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Antioxidant And Anti-Inflammatory Activities Of Boswellia Serrata Leaf Extract: A Pharmacological Perspective

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ABSTRACT
This study aimed to evaluate the antioxidant and anti-inflammatory activities of *Boswellia serrata* leaf extract using various in vitro mechanistic models. Building on its traditional use in managing inflammatory and oxidative stress-related disorders, *Boswellia serrata* has gained attention for its potential therapeutic benefits, largely attributed to its bioactive components, such as boswellic acids. The research employed several in vitro assays to assess the extract's ability to neutralize reactive oxygen species (ROS) and modulate the production of key pro-inflammatory cytokines, including IL-1β, TNF-α, and IL-6. Results demonstrated that the extract exhibits significant antioxidant activity, particularly at higher concentrations, effectively scavenging free radicals. Additionally, the extract showed a dose-dependent reduction in pro-inflammatory cytokine levels, suggesting its strong anti-inflammatory potential. These findings underscore the therapeutic promise of *Boswellia serrata* leaf extract as a natural remedy for conditions characterized by oxidative stress and chronic inflammation. The study provides a solid foundation for further research into the specific bioactive compounds responsible for these effects and their possible applications in clinical settings.

Keywords: *Boswellia serrata*, Antioxidant, Oxidative stress, anti-inflammatory

INTRODUCTION

Inflammation and oxidative stress are linked biological processes that are important in the aetiology of many chronic illnesses, such as diabetes, cancer, heart disease, and neurological disorders. Research on medical processes has shifted significantly to try to understand these mechanisms and discover solutions to reduce their negative impacts. Because of their wide range of bioactive chemicals, medicinal plants have attracted a lot of interest for their ability to fight inflammation and oxidative stress, providing a safe, easy way to prevent and cure these conditions [(1-4)]. When the body's capacity to detoxify these dangerous byproducts or heal the consequent damage is out of balance, reactive oxygen species (ROS) are produced at an excessive rate, leading to oxidative stress. Free radicals like superoxide and hydroxyl radicals are examples of ROS that are produced naturally during normal cellular metabolism. Nevertheless, the body's antioxidant defences may be overpowered by excessive ROS production, which is brought on by things like radiation, environmental pollutants, and persistent inflammation. This results in oxidative damage to proteins, lipids, and DNA, which aggravates cellular malfunction and promotes the emergence of chronic illnesses [(1-7)]. The body uses a sophisticated system of antioxidants to keep cells in a homeostasis and counteract reactive oxygen species. Superoxide dismutase (SOD) and glutathione peroxidase are examples of enzymatic antioxidants in this system. Non-enzymatic antioxidants include glutathione, vitamin C, and vitamin E. Oxidative stress, which is brought on by weakened defences, can activate signalling pathways that encourage inflammation and cellular damage [(8-11)].

The immune system being activated and pro-inflammatory cytokines, chemokines, and other mediators being released are the hallmarks of inflammation, which is a normal and necessary reaction to infection or damage. Acute inflammation aids in healing and is a protective reaction; however, persistent inflammation is harmful and linked to

several chronic illnesses. Unresolved infections, ongoing irritation, or immune response dysregulation can all lead to persistent inflammation [1-7]. Sustained oxidative stress is frequently associated with chronic inflammation, resulting in a vicious cycle in which the creation of ROS exacerbates inflammatory responses, which in turn raises ROS levels. The relationship between inflammation and oxidative stress is linked to the development of conditions including Alzheimer's disease, rheumatoid arthritis, and atherosclerosis [12-16].

Because of their therapeutic qualities, medicinal plants have been employed for ages in traditional medical systems around the world. The medicinal potential of these plants has been more and more supported by scientific study in recent years, especially when it comes to oxidative stress and inflammation. Phytochemicals with strong antioxidant and anti-inflammatory properties, such as polyphenols, flavonoids, terpenes, and alkaloids, are abundant in many therapeutic plants [17-19]. The oxidative stress caused by ROS can be decreased by the phytochemicals found in medicinal plants. For instance, flavonoids, which are abundant in plants like berries and green tea, are well-known for having potent antioxidant qualities. They can provide electrons to counteract free radicals and shield cells from oxidative damage. By upregulating the expression of antioxidant enzymes, polyphenols—an additional class of chemicals frequently present in medicinal plants like grape seeds and turmeric—enhance the body's natural antioxidant defences [20-24]. Many medicinal plants also possess anti-inflammatory properties, making them valuable in managing chronic inflammatory conditions. For instance, curcumin, a compound found in turmeric, has been shown to inhibit the activation of nuclear factor-kappa B (NF-κB), a key transcription factor that regulates the expression of pro-inflammatory genes. Similarly, boswellic acids from *Boswellia serrata* have been found to inhibit the production of pro-inflammatory cytokines, reducing inflammation [25, 26].

The dual role of medicinal plants in combating oxidative stress and inflammation underscores their importance in the prevention and treatment of chronic diseases. By harnessing the bioactive compounds found in these plants, it is possible to develop natural therapies that offer protection against the damaging effects of oxidative stress and chronic inflammation. As research continues to uncover the mechanisms underlying the therapeutic effects of medicinal plants, these natural resources will likely play an increasingly significant role in modern medicine, providing a complementary approach to conventional treatments for a wide range of diseases.

Boswellia serrata, commonly known as Indian frankincense, has been a vital component in traditional medicine systems, particularly in India, where it is referred to as "Salai guggul." In Ayurveda, the ancient Indian system of medicine, *Boswellia serrata* resin has been used for centuries to treat various ailments. It is highly valued for its anti-inflammatory properties and is traditionally used to alleviate conditions such as arthritis, asthma, and inflammatory bowel diseases [27, 28]. The resin, often called "shallaki," is also applied in treating wounds, promoting healing due to its antimicrobial properties. Beyond India, *Boswellia serrata* has been used in traditional practices across other cultures as well [28, 29]. In African and Middle Eastern medicine, the resin is burned as incense, valued for its ability to purify the air and ward off evil spirits, while also being used internally for digestive and respiratory ailments. In traditional Chinese medicine, *Boswellia* is utilized for its pain-relieving and anti-inflammatory effects, often in combination with other herbs to treat joint and muscle pain. These traditional uses of *Boswellia serrata* highlight its importance in natural medicine, reflecting a rich history of application across diverse cultures [29, 30].

Building upon the extensive literature review and the compelling evidence highlighted by previous research, this study was designed with the specific objective of evaluating the antioxidant and anti-inflammatory activities of *Boswellia serrata* leaf extract. The rich history of *Boswellia serrata* in traditional medicine, particularly its documented use in managing inflammatory conditions and oxidative stress-related disorders, provided a strong rationale for this investigation. Given the plant's known bioactive components, such as boswellic acids and other terpenoids, which have been reported to possess significant anti-inflammatory and antioxidant properties, it was deemed critical to explore these effects through a series of in vitro mechanistic models. The focus was not only on quantifying its ability to scavenge reactive oxygen species (ROS) but also on understanding how the extract modulates the production of pro-inflammatory cytokines and other mediators of inflammation. This approach was intended to provide a thorough understanding of the extract's therapeutic potential, laying the groundwork for future studies that could explore its efficacy in clinical settings. The anticipated outcomes of this research were expected to reinforce the traditional uses of *Boswellia serrata* and contribute to the growing body of evidence supporting its use as a natural remedy for conditions associated with oxidative stress and inflammation.

MATERIAL AND METHODS

Drugs, chemicals, and reagents

The extraction process utilized ethanol, methanol, and distilled water, all of which were high analytical grade and sourced from Loba Chemie, Mumbai, India. Enzyme-linked immunosorbent assay (ELISA) kits, which are particularly made to identify pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6, were critical reagents for the anti-inflammatory tests. RAW 264.7 macrophage cells, the required culture medium, foetal bovine serum (FBS), and antibiotics such streptomycin and penicillin were used in the in vitro cell culture investigations. To cause inflammation in these cells, lipopolysaccharide (LPS) was obtained from Himedia and Sigma Aldrich in India. All chemicals and

drugs were obtained from reliable suppliers to ensure their purity and consistency, contributing to reproducible and accurate experimental results.

Collection, authentication, and extraction of the plants

The *Boswellia serrata* plant was sourced from the Solan district of Himachal Pradesh. A herbarium specimen was prepared, and the plant's identity was confirmed by a botanist. The leaves were shade-dried and then ground into a coarse powder using a mechanical grinder. This powder underwent cold maceration in methanol for 10 days. Afterward, the extract was collected and dried in a vacuum desiccator. The final extract, designated as BSLE-M, was stored at -4°C for future use.

Preliminary Phytochemical study

The plant extract underwent preliminary phytochemical screening to identify the compounds that might contribute to the plant's medicinal properties. This essential step was crucial for understanding the bioactive components within the extract that could influence antioxidant and anti-inflammatory pathways. The phytochemical analysis included a series of qualitative tests aimed at detecting various classes of compounds, such as alkaloids, flavonoids, tannins, saponins, sterols, and phenolic compounds. Specific tests, including the Dragendorff test for alkaloids, the Shinoda test for flavonoids, and the Froth test for saponins, were utilized. These screenings were performed using both aqueous and methanolic solvents to ensure a wide range of phytochemicals was extracted, recognizing that different solvents can isolate different compounds. Because of their well-known anti-inflammatory and antioxidant characteristics, the presence of flavonoids and phenolic acids was of special interest. Overall, the phytochemical screening laid the foundation for additional pharmacological research by offering insightful information about the complex composition of the extracts.

Antioxidant activity

ABTS radical decolorization assay

An established technique for assessing the antioxidant activity of plant extracts and other compounds is the ABTS radical decolourisation test [31, 32]. First, distilled water is used to create a stock solution containing 7 mM ABTS. Separately, a 2.45 mM potassium persulfate solution is also made. After mixing these two solutions 1:1 and letting them react for 12–16 hours at room temperature in the dark, the resulting product is the blue-green ABTS radical cation (ABTS•+). To get an absorbance of 0.70 ± 0.02 at 734 nm, the ABTS•+ solution is diluted with methanol or another appropriate solvent before to use. After that, methanol or another suitable solvent is used to dissolve the plant extracts or test samples. In order to conduct the experiment, 10 µL of the sample or standard (Trolox) and 990 µL of the diluted ABTS•+ solution are combined in a test tube, well mixed, and allowed to sit in the dark at room temperature for six minutes. After that, a UV-Vis spectrophotometer is used to measure the absorbance of the reaction mixture at 734 nm. The percentage inhibition of the ABTS•+ radical cation is used to determine the antioxidant activity of the sample: Inhibition (%) = [(Absorbance of Control - Absorbance of Sample) / Absorbance of Control] × 100. The antioxidant activity can be expressed as Trolox Equivalent Antioxidant Capacity (TEAC), which is expressed in µmol Trolox equivalent per gramme of sample, by comparing this inhibition percentage to a Trolox standard curve. To assure accuracy and repeatability, the test is usually run in triplicate. This technique offers a dependable and effective means of evaluating the antioxidant capacity of different compounds.

Hydroxyl Radical Scavenging Activity (HRSA)

The Hydroxyl Radical Scavenging Activity (HRSA) assay is a commonly used method to evaluate the ability of a substance to scavenge hydroxyl radicals [33], which are highly reactive species capable of causing significant damage to biological molecules such as DNA, proteins, and lipids. The assay typically involves generating hydroxyl radicals through the Fenton reaction, where hydrogen peroxide (H_2O_2) is reacted with ferrous ions (Fe^{2+}) to produce hydroxyl radicals [33].

To begin the assay, a reaction mixture is prepared, usually consisting of a suitable buffer (such as phosphate buffer), ferrous sulfate ($FeSO_4$), hydrogen peroxide (H_2O_2), and the test sample or standard. The test sample is dissolved in an appropriate solvent, typically water or methanol, to the desired concentration. The reaction is initiated by adding hydrogen peroxide to the mixture, and the hydroxyl radicals generated in this process will interact with a scavenger or probe, often deoxyribose or a similar compound. The hydroxyl radicals degrade the deoxyribose, leading to the formation of products that can be measured spectrophotometrically. The degree of scavenging by the test sample is determined by measuring the decrease in absorbance at a specific wavelength (typically around 532 nm, depending on the probe used). The results are usually expressed as a percentage inhibition of hydroxyl radical formation, calculated using the formula: Inhibition (%) = [(Absorbance of Control - Absorbance of Sample) / Absorbance of Control] × 100. The antioxidant capacity of the test sample can also be compared to a standard antioxidant, such as ascorbic acid or Trolox, to express the results in terms of equivalent antioxidant capacity. Performing the assay in triplicate or more is recommended to ensure reproducibility and reliability of the results. The HRSA assay is particularly useful for assessing

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the potential of natural extracts, pharmaceuticals, and other substances to protect against oxidative damage by neutralizing hydroxyl radicals.

Anti-inflammatory activity

Anti-inflammatory activity using RAW 264.7 macrophages

To evaluate the anti-inflammatory activity of a plant extract using RAW 264.7 macrophages [34, 35], start by cultivating the RAW 264.7 cells in Dulbecco's Modified Eagle Medium (DMEM), which has 1% penicillin-streptomycin and 10% foetal bovine serum (FBS) added. The cells need to be kept in a humidified environment with 5% CO2 at 37°C. When the cells attain 70–80% confluence, more experiments can begin. Using a cell scraper or pipette, carefully separate the cells, and then count them with a hemocytometer. Following that, the cells are plated in 24-well plates at a density of 5 × 10^5 cells per well or in 96-well plates at a density of 1 × 10^5 cells per well. Allow the cells to adhere and stabilize in the incubator for 24 hours. To induce an inflammatory response in the cells, treat them with 1 µg/mL of lipopolysaccharide (LPS). The LPS should be prepared in sterile phosphate-buffered saline (PBS) or DMEM and added to the culture medium to reach the desired final concentration. Alongside LPS treatment, administer varying concentrations of the plant extract to the cells. The extract should be prepared in sterile PBS or dimethyl sulfoxide (DMSO) and then diluted with DMEM. It's crucial to ensure that the final DMSO concentration does not exceed 0.1% to avoid any cytotoxic effects. Controls should include untreated cells, cells treated only with LPS, and cells treated with LPS and a known anti-inflammatory agent [35]. Incubate the treated cells at 37°C in a 5% CO2 atmosphere for 24 hours. After the incubation period, carefully collect the culture supernatant from each well. This supernatant will be used to assess the levels of inflammatory markers. For nitric oxide (NO) measurement, use the Griess reagent. Mix the supernatant with an equal volume of Griess reagent in a 96-well plate, incubate at room temperature for 10 minutes, and then measure the absorbance at 540 nm using a microplate reader. The concentration of nitric oxide can be determined using a sodium nitrite standard curve. Use certain enzyme-linked immunosorbent assay (ELISA) kits to measure pro-inflammatory cytokines including TNF-α, IL-6, and IL-1β. Observe the guidelines provided by the manufacturer, which usually entail incubating the supernatant with certain antibodies and then using a secondary antibody and a colorimetric substrate for detection. The cytokine concentrations are computed using a standard curve, and the absorbance is measured at the designated wavelength, which is typically 450 nm. Lastly, compare the levels of nitric oxide and cytokines in the treated groups with those in the LPS-only group by analysing the data. The significance of any detected differences should be ascertained by statistical analysis, such as ANOVA followed by post-hoc testing. A significant reduction in these markers compared to the LPS-only group would suggest that the plant extract possesses anti-inflammatory properties. This detailed approach ensures a comprehensive evaluation of the anti-inflammatory potential of plant extracts using RAW 264.7 macrophages.

RESULTS AND DISCUSSION

Preliminary Phytochemical study

The phytochemical analysis of *Boswellia serrata* methanol leaf extracts revealed significant chemical diversity. The extract showed a rich presence of terpenes and steroids, confirmed by strong positive results in the Lieberman-Burchard and Salkowski tests, aligning with its known anti-inflammatory properties, particularly due to boswellic acids. The detection of carbohydrates, ketones, and glycosides suggests additional benefits related to energy metabolism and cellular signalling. These findings highlight the diverse phytochemical profile of *Boswellia serrata*, which underpins its medicinal properties. Further research is needed to identify the specific bioactive compounds and explore potential synergistic effects within the extract.

Table 1. Summarizing the phytochemical test results for *Boswellia serrata* methanol leaf extracts.

Phytochemical Test	Detected Compounds	Observation	Implications
Lieberman-Burchard Test	Terpenes and Steroids	Strong positive reaction	Indicates a rich presence of terpenes and steroids, which are linked to anti-inflammatory effects.
Salkowski Test	Steroids and Triterpenoids	Strong positive reaction	Confirms the presence of steroids, supporting its known medicinal properties.
Molisch's Test	Carbohydrates	Positive reaction	Suggests potential benefits in energy metabolism.
Benedict's Test	Reducing Sugars	Positive reaction	Indicates the presence of reducing sugars, important for cellular energy processes.
Keller-Kiliani Test	Glycosides	Positive reaction	Suggests potential roles in cellular signaling.
2,4-Dinitrophenylhydrazine Test	Ketones	Positive reaction	Indicates the presence of ketones, which may have roles in metabolic pathways.

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Antioxidant activity

ABTS radical decolorization assay

The ABTS radical decolorization assay was employed to assess the antioxidant activity of a plant extract, ascorbic acid, and quercetin at various concentrations (0, 50, 100, 150, 200, 250 µg/mL). The results were reported as mean inhibition percentages with standard deviations, providing a measure of the precision and reproducibility of the assay. At the baseline concentration of 0 µg/mL, no inhibition of the ABTS radical was observed, confirming the absence of intrinsic antioxidant activity without the presence of the test substances. Upon introducing the extract, it exhibited moderate antioxidant activity, starting with 22.89 ± 0.99% inhibition at 50 µg/mL and progressively increasing to 87.76 ± 1.08% at 250 µg/mL. This suggests that while the extract possesses antioxidant properties, higher concentrations are necessary to achieve substantial radical scavenging effects. In contrast, ascorbic acid, a well-known antioxidant, demonstrated a stronger effect, beginning at 32.91 ± 1.09% inhibition at 50 µg/mL and rising to 83.19 ± 0.87% at the highest concentration tested. The consistent performance of ascorbic acid across the concentration range reflects its established efficacy in neutralizing free radicals. Quercetin, however, showed the highest antioxidant activity among the substances tested. It began with an inhibition of 42.52 ± 0.69% at 50 µg/mL and peaked at 94.98 ± 1.01% at 250 µg/mL, indicating that quercetin is highly effective even at lower concentrations. The significant increase in quercetin's activity, particularly at concentrations between 100 µg/mL and 150 µg/mL, highlights its potency and suggests that it could be especially useful in therapeutic contexts where lower dosages are advantageous. The small standard deviations associated with these measurements underscore the reliability and accuracy of the assay, ensuring that the results are reproducible and robust. Overall, quercetin emerged as the most promising candidate due to its superior antioxidant activity at lower doses, making it a strong contender for therapeutic applications aimed at combating oxidative stress. Meanwhile, the extract, although demonstrating effectiveness, would require higher concentrations or further optimization to reach the antioxidant potency of ascorbic acid and quercetin. These findings lay a solid foundation for further research into the therapeutic potential of these antioxidants, with quercetin standing out as a particularly potent agent for clinical use.

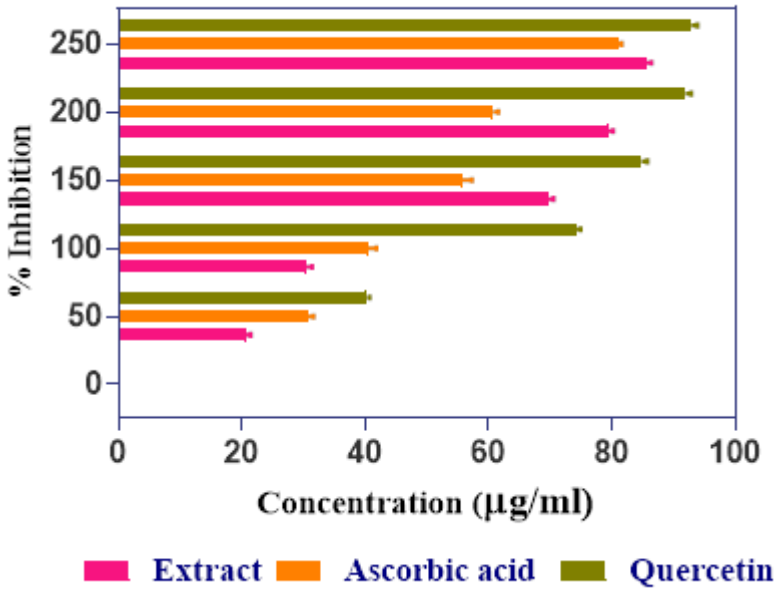

Figure 1. The extract's ABTS radical decolourisation test

Hydroxyl Radical Scavenging Activity (HRSA)

Figure 2 illustrates the Hydroxyl Radical Scavenging Activity (HRSA) of the extract, quercetin, and ascorbic acid across a range of concentrations (50, 100, 150, 200, 250 µg/ml), with the results presented as mean percentage inhibition ± standard deviation (SD). The data clearly show a dose-dependent increase in hydroxyl radical scavenging activity for all three substances, reflecting their capacity to neutralize these highly reactive species. The extract began with a modest inhibition of 18.06 ± 0.80% at 50 µg/ml, gradually increasing to 82.01 ± 1.01% at 250 µg/ml. This steady rise in activity indicates that while the extract is effective in scavenging hydroxyl radicals, it requires higher concentrations to achieve significant antioxidant effects. The data suggest that the extract's antioxidant potential could be harnessed effectively in formulations where higher dosages are feasible or where a gradual, sustained release of antioxidant activity is desired. Quercetin, on the other hand, demonstrated a much higher baseline effectiveness, with an initial inhibition of 43.98 ± 0.89% at 50 µg/ml, which escalated to 95.97 ± 1.02% at 250 µg/ml. This high level of

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activity even at lower concentrations highlights quercetin's potent antioxidant properties, making it a particularly effective agent for neutralizing hydroxyl radicals. The steep increase in quercetin's scavenging activity, especially at lower concentrations, underscores its potential as a powerful therapeutic antioxidant, potentially useful in clinical settings where precise dose management is critical. Ascorbic acid, a well-known antioxidant, also exhibited significant scavenging activity, beginning at 32.78 ± 0.92% inhibition at 50 µg/ml and rising to 97.02 ± 1.11% at the highest concentration tested. This consistent performance across all tested concentrations reaffirms ascorbic acid's established role in combating oxidative stress and its reliability as an antioxidant. The nearly complete inhibition of hydroxyl radicals at higher concentrations further solidifies ascorbic acid's position as a cornerstone in antioxidant therapy. The small standard deviations across all measurements indicate a high level of precision and reproducibility in the assay results, enhancing the confidence in these findings. This consistency suggests that the experimental procedures were robust and that the results are reliable indicators of each substance's antioxidant capacity. Overall, the results presented in Figure 2 provide compelling evidence of the strong therapeutic potential of quercetin and ascorbic acid due to their high efficacy at lower concentrations. Quercetin, in particular, stands out for its potent activity, even at minimal doses, making it an attractive candidate for further research and development in antioxidant therapies. The extract, while less potent, still demonstrates significant activity, particularly at higher concentrations, indicating its potential utility in formulations where a gradual or sustained antioxidant effect is desired. These findings support the potential application of these substances in dietary supplements and pharmaceuticals designed to enhance cellular antioxidant defense and mitigate oxidative stress-related conditions. Further research could delve into the specific mechanisms through which these substances interact with hydroxyl radicals and assess their potential clinical benefits in preventing or treating oxidative stress-related diseases.

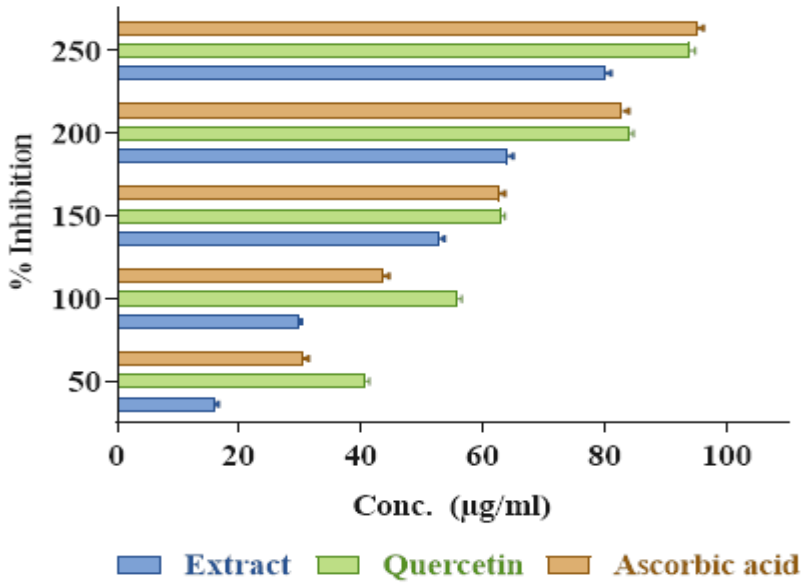

Figure 2. The extract's Hydroxyl Radical Scavenging Activity (HRSA)

Table 2. IC50 values of the extract and standards

Substance	Assay Type	IC50 Value (µg/mL)	Observations
Extract	ABTS Radical Decolorizing	128.91	Moderate potency in reducing ABTS radical formation by 50%
	Hydroxyl Radical Scavenging	147.32	Reduces hydroxyl radical formation by 50%
Ascorbic Acid	ABTS Radical Decolorizing	135.64	Comparable to Extract in ABTS activity
	Hydroxyl Radical Scavenging	120.19	Effective, but less than Quercetin in HRSA
Quercetin	ABTS Radical Decolorizing	68.40	Most potent in ABTS assay, lowest IC50 value
	Hydroxyl Radical Scavenging	84.77	Highly effective, lowest IC50 among substances in HRSA

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Anti-inflammatory activity

Evaluation of Anti-inflammatory activity using RAW 264.7 macrophages

The anti-inflammatory efficacy of different treatment doses utilising RAW 264.7 macrophages was demonstrated in the results, with particular attention to the production of pro-inflammatory cytokines including IL-1β, TNF-α, and IL-6. This arrangement was created to evaluate the treatment's capacity to lessen the inflammatory reactions brought on by lipopolysaccharide (LPS). The results unmistakably demonstrated that the concentration of the therapy has an effect on the levels of all three cytokines (IL-1β, TNF-α, and IL-6), demonstrating the treatment's effectiveness in reducing inflammatory reactions.

- **IL-1β:** Starting from a high with LPS induction at 222.56 ± 8.95 pg/mL, the level drops progressively across the treatment groups to 104.43 ± 1.27 pg/mL at the highest treatment concentration (120 µg/mL). This demonstrates a substantial reduction, nearing the baseline levels observed in the control group.
- **TNF-α:** Similarly, TNF-α levels show a marked reduction from 255.79 ± 9.27 pg/mL in the LPS-only group to 106.36 ± 1.78 pg/mL in the highest treatment group. This cytokine, known for its pivotal role in driving inflammation, is significantly mitigated by the treatment.
- **IL-6:** Reflecting the trends of the other cytokines, IL-6 decreases from 206.91 ± 8.95 pg/mL in the LPS group to 96.48 ± 1.75 pg/mL at the highest treatment concentration. This indicates effective control over this signaling molecule, which plays a crucial role in the acute phase response.

The findings indicate that, in a dose-dependent way, the therapy is quite successful in lowering the production of important inflammatory cytokines. The significant reduction in cytokine levels, particularly at increased treatment doses, highlights the therapy's potential as an anti-inflammatory drug. The treatment's broad-spectrum anti-inflammatory capabilities are further supported by the constancy of the drop in all three cytokines. This study highlighted the potential of the tested extract to significantly mitigate inflammatory responses in macrophages. Given the effectiveness at higher concentrations, further research could focus on understanding the mechanism of action, optimizing the dosage, and evaluating the treatment's efficacy in vivo. These findings could contribute to the development of new anti-inflammatory drugs or supplements, especially for conditions where cytokine storms or excessive inflammatory responses are a concern.

Table 3. Anti-inflammatory action measured by cytokine levels.

Serial No.	Treatment Groups	IL-1β (pg/mL)	TNF-α (pg/mL)	IL-6 (pg/mL)
1	Control	16.72±0.95	59.83±0.99	50.85±0.99
2	LPS only	226.74±9.12	259.97±9.73	210.94±8.66
3	15 µg/mL	187.26±6.77	229.95±8.75	190.76±7.90
4	30 µg/mL	167.84±5.63	189.92±6.91	160.95±5.66
5	60 µg/mL	138.24±5.84	169.87±6.59	140.98±2.91
6	120 µg/mL	108.68±2.93	110.58±2.91	100.69±2.86

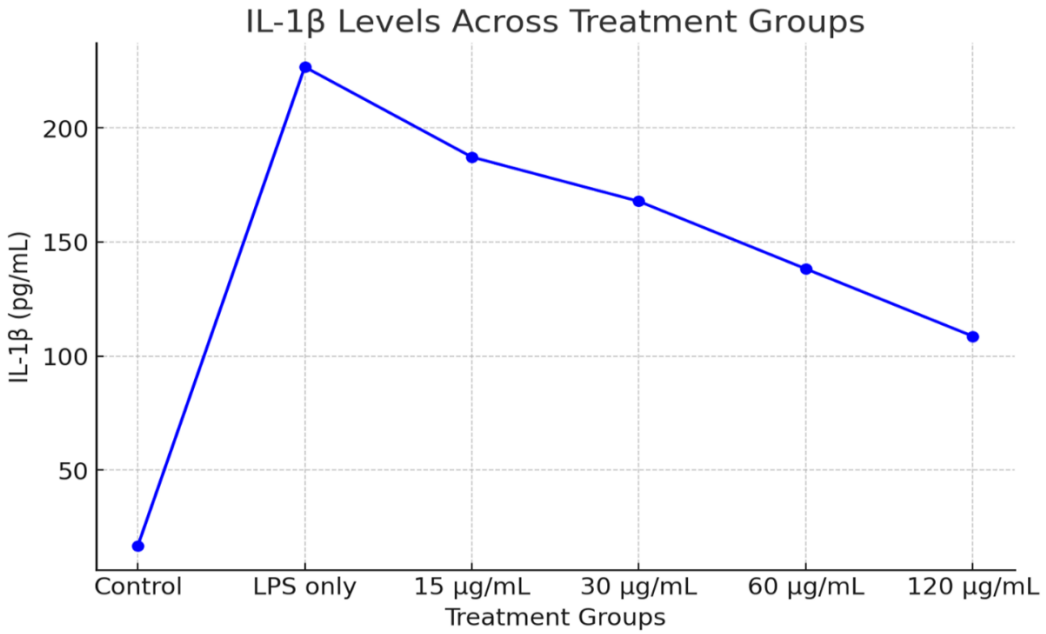

Figure 3. Anti-inflammatory activity in terms of IL-1β levels in RAW 264.7 macrophages.

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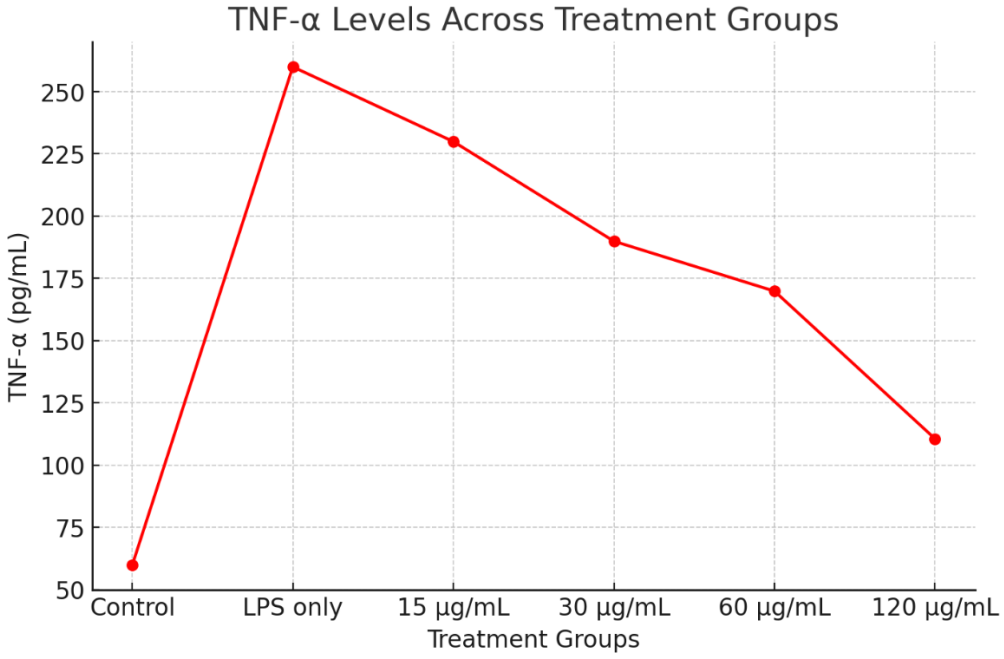

Figure 4. Anti-inflammatory activity in terms of TNF-α levels in RAW 264.7 macrophages.

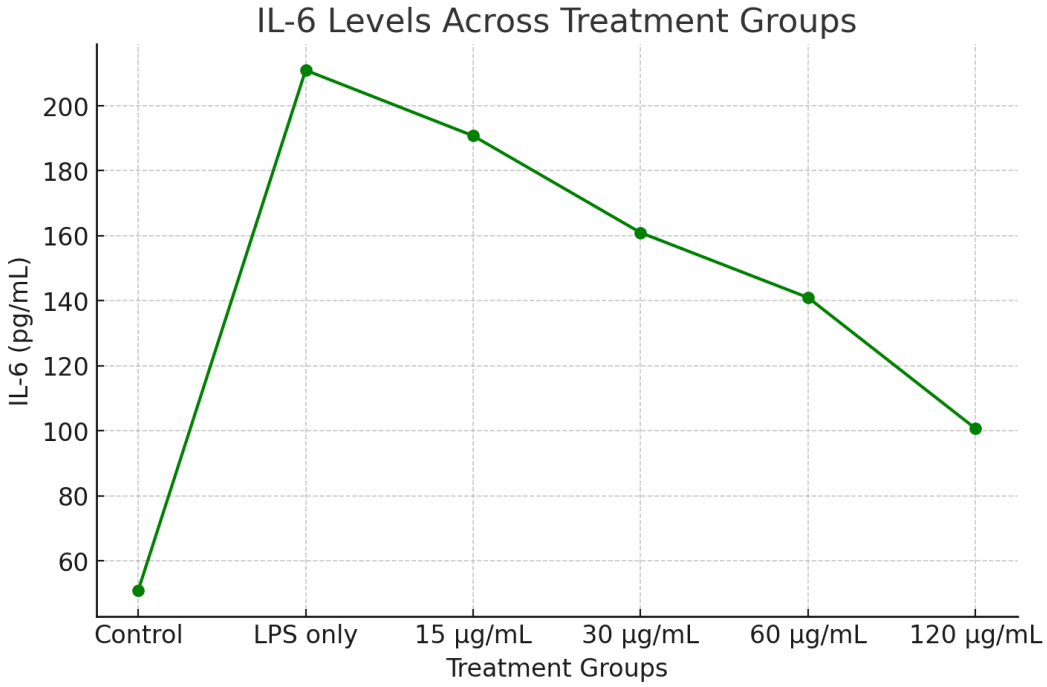

Figure 5. Anti-inflammatory activity in terms of IL-6 levels in RAW 264.7 macrophages.

CONCLUSIONS

The study provided compelling evidence that the extract, BSLE-M, which is composed of methanol leaves extracts from *Boswellia serrata*, possesses strong antioxidant and anti-inflammatory properties. Quercetin, a well-established antioxidant used as a reference in the study, demonstrated superior radical scavenging ability, affirming its robust antioxidant capacity. Although BSLE-M required higher concentrations to achieve similar effects, it still exhibited significant antioxidant activity, indicating its potential effectiveness in neutralizing free radicals. BSLE-M demonstrated a distinct, dose-dependent decrease in the generation of important pro-inflammatory cytokines, including IL-1β, TNF-α, and IL-6, in the anti-inflammatory tests. This reduction suggests that BSLE-M can effectively modulate inflammatory responses, making it a potential candidate for managing conditions characterized by chronic inflammation. The extract's ability to decrease cytokine levels in a manner comparable to standard anti-inflammatory agents highlights its potential utility in therapeutic applications.

These findings underscore the therapeutic promise of BSLE-M in addressing disorders linked to oxidative stress and inflammation. Its efficacy, which rivals that of established antioxidants and anti-inflammatory drugs, suggests that BSLE-M could be developed into a valuable treatment option in clinical settings. The findings show that more study is necessary in addition to indicating the extract's immediate medicinal potential. Future research should concentrate on clarifying the processes by which BSLE-M works as well as carrying out clinical trials to verify its safety and effectiveness in people. In conclusion, BSLE-M shows promise as a therapeutic option for the creation of novel treatments meant to counteract inflammation and oxidative stress. Its natural origin and dual antioxidant and anti-inflammatory qualities make it a desirable candidate for creating new medicines. Further investigation may result in the identification of BSLE-M as a very effective therapeutic agent, providing new therapeutic options for the management of illnesses linked to oxidative and inflammatory damage.

REFERENCES

1. Abad C, Vargas FR, Zoltan T, Proverbio T, Piñero S, Proverbio F, et al. Magnesium sulfate affords protection against oxidative damage during severe preeclampsia. Placenta. 2015;36(2):179-85.
2. Abrahamovych M, Abrahamovych O, Fayura O, Fayura L, Tolopko S. THE EFFECT OF OXIDATIVE STRESS ON THE AUTONOMIC NERVOUS SYSTEM IN PATIENTS WITH LIVER CIRRHOSIS. Georgian Med News. 2020(298):94-9.
3. Adamczyk B, Wawrzyniak S, Kasperczyk S, Adamczyk-Sowa M. The Evaluation of Oxidative Stress Parameters in Serum Patients with Relapsing-Remitting Multiple Sclerosis Treated with II-Line Immunomodulatory Therapy. Oxid Med Cell Longev. 2017;2017:9625806.
4. Adema AY, van Ittersum FJ, Hoenderop JG, de Borst MH, Nanayakkara PW, Ter Wee PM, et al. Reduction of Oxidative Stress in Chronic Kidney Disease Does Not Increase Circulating α-Klotho Concentrations. PLoS One. 2016;11(1):e0144121.
5. Akbulak O, Karadag AS, Akdeniz N, Ozkanli S, Ozlu E, Zemheri E, et al. Evaluation of oxidative stress via protein expression of glutathione S-transferase and cytochrome p450 (CYP450) ısoenzymes in psoriasis vulgaris patients treated with methotrexate. Cutan Ocul Toxicol. 2018;37(2):180-5.
6. Akhgarjand C, Vahabi Z, Shab-Bidar S, Anoushirvani A, Djafarian K. The effects of probiotic supplements on oxidative stress and inflammation in subjects with mild and moderate Alzheimer's disease: a randomized, double-blind, placebo-controlled study. Inflammopharmacology. 2024;32(2):1413-20.
7. Al-Sheikh YA, Al-Zahrani KY. The Status of Biochemical and Molecular Markers of Oxidative Stress in Preeclamptic Saudi Patients. Curr Mol Med. 2018;18(7):475-85.
8. Anraku M, Tanaka M, Hiraga A, Nagumo K, Imafuku T, Maezaki Y, et al. Effects of chitosan on oxidative stress and related factors in hemodialysis patients. Carbohydr Polym. 2014;112:152-7.
9. Arab A, Khorvash F, Karimi E, Heidari Z, Askari G. The effects of the dietary approaches to stop hypertension (DASH) diet on oxidative stress and clinical indices of migraine patients: a randomized controlled trial. Nutr Neurosci. 2022;25(11):2259-68.
10. Asemi Z, Samimi M, Tabassi Z, Shakeri H, Sabihi SS, Esmaillzadeh A. Effects of DASH diet on lipid profiles and biomarkers of oxidative stress in overweight and obese women with polycystic ovary syndrome: a randomized clinical trial. Nutrition. 2014;30(11-12):1287-93.
11. Atan RM, Ersoy G, Çakıcı Ç. Effects of Hardaliye, a Fermented Grape Drink, on Oxidative Stress, Lipid Profile, and Blood Pressure in Young Soccer Players: A Randomized Controlled Trial. J Am Nutr Assoc. 2024;43(4):356-64.
12. Abedini M, Ramezani-Jolfaie N, Ghasemi-Tehrani H, Tarrahi MJ, Amani R. The effect of concentrated pomegranate juice on biomarkers of inflammation, oxidative stress, and sex hormones in overweight and obese women with polycystic ovary syndrome: A randomized controlled trial. Phytother Res. 2023;37(6):2255-61.
13. Adams JM, Valentine CJ, Karns RA, Rogers LK, Murase M, Fowler GN, et al. DHA Supplementation Attenuates Inflammation-Associated Gene Expression in the Mammary Gland of Lactating Mothers Who Deliver Preterm. J Nutr. 2022;152(6):1404-14.
14. Ağbaş A, Canpolat N, Çalışkan S, Yılmaz A, Ekmekçi H, Mayes M, et al. Hemodiafiltration is associated with reduced inflammation, oxidative stress and improved endothelial risk profile compared to high-flux hemodialysis in children. PLoS One. 2018;13(6):e0198320.
15. Burbank AJ, Hernandez ML, Robinette C, Wang T, Zhou H, Alexis N, et al. Short course gamma tocopherol did not mitigate effects of ozone on airway inflammation in asthmatics. Inhal Toxicol. 2020;32(7):279-81.
16. Chai SC, Davis K, Zhang Z, Zha L, Kirschner KF. Effects of Tart Cherry Juice on Biomarkers of Inflammation and Oxidative Stress in Older Adults. Nutrients. 2019;11(2).
17. Silva FF, de Oliveira GAC, Costa HCM, Regis WCB. Royal Sun Culinary-Medicinal Mushroom, Agaricus brasiliensis (Agaricomycetes), Supplement in Training Capacity Improvement Parameters. Int J Med Mushrooms. 2017;19(9):759-66.

18. Spim SRV, Pistila AMH, Pickler TB, Silva MT, Grotto D. Effects of Shiitake Culinary-Medicinal Mushroom, Lentinus edodes (Agaricomycetes), Bars on Lipid and Antioxidant Profiles in Individuals with Borderline High Cholesterol: A Double-Blind Randomized Clinical Trial. Int J Med Mushrooms. 2021;23(7):1-12.
19. Ved DK, Goraya GS. Demand and supply of medicinal plants in India. New Delhi: National Medicinal Plants Board; 2008 2010 Nov 1.
20. Singh N, Ahuja V, Sachdev V, Upadhyay AD, Goswami R, Ramakrishnan L, et al. Antioxidants for Pancreatic Functions in Chronic Pancreatitis: A Double-blind Randomized Placebo-controlled Pilot Study. J Clin Gastroenterol. 2020;54(3):284-93.
21. Soto-Méndez MJ, Aguilera CM, Mesa MD, Campaña-Martín L, Martín-Laguna V, Solomons NW, et al. Strong Associations Exist among Oxidative Stress and Antioxidant Biomarkers in the Circulating, Cellular and Urinary Anatomical Compartments in Guatemalan Children from the Western Highlands. PLoS One. 2016;11(1):e0146921.
22. Souza AV, Giolo JS, Teixeira RR, Vilela DD, Peixoto LG, Justino AB, et al. Salivary and Plasmatic Antioxidant Profile following Continuous, Resistance, and High-Intensity Interval Exercise: Preliminary Study. Oxid Med Cell Longev. 2019;2019:5425021.
23. Stankiewicz B, Cieślicka M, Kujawski S, Piskorska E, Kowalik T, Korycka J, et al. Effects of antioxidant supplementation on oxidative stress balance in young footballers- a randomized double-blind trial. J Int Soc Sports Nutr. 2021;18(1):44.
24. Stenqvist A, Oleszczuk K, Leijonhufvud I, Giwercman A. Impact of antioxidant treatment on DNA fragmentation index: a double-blind placebo-controlled randomized trial. Andrology. 2018;6(6):811-6.
25. Guo Y, Zhang P, Liu Y, Zha L, Ling W, Guo H. A dose-response evaluation of purified anthocyanins on inflammatory and oxidative biomarkers and metabolic risk factors in healthy young adults: A randomized controlled trial. Nutrition. 2020;74:110745.
26. Singh S, Kaur R, Sharma SK. Antinociceptive, antiinflammatory and antipyretic activities of *Rumex hastatus* D. don stem and roots. Der Pharmacia Sinica. 2013;4(3):95-102.
27. Siddiqui MZ. Boswellia serrata, a potential antiinflammatory agent: an overview. Indian journal of pharmaceutical sciences. 2011;73(3):255-61.
28. Joshi SK, Sharma BD, Bhatia CR, Singh RV, Thakur RS. The wealth of India raw materials. Council of Scientific and Industrial Research Publication, New Delhi. 1992;3:270-1.
29. Chiavari G, Galletti GC, Piccaglia R, Mohamud MA. Differentiation between resins Boswellia carterii and Boswellia frereana (frankincense) of Somali origin. Journal of Essential Oil Research. 1991;3(3):185-6.
30. Khare CP. Botany, Indian Herbal Remedies: Rational Western Therapy, Ayurvedic, and Other Traditional Usage. Springer, New York; 2004.
31. Nenadis N, Wang L-F, Tsimidou M, Zhang H-Y. Estimation of scavenging activity of phenolic compounds using the ABTS•+ assay. Journal of agricultural and food chemistry. 2004;52(15):4669-74.
32. Lee BW, Lee JH, Gal SW, Moon YH, Park KH. Selective ABTS radical-scavenging activity of prenylated flavonoids from Cudrania tricuspidata. Bioscience, biotechnology, and biochemistry. 2006;70(2):427-32.
33. Tijani AA, Adekomi DA, Adewole SO. In vitro and in vivo determination of hydroxyl radical scavenging activity (HRSA) of fractions of aqueous extract of Moringa oleifera leaves (AEMOL). Eurasian J Med Oncol. 2018;2(4):209-16.
34. Moro C, Palacios I, Lozano M, D'Arrigo M, Guillamón E, Villares A, et al. Anti-inflammatory activity of methanolic extracts from edible mushrooms in LPS activated RAW 264.7 macrophages. Food chemistry. 2012;130(2):350-5.
35. Cherng S-C, Cheng S-N, Tarn A, Chou T-C. Anti-inflammatory activity of c-phycocyanin in lipopolysaccharide-stimulated RAW 264.7 macrophages. Life sciences. 2007;81(19-20):1431-5.