1H, *d*, J=2.5 Hz, H-6) and 6.31 ppm (1H, *d*, J=2.5 Hz, H-8). The presence of *O*-glycosidic anomeric signal at 5.2ppm (1H, *d,* J=7.5 Hz, H-1").A signal at *δ*=6.879ppm (2H, *s*, H-2', H-6') indicating the equivalent protons at H-2' and H-6'. A signal for methyl of rhamnose at 1.45 ppm (3H, *s*,). UV as well as ^{1}H-NMR chemical shifts were found to be similar to those reported for Myrecitin-3-*O*-*α*-L-$^1C_4$-rhamnoside [17].

Compound 3:

was obtained as yellow crystalline powder. It gave dark purple color on PC under short UV light. R_f values: 22.5 (BAW) and 7.5 (6% AcOH). Upon spraying with $FeCl_3$, dirty green colour is developed. Also, it exhibited UVspectrum which showed two bands at λ_{max}MeOH (360nm band I and 264 nm band II) which indicated the flavonol nucleus [16]. It showed a bathochromic shift (7 nm) in band II and (30 nm) in band I on addition of sodium methoxide. Similarly, upon using sodium acetate, bathochromic shift (10nm) in band II and (20nm) in band I to prove that the 3', 4' and 7 OH positions are free. Adding boric acid, no change occurred in band II indicating that no orthodihydroxy is present while a bathochromic shift (25 nm) in band I indicationg free ortho-dihydroxy at positions 3' an 4'.^{1}H-NMR spectrum of compound **3** showed the presence of aromatic proton signals at *δ* =7.199 (1H, *d*, J=2.5 *Hz*, H-2'), *δ*=6.909 (1H, *dd*, J=2.5 *&* 8 *Hz*, H-6') and *δ* =6.882 (1H, *d*, J=8 *Hz*, H-5'). Presence of *O*-glycosidic anomeric signal at δ=5.214 ppm (1H, *d,* J=7.5 Hz, H-1"). Signal for methyl of rhamnose at *δ*=1.242 ppm (3H, *s,* CH_3rhamnose). Signal sat δ=6.22ppm (1H, *d*, J=2.5 Hz, H-6) and δ=6.43ppm (1H, *d*, J=2.5 Hz, H-8). UV as well as ^{1}H-NMR chemical shifts were found to be similar

to those reported for Quercetin-3-*O*-α-L-$^{1}C_{4}$-rhamnoside [18].

3.2 Biological Results

3.2.1 Carrageenan-induced rat paw edema model

The anti-inflammatory activity was expressed as percentage inhibition of edema volume in treated animals in comparison with the control and carrageenan-treated groups. The percent edema inhibition was calculated as:

$$[(V_{car.} - V_t) / (V_{car.} - V_{con.}) \text{ X } 100]$$

where: $V_{car.}$, V_t and $V_{con.}$ are the paw volumes of carrageenan, extract treatment and control groups, respectively.

Intraplantar injection of carrageenan to rats resulted in severe inflammation and significant increase in the mean volume of the challenged paw compared with that of the untreated paws. As shown in Table 1, pretreatment with methanolic leaf extract of *S. brachypetalea* 50 mg/kg resulted in significant amelioration of carrageenan-induced paw edema only after 3 and 4 hours. However, the leaf extract of *S. brachypetalea* (100 mg/kg) significantly decreased carrageenan-induced paw edema starting after 2 hours, while pretreatment with the leaf extract (200 mg/kg) resulted in significant reduction in paw edema at all time intervals. In a similar pattern, indomethacin caused significant inhibition of carrageenan-induced paw edema by about 57.14%, 75.86%, 92.59% and 90% after 1, 2, 3 and 4 hrs, respectively. Carrageenan challenge resulted in more than 10-fold increase in PGE_2 concentration compared to animals in the control group. Animals receiving methanolic leaf extract of *S. brachypetalea* (50, 100 & 200 mg/kg) or indomethacin (10 mg/kg) showed significant reduction of carrageenan-induced PGE_2 production in the inflamed skin tissues Fig. 1. It is worthy noted that pre-treatment with leaf extract of *S. brachypetalea* at dose 200 mg/kg restored normal PGE_2 concentration with no significant difference from the control group.

3.2.2 Croton oil induced rat ear edema model

Application of croton oil to rat ears caused a significant increase in the weight of the ear punch from control animals by 53.95% as shown in Table 2 and Fig. 2. Indomethacin pretreatment caused insignificant increase of punch weight from control values by about 10.07%. Furthermore, pretreatment of rats with the methanolic leaf extract of *S. brachypetalea* (50, 100 & 200 mg/kg) significantly reduced the increase of punch weight from control ears to about 39.56%, 23.02% and 15.82%, respectively. It is worthy noted that pretreatment of rats with the methanolic leaf extract of *S. brachypetalea* 200 mg/kg show no significant difference from the reference group.

Assay of MPO activity in rat ears indicated that application of croton oil caused a significant (4-fold) increase in the activity compared to the control group, as shown in Fig. 3. Indomethacin pretreatment resulted in significant protection against croton oil–induced enhancement of MPO activity by about 66% of the induced ear. In addition, pretreatment of rats with the methanolic leaf extract of *S. brachypetalea* (50, 100 & 200 mg/kg) significantly reduced MPO activity by about 31.9%, 44%, and 63% of the induced ear, respectively.

A similar pattern of effect was detected with TNF-α level, as shown in Fig. 4. Application of croton oil significantly increased TNF-α level by about 20-folds, compared to control value. Indomethacin pretreatment resulted in significant protection against croton oil–induced enhancement of TNF-α level by about 81.4% of the induced ear. Pretreatment of rats with the methanolic leaf extract of *S. brachypetalea* (50, 100 & 200 mg/kg), however, significantly reduced TNF-α level by about 31.4%, 43.1%, and 71.3% of the induced ear, respectively.

The histopathological examination of the ear tissue revealed: Fig. 5 (A) shows normal histological structure of the epidermal and dermallayers as well as underlying cartilage with no obvious neutrophil infiltration. Fig. 5 (B) the croton oil–treated rats shows congestion of dermal blood vessels accompanied with perivascular edema. Also, the blood vessels shows large numbers of leukocytes mixed with RBCs, with the dermis shows large numbers of inflammatory cells mainly macrophages and lymphocytes. Indomethacin pretreatment, Fig. 5 (C), shows almost intact dermal and cartilaginous structures with no histopathological changes. Fig. 5 (D) which represents ear tissue treated with 50 mg/kg methanolic leaf extract of *S. brachypetalea*, shows few inflammatory cells mainly macrophage and lymphocytes in the dermis. Edema and congestion of dermal blood vessels is the most prominent lesion in the dermis of ear tissue treated with 100 mg/kg

methanolic leaf extract of *S. brachypetalea*, as shown in Fig. 5 (E). However, ear tissues treated with 200 mg/kg leaf extract of *S. brachypetalea* shows normal dermal structure with no histopathological changes, as shown in Fig. 5 (F).

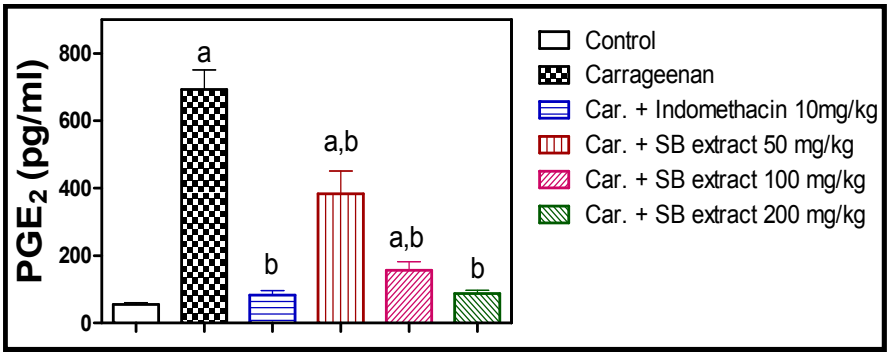

Fig. 1. Effect of the methanolic leaf extract of *S. brachypetalea* on the level of PGE_2 in carrageenan-induced rat paw edema model

**Data are presented as mean±S.D. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test. n=6, [a] statistically different from the control group at p<0.05, [b] statistically different from the carrageenan-treated group at p < 0.05*

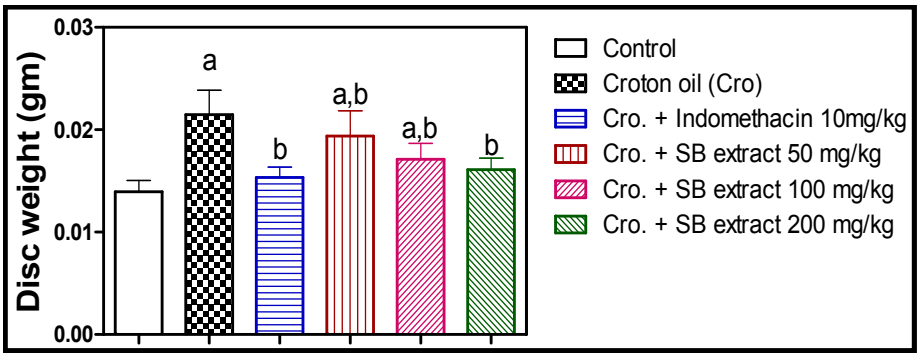

Fig. 2. Effect of the methanolic leaf extract of *S. brachypetalea* on the ear disc weight in croton oil-induced rat ear edema model

**Data are presented as mean ± S.D. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test.n=6, [a] statistically different from the control group at p<0.05, [b] statistically different from the carrageenan-treated group at p<0.05*

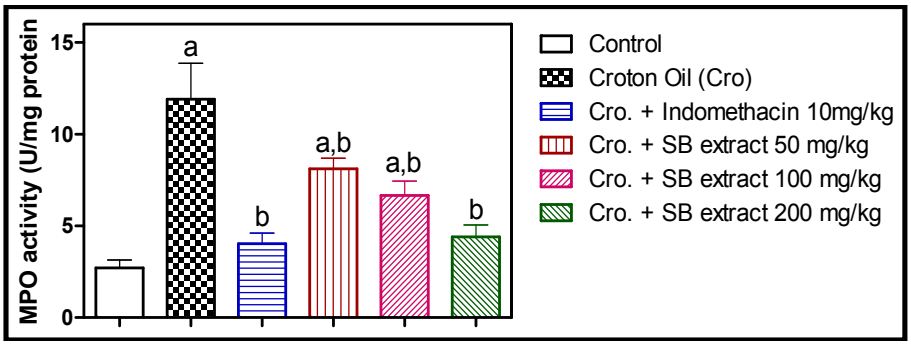

Fig. 3. Effect of the methanolic leaf extract of *S. brachypetalea* on MPO activity in croton oil-induced rat ear edema model

**Data are presented as mean ± S.D. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test. n=6, [a] statistically different from the control group at p < 0.05, [b] statistically different from the carrageenan-treated group at p < 0.05*

Table 1. The effect of the methanolic leaf extract of *S. brachypetalea* ($SB_{extract}$) on rat paw volume in carrageenan-induced rat paw edema model

Group	1 hour		2 hours		3 hours		4 hours	
	paw volume (ml)	% edema inhibition	paw volume (ml)	% edema inhibition	paw volume (ml)	% edema inhibition	paw volume (ml)	% edema inhibition
Control (I)	0.61±0.08	--	0.58±0.11	--	0.57±0.10	--	0.53±0.06	--
Carrageenin (II)	0.82±0.10	--	0.87±0.04	--	1.11±0.17	--	1.33±0.16	--
Indomethacin (III) 10 mg/kg	0.70^{b}±0.11	57.14%	0.65^{b}±0.09	75.86%	0.61^{b}± 0.07	92.59%	0.61^{b}± 0.07	90%
$SB_{extract}$(IV) 50mg/kg	0.81^{a}± 0.18	4.76%	0.82^{a}±0.08	17.24%	$0.97^{a,b}$±0.10	25.93%	$1.08^{a,b}$±0.09	31.25%
$SB_{extract}$(V) 100mg/kg	0.76^{a}±0.09	28.57%	$0.75^{a,b}$±0.05	41.37%	$0.84^{a,b}$±0.13	50%	$0.93^{a,b}$±0.07	50%
$SB_{extract}$(VI) 200mg/kg	0.68^{b}±0.12	66.66%	0.64^{b}±0.13	79.31%	0.65^{b}±0.10	85.19%	0.72^{b}±0.08	76.25%

**Data are presented as mean±S.D. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test. n=6, [a] statistically different from the corresponding control group at $p < 0.05$, [b] statistically different from the corresponding carrageenan-treated group at $p < 0.05$*

Table 2. The effect of the methanolic leaf extract of *S. brachypetalea* ($SB_{extract}$) on rat disc weight in croton oil-induced rat ear edema model

Group	Disc Weight (g)	Percentage increase in punch weight from control
Control (I)	0.0139±0.0011	--
Croton oil (Cro.) (II)	0.0214^{a}±0.0023	53.95%
Cro. + Indomethacin (10mg/kg) (III)	0.0153^{b}±0.001	10.07 %
Cro. + $SB_{extract}$(50mg/kg) (IV)	$0.0194^{a,b}$±0.0024	39.56%
Cro. + $SB_{extract}$(100mg/kg) (V)	$0.0171^{a,b}$±0.0015	23.02%
Cro. + $SB_{extract}$(200mg/kg) (VI)	0.0161^{b}±0.0011	15.82%

**Data are presented as mean±S.D. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test. n=6, [a] statistically different from the corresponding control group at $p<0.05$, [b] statistically different from the corresponding carrageenan-treated group at $p < 0.05$*

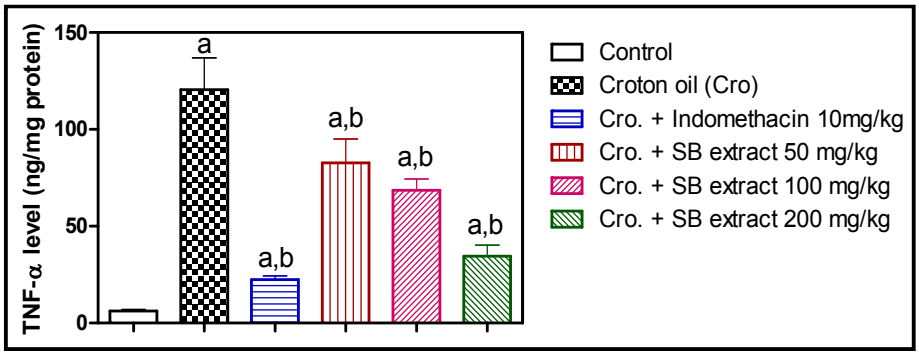

Fig. 4. Effect of the methanolic leaf extract of *S. brachypetalea* on TNF-α level in croton oil-induced rat ear edema model

**Data are presented as mean±S.D. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test. n=6, [a] statistically different from the control group at p<0.05 [b] statistically different from the carrageenan-treated group at p<0.05*

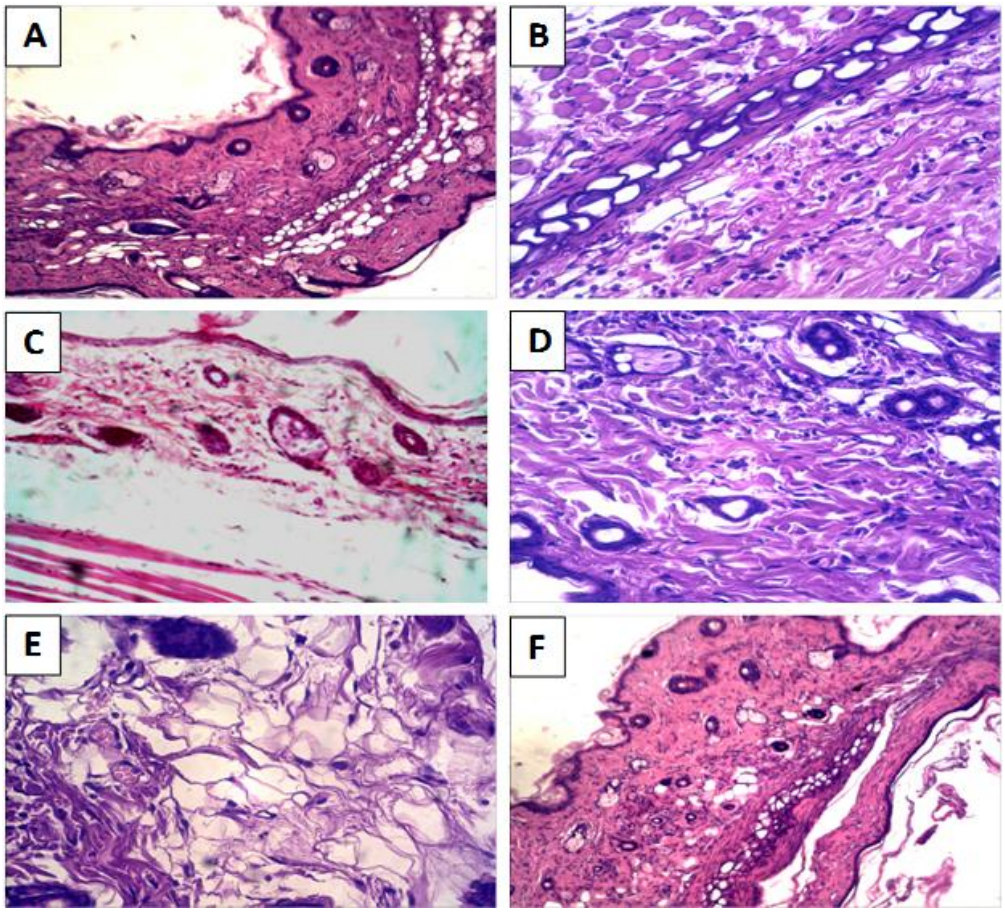

Fig. 5. Effect of the methanolic leaf extract of *S. brachypetalea* extract on histopathological changes in the croton oil–induced ear edema experiment

**Magnification X100. (A) normal histological structure of the epidermal and dermal layers as well as underlying cartilage with no obvious neutrophil infiltration. (B) croton oil–treated rats with massive neutrophil infiltration in the dermal layer. (C) ear tissue treated with indomethacin shows almost intact structures (D, E, F) ear tissues treated with 50, 100 & 200 mg/kg the methanolic leaf extract of S. brachypetalea extract shows pathological lesions that differ in severity according to the dose used*

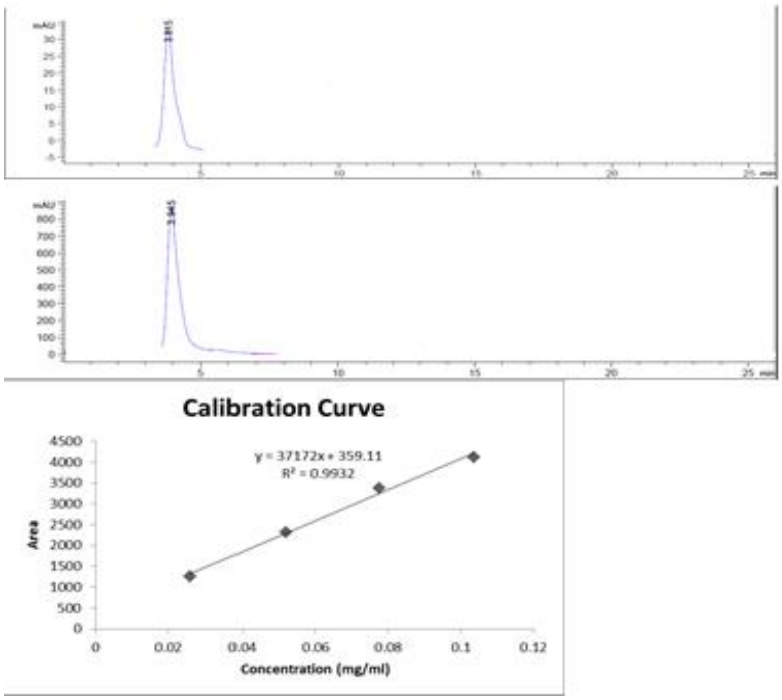

Fig. 6. HPLC chromatograms for *S. brachypetalea* methanolic leaf extract, gallic acid standard and calibration curve

3.3 HPLC Standardization of the Methanolic Leaf Extract of *S. brachypetalea*

The methanolic leaf extract of *S. brachypetalea* showed an intense peak at R_t 3.815 minutes. Spiking with gallic acid showed that this peak is identified as gallic acid. The standard solution of gallic acid was prepared and analyzed in the concentration range of 0.02592 to 0.1036 mg/ml. The regression equation of the curve was Y=37172X+359.1 as shown in Fig. 6. The regression coefficient (R^2) was 0.993 confirming the linearity of the method. The intraday precison for gallic acid was satisfactory with RSD of 3.14%. Accuracy was expressed as recovery showing 101%. Gallic acid content was calculated as 206 mg per one gram extract.

3.4 Total Phenolic Content and Total Flavonoid Content

Phenolic compounds are regarded as the molecules with the highest potential to neutralize free radicals. Therefore, their quantification is a common practice in different areas of food research. Total phenolic content of the methanolic leaf extract of *S. brachypetala* was 376 mg of caffeic acid equivalents (CAE) per one gram of the extract while the total flavonoidal content was 67.87 mg per one gram extract quercetin equivalent (QE).Various flavonoids have been found to be prominent anti-inflammatory activity by inhibiting cycloxygenase (COX) [19]. quercetin and myrecitin had shown inhibitory activity of the MPO [20]. Similarly phenolic acids *viz.* gallic acid had proved to inhibit histamine release and also derceased the release of TNF-α [21]. The anti-inflammatory activity of the leaf extract might be attributed to the richness of the plant with phenolic constituents.

4. CONCLUSION

In the present study, *S. brachypetalea* is a plant rich in phenolics and flavonoids with promising anti-inflammatory activity. The extract had decreased the inflammatory markers *viz.* PGE_2, TNF-α and MPO. It decreased also the paw volume and weight of ear disc. The anti-inflammatory activity was confirmed by the histopathological examination of the ear tissue. Three phenolic compounds were isolated. A validated HPLC method for the analysis of the methanolic leaf extract using gallic acid as a reference proved to be accurate and reliable.

CONSENT

It is not applicable

ETHICAL APPROVAL

“All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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