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ORIGINAL ARTICLE

Bioassay-guided Isolation, Identification, and Evaluation of Anti-inflammatory Activity of β-Boswellic Alcohol and 3-O-Acetyl-11-hydroxy-β-Boswellic Acid from the Leaves of *Boswellia serrata*

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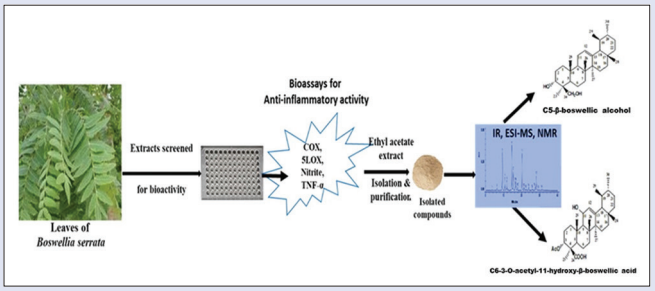
ABSTRACT

Background: There are no in-depth studies on the extracts of the leaves of *Boswellia serrata* (BS), which contain many bioactive phytoconstituents and responsible for their anti-inflammatory effects. **Objectives:** The goal of this research plan was the isolation and purification of most active compounds from the leaves of BS via bioassay-guided fractionation based on anti-inflammatory activity. **Materials and Methods:** The silica gel column chromatographic techniques with different solvent systems were used for the separation of the constituents of the ethyl acetate-soluble fraction of BS leaves. The structures of the isolated compounds were assigned based on various spectroscopic analysis (high-resolution mass spectrometry, ^{1}H NMR, and ^{13}C NMR) and comparison with literature data. The anti-inflammatory activity of all crude extracts, subfractions of ethyl acetate extract, and two isolated new compounds were evaluated in cell-free (cyclooxygenase and 5-lipoxygenase) and cell-based assays (nitrite and tumor necrosis factor-alpha) in RAW264.7. **Results:** Bioassay-guided fractionation of the extracts of the leaves of BS afforded two ursane-type triterpenoids (compound 5, β-boswellic alcohol, and compound 6, 3-O-acetyl-11-hydroxy-β-boswellic acid) and other ten known triterpene acids. Compound 5 and 6 exhibited strong anti-inflammatory activity with an IC_{50} value of a range 26.5–34.2 μM and 18.3–21.1 μM, respectively, in cell-free and cell-based assays. **Conclusion:** This is the first report on isolation and identification of β-boswellic alcohol and 3-O-acetyl-11-hydroxy-β-boswellic acid from the leaves of BS, which showed strong anti-inflammatory activity and holds great promise for the treatment of numerous inflammatory diseases.

Key words: Acetyl-11-hydroxy-β-boswellic acid, anti-inflammatory, *Boswellia serrata*, LC-ESI-MS, NMR, β-boswellic alcohol

SUMMARY

- Two ursane-type triterpenoids were isolated from *Boswellia serrata* leaves
- FT-IR, NMR, and HR-ESI-MS were used to establish the structure of the compounds
- β-boswellic alcohol and 3-O-acetyl-11-hydroxy-β-boswellic acid were established
- Both compounds exhibited significant anti-inflammatory activity.



Abbreviations used: BS: *Boswellia serrata*: CE: Chloroform extract; CF: Combined fraction; COX: Cyclooxygenase; EtoAcE: Ethyl acetate extract; *n*-hexE; *n*-hexane extract; 5-LOX: 5-lipoxygenase; LPS: Lipopolysaccharide; MEBS: Methanol extract of *Boswellia serrata*; TCM: Traditional Chinese medicine; TNF-α: Tumor necrosis factor-alpha.

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INTRODUCTION

Boswellia serrata (BS; Salai/Salai guggal) is grown in mountainous regions of India, Northern Africa, and the Middle East.[1] It is popularly known as Indian olibanum, Salai guggal, loban, or kundru.[2] Frankincense (Salai guggal) has been used in the Ayurveda system of medicine as an analgesic agent, anti-inflammatory, anti-arthritic, antiproliferative and eczema/dermatitis for the treatment of different diseases.[3] Likewise, frankincense of BS is commonly used in Traditional Chinese Medicine as a remedy for diarrhea, dysentery, ringworm, boils, fevers (antipyretic), skin and blood diseases, cardiovascular diseases, mouth sores, bad throat, bronchitis, asthma, cough, vaginal discharges, hair loss, jaundice, hemorrhoids, syphilitic diseases, irregular menses, and stimulation of liver improving vasodilation.[4] There are different types of compounds present in various *Boswellia* species. The composition of the essential oil and other chemical substances changes in various *Boswellia* species and also differs depending on the climate, harvest conditions, and geographic locations.[5]

The stem bark resin fraction of BS has been intensively studied.[6-8] There are no in-depth studies on the extracts of the leaves of BS, which also contain many bioactive phytoconstituents, which are also responsible for their pharmacological effects. Our preliminary results showed that the extract of the leaves of BS possessed strong anti-inflammatory activity. It

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helped us to design this research plan. The aims of the current study were the separation of the most bioactive constituents from the leaves of BS via bioassay-guided fractionation based on anti-inflammatory activity assay using a battery of *in vitro* anti-inflammatory activity tests both in cell-free (cyclooxygenase [COX] and 5-lipoxygenase [5-LOX]) and cell-based assays (nitrite and tumor necrosis factor-alpha [TNF-α] in RAW264.7). Then, we isolated, purified, and characterized the maximum of anti-inflammatory compounds from the bioactive fractions using high-performance liquid chromatography (HPLC), LC-ESI-MS, FT-IR, HR-MS, ^{1}H NMR, and ^{13}C NMR.

MATERIALS AND METHODS

Chemicals and reagents

All solvents were used of HPLC-grade and analytical-grade reagents. 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide, phosphate-buffered saline, chloroform-d (deuterochloroform, CDCl3), tetramethylsilane (TMS), NMR-grade solvent, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich, Munich, Germany. Penicillin/streptomycin (PS) and trypsin were purchased from Gibco, Life Technology, Grand Island, NY, USA. RPMI 1640 medium, trypsin, and heat-inactivated fetal bovine serum (HIFBS) were obtained from Gibco, UK. Dulbecco's modified Eagle's medium and L-glutamate were purchased from Gibco BRL (Grand Island, USA). The incubator was procured from Corning (Rochester, USA). Human recombinant COX, soybean LOX (type V), linoleic acid, Griess reagent, diclofenac, zileuton, and dexamethasone were purchased from Sigma Chemicals (St. Louis, MO). Lipopolysaccharide (LPS) was purchased from Sigma (St Louis, USA), and the ELISA kits for mouse TNF-α were purchased from R and D Systems (Minneapolis, MN, USA). All other chemicals used in the experiment were of analytical grade available in India.

Plant material

The leaves of BS Roxb. were collected from dry mountainous regions of Madhya Pradesh and Chhattisgarh in India and were taxonomically identified by a botanist from Barkatullah University, India. A voucher specimen of it was deposited in the Central Herbarium, Madhya Pradesh, India, with the voucher specimen number VSN/31/2017/2045.

Extraction, isolation, and purification of *Boswellia serrata* leaf extract

Approximately 3.50 kg of the leaves of BS was collected, thoroughly washed with tap water, and finally rinsed with distilled water. The leaves were shade dried for 2 weeks and grounded to form powder. The coarse powder of the BS leaves (500 g) was transferred to a conical glass flask (5 L). After that, 3.5 L of 95% methanol solution was added with stirring and put in an ultrasonic bath. The extraction conditions were 90 min at 45°C, ultrasound power of 100 W, and the frequency of 35 kHz. The extraction was repeated three times. The obtained extracts were filtered through a cellulose filter and combined. The filtrates were evaporated in vacuo at 50°C until dryness with the use of a rotary evaporator. The crude methanol extract of BS (MEBS; 60 g) was obtained and partitioned by liquid/liquid extraction between H_2O (1 L) and n-hexane/chloroform ($CHCl_3$)/ethyl acetate (EtOAc) (4 × 0.5 L), resulting to provide three extracts: n-hexanes, chloroform ($CHCl_3$), and EtOAc. The three extracts, such as *n*-hexane (*n*-hexE 18% w/w), chloroform ($CHCl_3$ E, 10% w/w), and ethyl acetate (EtoAcF, 72% w/w), were evaporated using a rotary evaporator at 40°C until dryness to give 10.8 g, 6 g, and 43.2 g, respectively. All four crude extracts (MEBS, *n*-hexE, $CHCl_3$ E, and EtoAcF) were subjected to different *in vitro* anti-inflammatory activity assays. The part of the ethyl acetate fraction (EtoAc, 20.0 g) was selected based on the bioactivity results and proceeded for the further separation using column chromatography (4.5 cm i.d. X 45 cm long; SiO_2 −500 g; 70–230 mesh size, Merk) and eluted in a gradient manner with *n*-hexane-ethyl acetate-methanol, in increasing polarity to collect eighteen fractions (labeled as subfractions [sub of EtoAc]: 1–18). The thin-layer chromatography (TLC) profiles of each collected fractions were developed and also all subfractions were tested for their anti-inflammatory activity *in vitro* assays. Those fractions with similar TLC profiles and anti-inflammatory activity were combined and their new codes assigned as combined fraction (CF). A total of six CFs (CF1 to CF6) were obtained.

Three CFs (CF3, CF4, and CF5) were further separated for their better anti-inflammatory activities among six CFs. CF 3 (CF3 −3.6 g) was chromatographed over silica gel CC (ethyl acetate:methanol step gradients: 90:10, 80:20, 70:30, and 50:50) to furnish five subfractions (Fr. CF_{3A} to CF_{3E}). Fractions CF_{3B}, CF_{3C}, CF_{3D}, and CF_{3E} were again purified by a ODS-C_{18} HPLC column (R_t 6.3, 9, 10.3, and 11.8 min, respectively) eluted with 65% MeOH/H_2O to furnish compound 1 (C1, 24 mg), compound 2 (C2, 22 mg), compound 3 (C3, 20 mg), and compound 4 (C4, 18 mg).

CF 4 (CF4 −3.0 g) was chromatographed over silica gel column chromatography [ethyl acetate:methanol (80:20) step gradients: 90:10, 75:25, 65:35, and 50:50) to furnish eight subfractions, designated CF_{4A-H} (Fr. CF_{4A} to CF_{4H}). Fractions CF_{4C}, CF_{4E}, and CF_{4F} were further purified by a ODS-C_{18} HPLC column (R_t 14.4, 15.2, and 17.5 min, respectively) eluted with 80% MeOH/H_2O to give compound 5 (C5, 22 mg), compound 6 (C6, 25 mg), and compound 9 (C8, 19 mg).

CF 5 (CF5 −3.5 g) was subjected to silica gel column chromatography (methanol:ethyl acetate [80:20] step gradients, 80:20–75:25) to offer nine subfractions designated CF_{5A-I}. Fractions CF_{5B}, CF_{5C}, CF_{5D}, CF_{5F}, and CF_{5I} were further purified by semipreparative HPLC (90% MeOH) with ODS-C_{18} HPLC column to afford compound 7 (C9, 15 mg, R_t 16.2 min), compound 8 (C10, 12 mg, R_t 16.4 min), compound 10 (C11, 10 mg, R_t 20.2 min), compound 11 (C9, 8 mg, R_t 21.5 min), and compound 12 (C13, 11 mg, R_t 28.1 min).

Structure elucidation of isolated compounds

The structural characterizations of the twelve triterpenoid compounds were carried out by spectroscopic techniques (FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectroscopy). The detailed information of spectra and spectral data are displayed in Supplementary Material S1-S14. The known structures of these twelve triterpenoids were identified by comparing their spectral data and physicochemical properties with those reported in the literature.[4,9-13]

Purity analysis by high-performance liquid chromatography

The stock solutions (0.2 mg/mL) of twelve isolated compounds (analytes) were prepared by precisely weighting and dissolving each in methanol. A mixture of 12 compounds stock solution was prepared by adding appropriate volume of each stock solution to produce a solution containing 1 µg/mL of each analyte. Similarly, a mixed standard stock solution of each compound reference standard was prepared by adding appropriate volume of each reference standard's stock solution to produce a solution containing 0.5 µg/mL of each analyte. All stock solutions were stored at 4°C and filtered through a 0.45 µm membrane filter before injection. The EtoAC extract (10.0 mg) of the leaves of BS was accurately weighted and ultrasonically dissolved with 10 mL methanol. The sample

solution was stored at 4°C and filtered through a 0.45-µm membrane filter before injection. The chromatographic analysis was carried out with Agilent 6540 LC-Q-T of mass spectrometer.

LC-MS/MS analysis

Reversed-phase HPLC with diode array detection and electrospray ionization mass spectrometry (RP-HPLC-DAD-ESI-TQ-MS/MS) procedure was used for the identification of the isolated compound. Mass spectra of the isolated compound were determined using liquid chromatography-mass spectrometry. MS experiments were performed on Agilent 6540 LC-Q-T of mass spectrometer. Positive-mode ESI was used for ionization of the compound. Agilent 6540 UHD accurate mass detector was used with AJS ESI (Agilent Jet Stream) as source. Agilent MassHunter Qualitative analysis (B.04.00) software was used for data analysis and Agilent MassHunter workstation software was used for data acquisition. The chromatography was performed using a Zorbax reverse-phase column (RRHD SB-C_{18} 3 × 150 mm, 1.8 µm) (Agilent Technologies, Santa Clara, CA). The compound was prepared in MS-grade methanol and 5 µL of the compound was injected through HPLC into ESI probe. The LC-MS conditions: Eluent A was water and eluent B was acetonitrile. The injection volume was 5 µL and elution flow was 200 µL/min. Gradient program: 0.0–4.0 min 5%–25% B, 4.0–8.0 min 25%–45% B, 8.0–12 min 45%–65% B, 12–24 min 65%–75% B, 24–36 min 65%–85% B, and 36–40 min 85%–95% B. The DAD acquisitions were performed in the range of 200–600 nm and chromatograms were integrated at 254 nm. For ESI-MS, the parameters were set as follows: temperature levels of ESI interface, desolvation line, and heat block were 200, 300, and 400°C, respectively; the flow levels of nebulizing gas (N_2), heating gas (air), and collision-induced dissociation gas (Ar) were 3, 10, and 0.3 mL/min, respectively. The capillary voltage was kept at −3.5 kV and in negative mode. ESI-MS spectra were recorded by scanning in the range of m/z 100–1000. The identification of compounds was done by analysis of their retention time, ultraviolet, and mass spectrometric data comparing the same parameters with the reference samples and/or literature data. HRESIMS was performed using a Waters Q-TOF 2 instrument. The parameters in the ESI negative mode were capillary voltage 4.8 kV, dry heater temperature 200°C, and infusion solvent methanol (MeOH).

FT-IR spectroscopy

The isolated compound as the powder was characterized by FTIR spectrophotometer (Perkin Elmer Spectrum one FTIR spectrometer, USA) using potassium bromide (KBr) disc method. The IR spectrum was scanned at infrared region of 400–4000 cm^{-1}.

NMR spectroscopy

^{1}H-NMR and ^{13}C-NMR spectra of isolated compound in $CDCl_3$ were acquired on a Bruker Avance 400 (400 MHz) spectrometer, using TMS, as an internal standard (chemical shift values were quoted in ppm, δ).

Cell culture and cell lines

RAW 264.7 (Mouse Macrophage cell line) was procured from the National Centre for Cell Science, Pune, India. RAW 264.7 macrophage cell was purchased from the American Type Culture Collection (VA, USA) and cultured in high-glucose Dulbecco's modified Eagle's medium (4500 mg/L D-glucose; Invitrogen, Carlsbad, CA) supplemented with 100 IU/mL PS and 10% fetal bovine serum. Cells were maintained in 75 cm^2 flasks, under 5% CO_2, and 70% humidity at 37°C until 70% confluent. Cells were cultured in RPMI-1640 (Invitrogen, Gibco, Waltham, MA, USA) medium containing L-glutamine at 5% CO_2-humidified atmosphere at 37°C growth medium supplemented with 10% HIFBS and 1% PS.

Anti-inflammatory activities of *Boswellia serrata* leave extracts

cyclooxygenase inhibition assay

The assay was performed using Colorimetric COX inhibitor screening assay kit. Briefly, the reaction mixture contains 150 µL of assay buffer, 10 µl of heme, 10 µL of enzyme (human recombinant COX, Sigma-Aldrich), and 10 µL of crude extracts (10 mg/mL in methanol) and isolated compound (1.0 mg/mL in methanol). The assay utilizes the peroxidase component of the COX catalytic domain. The peroxidase activity was assayed colorimetrically by monitoring the appearance of oxidized N, N, N, N'-tetramethyl-p-phenylenediamine (TMPD) at 590 nm. Diclofenac was used as a standard drug. The percentage COX inhibition was calculated using the following equation:

COX inhibition activity (%) = (1−T/C) × 100, where T = Absorbance of the inhibitor well at 590 nm and C = Absorbance of the 100% initial activity without inhibitor well at 590 nm.

5-Lipoxygenase assay

Briefly, the mixture contained 25 µL of each sample dissolved in dimethyl sulfoxide, 50 µL of linoleic acid (0.003 g/10 mL), and made up to 1 mL with 0.1 M borate buffer with Tween 80%–0.005%. The reaction was initiated with the addition of 1.5 µL 5-LOX (0.054 g/mL). The increase in absorbance at 234 nm was recorded for 5 min. The percentage inhibition of enzyme activity was calculated by comparison with the negative control: % = ([Ao − A1]/Ao) × 100, where Ao was the absorbance of the blank sample and A1 was the absorbance of the sample. The tests were carried out in triplicate. Sample concentration providing 50% inhibition (IC_{50}) was obtained plotting the inhibition percentage against sample concentrations. Zileuton was used as a positive control (PC).

Nitrite assay

The nitrite concentration was measured as an indicator of nitric oxide (NO) production, according to the Griess reaction. NO levels were determined by measuring the amount of nitrite in the cell culture supernatant using Griess reagent according to the manufacturer's protocol. The RAW 264.7 cells were stimulated with LPS (1 µg/mL) and samples for 24 h followed by centrifugation at 1500 rpm for 10 min. Next, 50 µL of cell culture supernatant was mixed with 50 µL of Griess reagent (consisting of 1% sulfanilamide, 0.1% N-(1-naphthylethylenediamine) dihydrochloride, and 2.5% ortho-phosphoric acid) and was incubated for 15 min at room temperature. The absorbance was measured using an ELISA reader at 540 nm and compared with a standard calibration curve prepared with sodium nitrite solution. Dexamethasone was used as a PC. Each experiment was performed at least in triplicate.

Measurement of cytokine tumor necrosis factor-alpha level

The RAW 264.7 cells were seeded at 5 × 104 cells/well in flat-bottomed 96-well plates. Samples and LPS (1 µg/mL) were added to the culture medium and were incubated at 37°C for 24 h. The medium was collected in a microcentrifuge tube and was centrifuged at 1500 rpm for 10 min. The supernatant was decanted into new microcentrifuge tubes and the level of TNF-α was determined using the commercial mouse ELISA kit. Dexamethasone was used as a PC.

Statistical analysis

All the data analyses were performed with SigmaPlot Statistical Software (Version 11.0) (USA) and GraphPad prism (version 5.0) (USA). The difference between the mean values (in triplicate) was assessed for statistical difference using one-way analysis of variance if any. $P < 0.05$ was considered statistically significant. The results are shown as the mean ± standard deviation.

RESULTS AND DISCUSSION

Assessment of anti-Inflammatory activity of *Boswellia serrata* leaf crude extracts and ethyl acetate subfractions

Figure 1 shows the bioassay-guided fractionation and isolation of the compounds from BS leaves. The anti-inflammatory activities of the extract of the leaves of BS were tested using different *in vitro* assays such as COX, 5-LOX, nitrite, and TNF-α assays. The results of the bioactivity of various crude extracts of BS leaves are shown in Table 1. Ethyl acetate extract showed significant percentage inhibition of COX (88.77%), 5-LOX (85.20%), nitrite (80.22%), and TNF-α (83.12%) at 250 µg/mL. Methanol, chloroform, and n-hexane extracts showed lower percentage inhibition (<50%) of anti-inflammatory effect as compared to the ethyl acetate extract. Diclofenac (10 µg/mL) and zileuton (10 µg/mL) were used as a PC for the inhibition of COX and 5-LOX, respectively; dexamethasone (10 µg/mL) was used as a PC for nitrite and TNF-α activity [Table 1]. PC data were well matched with the literature values. The ethyl acetate extract (EAE) showed the highest anti-inflammatory activity, followed by the methanol extract (ME), chloroform extract (CE), and hexane extract (HE) [Table 1]. Based on these results, one part of the ethyl acetate extract (EtoAc fraction) was selected and proceeded for the further targeted isolation and separation using column chromatography and eluted in a gradient manner with *n*-hexane-ethyl acetate–methanol (20:60:20) and collected total eighteen subfractions (designated as EAE_{1-18}) [Figure 1]. These subfractions were again tested for the evaluation of their anti-inflammatory activity *in vitro* assay. The results of the anti-inflammatory activities of these

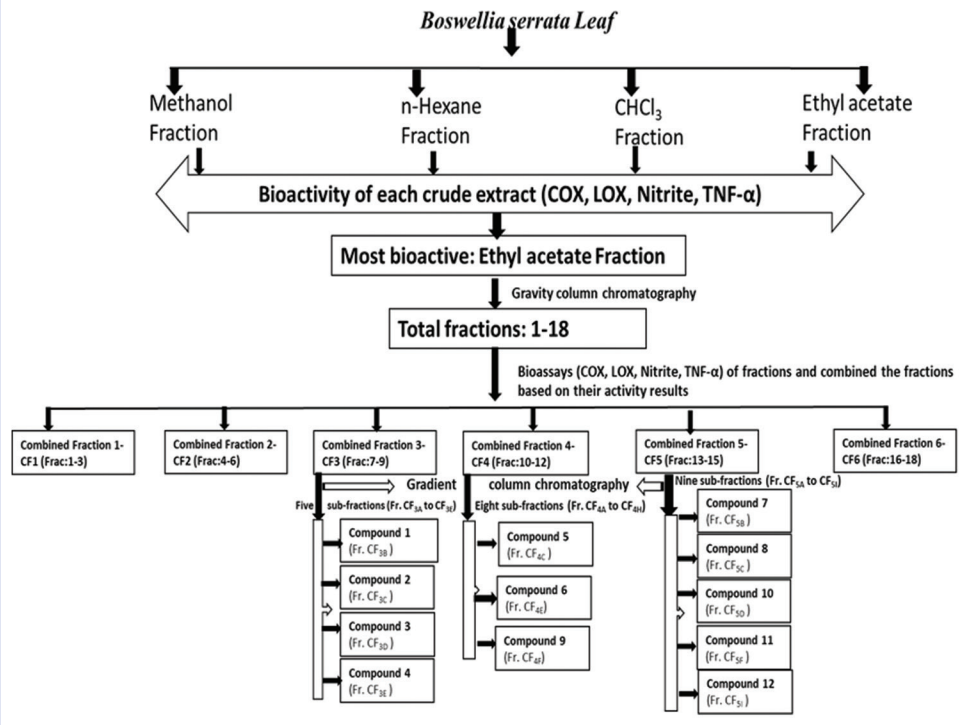

Figure 1: Flowchart for bioassay-guided fractionation and isolation of the compounds 1–12 from *Boswellia serrata* leaves

Table 1: Effects of the crude ethyl acetate extract and other three different solvent extracts (250 µg/mL) of *Boswellia serrata* leaves on the anti-inflammatory activity (cyclooxygenase-1, cyclooxygenase-2; 5-lipoxygenase, nitrite, and tumor necrosis factor-α)

Name of fraction (leaves of-BS)	Percentage inhibition of COX, 5-LOX, nitrite, and TNF-α			
	COX	5-LOX	Nitrite	TNF-α
Methanolic extract of BS	48.27±3.80	40.15±2.42	37.11±2.64	35.60±2.36
n-hexane extract of BS	22.20±1.36	18.70±2.40	20.25±2.10	17.55±1.23
Chloroform extract of BS	37.09±0.41	31.22±0.32	36.80±0.42	30.61±0.68
Ethyl acetate extract of BS	88.77±3.22	85.20±3.20	80.22±2.30	83.12±3.26
Diclofenac (PC-10 µg/mL)	95.32±3.70	-	-	-
Zileuton (PC-10 µg/mL)	-	99.64±4.18	-	-
Dexamethasone (PC-10 µg/mL)	-	-	97.99±3.52	98.34±3.22

Results are summarized with the mean values of *n*=3±SD. PC: Positive control; SD: Standard deviation; CF: Combined fraction; TNF: Tumor necrosis factor; LOX: 5-lipoxygenase; COX: Cyclooxygenase; EtoAC: Ethyl acetate extract; BS: *Boswellia serrata*

Table 2: Effects of the different subfractions (50 µg/mL of each fraction) of ethyl acetate (ethyl acetate extract) extract of *Boswellia serrata* leaves on the anti-inflammatory activity 5-lipoxygenase, nitrite, and tumor necrosis factor-alpha

Subfraction of ethyl acetate (EtoAC) extract of BS	Percentage inhibition of COX, 5-LOX, nitrite, and TNF-α				CF
	COX	5-LOX	Nitrite	TNF-α	
SubF1	8.32±0.21	10.11±0.17	11.34±0.16	14.34±0.58	CF1
SubF2	10.03±0.26	12.35±0.14	13.28±0.12	12.24±0.34	
SubF3	11.00±0.44	10.23±0.15	14.31±0.14	16.22±0.15	
SubF4	26.73±0.58	25.54±0.25	28.40±0.32	32.31±0.97	CF2
SubF5	28.23±0.62	30.27±0.21	25.66±0.24	34.43±0.62	
SubF6	25.12±0.40	31.44±0.24	30.11±0.36	39.89±0.80	
SubF7	54.10±1.25	52.13±0.94	53.62±0.82	55.00±1.00	CF3
SubF8	58.33±1.36	61.00±1.10	64.19±0.91	58.65±1.06	
SubF9	60.44±1.60	64.24±1.13	67.64±0.96	60.20±1.20	
SubF10	75.21±1.50	77.00±1.07	74.19±1.11	68.65±1.60	CF4
SubF11	79.21±2.01	79.24±1.11	76.64±1.14	74.20±1.53	
SubF12	82.21±1.92	80.00±1.93	82.19±1.26	78.65±1.58	
SubF13	80.21±2.03	84.24±1.82	85.64±1.57	84.20±1.29	CF5
SubF14	88.21±1.94	90.00±2.04	89.19±1.68	88.65±2.09	
SubF15	93.21±2.30	95.24±1.97	94.64±1.49	87.20±1.83	
SubF16	38.21±0.16	30.00±0.75	34.19±0.65	38.65±0.77	CF6
SubF17	27.21±0.21	17.24±0.45	19.64±0.50	20.20±0.64	
SubF18	13.21±0.30	10.00±0.32	11.19±0.46	10.65±0.71	
Diclofenac (PC-10 µg/mL)	98.38±2.27		-	-	-
Zileuton (PC-10 µg/mL)	-	99.04±1.55			-
Dexamethasone (PC-10 µg/mL)	-	-	98.58±2.40	97.37±2.12	-

Results are summarized with the mean values of n=3±SD. PC: Positive control; SD: Standard deviation; CF: Combined fraction; TNF: Tumor necrosis factor; LOX: 5-lipoxygenase; COX: Cyclooxygenase; EtoAC: Ethyl acetate extract; BS: *Boswellia serrata*

fractions are shown in Table 2. The subfractions which showed similar activities and similar Rf values from TLC data were combined and obtained the total six CFs, designated as CF_{1-6} of ethyl acetate extract. The order of anti-inflammatory activities showed by these CFs (CF_{1-6}) was $CF_5 > CF_4 > CF_3 > CF_2 > CF_6 > CF_1$ [Table 2].

Isolation and characterization of compounds

Three CFs (CF_3, CF_4, and CF_5) were considered for purification using a silica gel column chromatography with stepwise gradient elution with ethyl acetate–methanol (80:20 v/v) at the final step and further purified by semipreparative HPLC (65%–90% MeOH/H_2O) with ODS-C_{18} HPLC column to afford twelve compounds [Figure 2].

Compound 5 (peak 6, C5: 3α-hydroxy-24-hydroxymethyl-urs-12-ene-24-ol: β-boswellic alcohol)

Compound 5 (C5) was isolated as a white, amorphous powder, $[\alpha]_D$ +42.6 and gave a positive red coloration in the Liebermann–Burchard reaction, suggesting that C5 was a triterpenoid. Its IR spectrum showed absorption bands for hydroxy and carboxylic groups at 3306 and 1647 cm^{-1}, respectively [Figure S1]. It showed a single peak with R_t 14.4 min in HPLC [Figure 2d]. The high-resolution mass spectrometry (HR-ESI-MS) of compound 5 (C5) showed a molecular ion peak at m/z 441.7025 ([M-H]$^-$, calculated for m/z 441.7021), suggesting a molecular formula $C_{30}H_{50}O_2$ [Figure S2]. The ^{1}H NMR spectrum of compound 5 [Figure S3] displayed resonances for five singlet methyl groups (δ_H 0.84, 0.94, 1.07, 1.12, and 1.39), an oxygenated methine proton (δ_H 4.78, br s), an olefinic proton (δ_H 5.34, br s), and two oxymethylene protons (δ_H 4.78, 5.34, br s). In the ^{13}C NMR spectrum, 30 carbon signals were observed [Figure S4], including five methyl (δ_C 14.3, 17.9, 21.2, 24.7, and 28.8), an oxygenated methine (δ_C 70.8), two olefinic carbons (δ_C 128.5, 165.4), and one hydroxymethyl group (δ_C 181.0) [Table 3]. In the COSY spectrum [Figure S5], the methine proton resonance at δ_H 4.70 (br s) was assigned as being attached to the carbon that resonated at δ_C 70.8, suggesting that the hydroxyl group at C-3 might be R-substituted. These spectroscopic data were closely similar to those of boswellic acid,[14] which was first time isolated and reported from the leaves of plant BS.

Comparison of the ^{1}H and ^{13}C NMR spectra of compound 5 (C5) with those of β-boswellic acids (Belsner *et al.*, 2003) displayed close similarities, with notable difference being the substitution mode in ring A. The chemical shift at δc 70.8 in the ^{13}C-NMR spectra and HMBC [Figure S6] correlations of H-3 with CH_3-23 (δc 24.7) and quaternary carbon C-24 with hydroxymethyl group (δc 179.0) confirmed the presence of hydroxyl group in ring A at C-3 position, while the alpha orientation was further confirmed by NEOSY cross-peaks [Figures 3 and S7].[15] The presence of hydroxymethyl (CH_2OH) group was assumed to be located either at the C-23 or C-24 position. The key HMBC correlations of the methyl proton H-3 (δ_H 4.78) with C-3 (70.8) and C-4 (47.8) and CH_3-23 (24.7) with C-3 (70.8) and C-24 (179.0) proved that hydroxymethyl located either at C-23 or C-24 position. On the basis of the above results, compound 5 was deduced as urs-12-en-3α,24-diol, commonly named as boswellic alcohol (C5) [Figure 4]. This compound is isolated for the first time from the leaves of BS and has shown strong the anti-inflammatory activity [Table 4].

Compound 6 (peak 7, C6: 3-O-acetyl-11-hydroxy-β-boswellic acid)

Compound 6 (peak 7, C6: 3-O-acetyl-11-hydroxy-β-boswellic acid) was isolated as white amorphous powder. UV spectra of the compound displayed characteristic absorption bands for conjugated double bond 248 nm and its specific rotation $[\alpha]_D$ was +84.3°. It gave a positive red coloration in the Liebermann–Burchard reaction, suggesting that C6 was a triterpenoid. Its molecular formula was established as $C_{32}H_{50}O_5$ on the basis of its HR-ESI-MS (*m*/z 513.7389 [M-H]$^-$) from its HR-ESI-MS data (calculated m/z 513.7386) [Figure S8]. The IR spectrum of this compound [Figure S9] showed characteristic absorptions bands for

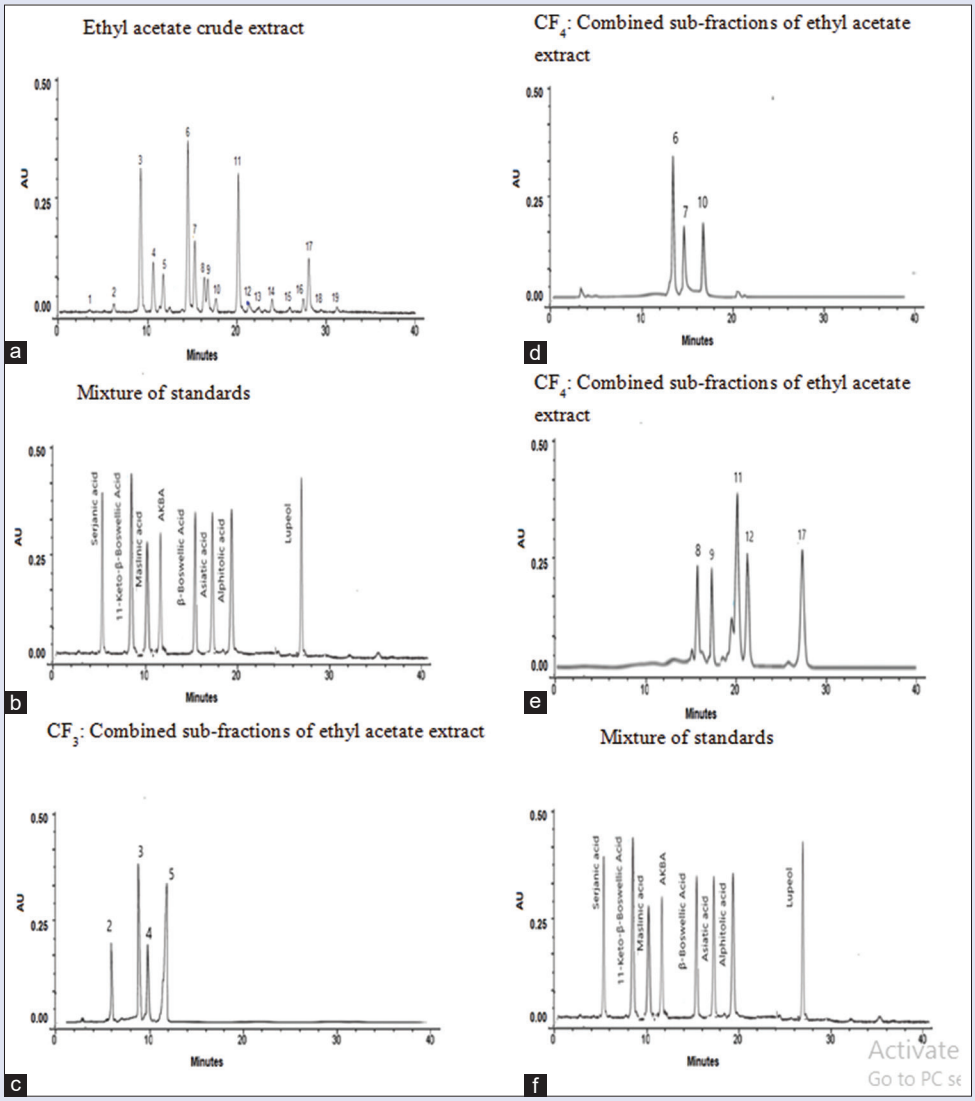

Figure 2: High-performance liquid chromatography chromatograms of ethyl acetate (EtoAc) extract of *Boswellia serrata* (BS) leaves and its most active combined fractions (CF_{3-5}), monitored at 260 nm. (a) Ethyl acetate crude extract, (b) mixture of standards, (c) CF_3: Combined subfractions of ethyl acetate extract, (d) CF_4: Combined subfractions of ethyl acetate extract, (e) CF_4: Combined subfractions of ethyl acetate extract, (f) mixture of standards

hydroxyl (3410 cm^{-1}), carboxylic acid (1702 cm^{-1}), and a double bond (1632 cm^{-1}) functional groups. FT-IR spectroscopy was carried out to ascertain functional groups. The FT-IR spectrum of the compound showed IR absorption bands in the high wave region at 3494 cm^{-1} (-OH), 3398 cm^{-1} (-CH of cyclic ring), 2934 cm^{-1} (C = C), and 2635 cm^{-1} (-CH-). In fingerprint region, the FT-IR spectrum of the compound represented dominant bands at 1606 cm^{-1} (-COOH), 1435 cm^{-1} (-C=C-C-O-), 1425 cm^{-1} (-CH2-), 1380 cm^{-1} (-CH3), 1056 cm^{-1} (-C=O), 636 cm^{-1} (α, β-unsaturated carbonyl), and many other bands of medium-to-weak intensity. The observed band at 1606 cm^{-1} can be assigned to C = O stretching of –COOH functional group. Other bands in the spectral range were assigned to bending vibrations of –OH,-CH_2 and CH_3 groups as well as to skeletal bending bonds. The band at 1260 cm^{-1} was due to C-O stretching among others. The intense band as 636 cm^{-1} in IR spectrum was due to vibration of the CH_2 in α, β-unsaturated carbonyl group. Theoretical wave numbers responsible for the functional groups were compared with observed wave numbers and closely matched with the structure of pentacyclic triterpenoid, i.e., boswellic acid derivative.[10] The ^{1}H NMR and ^{13}C NMR experiments of the compound were carried out, and the results are shown in Table 3. The ^{1}H-NMR spectrum [Figure S10] of the purified compound showed eight singlets at δ 0.79, 0.92, 1.02, 1.18, 1.20,1.32, 1.34, and 2.02 and one doublet at δ 0.82 for the nine methyl group signals. In addition, two olefinic protons were observed at δ 5.33 (m) and 5.57 (s), which are characteristic of ursane-type triterpenes related to boswellic acids.[10,12,16] Seven primary carbons (methyl signals; C-23 and C-25 to C-30) at δ 13.4, 17.6, 18.8, 19.4, 20.6, 24.2, and 28.4; nine secondary carbons (methylene signals) at δ 19.2, 21.4, 24.2, 27.6, 28.2, 30.4, 33.4, 34.3, and 43.6; seven tertiary carbons (methine signals) at δ 38.2, 40.2, 41.3, 48.64, 50.6, 60.8, 76.2, and 130.8; and eight quaternary carbons at δ 35.4, 38.8, 45.3, 47.2, 74.4, 130.8, 164.7, and 170.6 were assigned for the purified compound from its ^{13}C NMR spectrum [Table 3 and Figure S11]. The ^{13}C-NMR spectrum of 6 also showed the presence of an olefinic group at δc 130.8 and 164.7 (C-12, C-13), a carboxylic group at δc 180.6 (C-24) and two hydroxylated carbons at δc 74.4 and 76.2. On the other hand, the singlet peak at δ 5.57 (H-12) correlated with C-11 (δc 76.2), C-9 (δc

60.8), C-13 (δc 164.7), and C-14 (δc 43.6) in the HMBC spectrum [Figure S12] allowed its assignment to olefinic double bond between C-12 and C-13. One acetyl methyl in the range of δ 13 ppm and 29 ppm and one downfield quaternary carbon (C-24) at δ 180.6 along with one methine carbon (C-3) signals observed at δ 74.4, which indicated that the compound is likely to be a boswellic acid derivative. The olefinic moiety in the ring-C was confirmed by the presence of signals at δc 164.7 in the ^{13}C NMR spectrum for quaternary carbons (C-13) and the methine carbon (C-12) at δ 130.8. The downfield shift of C-13 and a signal at δ 196.4 in ^{13}C NMR spectrum indicated the presence of the α-β unsaturated system with alkene moiety. The three bond long range correlations from this signal to C-9 (δc 60.8), C-12 (δc 130.8), and C-13 (δc 164.7) in the NOESY spectrum [Figure S13] allowed its assignment to H-11. This proton was associated with a carbon signal at δc 76.2 in the NMR spectrum and showed COSY correlation with a signal at δ_H 2.48 (H-9) and 5.57 indicating the location of hydroxyl group at C-11 [Figures 3 and S14]. Furthermore, the quaternary carbon (C-31) at δ 170.6, coupled with the indication of acetyl group in ^{1}H NMR spectrum, also confirmed the presence of acetyl group in the compound. All the above data can be considered only on an urs-12-ene triterpenoid structure for compound C6 with a hydroxyl group at the C-3 α position and another secondary hydroxyl group attached to the C-l1 β position.[4] Thus, on the basis of spectroscopic data and also comparison with literature data, structure of the compound C6

Table 3: ^{1}H NMR and ^{13}C NMR spectrum of compounds 1–12

Atom	C1 (Serjanic acid)		C2 (KBA)		C3 (Maslinic acid)		C4 (AKBA)		C5 (β- boswellic alcohol)		C6 (3-acetyl-11-hydroxy-BA)	
	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C
C-1	–	38	1.32,2.51 (m)	34.4t	0.93. 1.95 (m)	47.3	1.19, 2.53 (m)	34.6t	1.48,1.35 (m)	33.5 (t)	1.43,2.46 (m)	35.4 (t)
C-2	–	26.9	1.55, 2.30 (m)	24.4t	3.64 (dt. 4.5, 10.0	68	1.58, 2.24 (m)	23.5t	2.22,1.70 (m)	26.5 (t)	1.54, 2.42 (m)	24.2 (t)
C-3	3.47 (m)	76.8	4.06 (dd)	70.4d	–	83.2	5.30 (dd)	73.1d	4.78 (m)	70.8 (d)	5.33 (m)	74.4 (d)
C-4	–	38.3	–	47.3s	0.86 (m)	39.4	–	46.5s	–	47.8 (s)	–	47.2 (s)
C-5	–	54.8	1.46 (m)	49.8d	1.45. 1.58 (m)	55.4	1.38 (m)	50.4d	1.56 (m)	49.4 (d)	1.64 (m)	50.6 (d)
C-6	–	18	1.71, 1.87 (m)	18.8t	1.52. 1.57 (m)	18.4	1.72, 1.87 (m)	18.7t	1.88,1.74 (m)	19.7 (t)	1.97, 2.24 (m)	19.2 (t)
C-7	–	32.4	1.66, 1.73 (m)	33.5t	–	32.8	1.64, 1.73 (m)	32.8t	1.68,1.44 (m)	33.2 (t)	1.48, 1.74 (m)	33.4 (t)
C-8	–	38.8	–	45.1s	1.65 (m)	39.3	–	45.2s	–	43.5 (s)	–	45.3 (s)
C-9	–	47.1	2.41 (s)	60.4d	–	47.7	2.39 (s)	60.3d	1.65 (m)	56.8 (d)	2.48 (s)	60.8 (d)
C-10	–	36.6	–	37.5s	1.92. 1.96 (m)	38	–	37.4s	–	37.5 (s)	–	38.2 (s)
C-11	–	22.8	–	199.7s	5.27 (t 3.5)	23.5	–	199.2s	1.97,1.18 (m)	73.4 (s)	4.56 (m)	76.2 (d)
C-12	5.52 (m)	121.9	5.53 (s)	130.4d	–	121.7	5.53 (s)	130.4d	5.34 (s)	128.5 (d)	5.57 (s)	130.8 (d)
C-13	–	143.5	–	165.2s	–	144.4	–	165.0s	–	149.4 (s)	–	164.7 (s)
C-14	–	41.2	–	43.8s	1.10, 1.79 (m)	41.7	–	43.8s	–	43.3 (s)	–	43.6 (s)
C-15	–	27.2	1.20,1.87 (m)	27.2t	1.62. 2.03 (m)	27.8	1.20,1.88 (m)	27.2t	1.84,1.02 (m)	27.2 (t)	1.23,1.78 (m)	27.6 (t)
C-16	–	22.7	0.99, 2.07 (m)	27.5t	–	23.3	0.99, 1.08 (m)	27.5t	2.02, 0.98 (m)	28.4 (t)	1.04,1.18 (m)	28.2 (t)
C-17	–	44.9	–	35.3s	2.87 (dd)	46.2	–	33.9s	–	34.8 (s)	–	34.3 (s)
C-18	2.66 (d)	42	1.53 (m)	59.0d	1.16. 1.51 (m)	41.5	1.53 (m)	58.8d	1.44 (m)	59.5 (d)	1.58 (m)	58.4 (d)
C-19	–	41.6	1.39 (m)	39.2d	–	46	1.39 (m)	39.3d	1.48 (m)	39.2 (d)	1.37 (m)	38.8 (d)
C-20	–	43.2	0.92 (m)	40.0d	1.22. 1.42 (m)	30.5	0.92 (m)	39.2d	0.98 (m)	49.6 (d)	1.02 (m)	40.2 (d)
C-21	–	29.7	1.42 (m)	30.9d	1.36. 1.76 (m)	33.8	1.45 (m)	30.9d	1.44,1.20 (m)	31.4 (d)	1.42 (m)	30.4 (d)
C-22	–	33.2	1.28, 2.57 (m)	40.9t	1.04 (s)	32.7	1.29, 2.57 (m)	40.9t	1.55,1.14 (m)	47.5 (t)	1.27, 2.57 (m)	41.3 (t)
C-23	1.26 (s)	28.2	1.33 (s)	24.3q	0.84 (s)	28.9	1.22 (s)	23.8q	1.39 (s)	24.7 (s)	1.32 (s)	24.2 (q)
C-24	1.04 (s)	16	–	182.5s	0.83 (s)	17.1	–	181.9s	4.70 (s)	181.0 (s)	–	180.6 (s)
C-25	0.91 (s)	15.1	1.12 (s)	13.5q	1.03 (s)	16.4	1.14 (s)	13.2q	0.94 (s)	14.3 (q)	1.18 (s)	13.4 (q)
C-26	1.07 (s)	16.7	1.17 (s)	18.5q	1.18 (s)	17.2	1.17 (s)	18.4q	1.07 (s)	17.9 (q)	1.20 (s)	18.8 (q)
C-27	1.25 (s)	25.5	1.30 (s)	20.5q	1.01 (s)	25.7	1.33 (s)	20.5q	1.12 (s)	21.2 (q)	1.34 (s)	20.6 (q)
C-28	–	178.1	0.81 (s)	28.8q	–	179	0.81 (s)	28.8q	0.84 (s)	28.8 (q)	0.79 (s)	28.4 (q)
C-29	0.88 (s)	27.8	0.78 (d)	18.5q	0.93 (s)	32.8	0.78 (d)	17.4q	0.82 (d)	17.4 (q)	0.82 (d)	17.6 (q)
C-30	0.97 (d)	176.2	0.93 (s)	21.1q	0.96(s)	23.3	0.93 (s)	21.3q	0.98 (d)	20.8 (q)	1.02 (s)	19.4 (q)
C-31							0.93 (s)	170.3q			0.92 (s)	170.6 (q)
C-32							2.07 (s)	21.1q			2.02 (s)	21.4 (q)

Contd...

Table 3: Contd...

Atom	C7 (Madecassic acid)		C8 (β-BA)		C9 (Asiatic acid)		C10 (Alphitolic acid)		C11 (β-amyrin)		C12 (Lupeol)	
	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C
C-1	1.99 (m)	48.2 (t)	1.49,1.33 (m)	33.9	1.04, 1.97 (m)	48.05	–	46.5	1.49, 1.55 (m)	38.79	1.51,1.72 (m)	38.7
C-2	3.58 (m)	70.0 (d)	2.24,1.60 (m)	26.2	3.72 (m)	69.7	3.80 (dd)	68.6	1.52, 1.55 (m)	27.44	1.50, 1.70 (m)	27.8
C-3	3.34 (d)	78.2 (d)	4.08 t (2.5)	70.8	3.38 (d)	78.24	3.29 (d)	83.2	3.20 (dd)	79.24	4.48 (dd)	81.3
C-4	–	44.2 (s)	–	47.4	–	43.4	–	40.6	–	38.99	–	38.4
C-5	1.19 (m)	48.5 (d)	1.50 (m)	49.1	1.31 (m)	48.04	–	55.2	0.71	55.37	0.70 (m)	55.7
C-6	4.41 (s)	19.1 (t)	1.83,1.70 (m)	19.7	1.42, 1.47 (m)	19.08	–	18.2	1.30, 1.53 (m)	18.58	1.44, 1.53 (m)	18.5
C-7	1.47, 1.78 (m)	34.6 (t)	1.58,1.41 (m)	33.1	1.33, 1.68 (m)	33.66	–	34.1	–	32.85	1.25,1.49 (m)	34.5
C-8	–	41.7 (s)	–	40	–	40.8	–	39.2	–	40.21	–	41.2
C-9	1.66 (m)	51.9 (d)	1.63 (m)	46.8	1.68 (m)	48.82	–	50.3	1.95 (m)	47.43	1.30 (m)	50.7
C-10	–	39.3 (s)	–	37.5	1.02 (m)	38.99	–	38.2	–	37.15	–	37.4
C-11	2.01, 2.08 (m)	22.4 (t)	1.92,1.18 (m)	23.4	2.0 (m)	24.46	–	20.9	1.84 (m)	23.75	1.25, 1.47 (m)	21.3
C-12	5.30 (t)	28.6 (t)	5.14 t (3.5)	124.5	5.26 (m)	126.61	5.19 (br s)	25.3	5.16t	121.9	1.51,1.61 (m)	25.4
C-13	–	44.5 (d)	–	139.6	–	139.89	–	38.1	–	145.4	1.63 (m)	38.1
C-14	–	42.4 (s)	–	42.3	–	43.4	–	40.6	–	41.92	–	43.2
C-15	1.06 (m)	28.1 (t)	1.86,1.02 (m)	26.5	1.11, 1.95 (m)	29.19	–	30.4	–	26.36	1.13,1.41 (m)	25.4
C-16	1.68, 2.00 (m)	33.2 (t)	2.02, 0.88 (m	28.1	1.66, 2.06 (m)	25.35	–	32.1	–	27.14	1.42,1.56 (m)	34.5
C-17	1.82 (m)	49.7 (5)	–	33.8	–	48.5	–	56.1	–	32.7		
C-18	2.26 (d)	49.5 (d)	1.34 (m)	59.2	2.23 (m)	54.41	2.75 (dd)	48.1	1.89 (m)	47.84	1.56 (m)	48.3
C-19	1.33 (m)	43.5 (d)	1.34 (m)	39.7	1.00 (m)	40.42	–	48.9	1.59 (m)	47.03	2.37 (dt)	48.3
C-20	1.01 (m)	86.2 (s)	0.94 (m)	39.6	1.40 (m)	40.4	–	150.7	–	31.3	–	151.4
C-21	1.28, 1.50 (m)	28.5 (t)	1.41,1.29 (m)	31.3	1.37, 1.52 (m)	31.8	–	29.5	1.66 (m)	37.35	0.87,1.08 (m)	30.2
C-22	1.59, 1.67 (m)	26.4 (t)	1.45,1.27 (m)	41.5	1.65, 1.72 (m)	38.14	–	37	–	34.94	1.33,1.46 (m)	40.3
C-23	3.26, 3.50 (d)	66.3 (t)	1.34 (s)	24.2	3.29, 3.53 (m)	66.38	1.06 (s)	28.2	0.77 (s)	15.71	0.85 (s)	28.3
C-24	0.67 (s)	13.8 (q)	–	183.1	0.72 (s)	13.91	0.82 (s)	16.4	0.98 (s)	28.31	0.88 (s)	16.8
C-25	0.95 (s)	18.5 (q)	0.91 (s)	13.3	1.07 (s)	17.67	0.77 (s)	17.1	0.92 (s)	15.8	0.88 (s)	16.5
C-26	0.96 (s)	16.3 (q)	1.05 (s)	16.9	0.88 (s)	17.88	0.68 (s)	15.7	0.94 (s)	17.01	1.03 (s)	16.3
C-27	0.98 (s)	14.7 (q)	1.11 (s)	23.2	1.16 (s)	24.14	1.17 (s)	14.5	1.11 (s)	26.21	0.94 (s)	14.8
C-28	–	180.1 (s)	0.83 (s)	28.8	–	180.52	–	179.3	0.81 (s)	28.62	0.79 (s)	18.3
C-29	1.03 (d)	19.0 (q)	0.80 (d)	17.4	0.91 (d	17.67	0.92 (s)	109.4	0.85 (s)	33.56	4.57 (m), 4.69 (	109.7
C-30	1.32 (s)	24.4 (q)	0.94 (d)	21.4	0.99 (s)	21.57	0.86 (s)	19.1	0.85 (s)	23.91	1.68 (s)	19.6

was deduced as 3-O-acetyl-11-hydroxy-β-boswellic acid, which was further corroborated by the physical and spectral data reported from the literature.[4,7,9] It is noteworthy mentioning that the 3-O-acetyl-11-hydroxy-β-boswellic acid (C6) [Figure 4] for the first time was isolated from the leaves of BS.

The known twelve pentacyclic triterpenoids were also identified from the leaves of BS. By comparison of their spectral data (NMR and MS) with those already reported in the literature, these compounds were identified as serjanic acid (C1),[17] 11-keto-β-boswellic acid (C2),[18,19] maslinic acid (C3),[20] 3-O-acetyl-11-keto-β-boswellic acid (C4),[10] madecassic acid (C7),[15] β-boswellic acid (C8),[18,19] asiatic acid (C9),[21] alphitolic acid,[22] β-Amyrin (11),[23] and lupeol (C12).[24,25] The structures of the compounds are shown in Figure 4. The detailed information of ^{1}H NMR and ^{13}C NMR spectrum of compounds (compounds 1–12) is displayed in Table 3.

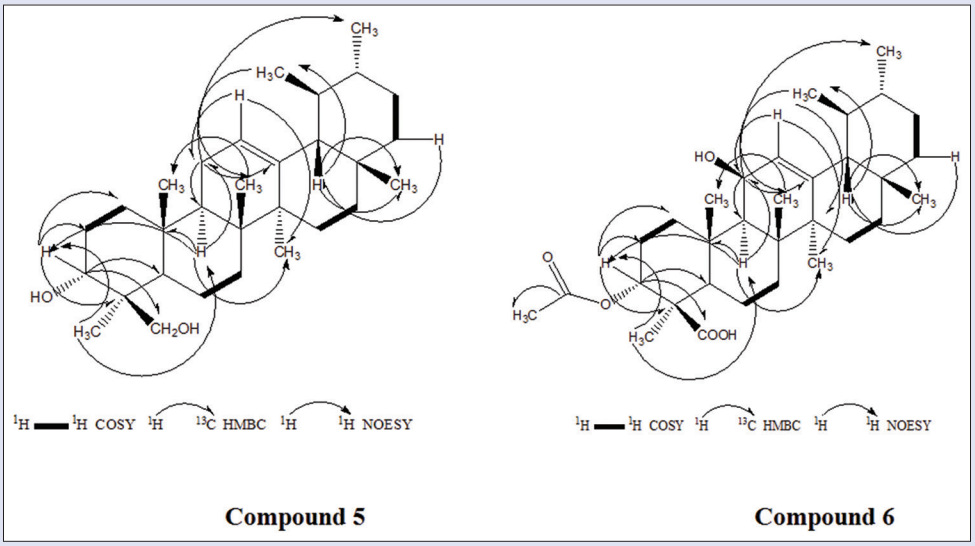

Figure 3: NMR assignments from salient signals of Compound 5 (β-boswellic alcohol: urs-12-en-3α,24-diol) and Compound 6 (3-O-acetyl-11-hydroxy-β-boswellic acid)

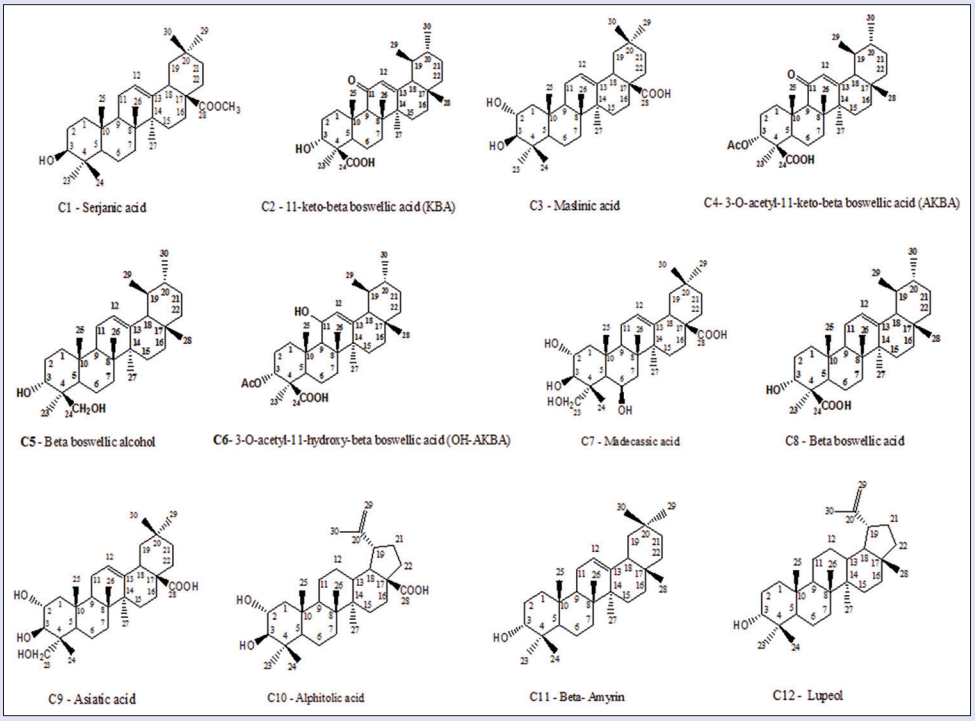

Figure 4: Structures of the isolated compounds 1 to 12. Serjanic acid (C1), 11-Keto-β-boswellic acid (KBA, C2), maslinic acid (C3), 3-O-acetyl-11-keto-β-boswellic acid (AKBA) (C4), Urs-12-ene-3α 24-ol (β-boswellic alcohol) (C5), 3-O-acetyl-11-hydroxy-β-boswellic acid (C6), madecassic acid (C7), β-boswellic acid (β-BA) (C8), asiatic acid (C9). Alphitolic acid (C10), β-Amyrin (C11), and Lupeol (C12)

Anti-inflammatory activities of isolated pure compound 5 and 6 (IC_{50} determination)

The isolated new compound C5, β-boswellic alcohol, showed significant percentage inhibition on COX (92.20%), 5-LOX (90.21%), NO (88.57%), and TNF-α (87.44%) at 25 μg/mL. Similarly, another isolated new compound C6 (3-O-acetyl-11-hydroxy-β-boswellic acid) also showed significant percentage inhibition on COX (97.42%), 5-LOX (93.68%), NO (96.37%), and TNF-α (95.22%) at 25 μg/mL. These two compounds demonstrated dose-dependent anti-inflammatory activity with different concentrations (1–25 μM). IC_{50} values of 3-O-acetyl-11-hydroxy-β-boswellic acid were 18.36 μM (COX), 21.10 μM (5-LOX), 18.53 μM (nitrite), and 15.33 μM (TNF-α) and it was better than β-boswellic alcohol [Table 4]. There was a significant enhancement of the inhibitory effects of ethyl acetate fraction on various inflammatory markers with the progress of purification with a decrease in IC_{50} from 80.20–90.30 to 25.0 μg/mL. The bioactivity-guided fractionation of the ethyl acetate extract indicated that the isolated two new compounds (β-boswellic alcohol and 3-O-acetyl-11

Table 4: IC_{50} of isolated compound 5 (β-boswellic alcohol) and compound 6 (3-O-acetyl-11-hydroxy-β-boswellic Acid) from the leaves *Boswellia serrata* on the anti-inflammatory activity (cyclooxygenase, 5-lipoxygenase, nitrite, and tumor necrosis factor-alpha)

Isolated new compound from the extract of the leaves of BS	IC_{50} value (μM±SEM)			
	COX	5-LOX	Nitrite	TNF-α
β-Boswellic Alcohol (C5)	32.31±1.57	26.52±1.28	30.30±1.27	34.25±1.33
3-O-acetyl-11-hydroxy-β-boswellic acid (C6)	18.36±1.10	21.10±1.06	18.53±1.32	15.33±1.65
Diclofenac (PC)	4.23±0.21	-	-	-
Zileuton (PC)	-	3.85±0.14	-	-
Dexamethasone (PC)	-	-	0.09±0.15	0.07±0.02

Results are summarized with the mean values of n=3±SD. SD: Standard deviation; BS: *Boswellia* serrate; COX: Cyclooxygenase; 5-LOX: 5-lipoxygenase; TNF-α: Tumor necrosis factor-alpha; SEM: Standard error of mean; PC: Positive control

-hydroxy-β-boswellic acid) were responsible for the anti-inflammatory activity.

CONCLUSION

To the best of our knowledge, the isolation of pentacyclic triterpenes from BS leaves has not been reported elsewhere. This is the first study on isolation and identification of a dozen of pentacyclic type triterpenoid compounds from BS leaves using bioactivity-guided fractionation and their structures were established. These compounds showed strong anti-inflammatory activity through inhibition of COX and 5-LOX enzyme activity and also exhibited inhibition of nitrite and TNF-α production in LPS-stimulated RAW 264.7 cells. It was found that 3-O-a cetyl-11-hydroxy-β-boswellic acid (11-hydroxy AKBA) had showed better anti-inflammatory than β-boswellic alcohol. Hence, these results suggest that 3-O-acetyl-11-hydroxy-β-boswellic acid possesses strong anti-inflammatory activity and holds great promise for the treatment of numerous inflammatory diseases.

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Conflicts of interest

There are no conflicts of interest.

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