Communication in Physical Sciences, 2026, 13(8):1330-1347

Isolation, Characterization and In Vitro Evaluation of Antioxidant and Anti-Inflammatory Properties of *Boswellia Serrata* Stem Bark from Jalingo, Taraba State

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Received: 24 April 2026/Accepted: 19 July 2026/Published: 28 July 2026
https://doi.org/10.4314/cps.v13i8.7

Abstract : Boswellia serrata is widely recognized in traditional medicine for its therapeutic properties, yet, limited data exist on its bioactivities in Taraba State, Nigeria. This study aimed to determine the phytochemical properties, anti-inflammatory and antioxidant properties of the methanolic stem bark extracts of Boswellia serrata obtained from Jalingo local government area of Taraba State. The anti-inflammatory activity of the crude extract was determined using albumin denaturation method while the antioxidant activity was determined using DPPH Method. Column chromatography was carried out to obtain the pure isolated compounds. FT-IR and GC-MS were used to determine the functional groups and identification of compounds respectively. The phytochemical screening revealed the presence of alkaloids, steroids, phenols and flavonoids. Other phytochemicals revealed include terpenoids, glycosides, quinones and phlobatanins. Three fractions coded BS001, BS002, BS003 were obtained from column chromatography of the crude extracts of Boswellia serrata. The assay for anti-inflammatory activity revealed that Boswellia serrata has very significant anti-inflammatory and antioxidant properties. The FT-IR analysis for B. serrata revealed peaks at 3395.6 cm^{-1}, 2935.1 cm^{-1}, 2872.2 cm^{-1}, 2227.2 cm^{-1}, 1717.0 cm^{-1} and 1462.1 cm^{-1} which were assigned to hydroxyl group, carboxylic group, alkanes, alkynes and carbonyl groups.

Keywords: *Isolation, Characterization, Antioxidant, Anti-Inflammatory, Boswellia Serrata*

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1.0 Introduction

Medicinal plants constitute an important source of biologically active compounds and have been used for centuries in traditional healthcare systems for the prevention and management of diverse diseases. Their therapeutic effects are associated with a wide range of secondary metabolites, including phenolic compounds, flavonoids, terpenoids, alkaloids, steroids, glycosides, and other phytoconstituents. Among these compounds, phenolics and terpenoids are particularly important contributors to the antioxidant properties of many medicinal plants. The distribution and concentration of these constituents vary considerably among different plant species, plant parts, geographical locations, environmental conditions, and stages of plant development. Consequently, leaves, stems,

roots, bark, flowers, fruits, and other plant parts may exhibit different phytochemical profiles and biological activities. In addition, the extraction solvent and extraction procedure can substantially influence the nature and concentration of compounds recovered from plant materials, thereby affecting the biological properties of the resulting extracts (Xu et al., 2017).

Oxidative stress and inflammation are closely interconnected biological processes that contribute to the development and progression of several chronic and degenerative disorders. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are continuously generated during normal cellular metabolism, and their levels are normally controlled by endogenous antioxidant defence mechanisms. However, excessive exposure to environmental pollutants, cigarette smoke, radiation, alcohol, and other oxidative stressors can result in an imbalance between free-radical generation and antioxidant defence, leading to oxidative damage to cellular lipids, proteins, nucleic acids, and other biomolecules (Li et al., 2015; Wang et al., 2016; Zhou et al., 2016). Exogenous antioxidants can complement endogenous defence mechanisms by scavenging free radicals, quenching reactive species, chelating pro-oxidant metals, and interrupting oxidative chain reactions (Baiano & Nobile, 2015). Similarly, inflammation is an essential component of the body's protective response to infection, tissue injury, and harmful stimuli. Although acute inflammation is important for tissue protection and repair, persistent or dysregulated inflammation can contribute to tissue damage and chronic disease. The close association between oxidative stress and inflammatory signalling has therefore increased interest in natural products with both antioxidant and anti-inflammatory properties.

The genus *Boswellia*, belonging to the family Burseraceae, comprises aromatic resin-producing trees that are widely recognized for their ethnomedicinal importance. Several *Boswellia* species have traditionally been used for the management of inflammatory conditions, pain, and other health disorders. The medicinal properties of *Boswellia* have been associated with diverse bioactive constituents, including terpenoids and boswellic acids. Extracts and essential oils obtained from different *Boswellia* species have been reported to exhibit antioxidant, anti-inflammatory, analgesic, cardioprotective, antimicrobial, and other pharmacological activities (Verhoff et al., 2014; Iram et al., 2017). The biological and chemical characteristics of *Boswellia* species may, however, vary according to species, plant part, geographical origin, climatic conditions, age of the plant, harvesting period, and extraction method. Di Stefano et al. (2020), for example, reported differences in the biochemical composition of *Boswellia* essential oils associated with different agroclimatic conditions, demonstrating the importance of geographical and environmental factors in determining the chemical characteristics of these plants.

Boswellia serrata Roxb. ex Colebr., commonly known as Indian frankincense, is one of the most extensively investigated species of the genus and has a long history of use in traditional medicine. Its therapeutic potential has been associated particularly with anti-inflammatory activity, while increasing evidence also indicates antioxidant and other pharmacological properties. The phytochemical constituents of *B. serrata* may vary among plant parts and extraction fractions. Kushwaha and Tyagi (2021) reported that methanolic extracts of *B. serrata* leaves exhibited considerable total phenolic content and antioxidant activity, while ethyl acetate and *n*-butanol fractions also demonstrated substantial phenolic content and antioxidant capacity. Their findings further suggested a positive association between phenolic content and antioxidant activity, emphasizing the

potential contribution of phenolic constituents to the biological activity of *B. serrata* extracts. Recent experimental evidence has further strengthened the reported antioxidant and anti-inflammatory potential of *B. serrata*. Biswas et al. (2024) demonstrated that *B. serrata* leaf extract exhibited significant free-radical-scavenging activity and dose-dependent anti-inflammatory effects in in vitro models. The extract was also reported to influence important inflammatory mediators, including interleukin-1 beta (IL-1β), tumour necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6). These findings provide further evidence that *B. serrata* contains constituents capable of simultaneously modulating oxidative and inflammatory processes. Similarly, Bertocchi et al. (2018) investigated two *B. serrata* extracts using cultured porcine aortic endothelial cells and reported anti-inflammatory effects following lipopolysaccharide-induced cellular stress. Their findings also demonstrated that the biological response depended on extract formulation and concentration, highlighting the importance of dose and chemical composition when evaluating the therapeutic properties of *B. serrata* extracts.

The antioxidant potential of *Boswellia* species is not restricted to *B. serrata*. Akomolafe et al. (2025) evaluated aqueous extracts of the leaves, stem bark, and root bark of *Boswellia dalzielii* Hutch., a species occurring in Northern Nigeria and the West African savannah. The researchers reported substantial differences in phytochemical composition and antioxidant activity among the different plant parts. Notably, the stem-bark extract demonstrated the highest ferric-reducing ability and DPPH radical-scavenging capacity among the plant parts examined for those parameters. UHPLC-DAD-MS analysis further revealed differences in the phytochemical profiles of the leaves, stem bark, and root bark. These findings are particularly relevant to the present investigation because they demonstrate that the biological activity and phytochemical composition of *Boswellia* can differ markedly according to plant part. In another study, Hamadjida et al. (2024) reported that *B. dalzielii* extract significantly reduced malondialdehyde and inflammatory markers while increasing antioxidant defence markers such as reduced glutathione, superoxide dismutase, and catalase in an alloxan-induced diabetic rat model. Collectively, these findings support the potential of *Boswellia* species as sources of natural antioxidant and anti-inflammatory agents.

The isolation and characterization of individual bioactive constituents from medicinal plants are important for linking observed biological activities with specific chemical components. Chromatographic separation combined with spectroscopic techniques can provide useful information about the chemical constituents responsible for the biological effects of plant extracts. Kushwaha and Tyagi (2021) recommended chromatographic and spectroscopic investigations of bioactive fractions of *B. serrata* based on their substantial phenolic content and antioxidant activity. Similarly, Sivapalan et al. (2024), in a study of *Vitex negundo*, demonstrated the value of combining antioxidant and anti-inflammatory assays with GC-MS analysis and bioassay-guided fractionation for the identification and characterization of bioactive constituents. The isolation of a specific compound following biological evaluation illustrates how fractionation and chemical characterization can complement pharmacological assays in medicinal-plant research. Such an integrated approach is relevant to the investigation of *B. serrata* because the biological activity of a crude extract may result from the combined or synergistic effects of several constituents rather than from a single compound.

Despite the considerable evidence supporting the medicinal importance of *B. serrata*, several aspects remain insufficiently investigated.

Existing studies have predominantly examined resin, gum-resin, leaves, essential oils, or extracts obtained from geographical locations outside Nigeria. Comparatively limited information is available on the phytochemical composition and biological activities of *B. serrata* stem bark obtained from Jalingo, Taraba State, Nigeria. In particular, the antioxidant and anti-inflammatory properties of the locally sourced stem-bark extract and the chemical characteristics of its chromatographically separated fractions have not been adequately documented. This constitutes an important geographical and phytochemical knowledge gap because differences in plant part, environmental conditions, geographical origin, extraction solvent, and processing procedures can influence both phytochemical composition and biological activity. Therefore, investigation of *B. serrata* collected from Jalingo is necessary to establish baseline information on the chemical constituents and biological properties of the plant material available in this locality.

The present study therefore aimed to isolate, characterize, and evaluate the in vitro antioxidant and anti-inflammatory activities of bioactive constituents from the methanolic stem-bark extract of *Boswellia serrata* collected from Jalingo Local Government Area, Taraba State, Nigeria. Specifically, the study sought to: (i) extract bioactive constituents from the stem bark of *B. serrata* using methanol; (ii) determine the phytochemical constituents present in the crude extract; (iii) evaluate the in vitro anti-inflammatory activity of the crude extract using an appropriate protein-denaturation assay; (iv) evaluate its antioxidant activity using the DPPH radical-scavenging assay; and (v) isolate and characterize selected constituents or fractions obtained from the crude extract using chromatographic and spectroscopic techniques, including column chromatography, FT-IR, and GC-MS.

The significance of this study lies in its potential to provide scientific evidence concerning the phytochemical composition and biological activities of *B. serrata* stem bark obtained from Jalingo, Taraba State. The findings may contribute to the growing body of knowledge on *Boswellia* species as potential sources of naturally occurring antioxidant and anti-inflammatory compounds. The study may also provide baseline information for subsequent investigations involving compound purification, structural elucidation, mechanistic studies, and in vivo evaluation. Furthermore, documenting the biological properties of locally available medicinal plants may contribute to the scientific validation of traditional medicinal resources and support future research into the development of plant-derived therapeutic agents. However, the observed in vitro activities should be regarded as preliminary evidence of biological potential and should not be interpreted as establishing clinical efficacy without further toxicological, mechanistic, and in vivo studies

2.0 Materials and Methods

2.1 Materials

The plant material used for this study was the stem bark of *Boswellia serrata*. The laboratory glassware and materials used included beakers, conical flasks, measuring cylinders, test tubes, pipettes, Petri dishes, glass rods, retort stands, burettes, and other routine laboratory equipment.

The major laboratory equipment and apparatus employed included an analytical weighing balance, Soxhlet extractor, rotary evaporator, water bath, heating mantle, chromatographic column, distillation apparatus, thin-layer chromatography (TLC) plates, and Fourier-transform infrared (FT-IR) spectrometer.

The reagents and solvents used included methanol, *n*-hexane, ethyl acetate, dimethyl sulfoxide (DMSO), silica gel, white sand, distilled water, Dragendorff's reagent, Wagner's reagent, 2,2-diphenyl-1-

picrylhydrazyl (DPPH), sodium hydroxide (NaOH), hydrochloric acid (HCl), ferric chloride, glacial acetic acid, concentrated sulphuric acid, and other analytical-grade reagents required for the phytochemical and biological assays.

2.2 Study Area

The study was conducted using plant material collected from Jalingo Local Government Area of Taraba State, Nigeria. Jalingo is the capital of Taraba State and is located in northeastern Nigeria. The area is characterized by a tropical climate and supports a variety of plant species with potential medicinal and ethnobotanical importance.

2.3 Sample Collection and Preparation

2.3.1 Collection and Authentication of Boswellia serrata

The stem bark of *Boswellia serrata* was collected from Jalingo Local Government Area of Taraba State, Nigeria. The plant material was collected from a mature plant and transported to the laboratory in a clean container for processing. The identity of the plant was initially recognized based on its morphological characteristics and its known medicinal use among local traditional medicine practitioners.

Following collection, a representative plant specimen was submitted to a qualified taxonomist for taxonomic identification and authentication. The authenticated plant material was subsequently used for the extraction and phytochemical investigations.

Note: The authors should insert the name of the institution, name of the taxonomist, and herbarium/voucher specimen number if these details are available. These details are important for reproducibility and botanical verification.

2.3.2 Washing and Drying

The collected stem-bark samples were thoroughly washed with distilled water to remove adhering soil particles and other extraneous materials. The samples were subsequently air-dried at room temperature in the Chemistry Laboratory of Modibbo Adama University, Yola, for approximately two weeks. Drying was continued until a constant dry condition was achieved. The dried samples were protected from direct sunlight during drying to minimize possible degradation of light-sensitive phytochemicals.

2.3.3 Pulverization

The dried stem bark was mechanically reduced to smaller pieces and subsequently pulverized using a clean mortar and pestle. The resulting powdered plant material was stored in a clean, dry, airtight container until extraction.

2.4 Extraction of Boswellia serrata Stem Bark

The powdered stem bark of *Boswellia serrata* was extracted using a Soxhlet extraction technique, following the general procedure described by Rao et al. (2024), with appropriate modifications where necessary. A measured quantity of the powdered plant material was placed in the Soxhlet apparatus and extracted continuously with methanol. The extraction was continued until exhaustive extraction of the soluble constituents was achieved.

The resulting methanolic extract was filtered and concentrated under reduced pressure using a rotary evaporator. The concentrated extract was further dried to obtain the crude methanolic extract. The percentage extraction yield was determined where the initial mass of the plant material and final mass of the dried crude extract were available, using equation 1

$$Percentage\ yeild\ (\%) = \frac{M_{dried}}{M_{powder}} \times \frac{100}{1} \quad (1)$$

where M_{dried} = Mass of dried crude and M_{powder} = Mass of powdered plant material

The dried crude extract was stored in a suitable airtight container until further phytochemical, antioxidant, anti-inflammatory, chromatographic, and spectroscopic analyses.

2.5 Preliminary Phytochemical Screening

Preliminary qualitative phytochemical screening of the methanolic stem-bark extract

was performed using standard chemical tests to determine the presence or absence of selected secondary metabolites, including alkaloids, flavonoids, saponins, tannins, phenols, glycosides, and quinones.

2.5.1 Test for Alkaloids

Approximately 3 mL of the plant extract was treated with 1 mL of 1% hydrochloric acid and heated gently for approximately 20 min. The mixture was allowed to cool and was subsequently filtered. About 1 mL of the filtrate was treated with two drops of Mayer's reagent. The formation of turbidity or a precipitate was taken as an indication of the presence of alkaloids (Narasimhan, 2012).

2.5.2 Test for Flavonoids

The alkaline reagent test was used for the qualitative detection of flavonoids. Approximately 1 mL of the plant extract was treated with 1 mL of 10% sodium hydroxide solution. The development of an intense yellow colour was taken as a positive indication for the presence of flavonoids. The observation was further assessed following acidification where necessary to confirm the colour response.

2.5.3 Test for Saponins

Approximately 2 mL of the plant extract was transferred into a test tube and vigorously shaken. The formation of persistent froth or foam was taken as evidence of the presence of saponins (Paul, 2016).

2.5.4 Test for Tannins

Approximately 0.5 mL of the plant extract was diluted with about 1 mL of distilled water, followed by the addition of 1–2 drops of ferric chloride solution. The development of a characteristic blue or blue-black colour was taken as evidence of the presence of tannins (Dutta, 2010).

2.5.5 Test for Phenols

A small portion of the plant extract was dissolved in distilled water, after which a few drops of ferric chloride solution were added. The development of a characteristic colour reaction was taken as evidence of the presence of phenolic compounds (Kakad, 2017).

2.5.6 Test for Glycosides

The extract was treated with glacial acetic acid followed by a few drops of ferric chloride solution. Concentrated sulphuric acid was then carefully introduced along the side of the test tube to form a separate layer. The development of a reddish-brown colour at the interface between the two layers, accompanied by a bluish-green colour in the upper layer, was taken as an indication of the presence of glycosides (Chhetri, 2008).

2.5.7 Test for Quinones

A small quantity of the plant extract was placed in a test tube and treated with concentrated hydrochloric acid. The formation of a yellow precipitate was considered indicative of the presence of quinones (Kakad, 2017).

2.6 Determination of Antioxidant Activity by DPPH Radical-Scavenging Assay

The antioxidant activity of the methanolic stem-bark extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay based on the method described by Amponsah et al. (2013), with appropriate modifications.

Five concentrations of the extract (30, 60, 90, 120, and 150 µg/mL) were prepared in methanol. For each concentration, 2.5 mL of the extract solution was mixed with 1.0 mL of freshly prepared 0.3 mM DPPH solution in methanol. The mixtures were incubated in the dark at room temperature for 30 min to allow the reaction to occur.

The absorbance of each reaction mixture was measured at 518 nm using a spectrophotometer. Methanol (1.0 mL) added to 2.5 mL of the corresponding extract concentration was used as the sample blank, while a mixture containing 1.0 mL of 0.3 mM DPPH solution and 2.5 mL of methanol served as the negative control. Ascorbic acid prepared

at the same concentrations as the test extract was used as the reference antioxidant.

The percentage inhibition of the DPPH radical was calculated using equation 2

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{A_c - A_s}{A_c} \times \frac{100}{1} \quad (2)$$

where A_c = absorbance of the control reaction; and A_s = absorbance of the sample reaction after appropriate blank correction.

The antioxidant activity was expressed as the percentage inhibition of DPPH radicals at each tested concentration.

2.7 In Vitro Anti-inflammatory Activity by Albumin Denaturation Assay

The in vitro anti-inflammatory activity of the methanolic stem-bark extract was evaluated using the inhibition of albumin denaturation method described by Suchita et al. (2017), with appropriate modifications.

The reaction mixture consisted of 0.2 mL of egg albumin, 2.8 mL of phosphate-buffered saline (PBS; pH 6.4), and 2.0 mL of the test extract at different concentrations (31.25, 62.5, 125, 250, 500, and 1000 µg/mL). A corresponding reaction mixture containing distilled water in place of the test extract served as the control.

The reaction mixtures were incubated at 37 °C for 15 min and subsequently heated at 70 °C for 5 min to induce protein denaturation. The mixtures were allowed to cool to room temperature, and the absorbance was measured at 660 nm using a spectrophotometer.

Diclofenac sodium was used as the reference anti-inflammatory drug. The reference drug was evaluated over the stated concentration range of 6.25–25 mg/mL under comparable experimental conditions.

The percentage inhibition of albumin denaturation was calculated using equation 3

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{A_c - A_s}{A_c} \times \frac{100}{1} \quad (3)$$

where A_c = absorbance of the control reaction; and A_s = absorbance of the reaction mixture containing the test sample after appropriate blank correction.

Higher percentage inhibition of albumin denaturation was interpreted as greater in vitro anti-inflammatory activity.

2.8 Isolation and Fractionation by Column Chromatography

The crude methanolic extract was subjected to column chromatographic separation to obtain fractions containing different groups of constituents. Approximately 5 g of the crude extract was loaded onto a silica-gel stationary phase packed in a chromatographic column. A solvent system consisting of *n*-hexane, ethyl acetate, and methanol in a ratio of 40:20:40 was used as the mobile phase.

As the mobile phase passed through the silica-gel column, the constituents of the crude extract were separated according to their differential interactions with the stationary and mobile phases. Compounds with different polarities migrated through the column at different rates. The eluates were collected in appropriately labelled fractions and monitored using thin-layer chromatography (TLC), where applicable, to assess their separation profiles.

Fractions exhibiting similar chromatographic characteristics were combined where appropriate and concentrated using a rotary evaporator or under suitable controlled conditions. The resulting fractions were coded and retained for subsequent spectroscopic characterization.

Important: If the study obtained fractions coded BS001, BS002, and BS003, this section should explicitly state how these fractions were obtained, their masses/yields, TLC profiles, and whether they were subsequently purified. Unless purity was established, they should be described as isolated fractions rather than pure compounds.

2.9 Characterization by Fourier-Transform Infrared Spectroscopy

The chromatographically obtained fractions of *Boswellia serrata* were subjected to Fourier-

transform infrared (FT-IR) spectroscopy to identify the major functional groups present in the samples.

A suitable quantity of each dried fraction was prepared for FT-IR analysis according to the operating procedure of the instrument. Where the potassium bromide (KBr) pellet technique was employed, the dried sample was thoroughly mixed with spectroscopic-grade KBr and compressed under pressure to form a translucent pellet. The pellet was then placed in the FT-IR sample holder, and the infrared spectrum was recorded over the operating spectral range of the instrument.

The characteristic absorption bands obtained from the spectra were compared with established infrared absorption ranges to assign the corresponding functional groups. Particular attention was given to absorption bands associated with hydroxyl (O–H), C–H, carbonyl (C=O), C=C, and other characteristic functional groups that may occur in the constituents of *B. serrata*.

Important correction: The original statement that the "sensor" shines "20 mL" of dilution through the sample is not appropriate for a KBr-pellet FT-IR procedure. The actual sample-preparation procedure and FT-IR operating range used in the laboratory should be stated accurately. If ATR-FTIR rather than KBr-pellet FTIR was used, this subsection should be rewritten accordingly.

2.10 Statistical Analysis

All antioxidant and anti-inflammatory experiments were performed in triplicate. The experimental results were expressed as mean ± standard deviation (SD). The data were analyzed using Statistical Package for the Social Sciences (SPSS), version 25. Where appropriate, concentration-dependent differences were evaluated using suitable statistical tests, and statistical significance was considered at $p < 0.05$. where IC_{50} values were determined for the DPPH assay, the concentration-response data were subjected to appropriate regression analysis to estimate the concentration of extract required to produce 50% inhibition of DPPH radical activity.

3.0 Results and Discussion

3.1 Preliminary Phytochemical Screening

The qualitative phytochemical screening of the methanolic stem-bark extract of *Boswellia serrata* revealed the presence of several classes of secondary metabolites, as presented in Table 1. Alkaloids, terpenoids, glycosides, phenols, flavonoids, and quinones were detected, whereas tannins and saponins were not detected under the experimental conditions employed.

Table 1 demonstrates that the methanolic extract contained a chemically diverse group of secondary metabolites. The detection of phenols and flavonoids is particularly relevant to the antioxidant activity subsequently observed in the DPPH assay because these classes of compounds commonly possess hydroxyl groups capable of donating hydrogen atoms or electrons to reactive species. Phenolic and flavonoid compounds can therefore contribute to free-radical scavenging through hydrogen-atom transfer and electron-transfer mechanisms. The presence of terpenoids is also noteworthy because terpenoid constituents are characteristic components of *Boswellia* species and have been associated with several pharmacological activities, including anti-inflammatory effects.

The detection of alkaloids and glycosides further indicates that the biological properties of the extract may result from the combined effects of several classes of phytochemicals rather than a single constituent. Quinones were also detected and may contribute to the biological activity of the extract through their redox properties. However, qualitative phytochemical screening only establishes the presence or absence of broad classes of compounds and does not provide information about their concentrations or individual molecular identities. Therefore, the phytochemical results should be interpreted as

preliminary evidence and complemented by chromatographic and spectroscopic characterization.

The present findings are broadly consistent with previous investigations of *Boswellia* species. Kushwaha and Tyagi (2021) reported substantial phytochemical constituents and antioxidant activity in *B. serrata* leaf extracts and observed a positive association between phenolic content and antioxidant capacity. Similarly, Biswas et al. (2024) reported significant antioxidant and anti-inflammatory activities in *B. serrata* leaf extract and attributed these effects to its bioactive phytochemical constituents. The findings also agree with Akomolafe et al. (2025), who demonstrated considerable variation in the phytochemical composition of different plant parts of *Boswellia dalzielii*, with stem-bark extracts showing particularly notable antioxidant activity in some of the assays investigated. These comparisons emphasize that the phytochemical composition of *Boswellia* is influenced by species and plant part.

The absence of tannins and saponins in the present extract does not necessarily indicate their complete absence from the plant. Qualitative reactions are affected by extraction solvent, concentration, detection sensitivity, sample preparation, and assay conditions.

Table 1: Qualitative Phytochemical Constituents of the Methanolic Stem-Bark Extract of *Boswellia serrata*

Phytochemical constituent	Result
Alkaloids	+
Tannins	−
Terpenoids	+
Glycosides	+
Saponins	−
Phenols	+
Flavonoids	+
Quinones	+

Key: + = Present; − = Absent.

Consequently, the observed phytochemical profile should be considered specific to the methanolic stem-bark extract examined in this study.

From the results, it is evidence that, the phytochemical profile presented in Table 1 provides a plausible chemical basis for the antioxidant and anti-inflammatory activities observed in the subsequent assays. Nevertheless, further quantitative phytochemical analysis and compound-level identification are required to establish which constituents are principally responsible for the observed activities.

3.2 Column Chromatographic Fractionation

The methanolic stem-bark crude extract of *Boswellia serrata* was subjected to silica-gel column chromatography to separate constituents according to their differential interactions with the stationary and mobile phases. Three major fractions, coded BS001, BS002, and BS003, were obtained. The reported chromatographic separation gave an (Rf) value of approximately 0.55 using a solvent system consisting of methanol:ethyl acetate:*n*-hexane in the ratio of 40:20:40.

The recovery of three chromatographic fractions indicates that the crude methanolic extract contained constituents with sufficiently different physicochemical properties to permit partial separation under the chromatographic conditions employed. This observation is consistent with the qualitative phytochemical screening in Table 1, which demonstrated the coexistence of several chemically distinct classes of metabolites in the crude extract.

Column chromatography is particularly useful for the preliminary fractionation of complex plant extracts because compounds of different polarity and affinity for silica gel migrate through the stationary phase at different rates. The resulting fractions can subsequently be subjected to spectroscopic and chromatographic characterization. Kushwaha and Tyagi (2021) similarly emphasized the usefulness of chromatographic fractionation

for separating potentially bioactive fractions of *B. serrata* extracts, particularly those associated with substantial phenolic content and antioxidant activity.

The present study should, however, distinguish clearly between fractions and pure compounds. Since the experimental results identify BS001, BS002, and BS003 as fractions, they should not be described as pure compounds unless further purification and analytical evidence demonstrate chemical homogeneity. This distinction is important because a fraction may contain several structurally related or unrelated constituents that can collectively contribute to its biological activity.

The chromatographic separation also provides an important basis for subsequent characterization. Sivapalan et al. (2024) demonstrated that combining biological assays, chromatographic fractionation, GC-MS analysis, and subsequent structural characterization can facilitate the identification of bioactive constituents from medicinal plants. Accordingly, the fractions obtained in the present study represent useful intermediate materials for future purification and detailed structural elucidation.

3.3 In Vitro Anti-inflammatory Activity of Boswellia serrata

The in vitro anti-inflammatory activity of the methanolic stem-bark extract of *Boswellia serrata* was evaluated using the inhibition of albumin denaturation assay. The results obtained for the extract and diclofenac sodium are presented in Table 2. The assay showed that the *B. serrata* extract exhibited measurable concentration-dependent protection against protein denaturation, although the magnitude of inhibition was substantially lower than that observed for diclofenac at most of the tested concentrations.

As shown in Table 2, the percentage inhibition produced by the *B. serrata* extract increased from 32.16 ± 0.24% at 31.25 µg/mL to 46.82 ± 0.46% at 1000 µg/mL. Although some fluctuations occurred between 62.5 and 250 µg/mL, the overall response showed an increasing tendency with increasing extract concentration. This suggests that increasing concentrations of the extract progressively enhanced its ability to prevent heat-induced albumin denaturation. The relatively small standard deviations at most concentrations also indicate reasonable repeatability among the triplicate measurements.

In comparison, diclofenac produced substantially higher inhibition at all concentrations investigated, increasing from 60.85 ± 0.31% at 31.25 µg/mL to 71.75 ± 0.24% at 1000 µg/mL. Thus, based on the observed percentage inhibition values, diclofenac demonstrated stronger activity than the crude *B. serrata* extract under the experimental conditions used. This comparison is important because the crude extract contains a complex mixture of compounds, whereas diclofenac is a defined pharmaceutical compound with established pharmacological activity.

Table 2: In Vitro Anti-inflammatory Activity of the Methanolic Stem-Bark Extract of *Boswellia serrata*

Concentration (µg/mL)	Diclofenac (% inhibition)	*B. serrata* extract (% inhibition)
31.25	60.85 ± 0.31	32.16 ± 0.24
62.50	64.00 ± 1.50	34.57 ± 0.34
125	67.62 ± 0.29	33.55 ± 0.47
250	69.75 ± 0.21	34.90 ± 0.86
500	71.38 ± 0.11	39.70 ± 0.90
1000	71.75 ± 0.24	46.82 ± 0.46

The observed anti-inflammatory activity of the extract is nevertheless significant from a phytochemical perspective because the extract

contains terpenoids, flavonoids, alkaloids, phenols, and glycosides (Table 1), several of which may contribute to modulation of inflammatory processes. The result is also consistent with previous reports demonstrating anti-inflammatory activity in *B. serrata*. Bertocchi et al. (2018) reported that *B. serrata* extracts exhibited anti-inflammatory activity in porcine aortic endothelial cells exposed to lipopolysaccharide-induced stress. Importantly, their findings also showed that the magnitude of the response depended on the formulation and concentration of the extract, emphasizing that the biological effects of *Boswellia* preparations cannot necessarily be attributed to a single constituent.

Biswas et al. (2024) likewise reported significant anti-inflammatory activity of *B. serrata* leaf extract in vitro, including concentration-dependent modulation of inflammatory mediators such as TNF-α, IL-6, and IL-1β. Although their study investigated leaves rather than stem bark and employed different mechanistic assays, the findings support the broader anti-inflammatory potential of *B. serrata*. The differences in activity between the present stem-bark extract and previously studied leaf or resin preparations may reflect differences in plant part, phytochemical composition, extraction conditions, and assay methodology.

The presence of terpenoids in the present extract is particularly relevant because terpenoid-rich fractions are characteristic of *Boswellia* preparations and have been associated with anti-inflammatory effects. The detected phenols and flavonoids may also contribute through antioxidant mechanisms, since oxidative stress can amplify inflammatory signalling. Thus, the antioxidant and anti-inflammatory activities observed in this study may be chemically interconnected rather than independent phenomena.

A critical issue concerns the reported IC_{50} value of 32.17 µg/mL for the *B. serrata* extract and 71.30 µg/mL for diclofenac. These values, as currently presented, are not consistent with the inhibition data in Table 2. The *B. serrata* extract produced only 46.82% inhibition at the highest tested concentration of 1000 µg/mL; therefore, an IC_{50} of 32.17 µg/mL cannot be derived directly from these displayed data without an alternative calculation or a different underlying dataset. Moreover, the extract would need to produce at least 50% inhibition within or around the tested concentration range for a conventional experimental IC_{50} to be directly established. Consequently, the reported IC_{50} values should be recalculated from the original absorbance data before publication.

Based on the observed inhibition percentages alone, the present results support anti-inflammatory activity of the extract but do not support the conclusion that the crude extract was more potent than diclofenac. This distinction is important for scientific interpretation and should be corrected in the manuscript.

The findings nevertheless have potential implications for medicinal-plant research. The moderate activity of the crude extract suggests that the active constituents may occur at relatively low concentrations or may require further enrichment through chromatographic fractionation. Future bioassay-guided fractionation of BS001–BS003 could therefore determine whether individual fractions possess stronger activity than the crude extract. Such an approach could identify constituents with greater activity and provide a basis for subsequent mechanistic and toxicological studies.

3.4 DPPH Radical-Scavenging Activity

The antioxidant activity of the methanolic stem-bark extract of *Boswellia serrata* was evaluated using the DPPH radical-scavenging assay. The assay measures the ability of antioxidant constituents to donate hydrogen atoms or electrons to the stable DPPH radical, resulting in a reduction in its characteristic purple colour. The percentage inhibition values

obtained for the extract and ascorbic acid are presented in Table 3.

The results demonstrate that the *B. serrata* extract possessed measurable antioxidant activity, which increased progressively with concentration. DPPH inhibition increased from 28.59 ± 8.30% at 30 µg/mL to 56.44 ± 0.39% at 150 µg/mL. This concentration-dependent increase indicates that the extract contains constituents capable of donating hydrogen atoms or electrons to DPPH radicals. The relatively large standard deviation at the lowest concentration compared with the higher concentrations may reflect greater experimental variability when the magnitude of radical scavenging is relatively low.

As shown in Table 3, ascorbic acid produced considerably greater DPPH inhibition than the *B. serrata* extract at every tested concentration, with values increasing from 71.64 ± 0.21% at 30 µg/mL to 90.31 ± 0.02% at 150 µg/mL. Thus, contrary to the original interpretation, the displayed experimental data indicate that ascorbic acid exhibited stronger radical-scavenging activity than the crude *B. serrata* extract under the conditions employed.

Table 3: DPPH Radical-Scavenging Activity of the Methanolic Stem-Bark Extract of *Boswellia serrata*

Concentration (µg/mL)	Ascorbic acid (% inhibition)	*B. serrata* extract (% inhibition)
30	71.64 ± 0.21	28.59 ± 8.30
60	76.54 ± 0.15	35.26 ± 0.43
90	82.52 ± 0.04	44.51 ± 0.27
120	87.43 ± 0.06	53.49 ± 0.44
150	90.31 ± 0.02	56.44 ± 0.39

The observed antioxidant activity of the extract can be related to the phytochemical profile presented in Table 1. In particular, the presence of phenols and flavonoids provides a plausible explanation for the radical-scavenging activity because these compounds commonly possess functional groups capable of donating hydrogen atoms or electrons to free radicals. Terpenoid constituents may also contribute to the antioxidant response. However, because the phytochemical analysis was qualitative, it is not possible to determine from the present data which specific constituents are principally responsible for the observed activity.

The present findings are consistent with the observations of Kushwaha and Tyagi (2021), who reported appreciable antioxidant activity in *B. serrata* leaf extracts and fractions, with higher phenolic content being associated with stronger antioxidant capacity. Biswas et al. (2024) also demonstrated significant antioxidant activity in *B. serrata* leaf extract, particularly at higher concentrations. Although these studies investigated leaves rather than stem bark, the agreement supports the presence of antioxidant constituents within different parts of *B. serrata*.

The findings can also be compared with those reported for *B. dalzielii*. Akomolafe et al. (2025) found that different plant parts displayed substantially different antioxidant properties, with the stem bark demonstrating notable DPPH radical-scavenging and ferric-reducing activities. This comparison reinforces the importance of plant part in determining antioxidant potential. Differences between the present results and those reported for other *Boswellia* preparations may also arise from differences in species, solvent, extraction conditions, geographical origin, phytochemical concentration, and assay methodology.

The reported IC_{50} values were 65.84 µg/mL for the *B. serrata* extract and 67.22 µg/mL for ascorbic acid. If these values were calculated by regression from the full concentration-response dataset, they should be retained only after verification against the original absorbance values and regression model. Based

solely on the percentage inhibition data displayed in Table 3, ascorbic acid is clearly more active than the crude extract across the tested concentration range. Therefore, the original statement that the extract possessed higher antioxidant activity than ascorbic acid merely because it had a lower reported IC_{50} should be reconsidered.

The relatively high antioxidant activity observed at 150 µg/mL, where the extract achieved 56.44% inhibition, nevertheless indicates that the stem-bark extract possesses meaningful radical-scavenging capacity. This has potential implications for further fractionation because concentration or enrichment of phenolic, flavonoid, or terpenoid constituents may enhance the activity of individual fractions. The observed activity therefore provides a useful basis for further phytochemical isolation and mechanistic investigation.

The present results are also broadly consistent with the general observation of Ibrahim et al. (2015) that plant extracts can exhibit appreciable DPPH radical-scavenging activity, although direct quantitative comparison should be made cautiously because different plant species, extraction procedures, concentrations, assay conditions, and reference standards may have been used.

3.5 FT-IR Characterization of Boswellia serrata Extract

The FT-IR spectrum of the methanolic *Boswellia serrata* extract provided information on the principal functional groups present in the extract. As illustrated in Figure 1, prominent absorption bands occurred at 3395.6, 2935.1, 2872.2, 2227.2, 1717.0, and 1462.1 cm^{-1}. These absorption bands indicate the presence of several important chemical functionalities and provide spectroscopic evidence supporting the diverse phytochemical composition observed during preliminary screening.

Fig. 1 shows a broad absorption band at 3395.6 cm^{-1}, which can be assigned primarily to O–H stretching vibrations. This band is consistent with hydroxyl-containing compounds such as alcohols and phenolic constituents. Its presence is particularly relevant to the detection of phenols in Table 1 and may also be associated with other hydroxyl-containing metabolites. Hydroxyl groups are capable of participating in hydrogen donation and may therefore contribute to the antioxidant activity of the extract.

The absorption band at 2935.1 cm^{-1} is more appropriately assigned to aliphatic C–H stretching, particularly asymmetric stretching of CH_2/CH_3 groups, rather than directly to a carboxylic group. The band at 2872.2 cm^{-1} can similarly be attributed to aliphatic C–H stretching vibrations. These bands suggest the presence of hydrocarbon chains and aliphatic structural components, which are compatible with the terpenoid-rich chemistry commonly associated with *Boswellia* species.

The absorption band at 2227.2 cm^{-1} lies within a region associated with C≡C stretching of alkynes and, depending on the precise spectral profile, may also overlap with nitrile (C≡N) stretching. Therefore, assignment of this band specifically to an alkyne should be considered tentative unless supported by additional spectroscopic or chromatographic evidence. FT-IR data alone are generally insufficient to establish the precise identity of a particular compound.

The strong band at 1717.0 cm^{-1} is consistent with C=O stretching vibrations, indicating the presence of carbonyl-containing compounds such as ketones, aldehydes, esters, acids, or related oxygenated metabolites. Carbonyl-containing constituents are chemically plausible in complex plant extracts and may contribute to the biological properties of the sample. The absorption at 1462.1 cm^{-1} is consistent with C–H bending vibrations, particularly deformation of CH_2 and/or CH_3 groups.

The results is broadly compatible with the qualitative phytochemical results presented in Table 1. The hydroxyl band at 3395.6 cm^{-1} supports the possible presence of phenolic and other hydroxyl-containing compounds, while the aliphatic C–H bands are consistent with terpenoid or other hydrocarbon-containing constituents. The carbonyl absorption at 1717.0 cm^{-1} further indicates the presence of oxygenated compounds. These observations provide a chemical basis for the antioxidant and anti-inflammatory activities reported in Tables 2 and 3, although FT-IR cannot by itself establish which individual compound is responsible for these activities.

Table 4: Major FT-IR Absorption Bands and Tentative Functional-Group Assignments

Wavenumber (cm^{-1})	Tentative assignment	Possible structural implication
3395.6	O–H stretching	Alcohols/phenols; hydrogen-bonded hydroxyl groups
2935.1	Aliphatic C–H stretching	CH_2/CH_3-containing aliphatic structures
2872.2	Aliphatic C–H stretching	CH_2/CH_3 groups
2227.2	C≡C and/or C≡N stretching	Possible unsaturated or nitrile-containing functionality; requires confirmation
1717.0	C=O stretching	Carbonyl-containing compounds
1462.1	C–H bending	CH_2/CH_3 deformation

The functional groups identified by FT-IR are The FT-IR findings also complement the chromatographic fractionation described in Section 3.2. The presence of several distinct functional groups confirms that the crude extract contains chemically diverse constituents, supporting the need for chromatographic separation before detailed compound identification. In this regard, the use of GC-MS in the broader characterization strategy would be particularly valuable because it can provide molecular-level information about volatile and semi-volatile constituents that cannot be conclusively identified from FT-IR alone.

Figure 1: FT-IR Spectrum of the Methanolic Stem-Bark Extract of *Boswellia serrata*

The spectrum in Figure 1 therefore provides preliminary structural information rather than definitive compound identification. Further characterization using GC-MS, NMR spectroscopy, or other complementary analytical techniques would be necessary to establish the molecular identities of the constituents associated with the observed absorption bands.

3.6 Integrated Interpretation and Implications of the Findings

Taken together, the findings demonstrate that the methanolic stem-bark extract of *Boswellia serrata* contains several classes of secondary metabolites and possesses measurable antioxidant and anti-inflammatory activities. The phytochemical screening in Table 1 identified alkaloids, terpenoids, glycosides, phenols, flavonoids, and quinones, providing a plausible chemical basis for the biological activities observed. The chromatographic separation further demonstrated that the crude extract could be separated into at least three fractions, BS001, BS002, and BS003, indicating chemical heterogeneity within the extract.

The DPPH assay in Table 3 demonstrated a clear increase in radical-scavenging activity with increasing extract concentration, from 28.59 ± 8.30% at 30 µg/mL to 56.44 ± 0.39% at 150 µg/mL. This concentration-response pattern indicates that the extract contains antioxidant constituents capable of reacting with DPPH radicals. Nevertheless, the activity remained lower than that of ascorbic acid at all tested concentrations. The finding should therefore be interpreted as evidence of appreciable antioxidant potential rather than superiority over the reference antioxidant.

Similarly, the albumin-denaturation assay in Table 2 demonstrated measurable anti-inflammatory activity, with inhibition increasing overall from 32.16 ± 0.24% at 31.25 µg/mL to 46.82 ± 0.46% at 1000 µg/mL. However, diclofenac produced substantially higher inhibition across the concentration range. The extract therefore demonstrated moderate in vitro anti-inflammatory potential, but the available data do not support the claim that it was more potent than diclofenac.

The combined antioxidant and anti-inflammatory activities are scientifically relevant because oxidative stress and inflammation are biologically interconnected processes. Constituents capable of scavenging reactive species may indirectly reduce oxidative amplification of inflammatory pathways, while anti-inflammatory phytochemicals may attenuate cellular responses associated with oxidative damage. Previous studies of *B. serrata* and related *Boswellia* species support this general relationship (Bertocchi et al., 2018; Biswas et al., 2024; Hamadjida et al., 2024).

The FT-IR spectrum in Fig, 1 provides additional evidence of chemical diversity, with hydroxyl, aliphatic C–H, carbonyl, and other characteristic absorption bands. These observations correspond broadly with the phytochemical classes detected in Table 1. However, the results should not be interpreted as proof that a particular functional group or phytochemical is solely responsible for the measured biological activities. Plant extracts contain complex mixtures in which additive or synergistic interactions may occur.

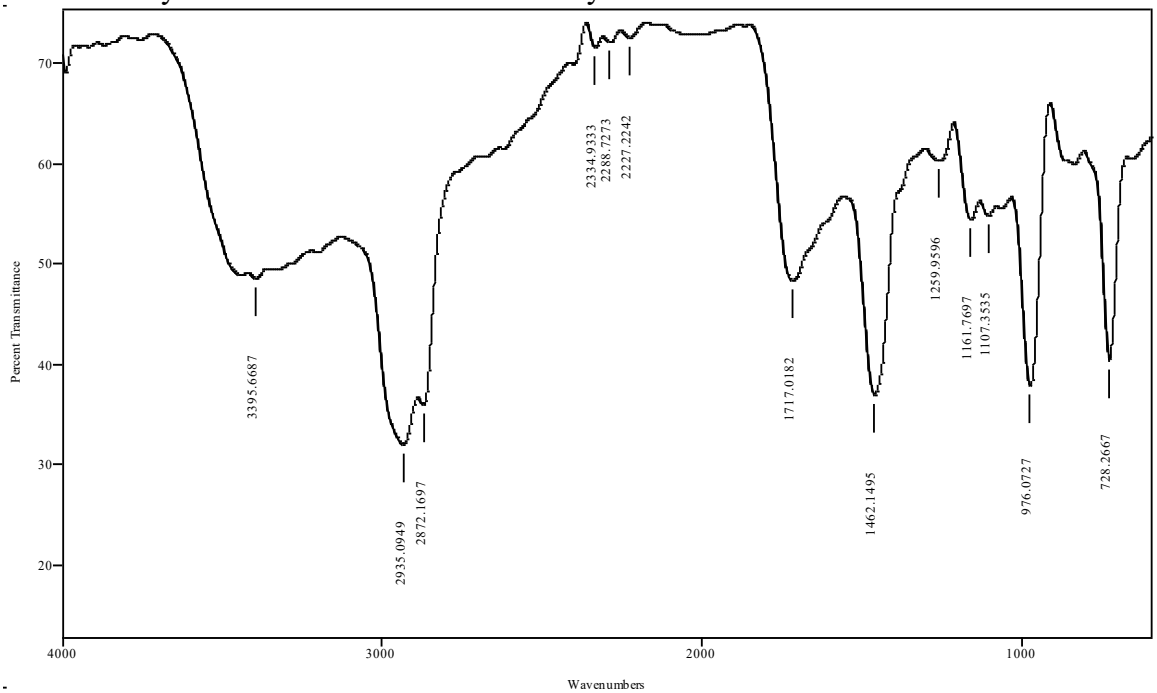

Fig. 1: The FTIR Spectral of the extract of *Boswellia serrata*

From an applied perspective, the findings support the potential of *B. serrata* stem bark as a source of natural antioxidant and anti-inflammatory constituents. The results also justify further bioassay-guided fractionation of BS001–BS003 to determine whether individual fractions possess greater biological activity than the crude extract. This approach could potentially lead to the identification of constituents with enhanced activity and provide information for subsequent mechanistic, toxicological, and in vivo studies. However, the present findings should be regarded as preliminary in vitro evidence rather than confirmation of therapeutic efficacy. DPPH radical-scavenging activity does not necessarily translate directly into antioxidant activity in biological systems, while albumin denaturation is a preliminary model of anti-inflammatory potential and does not reproduce the complex cellular pathways involved in inflammation. Consequently, further investigations using additional antioxidant assays, cellular inflammatory models, quantitative phytochemical analysis, GC-MS and/or NMR characterization, cytotoxicity studies, and appropriately designed in vivo experiments are required before any therapeutic conclusions can be drawn.

Finally, the results demonstrate that *B. serrata* stem bark collected from Jalingo, Taraba State, contains diverse phytochemical constituents and exhibits measurable antioxidant and anti-inflammatory properties. These findings provide preliminary scientific support for the medicinal potential of the plant material and establish a basis for further isolation, structural characterization, and biological evaluation of its active constituents.

4.0 Conclusion

This study demonstrated that the methanolic stem-bark extract of *Boswellia serrata* collected from Jalingo Local Government Area of Taraba State, Nigeria, contains diverse phytochemical constituents, including alkaloids, terpenoids, glycosides, phenols, flavonoids, and quinones. The extract exhibited measurable antioxidant activity in the DPPH radical-scavenging assay, with activity increasing with increasing extract concentration. It also demonstrated in vitro anti-inflammatory activity through inhibition of albumin denaturation. FT-IR analysis further revealed functional groups associated with hydroxyl, aliphatic C–H, carbonyl, and other chemical functionalities, supporting the presence of structurally diverse constituents in the extract. Chromatographic fractionation also yielded three fractions, designated BS001, BS002, and BS003.

The findings indicate that *B. serrata* stem bark possesses promising antioxidant and anti-inflammatory potential, which may be associated with the combined effects of its phytochemical constituents, particularly phenolic, flavonoid, and terpenoid compounds. However, the activities observed in the present study are based on in vitro assays and should not be interpreted as direct evidence of therapeutic efficacy in humans. Further chemical and biological investigations are therefore necessary to identify the specific active constituents and establish their mechanisms of action, safety, and therapeutic potential.

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Declarations

Consent for Publication

Not applicable.

Availability of Data

The data used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare that they have no competing interests and no conflict of interest regarding the publication of this manuscript.

Ethical Consideration

Not applicable.

Funding

The authors declared no source of external funding.

Authors' Contributions

All the authors were involved collectively in all aspect of the work