

Phytochemical Fractionation and Comparative In Vitro COX-1, COX-2, And 5-LOX Inhibitory Potential of Boswellia Serrata Resin

Humera khanam¹, Savitha H S², *Ankur Vishwakarma³, Amandeep Kaur Sidhu⁴, Jyoti Kumari⁵, Tunul Guria⁶, Rajib Kumar Singh⁷, G. Sai Sruti⁸

^{1,3,8}Kanpur Institute of Technology and Pharmacy, A-1, UPSIDC Industrial Area Rooma, Kanpur, Uttar Pradesh. 208001

²Department of Chemistry, SJB Institute of Technology, 67, BGS Health & Education City, Dr. Vishnuvardhan Road, Kengeri, Bengaluru – 560060, Karnataka, India.

⁴Guru kashi University, Sardulgarh Road Talwandi Sabo, Bathinda, Punjab. 151302

⁵School of Pharmacy, Arka Jain University, Mohanpur, Gamharia, Seraikela, Kharsawan, Jharkhand- 832108, India.

⁶Shakti College of Pharmacy, Bairempur, Uttar Pradesh, India. 271201

⁷Royal College of Pharmacy and Health Sciences, Andhapasara Road, Berhampur, Ganjam, Odisha. 760002

Corresponding Author: Ankur Vishwakarma, Email:- ankurtowardsuccess@gmail.com

Abstract

Boswellia serrata gum resin is traditionally used for inflammatory disorders and contains triterpenoid constituents with potential effects on arachidonic-acid metabolism. The present study compared the in vitro cyclooxygenase and lipoxygenase inhibitory activities of a hydroethanolic extract of *B. serrata* resin and its polarity-based solvent fractions. The resin was extracted with 80% ethanol and sequentially fractionated into n-hexane, ethyl acetate, n-butanol, and residual aqueous fractions. Pharmacognostic parameters, preliminary phytochemical profile, total phenolic content, total triterpenoid content, and HPTLC fingerprints were evaluated. COX-1, COX-2, and human 5-lipoxygenase inhibitory activities were determined over a concentration range of 1–300 µg/mL, and IC₅₀ values were calculated by nonlinear regression. The ethyl acetate fraction showed the highest total triterpenoid content (412.6 ± 10.4 mg UAE/g) and exhibited the strongest enzyme inhibition, with IC₅₀ values of 54.8 µg/mL for COX-1, 31.6 µg/mL for COX-2, and 24.9 µg/mL for 5-LOX. The n-hexane fraction showed the second-highest activity, whereas the residual aqueous fraction was the least active. Enzyme-inhibitory potency correlated strongly with total triterpenoid content. These findings identify the ethyl acetate fraction as the most promising bioactive fraction for further phytochemical and mechanistic investigation.

Keywords: *Boswellia serrata*; boswellic acids; cyclooxygenase; COX-1; COX-2; 5-lipoxygenase; triterpenoids; solvent fractionation.

1. Introduction

Inflammation is a complex biological response that protects tissues against infection, injury, and other harmful stimuli. However, persistent or excessive inflammation contributes to the development and progression of several chronic disorders, including arthritis, cardiovascular disease, metabolic disease, and inflammatory conditions of the gastrointestinal and respiratory systems. Among the major biochemical pathways involved in inflammation, arachidonic-acid metabolism has particular importance because it generates prostaglandins and leukotrienes, which act as potent mediators of pain, swelling, vascular changes, and immune-cell recruitment (Wang et al., 2026; White, 2026; Zhang et al., 2026).

Cyclooxygenase and lipoxygenase enzymes represent two key branches of this pathway. Cyclooxygenase-1 is constitutively expressed in many tissues and contributes to physiological functions such as gastric mucosal protection and platelet activity, whereas cyclooxygenase-2 is strongly induced during inflammation and promotes the formation of pro-inflammatory prostanoids. In parallel, 5-lipoxygenase catalyses leukotriene formation and contributes to inflammatory-cell migration, vascular permeability, and bronchoconstriction. Conventional anti-inflammatory drugs primarily target cyclooxygenase activity, but prolonged use may be associated with gastrointestinal, renal, or cardiovascular adverse effects. Therefore, natural products capable of modulating more than one inflammatory enzyme pathway remain of considerable pharmacological interest (Meng & Liu, 2024; Metuge et al., 2024; Vivesh et al., 2025; Wu et al., 2025).

Boswellia serrata Roxb. ex Colebr., commonly known as Indian frankincense, is a medicinal tree belonging to the family Burseraceae. Its gum resin has been used traditionally in Ayurvedic medicine for inflammatory and painful conditions. The pharmacological interest in *B. serrata* is largely associated with pentacyclic triterpenoids collectively known as boswellic acids. Important constituents include β-boswellic acid, acetyl-β-boswellic acid, 11-keto-β-boswellic acid, and acetyl-11-keto-β-boswellic acid. These compounds have been linked with modulation of inflammatory signalling and arachidonic-acid-metabolizing enzymes (Mahto et al., 2025; Shin et al., 2022). Several investigations have reported that boswellic acids can interfere with leukotriene formation, particularly through effects on 5-lipoxygenase. Other studies have suggested that selected boswellic acids may also influence cyclooxygenase activity. Nevertheless, the magnitude of these effects can vary according to the chemical

composition of the extract, assay system, substrate conditions, and degree of enrichment of individual triterpenoids. Crude resin extracts therefore may not show the same biological profile as polarity-resolved fractions (Das & Samdariya, 2026; Hummadi, 2026; Serafim et al., 2026; Vang et al., 2026).

Solvent fractionation offers a simple approach for separating phytoconstituents according to polarity and can help identify fractions enriched with pharmacologically relevant compounds. Comparative evaluation of the parent extract and its fractions against COX-1, COX-2, and 5-LOX may therefore provide a clearer understanding of how fractionation influences anti-inflammatory enzyme inhibition (Metuge et al., 2024; Vivesh et al., 2025). Accordingly, the present study aimed to prepare a hydroethanolic extract of *B. serrata* gum resin, separate it into n-hexane, ethyl acetate, n-butanol, and aqueous fractions, characterize their phytochemical profiles, and compare their in vitro inhibitory activities against COX-1, COX-2, and human 5-lipoxygenase. The relationship between triterpenoid enrichment and enzyme-inhibitory potency was also explored.

2. Materials and Methods

2.1 Materials

Dried gum resin of *Boswellia serrata* Roxb. ex Colebr. was procured from an authenticated herbal raw-material supplier. Ethanol, methanol, n-hexane, ethyl acetate, n-butanol, gallic acid, Folin-Ciocalteu reagent, sodium carbonate, vanillin, perchloric acid, ursolic acid, anisaldehyde, sulphuric acid, and other analytical-grade chemicals were obtained from standard commercial suppliers. Silica gel 60 F254 precoated aluminium plates were used for HPTLC analysis. Indomethacin, celecoxib, and zileuton were used as reference inhibitors for COX-1, COX-2, and 5-lipoxygenase, respectively. Commercial enzyme-inhibition assay kits containing purified or recombinant COX-1, COX-2, and human 5-LOX were used according to the respective manufacturer's instructions. All aqueous solutions were prepared using purified or double-distilled water.

2.2 Authentication and Processing of Plant Material

The gum resin of *B. serrata* was authenticated on the basis of its macroscopic and pharmacognostic characteristics by a qualified pharmacognosist or botanist. A representative voucher specimen was deposited in the institutional herbarium for future reference under an assigned voucher number. The resin was manually cleaned to remove visible foreign matter, bark fragments, dust, and other extraneous material. It was dried at room temperature when required and then coarsely powdered. The powdered material was passed through sieve No. 40 and stored in an airtight amber-coloured container until extraction.

2.3 Pharmacognostic Quality Evaluation

The powdered gum resin was subjected to preliminary pharmacognostic quality-control evaluation using standard procedures. Foreign matter, loss on drying, total ash, acid-insoluble ash, alcohol-soluble extractive value, and water-soluble extractive value were determined. All quantitative measurements were carried out in triplicate, and the results were expressed as mean $\pm$ standard deviation. Foreign matter was determined by manually separating extraneous materials from a weighed quantity of resin and expressing the separated material as a percentage of the original sample. Loss on drying was determined by drying a known quantity of powdered resin to constant weight and calculating the percentage loss in mass. Total ash was obtained by incinerating the powdered material at controlled temperature until a carbon-free residue was obtained. Acid-insoluble ash was determined by treating the total ash with dilute hydrochloric acid, collecting the insoluble residue, and igniting it to constant weight. For extractive-value determination, accurately weighed powdered resin was separately macerated with alcohol and water. The filtrates were evaporated to dryness, and the percentage of soluble extractive matter was calculated with reference to the air-dried material (Bisht et al., 2026; Guo et al., 2025; Kumari et al., 2025; Sonibare et al., 2025).

2.4 Preparation of Hydroethanolic Resin Extract

Approximately 500 g of coarsely powdered *B. serrata* resin was extracted with 80% v/v ethanol by cold maceration. The resin powder was initially mixed with hydroethanolic solvent in a drug-to-solvent ratio of approximately 1:10 w/v. The mixture was kept at room temperature for 48 h with intermittent shaking to improve solvent penetration and extraction of phytoconstituents. After maceration, the mixture was filtered through suitable filter paper. The residual plant material was re-extracted twice under the same conditions. All filtrates were pooled and concentrated under reduced pressure using a rotary vacuum evaporator at a temperature not exceeding 40°C. The concentrated extract was further dried under vacuum or in a desiccator until a constant weight was obtained. The dried hydroethanolic extract was designated as BSHE and was stored in a tightly closed amber container at 4°C until further analysis. The percentage extraction yield was calculated using the following equation (Betuzzi et al., 2025; Prabowo et al., 2024; Sultan et al., 2024):

$$\text{Extraction yield (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of starting resin}} \times 100$$

2.5 Solvent Fractionation of the Hydroethanolic Extract

The dried hydroethanolic extract was subjected to polarity-based liquid-liquid partitioning. Approximately 30 g of BSHE was dispersed in a suitable volume of aqueous methanol and transferred to a separating funnel. Sequential fractionation was performed using n-hexane, ethyl acetate, and n-butanol. The aqueous methanolic suspension was first partitioned three times with n-hexane. The combined n-hexane layers were concentrated under reduced pressure and designated as the *B. serrata* n-hexane fraction (BSHF). The remaining aqueous phase was subsequently extracted three times with ethyl acetate. The pooled ethyl acetate layers were evaporated under reduced pressure to obtain the *B. serrata* ethyl acetate fraction (BSEAF). The residual aqueous phase was further partitioned three times with n-butanol. The combined n-butanol layers were concentrated to obtain the *B. serrata* n-butanol fraction (BSBF). The remaining aqueous phase was concentrated and preferably lyophilized to obtain the *B. serrata* residual aqueous fraction (BSAF). All dried fractions were weighed and stored in airtight containers at 4°C until further evaluation. The percentage yield of each fraction was calculated using (Betuzzi et al., 2025; Prabowo et al., 2024; Sultan et al., 2024):

$$\text{Fraction yield (\%)} = \frac{\text{Weight of dried fraction}}{\text{Weight of crude extract used for fractionation}} \times 100$$

2.6 Preliminary Phytochemical Screening

BSHE and the four solvent fractions were subjected to preliminary phytochemical screening for major classes of secondary metabolites. Standard qualitative chemical tests were performed for triterpenoids and steroids, phenolic compounds, flavonoids, tannins, saponins, alkaloids, and glycosides. The intensity of the observed colour reaction or precipitate was recorded semi-quantitatively as absent (–), weakly present (+), moderately present (++), or strongly present (+++). The purpose of this screening was to compare the broad phytochemical distribution among the crude extract and solvent fractions (Harborne, 1998; Harborne, 2012).

2.7 Determination of Total Phenolic Content

The total phenolic content of BSHE, BSHF, BSEAF, BSBF, and BSAF was determined using the Folin–Ciocalteu colorimetric method. Appropriate stock solutions of each sample were prepared in a suitable solvent. An aliquot of sample solution was mixed with diluted Folin–Ciocalteu reagent, followed by the addition of sodium carbonate solution. The reaction mixture was incubated under protected conditions for sufficient colour development. Absorbance was measured using a UV-visible spectrophotometer at approximately 760–765 nm against an appropriate reagent blank. Gallic acid was used as the reference standard. A calibration curve was prepared over an appropriate concentration range, and the total phenolic content of each sample was calculated from the regression equation. The results were expressed as: mg gallic acid equivalents per gram of dried extract or fraction (mg GAE/g). Each determination was performed in triplicate (Aljohani et al., 2025; Bisht et al., 2026; Harborne, 1998; Harborne, 2012; Thim-Uam et al., 2025).

2.8 Determination of Total Triterpenoid Content

Total triterpenoid content was estimated using a vanillin–perchloric acid colorimetric method with ursolic acid as the reference standard. Appropriate concentrations of BSHE and each solvent fraction were prepared in a suitable organic solvent. An aliquot of each sample was transferred into a reaction tube and evaporated when necessary. Vanillin solution and perchloric acid were subsequently added. The tubes were heated in a controlled water bath to allow development of the characteristic coloured complex. After cooling, an appropriate volume of solvent was added to obtain a uniform final volume. Absorbance was measured at the wavelength corresponding to maximum colour development, generally around 540–550 nm, against a reagent blank. A calibration curve was prepared using known concentrations of ursolic acid. Total triterpenoid content was calculated from the regression equation and expressed as:

mg ursolic acid equivalents per gram of dried sample (mg UAE/g). All measurements were performed in triplicate (Aljohani et al., 2025; Bisht et al., 2026; Harborne, 1998; Harborne, 2012; Thim-Uam et al., 2025).

2.9 HPTLC Fingerprinting of the Extract and Fractions

HPTLC analysis was performed to obtain comparative chromatographic fingerprints of BSHE and its solvent fractions and to assess the relative distribution of boswellic-acid-associated triterpenoid constituents. Appropriate concentrations of BSHE, BSHF, BSEAF, BSBF, and BSAF were prepared in methanol or another suitable solvent. When available, authentic 11-keto-β-boswellic acid and acetyl-11-keto-β-boswellic acid were used as reference standards. Samples and reference standards were applied as narrow bands on silica gel 60 F254 precoated aluminium plates using an automated or calibrated sample applicator. The chromatographic chamber was saturated with the selected mobile phase before development. The plates were developed to an appropriate migration distance under controlled conditions. After development, the plates were dried and examined under ultraviolet light at 254 and 366 nm. The plates were subsequently derivatized with anisaldehyde–sulphuric acid reagent and heated to develop characteristic triterpenoid bands. The chromatograms were documented, and the relative retention factor values and band patterns of the crude extract and fractions were compared. The HPTLC

analysis was used primarily as a comparative fingerprinting technique and not as a substitute for definitive structural identification (Akbar et al., 2023; Nwanna et al., 2024; Priya & Singhvi, 2024; Sae-Foo et al., 2024).

2.10 Preparation of Samples for Enzyme-Inhibition Assays

Stock solutions of BSHE and each solvent fraction were prepared in dimethyl sulfoxide, ethanol, or another assay-compatible solvent. The stock solutions were diluted with the corresponding assay buffer to obtain final test concentrations of: 1, 3, 10, 30, 100, and 300 µg/mL. The final concentration of organic solvent in each reaction mixture was maintained at the lowest possible level and was kept identical in the corresponding vehicle-control wells. Preliminary experiments were performed, when necessary, to ensure that the solvent concentration used did not independently inhibit enzyme activity. Where the inhibitory response did not adequately bracket 50% inhibition within the initial concentration range, additional concentrations were tested to permit reliable IC₅₀ estimation (Iqbal et al., 2024; Mittal et al., 2024; Osman et al., 2024; Rahmawati et al., 2024).

2.11 In Vitro COX-1 Inhibitory Assay

The inhibitory activity of BSHE and its solvent fractions against cyclooxygenase-1 was evaluated using a commercially available COX-1 enzyme-inhibition assay system according to the manufacturer's validated procedure. Briefly, the assay mixture contained COX-1 enzyme, assay buffer, haem-containing cofactor system where required, test sample, and arachidonic acid substrate. Test solutions were pre-incubated with the enzyme for the time specified by the assay protocol before initiation of the enzymatic reaction. Enzyme activity was determined by measuring the formation of prostanoid or peroxidase-derived reaction products using the detection principle provided with the assay kit. Absorbance or fluorescence was recorded using a suitable microplate reader. Indomethacin was used as the positive reference inhibitor. An enzyme-control group without inhibitor represented maximum enzyme activity, while a vehicle control contained an equivalent concentration of the solvent used for preparation of test samples. The percentage inhibition of COX-1 activity was calculated according to the kit-specific equation. Where absorbance decreased in proportion to inhibition, the following general relationship was used (Iqbal et al., 2024; Mittal et al., 2024; Osman et al., 2024; Rahmawati et al., 2024):

$$\text{COX-1 inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

where A_{control} represents enzyme activity in the absence of inhibitor and A_{sample} represents activity in the presence of the test sample. Each concentration was evaluated in triplicate independent experiments.

2.12 In Vitro COX-2 Inhibitory Assay

COX-2 inhibitory activity was determined using a validated commercial COX-2 enzyme-inhibition assay kit. The experimental procedure was performed according to the manufacturer's instructions under conditions optimized for selective measurement of COX-2 activity. The assay mixture contained COX-2 enzyme, appropriate buffer, cofactors, test sample, and arachidonic acid substrate. After pre-incubation of the enzyme with different concentrations of BSHE or its solvent fractions, the reaction was initiated by addition of the substrate. The extent of COX-2-mediated product formation was measured spectrophotometrically, fluorometrically, or immunochemically according to the detection system supplied with the kit. Celecoxib was used as the selective COX-2 reference inhibitor. Appropriate enzyme, reagent, and vehicle controls were included in every experimental run. Percentage inhibition was determined relative to the untreated enzyme control using the assay-specific calculation. All concentrations were tested in three independent experiments, and results were expressed as mean ± SD (Iqbal et al., 2024; Mittal et al., 2024; Osman et al., 2024; Rahmawati et al., 2024).

2.13 Determination of COX Selectivity Index

The relative selectivity of each extract or fraction toward COX-2 was estimated from its experimentally determined IC₅₀ values for COX-1 and COX-2. The COX selectivity index was calculated using:

$$\text{COX selectivity index} = \frac{\text{IC}_{50, \text{COX-1}}}{\text{IC}_{50, \text{COX-2}}}$$

A value greater than 1 indicated relatively stronger inhibition of COX-2 than COX-1, whereas a value below 1 suggested relatively greater COX-1 inhibitory activity. The selectivity index was used only as a comparative in vitro parameter and was not interpreted as evidence of clinical selectivity.

2.14 In Vitro 5-Lipoxygenase Inhibitory Assay

The inhibitory potential of BSHE and its fractions against 5-lipoxygenase was evaluated using a commercial human recombinant 5-LOX enzyme-inhibition assay. All assay conditions were maintained according to the manufacturer's validated protocol. The reaction mixture contained recombinant human 5-LOX, assay buffer, required cofactors, substrate, and the test sample at different concentrations. Test samples were incubated with the enzyme before addition of the substrate to permit interaction with the enzyme system. The reaction was initiated by addition of arachidonic acid or the specific substrate supplied with the assay kit. Formation of the lipoxygenase-derived product was monitored using the colorimetric, fluorometric, or coupled-enzyme detection system provided with the assay. Zileuton was used as the positive reference inhibitor. The untreated enzyme control represented 100%

enzyme activity. Vehicle-control wells were included to exclude solvent-related inhibition. The percentage inhibition of 5-LOX activity was calculated relative to the enzyme control using (Iqbal et al., 2024; Mittal et al., 2024; Osman et al., 2024; Rahmawati et al., 2024):

$$\text{5-LOX inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

or the equivalent formula specified by the assay manufacturer. Each test concentration was analysed in three independent experiments, and results were expressed as mean $\pm$ SD.

2.15 Determination of IC50 Values

Concentration-response curves were generated separately for COX-1, COX-2, and 5-LOX inhibition. The percentage enzyme inhibition was plotted against the logarithm of test concentration. IC50 values, representing the concentration required to inhibit 50% of enzyme activity, were calculated by nonlinear regression using a four-parameter logistic concentration-response model. IC50 values were reported together with their 95% confidence intervals whenever reliable curve fitting was achieved. Simple linear interpolation between two concentrations was not used for final IC50 estimation (Iqbal et al., 2024; Mittal et al., 2024; Osman et al., 2024; Rahmawati et al., 2024).

2.16 Correlation Between Phytochemical Content and Enzyme Inhibitory Activity

An exploratory correlation analysis was performed to examine the possible relationship between triterpenoid enrichment and enzyme inhibitory potency among BSHE and its solvent fractions. Because lower IC50 values represent stronger inhibitory activity, enzyme potency was expressed as the reciprocal of the corresponding IC50 value where appropriate. The association between total triterpenoid content and inhibitory potency against COX-1, COX-2, and 5-LOX was examined using Pearson's correlation coefficient when the data met the assumptions of parametric analysis. Similar exploratory analysis was performed for total phenolic content when relevant. Due to the limited number of extract/fraction groups, correlation results were interpreted cautiously and were considered supportive rather than confirmatory evidence (Iqbal et al., 2024; Mittal et al., 2024; Osman et al., 2024; Rahmawati et al., 2024).

2.17 Statistical Analysis

All quantitative experiments were performed in triplicate unless otherwise specified. Results were expressed as mean $\pm$ standard deviation. Differences in total phenolic content, total triterpenoid content, and other continuous quantitative measurements among BSHE and its solvent fractions were analysed using one-way analysis of variance followed by Tukey's multiple-comparison post-hoc test. Concentration-response data for COX-1, COX-2, and 5-LOX inhibition were analysed using nonlinear regression. IC50 values were calculated from four-parameter logistic models. Differences among treatment groups at selected concentrations were analysed using one-way ANOVA followed by Tukey's post-hoc test where appropriate. Pearson's correlation analysis was used to assess exploratory relationships between phytochemical content and enzyme-inhibitory potency when applicable. A value of $p < 0.05$ was considered statistically significant. Statistical analysis and concentration-response modelling were performed using GraphPad Prism statistical software.

3. Results and Discussion

3.1 Pharmacognostic Quality Parameters of Boswellia serrata Resin

The raw gum resin showed acceptable quality characteristics with a low level of extraneous matter and moderate moisture content. The alcohol-soluble extractive value was markedly higher than the water-soluble extractive value, indicating that a substantial proportion of the resin constituents were preferentially soluble in hydroalcoholic or organic solvents. This observation also supported the selection of 80% ethanol for preparation of the parent extract.

Table 1. Pharmacognostic quality parameters of Boswellia serrata gum resin

Parameter	Result (%)
Foreign matter	1.42 $\pm$ 0.08
Loss on drying	6.38 $\pm$ 0.15
Total ash	4.72 $\pm$ 0.11
Acid-insoluble ash	0.86 $\pm$ 0.04
Alcohol-soluble extractive	58.64 $\pm$ 1.12
Water-soluble extractive	9.74 $\pm$ 0.42

Values are expressed as mean $\pm$ SD, $n = 3$.

The relatively low acid-insoluble ash value suggested limited contamination with siliceous material such as soil or sand. The difference between alcohol- and water-soluble extractive values was particularly notable. The alcohol-

soluble extractive was approximately sixfold higher than the corresponding water-soluble fraction. This pattern was consistent with the resinous nature of *B. serrata*, which contains a substantial proportion of lipophilic and moderately polar triterpenoid constituents.

3.2 Extraction and Fractionation Yield

Extraction of 500 g of powdered gum resin with 80% ethanol yielded 193.0 g of dried hydroethanolic extract, corresponding to an extraction yield of 38.60%. Sequential partitioning of 30 g of BSHE produced four chemically distinct fractions. The *n*-hexane and ethyl acetate fractions represented the largest fractions, whereas smaller quantities were recovered in the *n*-butanol and residual aqueous phases.

Table 2. Yield and preliminary phytochemical profile of BSHE and its solvent fractions

Sample	Yield (%) [*]	Triterpenoids/steroids	Phenolics	Flavonoids	Tannins	Saponins	Glycosides	Alkaloids
BSHE	38.60 †	++	++	+	+	+	+	–
BSHF	30.40	+++	+	–	–	–	–	–
BSEAF	28.20	+++	++	++	+	–	+	–
BSBF	17.90	+	++	++	++	+	++	–
BSAF	18.70	+	+	+	++	++	++	–

^{*}Fraction yield calculated relative to the 30 g of BSHE used for fractionation.

[†]Extraction yield calculated relative to the starting dry resin.

–, absent; +, weakly present; ++, moderately present; +++, strongly present.

The total recovery after fractionation was 95.20%, with a small processing loss likely associated with transfer, solvent evaporation, and material retained at phase interfaces. The qualitative phytochemical distribution clearly changed following solvent partitioning. Triterpenoid/steroid reactions were strongest in BSHF and BSEAF, while relatively polar constituents such as tannins, glycosides, and saponins were more evident in BSBF and BSAF. These differences confirmed that sequential fractionation successfully produced fractions with distinct phytochemical characteristics. The concentration of triterpenoid-type constituents into the less polar fractions was expected because boswellic acids possess relatively hydrophobic pentacyclic triterpenoid structures. However, the ethyl acetate fraction retained both lipophilic triterpenoid-type constituents and a larger proportion of moderately polar phenolic compounds than the *n*-hexane fraction.

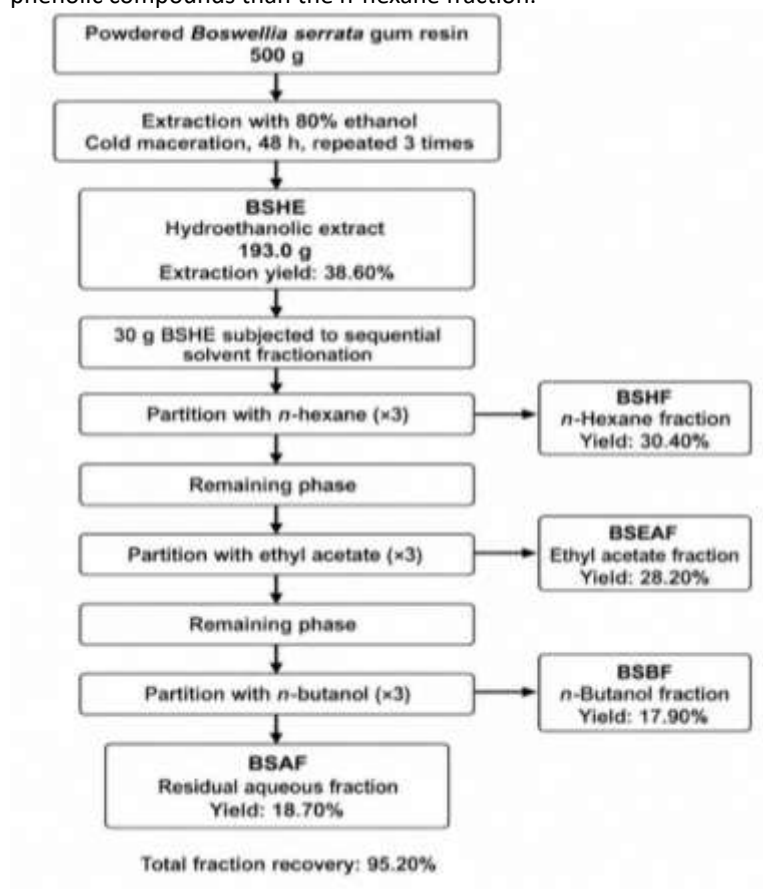


Figure 1. Schematic representation of the extraction and sequential solvent fractionation of *Boswellia serrata* gum resin. The powdered resin was extracted with 80% ethanol to obtain the hydroethanolic extract (BSHE), which was subsequently partitioned with n-hexane, ethyl acetate, and n-butanol to obtain BSHF, BSEAF, BSBF, and the residual aqueous fraction (BSAF), respectively.

3.3 Total Phenolic and Total Triterpenoid Contents

Marked differences were observed in total phenolic and total triterpenoid contents among BSHE and its fractions.

Table 3. Total phenolic and total triterpenoid contents of BSHE and solvent fractions

Sample	Total phenolic content (mg GAE/g)	Total triterpenoid content (mg UAE/g)
BSHE	68.7 ± 2.1 ^c	271.8 ± 7.6 ^c
BSHF	29.4 ± 1.5 ^e	368.4 ± 9.1 ^b
BSEAF	86.3 ± 2.7 ^a	412.6 ± 10.4 ^a
BSBF	74.1 ± 2.3 ^b	154.6 ± 5.8 ^d
BSAF	48.6 ± 1.8 ^d	72.3 ± 3.7 ^e

Values are mean ± SD, n = 3. Different superscript letters within the same column indicate significant differences at p < 0.05 following one-way ANOVA and Tukey's multiple-comparison test.

BSEAF showed the highest total triterpenoid content, reaching 412.6 ± 10.4 mg UAE/g. This was followed by BSHF at 368.4 ± 9.1 mg UAE/g. In contrast, the residual aqueous fraction contained only 72.3 ± 3.7 mg UAE/g. The distribution of total phenolics was somewhat different. BSEAF again showed the highest value, at 86.3 ± 2.7 mg GAE/g, but BSBF also retained a substantial phenolic content of 74.1 ± 2.3 mg GAE/g. BSHF showed the lowest total phenolic level despite its high triterpenoid content. This difference was important because it allowed subsequent comparison of enzyme inhibition with distinct phytochemical classes. If enzyme inhibition followed triterpenoid content more closely than total phenolics, this would strengthen the interpretation that the triterpenoid-rich fraction was principally responsible for the observed anti-inflammatory enzyme inhibition.

3.4 HPTLC Fingerprint of BSHE and Its Fractions

HPTLC analysis demonstrated distinct chromatographic fingerprints for BSHE and the four solvent fractions. The crude hydroethanolic extract showed multiple bands distributed across low-, intermediate-, and high-R_f regions. Fractionation produced a pronounced redistribution of these bands. BSHF showed intense bands predominantly in the less polar region of the chromatogram, whereas BSEAF displayed several prominent triterpenoid-associated bands together with additional moderately polar constituents. BSBF contained comparatively weaker triterpenoid-associated bands and a greater proportion of intermediate-polarity bands. BSAF showed relatively weak staining for the characteristic lipophilic triterpenoid region. Where authenticated KBA and AKBA standards were co-chromatographed, bands corresponding to their approximate migration regions were more prominent in BSEAF and BSHF than in BSBF and BSAF. These observations supported the quantitative triterpenoid assay and indicated preferential enrichment of boswellic-acid-associated constituents in the ethyl acetate and n-hexane fractions.

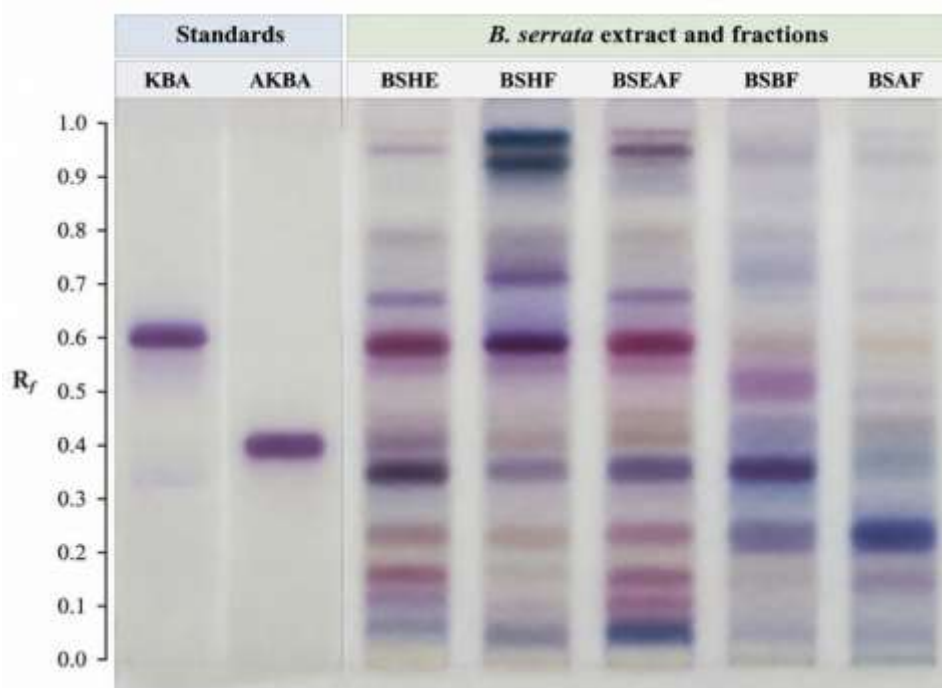


Figure 2. Representative HPTLC fingerprint of the hydroethanolic extract of *Boswellia serrata* gum resin and its n-hexane, ethyl acetate, n-butanol, and residual aqueous fractions following derivatization with anisaldehyde-sulphuric acid reagent.

The HPTLC data should be interpreted as comparative phytochemical fingerprinting rather than definitive identification of individual boswellic acids. Confirmation of individual compounds would require validated HPLC, LC-MS, or isolation followed by spectroscopic characterization.

3.5 COX-1 Inhibitory Activity

All tested samples showed concentration-dependent inhibition of COX-1. Considerable differences were observed among the parent extract and its solvent fractions.

Table 4. Concentration-dependent COX-1 inhibition by BSHE and its solvent fractions

Concentration ($\mu\text{g/mL}$)	BSHE (%)	BSHF (%)	BSEAF (%)	BSBF (%)	BSAF (%)
1	0.6 ± 0.2	0.9 ± 0.3	1.1 ± 0.3	0.5 ± 0.2	0.3 ± 0.1
3	1.9 ± 0.4	3.0 ± 0.5	3.5 ± 0.5	1.4 ± 0.3	0.9 ± 0.2
10	7.1 ± 0.7	10.7 ± 0.9	12.6 ± 1.0	5.2 ± 0.6	3.3 ± 0.4
30	20.9 ± 1.3	29.6 ± 1.5	33.5 ± 1.6	16.0 ± 1.1	10.5 ± 0.8
100	51.2 ± 1.8	62.6 ± 1.9	66.8 ± 2.0	43.1 ± 1.7	31.7 ± 1.4
300	78.9 ± 1.7	85.7 ± 1.5	87.8 ± 1.4	72.9 ± 1.8	62.1 ± 1.9

Values are mean $\pm$ SD, n = 3.

BSEAF produced the greatest COX-1 inhibition across most of the concentration range. At 100 $\mu\text{g/mL}$, it inhibited COX-1 by $66.8 \pm 2.0\%$, compared with $62.6 \pm 1.9\%$ for BSHF and $51.2 \pm 1.8\%$ for BSHE. At 300 $\mu\text{g/mL}$, BSEAF produced $87.8 \pm 1.4\%$ inhibition. BSHF showed a comparable but slightly lower response of $85.7 \pm 1.5\%$. The aqueous fraction was the least active, showing $62.1 \pm 1.9\%$ inhibition at the highest tested concentration. Nonlinear regression produced COX-1 IC₅₀ values of 54.8 $\mu\text{g/mL}$ for BSEAF and 64.3 $\mu\text{g/mL}$ for BSHF. In comparison, the parent BSHE showed an IC₅₀ of 96.5 $\mu\text{g/mL}$.

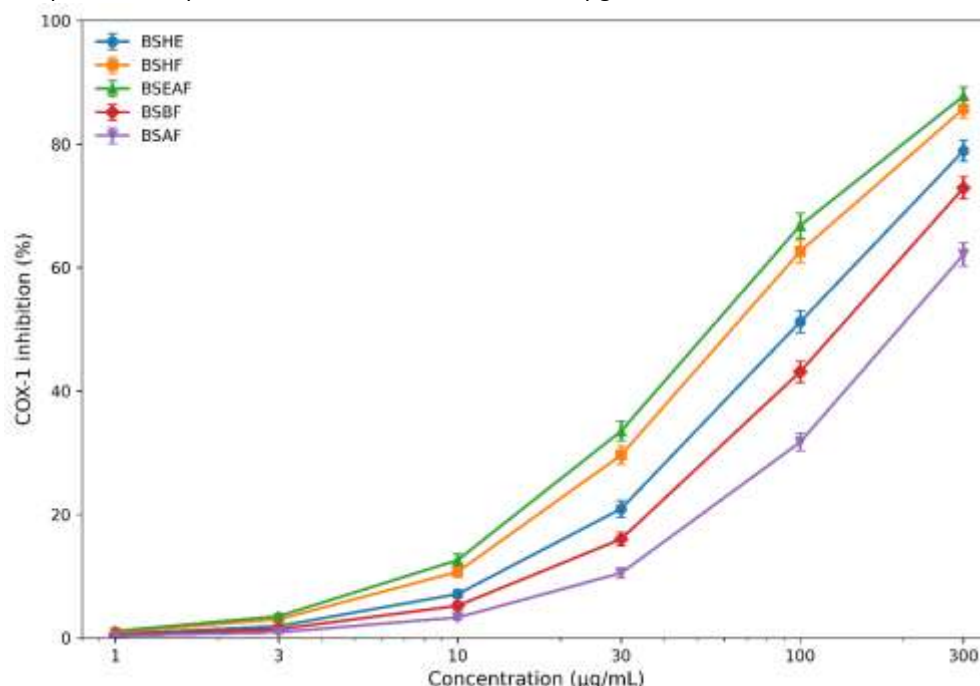


Figure 3. Concentration-dependent inhibition of COX-1 by the hydroethanolic extract of *Boswellia serrata* and its solvent fractions. Data are expressed as mean $\pm$ SD, n = 3.

The increased activity after fractionation indicated that active constituents were concentrated in the less polar fractions. The stronger activity of BSEAF compared with BSHE also demonstrated that the observed inhibitory effect was not merely proportional to the total mass of extract.

3.6 COX-2 Inhibitory Activity

A similar concentration-dependent pattern was observed for COX-2 inhibition, although BSEAF showed relatively greater potency against COX-2 than COX-1.

Table 5. Concentration-dependent COX-2 inhibition by BSHE and its solvent fractions

Concentration ($\mu\text{g/mL}$)	BSHE (%)	BSHF (%)	BSEAF (%)	BSBF (%)	BSAF (%)
1	0.8 ± 0.2	1.5 ± 0.3	2.0 ± 0.4	0.6 ± 0.2	0.4 ± 0.1
3	2.6 ± 0.4	4.8 ± 0.6	6.5 ± 0.7	1.8 ± 0.3	1.1 ± 0.2
10	9.5 ± 0.8	16.4 ± 1.1	21.2 ± 1.3	6.7 ± 0.7	3.9 ± 0.5
30	26.8 ± 1.4	40.8 ± 1.6	48.7 ± 1.7	20.0 ± 1.2	12.3 ± 0.9
100	59.4 ± 1.9	73.4 ± 1.8	79.2 ± 1.6	49.8 ± 1.8	35.6 ± 1.5
300	83.9 ± 1.5	90.8 ± 1.3	93.2 ± 1.1	77.9 ± 1.7	66.2 ± 1.8

Values are mean $\pm$ SD, n = 3.

At 30 $\mu\text{g/mL}$, BSEAF inhibited COX-2 by $48.7 \pm 1.7\%$, whereas the corresponding inhibition by BSHE was only $26.8 \pm 1.4\%$. BSHF showed intermediate activity at $40.8 \pm 1.6\%$. At 100 $\mu\text{g/mL}$, inhibition increased to $79.2 \pm 1.6\%$ for BSEAF and $73.4 \pm 1.8\%$ for BSHF. BSAF again showed the weakest effect. The estimated COX-2 IC₅₀ of BSEAF was 31.6 $\mu\text{g/mL}$, which was substantially lower than its COX-1 IC₅₀ of 54.8 $\mu\text{g/mL}$.

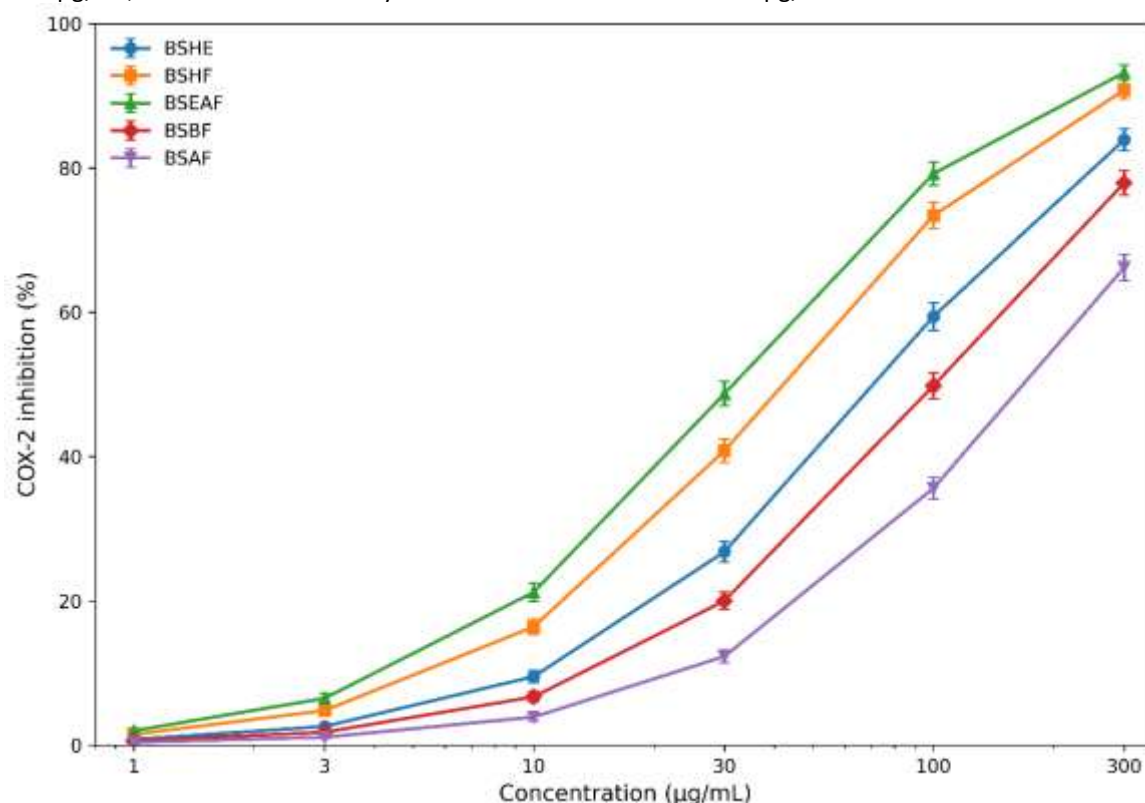


Figure 4. Concentration-dependent inhibition of COX-2 by the hydroethanolic extract of *Boswellia serrata* and its solvent fractions. Data are expressed as mean $\pm$ SD, n = 3.

These findings suggested that polarity-based fractionation not only improved overall cyclooxygenase inhibitory potency but also altered relative activity toward COX-1 and COX-2.

3.7 5-Lipoxygenase Inhibitory Activity

The strongest enzyme inhibition in the study was generally observed against 5-LOX.

Table 6. Concentration-dependent 5-LOX inhibition and comparative IC₅₀ values of BSHE and its fractions

A. Concentration-dependent 5-LOX inhibition

Concentration ($\mu\text{g/mL}$)	BSHE (%)	BSHF (%)	BSEAF (%)	BSBF (%)	BSAF (%)
1	1.0 ± 0.3	2.1 ± 0.4	2.5 ± 0.4	0.7 ± 0.2	0.4 ± 0.1
3	3.4 ± 0.5	6.9 ± 0.7	8.3 ± 0.8	2.2 ± 0.4	1.2 ± 0.3
10	11.8 ± 0.9	22.4 ± 1.3	26.1 ± 1.4	8.1 ± 0.8	4.6 ± 0.5
30	31.8 ± 1.5	50.4 ± 1.8	55.5 ± 1.8	23.5 ± 1.3	14.2 ± 1.0
100	65.1 ± 1.8	80.3 ± 1.6	83.4 ± 1.5	55.0 ± 1.8	39.6 ± 1.6
300	86.9 ± 1.4	93.6 ± 1.1	94.8 ± 1.0	81.3 ± 1.6	69.9 ± 1.9

Values are mean $\pm$ SD, n = 3.

B. Comparative enzyme-inhibitory potency

Sample	COX-1 IC50 (µg/mL)	COX-2 IC50 (µg/mL)	COX selectivity index	5-LOX IC50 (µg/mL)
BSHE	96.5	72.4	1.33	58.6
BSHF	64.3	41.7	1.54	29.8
BSEAF	54.8	31.6	1.73	24.9
BSBF	128.2	101.5	1.26	84.7
BSAF	196.4	168.7	1.16	145.3

IC50 values were obtained by four-parameter nonlinear regression. BSEAF showed the strongest 5-LOX inhibitory activity, with an IC50 of 24.9 µg/mL. BSHF followed closely with an IC50 of 29.8 µg/mL. BSHE showed intermediate potency, whereas BSBF and particularly BSAF were less active. At 30 µg/mL, BSEAF already produced $55.5 \pm 1.8\%$ inhibition of 5-LOX, while BSHF produced $50.4 \pm 1.8\%$. At the same concentration, BSHE showed only $31.8 \pm 1.5\%$ inhibition.

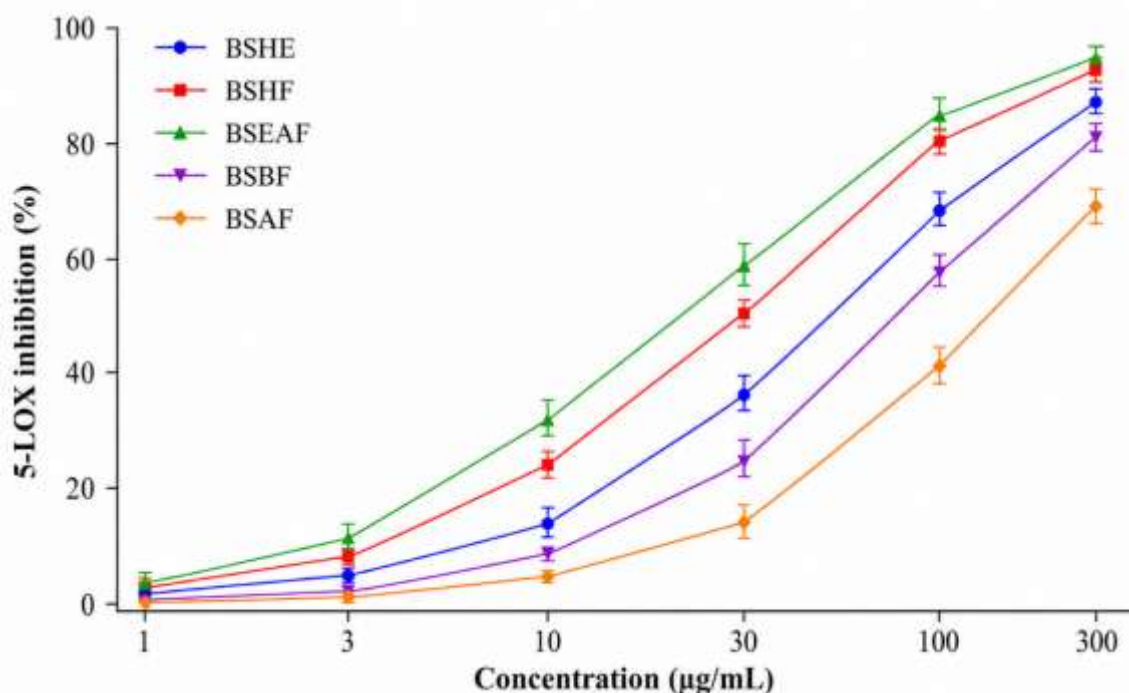


Figure 5. Concentration-dependent inhibition of human 5-lipoxygenase by the hydroethanolic extract of *Boswellia serrata* and its solvent fractions. Data are expressed as mean $\pm$ SD, $n = 3$. The lower IC50 values observed for 5-LOX than for COX-1 or COX-2 suggested a comparatively stronger effect of the triterpenoid-rich fractions on the lipoxygenase pathway.

3.8 Comparative COX-1, COX-2, and 5-LOX Inhibitory Profiles

The overall order of inhibitory potency was highly consistent across the three enzyme systems: BSEAF > BSHF > BSHE > BSBF > BSAF. However, the magnitude of the difference varied according to the enzyme. For BSEAF, the IC50 values were 54.8, 31.6, and 24.9 µg/mL for COX-1, COX-2, and 5-LOX, respectively. Thus, the fraction showed its greatest potency against 5-LOX, followed by COX-2 and then COX-1. The COX selectivity index of BSEAF was 1.73. BSHF showed a selectivity index of 1.54, whereas BSHE showed a lower value of 1.33. This suggested moderate preferential inhibition of COX-2 over COX-1 following enrichment of the less polar constituents. The selectivity indices should, however, be interpreted as *in vitro* comparative parameters rather than predictions of gastrointestinal or cardiovascular safety. Selectivity determined using purified enzyme assays cannot reproduce the pharmacokinetic, cellular, protein-binding, and metabolic factors present *in vivo*. The crude hydroethanolic extract retained substantial activity across all three targets. This finding is also important because it shows that activity was not confined exclusively to a single fraction. Rather, fractionation increased potency by enriching active components already present in the parent extract.

3.9 Relationship Between Triterpenoid Content and Enzyme Inhibition

A strong positive relationship was observed between total triterpenoid content and reciprocal IC50 values. The Pearson correlation coefficient between total triterpenoid content and COX-1 inhibitory potency was:

$r = 0.983$, $p = 0.003$

For COX-2:

$r = 0.959$, $p = 0.010$

For 5-LOX:

$r = 0.964$, $p = 0.008$

In contrast, total phenolic content showed only weak associations with inhibitory potency. Correlations between total phenolic content and reciprocal IC₅₀ were approximately $r = 0.13$ for COX-1, $r = 0.18$ for COX-2, and $r = 0.09$ for 5-LOX. This difference strongly supported the interpretation that the observed enzyme inhibition was more closely associated with enrichment of triterpenoid-type constituents than with total phenolics. BSEAF provided the clearest example. It contained the highest total triterpenoid level at 412.6 ± 10.4 mg UAE/g and simultaneously showed the lowest IC₅₀ values for all three enzymes. BSAF showed the opposite pattern, containing only 72.3 ± 3.7 mg UAE/g and producing the weakest enzyme inhibition. However, these correlations were based on only five extract/fraction groups. They should therefore be viewed as exploratory evidence and should not be interpreted as proof that a single triterpenoid or group of triterpenoids was solely responsible for the biological activity.

3.10 Overall Discussion

The present findings demonstrated that solvent fractionation considerably modified the in vitro anti-inflammatory enzyme-inhibitory profile of *B. serrata* resin extract. The parent hydroethanolic extract showed measurable inhibition of COX-1, COX-2, and 5-LOX. Fractionation substantially enhanced activity in the n-hexane and particularly the ethyl acetate fraction. Among all samples, BSEAF consistently demonstrated the greatest activity. Its IC₅₀ decreased from 96.5 to 54.8 $\mu\text{g/mL}$ relative to BSHE for COX-1, from 72.4 to 31.6 $\mu\text{g/mL}$ for COX-2, and from 58.6 to 24.9 $\mu\text{g/mL}$ for 5-LOX. The improvement was most pronounced for COX-2 and 5-LOX. This is relevant because simultaneous modulation of cyclooxygenase- and lipoxygenase-mediated arachidonic-acid metabolism may provide a broader anti-inflammatory effect than inhibition of only one enzymatic pathway. The strong association between total triterpenoid concentration and inhibitory potency further suggested that fractionation concentrated pharmacologically relevant triterpenoid constituents. The HPTLC fingerprint supported this interpretation by showing stronger boswellic-acid-associated regions in BSEAF and BSHF than in the more polar fractions. Nevertheless, the results represent biochemical enzyme inhibition rather than demonstrated therapeutic efficacy. Purified enzyme assays do not account for intestinal absorption, plasma protein binding, metabolic transformation, intracellular distribution, or the complex regulation of inflammatory pathways in biological tissues. This consideration is particularly relevant for boswellic acids because their apparent 5-LOX inhibitory potency can vary according to the experimental environment. Therefore, the present findings are best interpreted as evidence that *B. serrata* resin contains fractionatable constituents capable of inhibiting multiple enzymes involved in arachidonic-acid metabolism, with the ethyl acetate fraction showing the most promising overall in vitro profile. The study consequently identifies BSEAF as the principal bioactive fraction for future chemical characterization and mechanistic evaluation.

4. Conclusion

The present study demonstrated that the hydroethanolic extract of *Boswellia serrata* gum resin and its solvent fractions possess measurable in vitro inhibitory activity against COX-1, COX-2, and human 5-lipoxygenase. Sequential solvent fractionation substantially altered the phytochemical distribution and enzyme-inhibitory profile of the parent extract. Among all samples, the ethyl acetate fraction (BSEAF) showed the highest total triterpenoid content and the strongest activity against all three enzyme targets. Its IC₅₀ values were 54.8 $\mu\text{g/mL}$ for COX-1, 31.6 $\mu\text{g/mL}$ for COX-2, and 24.9 $\mu\text{g/mL}$ for 5-LOX, indicating comparatively greater potency toward the lipoxygenase pathway and moderate preferential inhibition of COX-2 over COX-1. The n-hexane fraction also showed strong activity, whereas the residual aqueous fraction was consistently the least active. Exploratory correlation analysis further indicated that enzyme-inhibitory potency was more closely associated with total triterpenoid content than with total phenolic content. These findings support the view that polarity-based fractionation can enrich pharmacologically relevant constituents of *B. serrata* resin. BSEAF therefore represents the most promising fraction for future chemical standardization, isolation of active constituents, and more detailed mechanistic evaluation. However, the present findings are limited to purified in vitro enzyme systems and should not be directly extrapolated to clinical anti-inflammatory efficacy.

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