https://doi.org/10.33472/AFJBS.6.6.2024.1626-1638

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Pharmacological Assessment of *Boswellia Serrata* and *Urtica Dioica* Leaf Extract Mixture: Implications for Oxidative Stress and Inflammation

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Article Info

Volume 6, Issue 6, May 2024

Received: 08 March 2024

Accepted: 23 April 2024

Published: 27 May 2024

doi: 10.33472/AFJBS.6.6.2024. 1626-1638

ABSTRACT:

This study evaluated the antioxidant and anti-inflammatory activities of an extract mixture containing the *Boswellia serrata* and *Urtica dioica* methanol leaves extract, codenamed as M-BSUD, using various in vitro mechanistic models. The antioxidant capacity was assessed through ABTS radical decolorizing and Hydroxyl Radical Scavenging Activity (HRSA) assays, while anti-inflammatory efficacy was determined using RAW 264.7 macrophages exposed to LPS to measure cytokine production. For the ABTS assay, quercetin demonstrated superior antioxidant activity with the lowest IC50 value of 64.31 µg/mL, indicating its potent radical scavenging ability. Ascorbic acid and the extract also showed significant antioxidant properties but required higher concentrations to achieve similar effects, with IC50 values of 131.04 µg/mL and 124.87 µg/mL, respectively. Similar trends were observed in the HRSA assay, where quercetin again showed the highest effectiveness with an IC50 of 80.68 µg/mL, followed by ascorbic acid and the extract with IC50 values of 116.96 µg/mL and 143.77 µg/mL, respectively. In the anti-inflammatory tests, a dose-dependent decrease in the production of IL-1β, TNF-α, and IL-6 was observed across all treatment concentrations in RAW 264.7 macrophages. The highest concentration tested (120 µg/mL) resulted in cytokine levels approaching those of the untreated control, underscoring the potential anti-inflammatory benefits of the treatments. These findings highlighted the robust antioxidant and anti-inflammatory properties of the extract mixture, M-BSUD, comparable to the standards showing the most promise as both an antioxidant and anti-inflammatory agent. This suggested its potential utility in therapeutic settings where oxidative stress and inflammation are implicated.

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

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1. Introduction

Oxidative stress represents a critical biological phenomenon, where an imbalance between free radicals and antioxidants in the body culminates in potential cellular and tissue damage. This

concept is intricately linked to the process of inflammation, a fundamental biological response to harmful stimuli, which plays a dual role both as a healing and a potentially damaging process. Understanding the complex relationship between oxidative stress and inflammation is pivotal for developing strategies to manage numerous chronic diseases. This introduction explores the nature of oxidative stress, its significance in the inflammatory process, and the potential implications for human health [1-5].

Oxidative stress occurs when there is an excess of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the body that the antioxidant defences cannot adequately neutralize. These reactive species are typically produced in the body as byproducts of oxygen metabolism and play essential roles in cell signalling and homeostasis. However, excessive ROS and RNS production can lead to cellular components' damage, including lipids, proteins, and DNA. The body's antioxidant defences include enzymatic antioxidants such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, and non-enzymatic antioxidants such as vitamin C, vitamin E, and glutathione. These systems work together to mitigate the effects of excess free radicals, thus protecting the body from oxidative damage. The balance between oxidative agents and antioxidants is crucial for maintaining cellular function and integrity. Oxidative stress can be induced by various external and internal factors [6-9]. External sources include exposure to pollutants, tobacco smoke, pesticides, and radiation, which can all lead to increased ROS production. Internally, oxidative stress may be produced by inflammatory processes, mitochondrial dysfunction, excessive exercise, or ischemia-reperfusion injury. These sources contribute significantly to the oxidative burden that the body's cells must counteract [6, 10-13].

Inflammation is the body's natural response to injury or infection, characterized by redness, heat, swelling, pain, and loss of function. While acute inflammation is a protective mechanism that facilitates tissue healing and recovery, chronic inflammation can lead to various diseases, including atherosclerosis, asthma, arthritis, and even cancer. Oxidative stress and inflammation are deeply intertwined, with ROS playing a critical role in initiating and sustaining inflammatory pathways. ROS are known to activate nuclear factor-kappa B (NF-κB), a key transcription factor that regulates the expression of inflammatory cytokines, chemokines, and adhesion molecules. The activation of NF-κB leads to the recruitment of inflammatory cells to the site of injury, which in turn produces more ROS, thus perpetuating the cycle of inflammation and oxidative stress. Additionally, ROS can activate other signaling pathways such as MAPKs (mitogen-activated protein kinases), which also contribute to inflammatory responses. The chronic interplay between oxidative stress and inflammation is central to the pathogenesis of numerous diseases. For instance, in atherosclerosis, oxidative stress leads to the oxidation of low-density lipoprotein (LDL) in blood vessels, which triggers an inflammatory response, culminating in plaque formation. In diabetes, elevated blood sugar levels can lead to oxidative stress, which in turn promotes inflammatory processes that damage pancreatic cells [6, 10-14]. Similarly, in neurodegenerative diseases such as Alzheimer's and Parkinson's, oxidative stress is known to induce neuroinflammation, which contributes to the progression of disease pathology. Moreover, oxidative stress is implicated in the aging process and the decline in organ function seen with age. It affects the skin, cardiovascular system, eyes, and other organs, underscoring the need for effective antioxidant strategies to mitigate these effects. Given the critical role of oxidative stress in inflammation and disease, therapeutic strategies that target this pathway could potentially alleviate many chronic conditions. Antioxidants sourced from diet and supplements—like vitamins C and E, polyphenols, and flavonoids—have shown promise in neutralizing ROS and blunting the inflammatory response. Additionally, drugs that specifically target oxidative pathways or enhance the body's own antioxidant capacity are currently under research and development. Emerging therapies also include lifestyle modifications such as diet changes, physical activity, and avoiding exposure to oxidative stress inducers like smoking and excessive sun exposure [14-18]. These

approaches aim to enhance the body's inherent antioxidant defences and reduce the burden of chronic diseases associated with oxidative stress and inflammation. The relationship between oxidative stress and inflammation is a cornerstone of many pathological processes. Understanding and manipulating this relationship holds the key to developing preventive and therapeutic strategies for a variety of inflammatory and oxidative stress-related conditions. Continued research in this area remains crucial for uncovering new insights into how oxidative stress influences inflammation and identifying novel therapeutic targets that could lead to better management of chronic diseases and overall health improvement [19-21].

Medicinal plants have been integral to human health and wellness since ancient times, offering a rich source of bioactive compounds with therapeutic potential. Among their myriad benefits, medicinal plants play a crucial role in combating oxidative stress and inflammation, two processes implicated in numerous chronic diseases and age-related conditions. Understanding the importance and significance of medicinal plants in addressing these physiological challenges sheds light on their profound impact on human health and the potential avenues for therapeutic intervention [21, 22]. Oxidative stress arises from an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify these reactive molecules or repair the resulting damage. ROS, including free radicals and other oxygen-derived species, can inflict oxidative damage to cellular structures such as lipids, proteins, and nucleic acids, leading to cellular dysfunction and contributing to the pathogenesis of various diseases, including cancer, cardiovascular disorders, neurodegenerative diseases, and aging processes. Medicinal plants offer a diverse array of antioxidant compounds, including polyphenols, flavonoids, carotenoids, and vitamins, which scavenge free radicals, inhibit oxidative reactions, and bolster endogenous antioxidant defences, thereby mitigating oxidative stress and its associated deleterious effects [22].

Inflammation, on the other hand, is a complex biological response mounted by the immune system to protect the body from harmful stimuli, including pathogens, irritants, and damaged cells. While acute inflammation serves a protective role in tissue repair and pathogen clearance, chronic inflammation can fuel the progression of various diseases, including arthritis, inflammatory bowel disease, cardiovascular diseases, and certain cancers. Medicinal plants harbor an array of anti-inflammatory constituents, such as polyphenols, alkaloids, terpenoids, and fatty acids, which modulate key inflammatory pathways and mediators, including cytokines, chemokines, and inflammatory enzymes. These bioactive compounds exert their anti-inflammatory effects by suppressing pro-inflammatory signaling cascades, inhibiting the expression of inflammatory genes, and enhancing the production of anti-inflammatory molecules, thereby attenuating chronic inflammation and its associated pathological consequences [5, 6, 14, 19]. The importance of medicinal plants in addressing oxidative stress and inflammation extends beyond their antioxidant and anti-inflammatory properties. Many phytochemicals present in medicinal plants exhibit pleiotropic effects, targeting multiple cellular pathways and biological processes implicated in disease pathogenesis. For instance, some plant-derived compounds possess anti-cancer properties by inducing apoptosis (programmed cell death), inhibiting angiogenesis (blood vessel formation), and suppressing metastasis (spread of cancer cells). Moreover, certain phytochemicals exhibit neuroprotective effects by enhancing neuronal survival, promoting synaptic plasticity, and mitigating neuroinflammation, offering promising avenues for the management of neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease.

Furthermore, medicinal plants represent a vast reservoir of novel bioactive compounds with untapped therapeutic potential, offering a source of inspiration for drug discovery and development. Modern pharmacological research has increasingly focused on identifying and characterizing bioactive compounds from medicinal plants, elucidating their mechanisms of action, and evaluating their efficacy and safety profiles through preclinical and clinical studies.

Indeed, several pharmaceutical drugs currently in clinical use or undergoing development are derived from natural products or inspired by natural compounds found in medicinal plants, underscoring the enduring significance of nature as a source of healing and innovation in medicine [7-10, 13].
In assumption, medicinal plants play a pivotal role in combating oxidative stress and inflammation, offering a natural and sustainable approach to promoting health and combating disease. Their rich repertoire of bioactive compounds, encompassing antioxidants, anti-inflammatory agents, and multifunctional phytochemicals, underscores their importance as invaluable resources in the quest for novel therapeutic interventions. By harnessing the therapeutic potential of medicinal plants, researchers and healthcare practitioners can advance our understanding of disease mechanisms, expand the armamentarium of treatment options, and ultimately improve the quality of life for individuals worldwide [7-10, 13]. Therefore, considering the literature review and the above facts, the present study aimed to evaluate the antioxidant and anti-inflammatory activities of an extract mixture containing the *Boswellia serrata* and *Urtica dioica* methanol leaves extract in various in vitro mechanistic models.

2. Material and Methods

Drugs, chemicals, and reagents

Chemicals and reagents for the extraction process included ethanol, methanol, and distilled water, all of high analytical grade and obtained for Loba Chemie, Mumbai, India. For the anti-inflammatory assays, essential reagents included enzyme-linked immunosorbent assay (ELISA) kits designed to measure pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6. In vitro cell culture studies utilized RAW 264.7 macrophage cells, with necessary culture media, fetal bovine serum (FBS), and antibiotics (penicillin and streptomycin). Lipopolysaccharide (LPS) which were employed to induce inflammation in these cells and were obtained from Himedia and Sigma Aldrich, India. All drugs and chemicals were sourced from reputable suppliers to ensure their purity and reliability for consistent and reproducible results.

Collection, authentication, and extraction of the plants

The plants, *Boswellia serrata* and *Urtica dioica* were collected from Kullu district of Himachal Pradesh. Herbariums were made and both plants were authenticated by a botanist. The whole plants were dried in shade and made to a coarse powder in a mechanical grinder. The coarse powder for each plant was subjected to a cold maceration in methanol for 7 days. After 7 days of cold maceration, the extracts were obtained and dried in vacuum desiccator. Both the extracts were combined in 1:1 ratio to get the extract mixture for further evaluation. The extract mixture containing the *Boswellia serrata* and *Urtica dioica* extract was codenamed as M-BSUD and stored at -4 ^{0}C until further use.

Preliminary Phytochemical study

The plant extracts were subjected to preliminary phytochemical screening to identify the constituent compounds that potentially contribute to the plant's medicinal efficacy. This foundational step was critical for understanding the bioactive components within the extracts, which may play a role in modulating antioxidant and inflammatory pathways. The phytochemical analysis involved a series of qualitative tests to detect the presence of various classes of compounds. These included alkaloids, flavonoids, tannins, saponins, sterols, and phenolic compounds. Specific tests such as the Dragendorff test for alkaloids, the Shinoda test for flavonoids, and the Froth test for saponins were employed. These preliminary screenings were conducted using both aqueous and methanolic solvents to extract a broad spectrum of phytochemicals, considering that different solvents can extract different compounds [23]. The presence of flavonoids and phenolic acids was particularly noted due to their well-documented

antioxidant and anti-inflammatory properties. Overall, the phytochemical screening provided a comprehensive insight into the complex makeup of the extracts, setting the stage for further pharmacological evaluations.

Antioxidant activity

ABTS radical decolorization assay

The ABTS radical decolorization assay is widely used to evaluate the antioxidant capacity of substances such as plant extracts [24]. This method employs ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)), a stable free radical, whose blue-green color diminishes in the presence of antioxidants. Below is a detailed procedure to carry out this assay: First, prepare a 7 mM ABTS stock solution in water and a 2.45 mM potassium persulfate solution. These two solutions should be mixed in equal volumes to form the ABTS radical cation solution. Allow this mixture to stand in the dark at room temperature for 12-16 hours to complete the radical generation. Before use, adjust the absorbance of the ABTS solution to about 0.70 ± 0.02 at 734 nm by diluting it with ethanol or phosphate-buffered saline (PBS). This step is crucial to ensure the consistency of the radical's concentration across assays. To assess the antioxidant capacity, mix a known volume of your test extract with the ABTS radical solution, typically in a 10:1 ratio of ABTS solution to extract. Allow the mixture to react for about 6 minutes at room temperature. Following incubation, measure the absorbance at 734 nm using a spectrophotometer. A decrease in absorbance is indicative of the antioxidant activity of the extract, as it neutralizes the ABTS radical. For quantification, it's important to create a calibration curve using Trolox, a water-soluble vitamin E analogue. several concentrations of Trolox were prepared and ran them through the same assay conditions as your test sample. Plotted the inhibition percentage against Trolox concentrations to establish a standard curve. The antioxidant activity of the test extract can then be expressed in Trolox equivalents by comparing it to this curve. The percentage reduction in absorbance is calculated using the formula:

$$Percentage\ Inhibition\ (\%) = \frac{Abs_{Control} - Abs_{Sample}}{Abs_{control}} \times 100$$

Hydroxyl Radical Scavenging Activity (HRSA)

The Hydroxyl Radical Scavenging Activity (HRSA) assay is an effective method used to evaluate the antioxidant capacity of various substances by assessing their ability to neutralize hydroxyl radicals [25]. These radicals are extremely reactive and can damage essential biomolecules, including DNA, proteins, and lipids. To conduct this assay, it was needed reagents such as ferrous sulphate and hydrogen peroxide, which reacted to produce hydroxyl radicals through the Fenton reaction, and salicylic acid, which served as a probe by reacting with hydroxyl radicals to form a coloured complex. This coloured complex was measured in spectroscopically. That was started by setting up the reaction mixture in a phosphate buffer containing ferrous sulphate and hydrogen peroxide. Added salicylic acid to this mixture to enable detection of hydroxyl radicals through the formation of a dihydroxybenzoic acid (DHBA) complex, which was detected and measured spectrophotometrically. Then introduced the test samples in varying concentrations to different aliquots of the reaction mixture (Extract mixture), along with a control sample without any test substance to establish the baseline radical activity. Then allowed these mixtures to incubate for about 30 minutes at room temperature before measuring the absorbance at around 510 nm using a spectrophotometer. To quantify the hydroxyl radical scavenging activity, calculated the percentage reduction in absorbance between the control and test samples using the formula:

$$Percentage\ Inhibition\ (\%) = \frac{Abs_{Control} - Abs_{Sample}}{Abs_{control}} \times 100$$

Anti-inflammatory activity

Anti-inflammatory activity using RAW 264.7 macrophages

To evaluate the anti-inflammatory activity of an extract using RAW 264.7 macrophages, a detailed experimental setup was required [26]. Started by culturing the RAW 264.7 cells in a suitable medium, such as DMEM supplemented with 10% FBS, until they reached the necessary confluence. The cells were maintained at standard conditions, typically at 37°C in a 5% CO2 atmosphere. Once the cells have stabilized, plate them in either 96-well or 24-well plates, depending on the scale of your assays, and allowed them to adhere for 24 hours. For the induction of inflammation, treated the cells with a non-toxic concentration of lipopolysaccharide (LPS), usually around 1 µg/mL. This step simulated an infection-like inflammatory response. Concurrently, administered various concentrations of the test extract mixture, prepared in appropriate dilutions. It was essential to determine these concentrations through preliminary cytotoxicity testing, such as an MTT assay, to ensure they were non-toxic. Several controls were included in this experiment: cells without any treatment, cells treated only with LPS, and cells treated with a known anti-inflammatory agent as a positive control. After treating the cells with both LPS and the extract for 24 hours, collected the supernatant from each well to measure inflammatory markers. Assessed nitric oxide production using the Griess reagent and measured cytokine levels (TNF-α, IL-6, IL-1β) using ELISA method. A reduction in these markers compared to the LPS-only treated cells indicated the anti-inflammatory effectiveness of the extract mixture.

Statistical analysis

Each experiment was run three times, and the results were reported as the mean of the three analyses ± standard deviations (SD). GraphPad Prism Software (V 8.0, USA) was used to calculate the correlation coefficient between variables, means, standard deviations, standard errors, standard curve, IC 50 values, and one-way ANOVA followed by Bonferroni's post hoc test. GraphPad Prism Software is used to prepare figures (USA). P-values less than 0.05 were regarded as statistically significant.

3. Results and Discussion

Preliminary Phytochemical study

The results of the phytochemical tests conducted on methanol leaves extracts of *Boswellia serrata* and *Urtica dioica* revealed significant variations in their chemical composition. *Boswellia serrata* demonstrates a rich presence of terpenes and steroids, as evidenced by strong positive reactions in Lieberman – Buchard's and Salkwoski's tests. This aligned with its known medicinal properties, particularly its anti-inflammatory effects attributed to boswellic acids. Additionally, the presence of carbohydrates, ketones, and glycosides suggests potential benefits in terms of energy metabolism and cellular signalling. Conversely, *Urtica dioica* displays a diverse profile, characterized by the presence of tannins, flavonoids, alkaloids, and carbohydrates. The presence of tannins and flavonoids, confirmed by positive reactions in chlorogenic and sodium hydroxide tests, indicates antioxidant and anti-inflammatory potential. However, the absence of terpenes and steroids suggests a different mechanism of action compared to *Boswellia serrata*. Overall, these findings underscored the diverse phytochemical compositions of *Boswellia serrata* and *Urtica dioica*, reflecting their distinct medicinal properties. Further research is warranted to elucidate the specific bioactive compounds responsible for their therapeutic effects and explore potential synergistic interactions within these botanicals.

Antioxidant activity

ABTS radical decolorization assay

This study assessed the antioxidant activity of an extract, ascorbic acid, and quercetin using the ABTS radical scavenging assay, presenting results as mean inhibition percentages ± standard deviation across varying concentrations (0, 50, 100, 150, 200, 250 µg/mL). Such antioxidant assays are crucial for identifying potential therapeutic agents that can mitigate oxidative stress implicated in various chronic diseases. Initially, at 0 µg/mL, no inhibition was observed for any substances, serving as a baseline indicating no intrinsic antioxidant activity without the presence of the test substances. Upon introduction of the substances, a clear dose-response relationship was established, which is characteristic of antioxidant assays and provides insight into the potency and efficacy of the substances at neutralizing free radicals. The extract demonstrated a moderate antioxidant capacity, starting with 20.67 ± 0.78% inhibition at 50 µg/mL and increasing to 85.54 ± 0.89% at the highest concentration tested (250 µg/mL). This suggests that while the extract possesses antioxidant properties, higher concentrations are required to achieve substantial radical scavenging activity. Ascorbic acid, recognized for its potent antioxidant effects, exhibited stronger activity than the extract, beginning at 30.7 ± 0.935% and escalating to 80.96 ± 0.65% at 250 µg/mL. The consistency and reliability of ascorbic acid as an antioxidant are well-documented and reflected in the results, which show significant activity across all tested concentrations. Quercetin displayed the highest antioxidant activity among the tested substances, with inhibition starting at 40.3 ± 0.47% at 50 µg/mL and peaking at 92.88 ± 0.99% at 250 µg/mL. The marked increase in activity, particularly between the concentrations of 100 µg/mL and 150 µg/mL, underscores quercetin's effectiveness at lower doses compared to the other substances. This suggests that quercetin could be particularly useful in clinical settings where effective dose management is crucial. The small standard deviations across the measurements underscore the assay's precision and the reproducibility of the results, enhancing the reliability of these findings. This study not only confirms the potent antioxidant capabilities of quercetin and ascorbic acid but also demonstrates the utility of the extract at higher doses. In summary, these results provide a robust foundation for further research into the therapeutic applications of these antioxidants. Quercetin, with its high efficacy at lower concentrations, may offer considerable benefits for dietary supplements and pharmaceuticals aimed at combating oxidative stress-related diseases. Meanwhile, the extract also shows promise but would require higher dosages or further refinement to increase its antioxidant potency to levels comparable with ascorbic acid and quercetin.

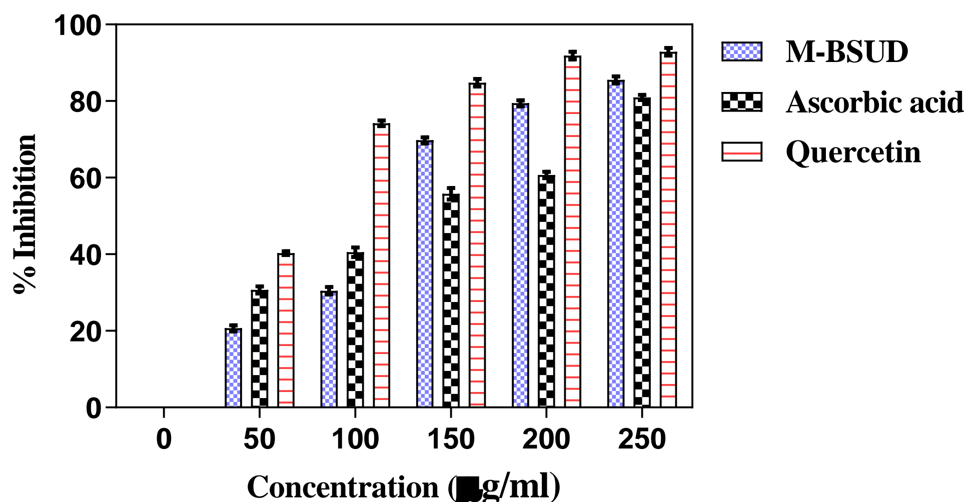

Figure 1. ABTS radical decolorization assay of the extract (M-BSUD)

Hydroxyl Radical Scavenging Activity (HRSA)

The results demonstrated the Hydroxyl Radical Scavenging Activity (HRSA) of the extract mixture (M-BSUD), quercetin, and ascorbic acid, measured across a range of concentrations (50, 100, 150, 200, 250 µg/ml). Results are presented as mean percentage inhibition ± standard deviation (SD), reflecting the effectiveness and consistency of each substance's ability to neutralize hydroxyl radicals, which are highly reactive species capable of causing cellular damage. The results clearly indicated a dose-dependent increase in hydroxyl radical scavenging activity for all three substances tested. Starting at a concentration of 50 µg/ml, the extract showed a scavenging activity of 15.94 ± 0.59%, which steadily increases to 79.89 ± 0.98% at 250 µg/ml. This suggests that while the extract is effective in scavenging hydroxyl radicals, higher concentrations are necessary to achieve more substantial antioxidant effects. Quercetin exhibited a higher baseline effectiveness at the lowest concentration (40.77 ± 0.58%) and showed the greatest increase in activity among the substances, reaching up to 93.76 ± 0.89% at 250 µg/ml. This high level of activity at even lower concentrations underscoreed quercetin's potent antioxidant properties, making it highly effective against hydroxyl radicals. Ascorbic acid also displayed significant scavenging activity, starting at 30.44 ± 0.87% at 50 µg/ml and increasing to 94.98 ± 0.99% at the highest concentration tested. This pattern reflected ascorbic acid's well-known antioxidant capabilities and its reliability in neutralizing reactive oxygen species. The standard deviations across all concentrations for each substance were relatively small, indicating that the assay results were consistent, and the experimental procedures were reproducible. These low SD values enhanced the reliability of the findings and suggested that the results could be confidently used to assess the antioxidant capacity of these substances. This study provided robust evidence of the dose-dependent antioxidant activities of the extract (M-BSUD), quercetin, and ascorbic acid in scavenging hydroxyl radicals. Quercetin and ascorbic acid demonstrated excellent potential for therapeutic use due to their high efficacy at lower concentrations. The extract (M-BSUD), although less potent, still showed significant activity at higher concentrations. These findings highlighted the importance of these substances in formulations aimed at mitigating oxidative stress and suggest potential applications in dietary supplements and pharmaceuticals designed to enhance cellular antioxidant defence. Further research could explore the mechanisms through which these substances interact with hydroxyl radicals and assess their potential benefits in clinical settings.

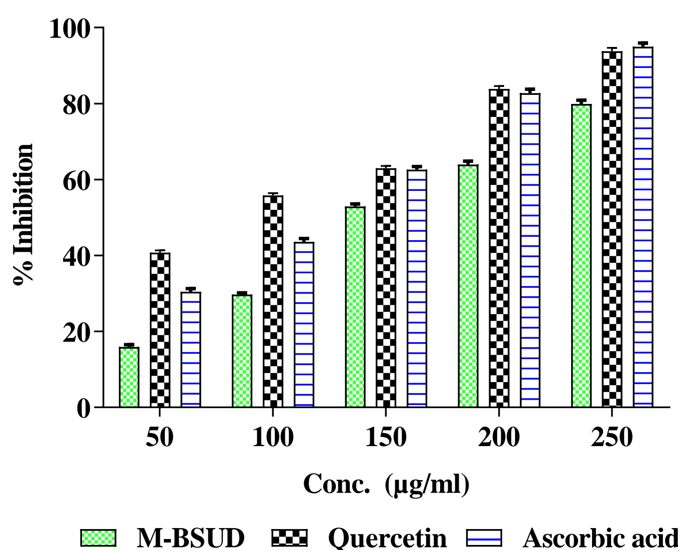

Figure 2. Hydroxyl Radical Scavenging Activity (HRSA) of the extract (M-BSUD)

Table 2. IC50 values of the extract and standards

Substance	Assay Type	IC50 Value (µg/mL)	Observations
Extract (M-BSUD)	ABTS Radical Decolorizing	124.87	Moderate potency in reducing ABTS radical formation by 50%
	Hydroxyl Radical Scavenging	143.77	Reduces hydroxyl radical formation by 50%
Ascorbic Acid	ABTS Radical Decolorizing	131.04	Comparable to Extract in ABTS activity
	Hydroxyl Radical Scavenging	116.96	Effective, but less than Quercetin in HRSA
Quercetin	ABTS Radical Decolorizing	64.31	Most potent in ABTS assay, lowest IC50 value
	Hydroxyl Radical Scavenging	80.68	Highly effective, lowest IC50 among substances in HRSA

Anti-inflammatory activity

Anti-inflammatory activity using RAW 264.7 macrophages

The results presented the anti-inflammatory activity of various concentrations of a treatment using RAW 264.7 macrophages, focusing on the production of pro-inflammatory cytokines IL-1β, TNF-α, and IL-6. This setup was designed to assess the potential of the treatment to reduce inflammatory responses induced by lipopolysaccharide (LPS). The data clearly showed a concentration-dependent decrease in the levels of all three cytokines (IL-1β, TNF-α, IL-6) as the treatment concentration increases, indicating the efficacy of the treatment in modulating inflammatory responses.

- IL-1β: Starting from a high with LPS induction at 222.56 ± 8.95 pg/mL, the level dropped progressively across the treatment groups to 104.43 ± 1.27 pg/mL at the highest treatment concentration (120 µg/mL). This demonstrated a substantial reduction, nearing the baseline levels observed in the control group.
- TNF-α: Similarly, TNF-α levels showed 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, was 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 indicated effective control over this signalling molecule, which plays a crucial role in the acute phase response.

The results suggested that the treatment is highly effective in reducing the production of key inflammatory cytokines in a dose-dependent manner. The substantial decreased in cytokine levels, especially at higher concentrations of the treatment, underscored its potential as an anti-inflammatory agent. The consistency in the reduction across all three cytokines further supported the broad-spectrum anti-inflammatory properties of the treatment. This study highlighted the potential of the tested extract (M-BSUD) 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 activity of extract mixture (M-BSUD) in terms of levels of cytokines.

Serial No.	Treatment Groups	IL-1β (pg/mL)	TNF-α (pg/mL)	IL-6 (pg/mL)
1	Control	12.6±0.82	55.61±0.98	46.68±0.99
2	LPS only	222.56±8.95	255.79±9.27	206.91±8.95
3	15 µg/mL	183.12±6.57	225.74±8.58	186.57±6.58
4	30 µg/mL	163.68±5.69	185.98±6.47	156.76±4.36
5	60 µg/mL	134.12±4.98	166.64±5.37	136.93±2.27
6	120 µg/mL	104.43±1.27	106.36±1.78	96.48±1.75

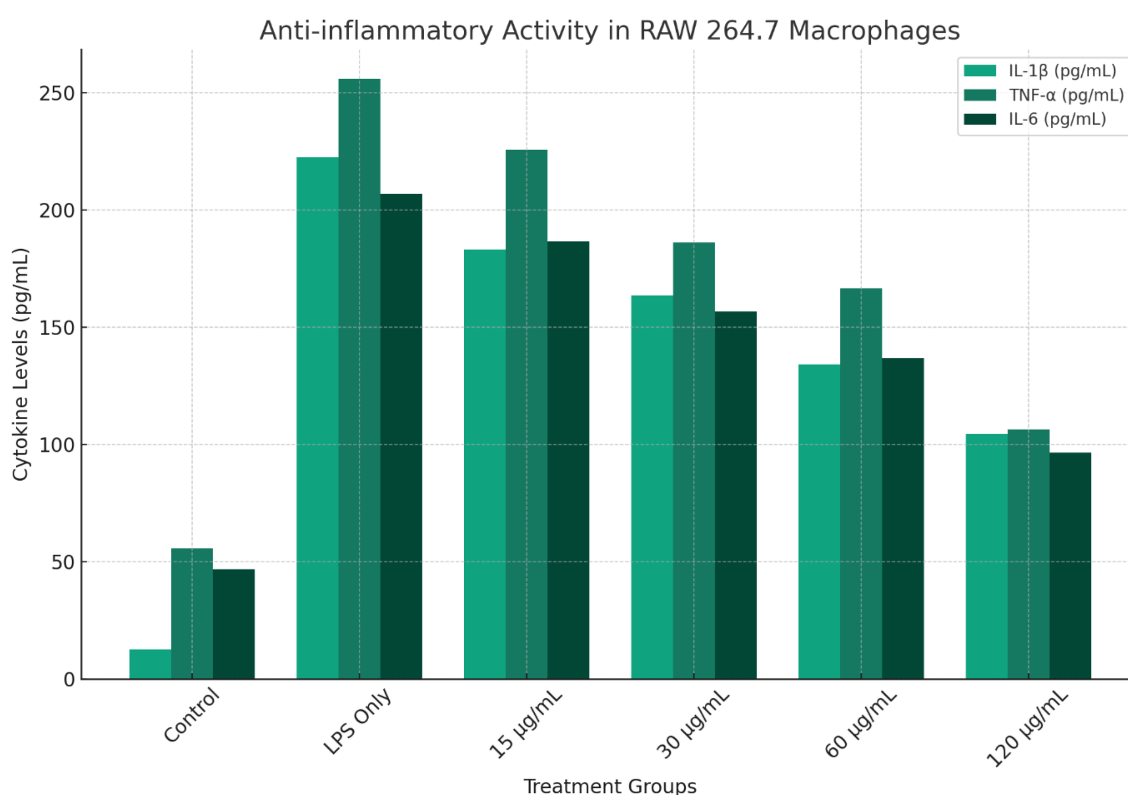

Figure 3. Anti-inflammatory activity of extract mixture (M-BSUD) in terms of levels of cytokines in RAW 264.7 macrophages.

4. Conclusions

The study demonstrated that the extract mixture, M-BSUD, containing *Boswellia serrata* and *Urtica dioica* methanol leaves extract, exhibits potent antioxidant and anti-inflammatory activities. Quercetin, a known antioxidant, displayed superior radical scavenging ability, while the extract showed significant antioxidant properties, albeit at higher concentrations. In the anti-inflammatory tests, M-BSUD exhibited a dose-dependent decrease in pro-inflammatory cytokine production, suggesting its potential in mitigating inflammation. These results underscore the therapeutic potential of M-BSUD in conditions associated with oxidative stress and inflammation. Its effectiveness comparable to standard antioxidants and anti-inflammatory agents indicated promising prospects for its use in therapeutic and clinical settings. Further research exploring its mechanisms of action and clinical efficacy is warranted to fully exploit

its therapeutic benefits. Overall, M-BSUD represented a promising candidate for the development of novel antioxidant and anti-inflammatory therapies.

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