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ANTIOXIDANT AND ANTI-INFLAMMATORY PROPERTIES OF BOSWELLIA SERRATA EXTRACT: IMPLICATIONS FOR NEUROPROTECTION

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ABSTRACT

This study investigated the antioxidant and neuroprotective properties of the methanolic extract of whole plant of*Boswellia serrata*codenamed asBSWP-M. The antioxidant capacity was assessed using the ABTS radical decolorization assay and the hydroxyl radical scavenging activity (HRSA) assay. BSWP-M exhibited significant antioxidant activity, with an IC50 value of 133.87 µg/mL in the ABTS assay, indicating its capability to scavenge free radicals effectively, though it was slightly less potent than ascorbic acid and quercetin, which had IC50 values of 109.07 µg/mL and 99.76 µg/mL, respectively. In the HRSA assay, BSWP-M showed an IC50 value of 122.87 µg/mL, demonstrating considerable hydroxyl radical scavenging activity.The anti-inflammatory neuroprotective potential of BSWP-M was evaluated by measuring its effects on intercellular ROS levels as well as the production of pro-inflammatory cytokines IL-6, IL-1, and TNF-α in RAW264.7 cells. Treatment with 100 mg/mL BSWP-M resulted in significant inhibition of cytokine production, with inhibition rates of 69.87%, 55.93%, and 61.84% for IL-6, IL-1, and TNF-α, respectively ($p < 0.001$).These findings highlightedBSWP-M as a promising natural agent with substantial antioxidant and neuroprotectiveactivities.

Keywords: Oxidative stress, Antioxidant, Neuroprotective, *Boswellia serrata,* Pro-inflammatory cytokines

INTRODUCTION

Oxidative stress, neuroprotection, and inflammation are intricately interconnected processes that play crucial roles in the pathophysiology of various neurodegenerative diseases and other health conditions. Understanding the interrelation among these factors is essential for developing effective therapeutic strategies to mitigate neuronal damage and promote brain health(Khan et al., 2019, Tsui et al., 2019, Koh et al., 2020, Azeez and Lunghar, 2021, Nathan and Ding, 2010).Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify these harmful compounds or repair the resulting damage. ROS are highly reactive molecules that can damage cellular components, including lipids, proteins, and DNA. In the brain, which is particularly susceptible to oxidative damage due to its high oxygen consumption and

abundant lipid content, oxidative stress is a key factor in the development and progression of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS)(Kataki et al., 2014).The accumulation of oxidative damage in neuronal cells leads to dysfunction and cell death, contributing to the characteristic cognitive and motor deficits observed in these disorders. Antioxidant defenses, including enzymatic systems like superoxide dismutase (SOD) and non-enzymatic molecules like glutathione, play a vital role in neutralizing ROS. However, in neurodegenerative diseases, these defenses are often overwhelmed, leading to chronic oxidative stress and progressive neuronal damage(Hassan et al., 2017, Zafar et al., 2019, Kang and Yang, 2020, Sies, 2020).

Inflammation is another critical factor in neurodegeneration, often closely linked with oxidative stress. Inflammatory responses in the brain, typically mediated by microglia and astrocytes, are essential for maintaining homeostasis and responding to injury or infection. However, chronic inflammation can have detrimental effects on neuronal health. Microglia, the brain's resident immune cells, become activated in response to injury or disease, releasing pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α). While these cytokines are crucial for initiating repair processes, their prolonged release can lead to sustained inflammation and further neuronal damage(Hassan et al., 2017, Pisoschi et al., 2021, Wang et al., 2021, Barmoudeh et al., 2022, Batty et al., 2022).Oxidative stress can exacerbate inflammation by activating signaling pathways that promote the production of pro-inflammatory mediators. For instance, ROS can activate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, a key regulator of inflammatory responses. Conversely, inflammation can enhance oxidative stress through the production of nitric oxide and other reactive nitrogen species by activated microglia and astrocytes. This bidirectional relationship creates a vicious cycle where oxidative stress and inflammation perpetuate each other, amplifying neuronal injury(Hassan et al., 2017, Pisoschi et al., 2021, Wang et al., 2021, Barmoudeh et al., 2022, Batty et al., 2022, Schmid-Schönbein, 2006).

Neuroprotection aims to preserve neuronal function and viability in the face of pathological conditions. Given the interconnected nature of oxidative stress and inflammation, effective neuroprotective strategies often target both processes simultaneously. Antioxidants, which neutralize ROS and restore redox balance, are a cornerstone of neuroprotective approaches(Schmid-Schönbein, 2006, Federico et al., 2007,

Nathan and Ding, 2010, Hassan et al., 2017).Compounds such as vitamin E, coenzyme Q10, and various polyphenols have shown promise in reducing oxidative damage and improving neuronal survival in preclinical models.In addition to antioxidants, anti-inflammatory agents are critical for neuroprotection. Non-steroidal anti-inflammatory drugs (NSAIDs) and specific inhibitors of inflammatory pathways, such as NF-κB inhibitors, can reduce the inflammatory response and mitigate neuronal damage. Furthermore, natural extracts with combined antioxidant and anti-inflammatory properties, like those from Boswellia serrata, have gained attention for their potential to address both oxidative stress and inflammation synergistically(Schmid-Schönbein, 2006, Trejo-Hurtado et al., 2023, Voufo et al., 2023, Wang et al., 2023, Yazdi et al., 2023).

The complex interplay between oxidative stress, neuroprotection, and inflammation underscores the need for integrated therapeutic approaches. Targeting multiple pathways involved in neurodegeneration can provide more comprehensive protection against neuronal injury. For instance, combining antioxidants with anti-inflammatory agents may offer synergistic benefits, enhancing their individual effects and providing more robust neuroprotection(Kakoti et al., 2015, Kataki et al., 2014)..In closing, oxidative stress and inflammation are key contributors to neurodegeneration, and their interrelationship plays a critical role in the progression of neuronal damage. Neuroprotective strategies that address both oxidative stress and inflammation hold promise for mitigating neuronal injury and improving outcomes in neurodegenerative diseases. Understanding and targeting the complex interactions among these processes is essential for developing effective treatments that preserve brain health and function(Federico et al., 2007, Chaudhari et al., 2014, Kakoti et al., 2015).

Boswellia serrata, commonly known as Indian frankincense, is a tree native to India, North Africa, and the Middle East. It has been used for centuries in traditional Ayurvedic medicine for its wide range of therapeutic properties. The resin extracted from *Boswellia serrata* contains a variety of bioactive compounds, most notably boswellic acids, which are credited with the plant's anti-inflammatory, antioxidant, and analgesic effects(Siddiqui, 2011, Khan et al., 2016).In recent years, scientific interest in Boswellia serrata has grown due to its potential benefits in treating inflammatory and neurodegenerative diseases. Studies have shown that boswellic acids inhibit key inflammatory enzymes, such as 5-lipoxygenase, thereby reducing the production of pro-inflammatory mediators. This anti-inflammatory action is complemented by the resin's ability to scavenge free radicals and

protect against oxidative stress, which is a significant factor in the pathogenesis of conditions like arthritis, asthma, and neurodegenerative disorders(Upaganlawar and Ghule, 2009, Alam et al., 2012).*Boswellia serrata*'s dual role in mitigating inflammation and oxidative damage makes it a promising candidate for integrative medicine approaches. Ongoing research aims to further elucidate its mechanisms of action and therapeutic potential, paving the way for its inclusion in modern pharmacological treatments(Alam et al., 2012). In light of the aforementioned information and the literature review, the current study's objective was to assess the antioxidant and neuroprotective properties of *Boswellia serrata* whole plant extract using a variety of in vitro mechanistic models.

MATERIAL AND METHODS

Drugs, chemicals, and reagents

The extraction method involved the use of chemicals and reagents from Loba Chemie, Mumbai, India, such as distilled water, methanol, and high analytical grade ethanol. Essential reagents for the anti-inflammatory assays included enzyme-linked immunosorbent assay (ELISA) kits, which measure pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6. RAW 264.7 macrophage cells were used in in vitro cell culture experiments, together with the required culture media, foetal bovine serum (FBS), and antibiotics (streptomycin and penicillin). To cause inflammation in these cells, lipopolysaccharide (LPS) was procured from Himedia and Sigma Aldrich in India. In order to guarantee high purity and dependability and to enable consistent and repeatable results, all chemicals and medications were purchased from reliable providers.

Collection, authentication, and extraction of the plants

The plant, *Boswellia serrata*, was gathered in the Kulluareaof Himachal Pradesh, India. A herbologist and a botanist created and verified herbariums. The entire plant was dried in the shade and ground into a coarse powder using a mechanical machine grinder. The coarse powder of the whole plant underwent a ten-day cold maceration in methanol. The extract was collected after ten days of cold maceration, and it was then dried in a vacuum desiccator. Codenamed BSWP-M, the final extract of the whole *Boswellia serrata* plant was kept at -4 ^{0}C until needed again.

Preliminary Phytochemical study

A preliminary phytochemical study was conducted to identify the presence of various bioactive compounds in the plant extracts. The study involved qualitative tests to detect alkaloids, flavonoids, tannins, saponins, terpenoids, and glycosides as per standard

protocols (Harborne, 1998). Alkaloids were identified using Mayer's and Dragendorff's reagents, which produced a cream or reddish-brown precipitate, respectively. Flavonoids were detected using the Shinoda test, where a pink or red coloration indicated their presence after the addition of magnesium turnings and hydrochloric acid. Tannins were confirmed by adding ferric chloride solution to the extract, resulting in a blue-black or greenish-black coloration. Saponins were detected through the froth test, where persistent frothing upon shaking the extract with water indicated their presence. Terpenoids were identified using the Salkowski test, where the formation of a reddish-brown coloration upon adding sulfuric acid indicated their presence. Glycosides were detected by adding glacial acetic acid and ferric chloride followed by sulfuric acid, leading to the formation of a brown ring at the interface. These qualitative tests provided a preliminary understanding of the phytochemical profile of the plant extracts, laying the groundwork for further quantitative analyses and bioactivity studies.

Antioxidant activity

ABTS radical decolorization assay

The ABTS radical decolorization assay is a commonly employed method for assessing the antioxidant potential of materials, including plant extracts (Re et al., 1999).This method is based on the ability of antioxidants to quench the ABTS radical cation (ABTS•+), a blue-green chromophore, leading to its decolorization. The procedure involved the generation of the ABTS•+ by reacting ABTS stock solution with potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12-16 hours before use. The resulting ABTS•+ solution was then diluted with ethanol to an absorbance of approximately 0.700 at 734 nm.Plant extracts were prepared in varying concentrations and added to the ABTS•+ solution. The reaction mixture was incubated at room temperature for a specific period, typically 30 minutes. The decrease in absorbance at 734 nm was measured using a spectrophotometer. The percentage inhibition of the ABTS•+ was calculated using the following formula:

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

The antioxidant activity of the extracts was expressed as IC50 values, which represent the concentration of the extract required to inhibit 50% of the ABTS•+ radicals. Lower IC50 values indicate higher antioxidant activity. This assay provided a reliable and rapid measure of the antioxidant potential of the plant extracts, contributing valuable data for further investigation of their therapeutic properties.

Hydroxyl Radical Scavenging Activity (HRSA)

The hydroxyl radical scavenging activity (HRSA) assay was utilized to assess the ability of plant extracts to scavenge hydroxyl radicals, which are highly reactive species contributing to oxidative stress and cellular damage(Tijani et al., 2018). This assay is based on the Fenton reaction, where hydroxyl radicals are generated through the reaction of ferrous ions (Fe^{2+}) with hydrogen peroxide (H_2O_2).In the HRSA assay, the reaction mixture typically consisted of the following components: plant extract at various concentrations, 1 mM $FeSO_4$ (ferrous sulfate), 1 mM EDTA (ethylenediaminetetraacetic acid), 20 mM H_2O_2, and 30 mM deoxyribose. The mixture was incubated at 37°C for 1 hour. During this incubation period, hydroxyl radicals are generated, which subsequently degrade deoxyribose into malondialdehyde (MDA).The resulting MDA reacts with thiobarbituric acid (TBA) under acidic conditions to form a pink chromogen, which can be measured spectrophotometrically at 532 nm. The extent of deoxyribose degradation is inversely proportional to the hydroxyl radical scavenging activity of the plant extracts.The percentage inhibition of hydroxyl radical-induced deoxyribose degradation was calculated using the formula:

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

The antioxidant capacity of the plant extracts was expressed as IC50 values, indicating the concentration of the extract required to inhibit 50% of hydroxyl radical activity. Lower IC50 values denote higher hydroxyl radical scavenging activity. This assay provided critical insights into the antioxidant properties of the plant extracts, highlighting their potential for therapeutic applications in mitigating oxidative stress.

Neuroprotective activity

Cell culture and treatment

To explore the neuroprotective effects of extract, SK-N-SH human neuroblastoma cells (sourced from the American Type Culture Collection, ATCC) were cultured in Dulbecco's Modified Eagle Medium (DMEM). This medium was enriched with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin to ensure optimal cell growth and maintenance. Cells were seeded into 96-well plates at a density of 1.5 × 10^4 cells per well and incubated at 37°C in a 5% CO2 humidified atmosphere for 24 hours to allow adherence and growth.To synchronize the cells and reduce nutrient variability, the medium was then replaced with DMEM containing antibiotics but devoid of serum, inducing a nutrient-deprivation stress condition. Following serum deprivation, the cells

were treated with 20 µM acrolein, a toxic aldehyde used to induce oxidative stress and mimic neurodegenerative disease conditions. The acrolein treatment lasted for 24 hours, permitting sufficient time for oxidative stress and cellular damage to occur.This setup provided a robust model to assess extract's neuroprotective properties by evaluating cell viability, morphological changes, and biochemical markers of oxidative stress post-treatment. Controls and replicates were included to ensure the reliability and reproducibility of the data, thereby validating the potential neuroprotective efficacy of the extract in mitigating acrolein-induced neurotoxicity(Thummayot et al., 2016, Thummayot et al., 2014).

Intracellular reactive oxygen species level

We evaluated the ability of the methanolic extract (BSWP-M) to scavenge reactive oxygen species (ROS) in SK-N-SH cells using the fluorescent dye 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) in order to confirm its antioxidant activity. This technique, which measures intracellular ROS levels reliably, is based on known methods. The process started with SK-N-SH cells being cultured in Dulbecco's Modified Eagle Medium (DMEM), which was supplemented with 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin. The cells were then incubated at 37°C in a humidified environment with 5% CO2. To test ASLE-M's ability to scavenge ROS, cells were treated with various doses of the compound once they had attained the proper confluence. After being treated with BSWP-M, the cells were incubated at 37°C for 30 minutes with 10 µM DCF-DA dye. In its natural state, DCF-DA is non-fluorescent and cell-permeable. It is enzymatically cleaved into 2',7'-dichlorodihydrofluorescein (DCFH), a non-fluorescent molecule, inside the cells by intracellular esterases. Next, the ROS in the cells quickly oxidise DCFH to produce the highly fluorescent compound 2',7'-dichlorofluorescein (DCF). Using a fluorescent microplate reader set to an excitation wavelength of 485 nm and an emission wavelength of 535 nm, the fluorescence intensity of DCF—which is directly proportional to the amount of ROS within the cells—was quantified. While decreased fluorescence intensity revealed that the antioxidant chemical under investigation was effectively scavenging ROS, higher fluorescence intensity indicated higher ROS levels. We evaluated the degree to which BSWP-M decreased intracellular ROS levels by contrasting the fluorescence intensity of BSWP-M-treated cells with untreated control cells. This comparison investigation demonstrated BSWP-M's ability to neutralise ROS and shield cells from oxidative stress, hence confirming its antioxidant capability. This technique offers a reliable and sensitive way to assess a compound's antioxidant activity in a cellular

setting, providing important information on its therapeutic uses in reducing damage caused by oxidative stress (Thummayot et al., 2014, Ramassamy and Singh, 2017).

Cytokines profiling(IL-1β, IL-6 and TNF-α)

Cytokine profiling was performed to evaluate the levels of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α in cell culture supernatants following treatment with the methanolic extract (BSWP-M). The procedure utilized enzyme-linked immunosorbent assay (ELISA) kits designed for high sensitivity and specificity for each cytokine(Lee et al., 2006).SK-N-SH cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin. The cells were seeded in 6-well plates at an appropriate density and incubated at 37°C in a humidified atmosphere containing 5% CO2. Once the cells reached approximately 70-80% confluence, they were treated with varying concentrations of BSWP-Mfor 24 hours.To induce an inflammatory response, lipopolysaccharide (LPS) at a concentration of 1 µg/ml was added to the cell cultures during the last 6 hours of BSWP-Mtreatment. This exposure aimed to simulate an inflammatory condition and elicit cytokine production.After treatment, the cell culture supernatants were collected and centrifuged at 1500 rpm for 10 minutes to remove any cell debris. The clarified supernatants were stored at -80°C until further analysis.The levels of IL-1β, IL-6, and TNF-α in the supernatants were quantified using commercial ELISA kits (e.g., from R&D Systems or Thermo Fisher Scientific). The assays were performed according to the manufacturer's instructions. Briefly, 100 µl of standards, samples, and controls were added to the wells of a 96-well microplate pre-coated with specific antibodies against each cytokine. After incubation, the wells were washed to remove unbound substances.A biotinylated antibody specific for each cytokine was added to the wells, followed by a streptavidin-HRP conjugate. The plate was incubated to allow binding, and then washed to remove any unbound antibody-enzyme reagent. A substrate solution was added to the wells, and colour development was monitored. The reaction was stopped with a stop solution, and the absorbance was measured at 450 nm using a microplate reader.The concentration of each cytokine in the samples was calculated by comparing the absorbance readings to a standard curve generated using known concentrations of the cytokines. The data were expressed as pg/ml of cytokine in the supernatants.This method provided a quantitative assessment of the inflammatory response, allowing for the evaluation of the anti-inflammatory potential of BSWP-Mby comparing cytokine levels in treated versus control samples.

Statistical Analysis

Statistical analysis was conducted to ensure the validity and reliability of the experimental results. All data were expressed as mean ± standard deviation (SD) from at least three independent experiments. The statistical significance of differences between treated and control groups was evaluated using one-way analysis of variance (ANOVA) followed by post-hoc tests, such as Tukey's multiple comparisontest, to compare multiple groups. For assays measuring intracellular reactive oxygen species (ROS) levels, cell viability, and other biochemical parameters, the fluorescence intensity or absorbance values were normalized to the control group and expressed as percentages. P-values less than 0.05 were considered statistically significant, indicating a less than 5% probability that the observed differences were due to random chance. Statistical analyses were performed using software tools such as GraphPad Prism or SPSS to facilitate robust data handling and interpretation.

RESULTS AND DISCUSSION

Preliminary Phytochemical study

The preliminary phytochemical study of the methanolic extract of whole plant of*Boswellia serrata* (BSWP-M) revealed the presence of a diverse range of bioactive compounds. The extract tested positive for phenols, saponins, tannins, sterols, carbohydrates, alkaloids, terpenoids, fatty acids, flavonoids, and glycosides. This rich phytochemical profile suggests that BSWP-M possesses multiple therapeutic properties, contributing to its antioxidant, anti-inflammatory, and neuroprotective effects. The presence of these compounds supports the traditional use of Boswellia serrata in herbal medicine and highlights its potential for developing new pharmacological treatments.

Table 1. Results of Preliminary Phytochemical study

Phytoconstituents	**BSWP-M**
Phenols	√
Saponins	√
Tannins	√
Sterols	√
Carbohydrates	√
Alkaloids	√
Terpenoid	√
Fatty acid	√
Flavanoids	√
Glycosides	√

Antioxidant activity

ABTS radical decolorization assay

The ABTS radical decolorization assay was conducted to evaluate the antioxidant capacity of the methanolic extract of BSWP-M, with ascorbic acid and quercetin serving as standard references. The results are summarized in the following figure 1, showing the percentage inhibition of ABTS radicals at various concentrations (µg/ml). The antioxidant activity of BSWP-M increases with concentration, indicating a dose-dependent relationship. At the highest tested concentration (250 µg/ml), BSWP-M achieves an inhibition of 90.87%, suggesting strong antioxidant properties.While BSWP-M shows considerable antioxidant activity, it is slightly less effective than ascorbic acid and quercetin at higher concentrations. Ascorbic acid and quercetin reach near-maximal inhibition levels of 85.93% and 97.89% respectively at 250 µg/ml. Notably, quercetin consistently shows the highest antioxidant activity among the tested substances.At lower concentrations (50-100 µg/ml), BSWP-M shows moderate antioxidant activity compared to ascorbic acid and quercetin. However, its efficacy increases substantially at concentrations above 100 µg/ml, narrowing the gap with the standards.The results indicated that BSWP-M had significant antioxidant potential, especially at higher concentrations. Its ability to scavenge ABTS radicals effectively demonstrates its capability to neutralize free radicals, which can help mitigate oxidative stress-related cellular damage. The dose-dependent increase in antioxidant activity suggests that BSWP-M contains potent bioactive compounds that become more effective at higher doses.While BSWP-M is slightly less potent than ascorbic acid and quercetin, its performance is commendable, particularly when considering the broader context of its natural extract composition. The strong antioxidant activity observed at higher concentrations indicates that BSWP-M could be a valuable natural antioxidant source.Future studies could focus on isolating and identifying the specific phytochemicals responsible for this activity in BSWP-M. Additionally, exploring the synergistic effects of these compounds with other antioxidants could further enhance its therapeutic potential. The findings support the potential application of BSWP-M in formulations aimed at preventing or managing oxidative stress-related conditions

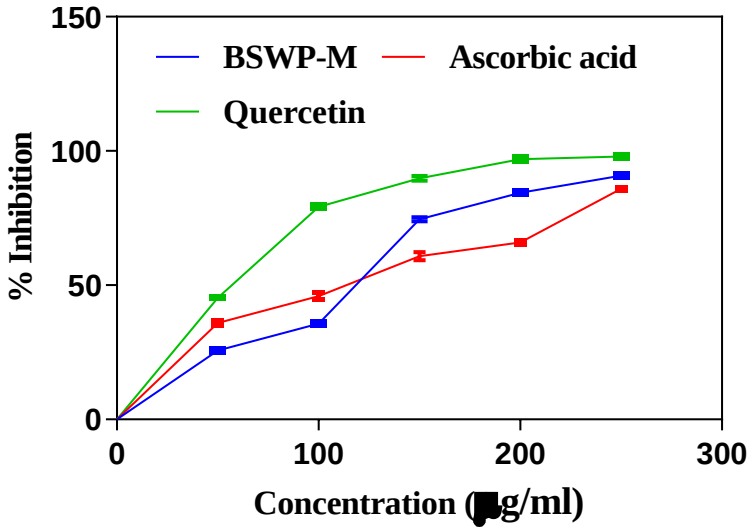

Figure 1. ABTS radical decolorization assay of the extract (BSWP-M)

Hydroxyl Radical Scavenging Activity (HRSA)

The hydroxyl radical scavenging activity (HRSA) of the extract BSWP-M was evaluated and compared to the standard antioxidants Quercetin and Ascorbic acid across various concentrations (0-250 µg/ml). The results indicate that BSWP-M exhibits a dose-dependent increase in HRSA. At the lowest concentration tested (50 µg/ml), BSWP-M showed moderate scavenging activity (17.9%), which was significantly lower than Quercetin (42.73%) and Ascorbic acid (32.4%). As the concentration increased, the HRSA of BSWP-M improved notably. At 150 µg/ml, BSWP-M achieved 54.84% activity, closely approaching the levels of Quercetin (64.93%) and Ascorbic acid (64.53%). At the highest concentration (250 µg/ml), BSWP-M reached 81.85% HRSA, nearing the efficacy of Quercetin (95.72%) and Ascorbic acid (96.94%). While Quercetin exhibited the highest HRSA across all concentrations, starting at 42.73% at 50 µg/ml and reaching 95.72% at 250 µg/ml, Ascorbic acid also demonstrated strong HRSA, slightly outperforming Quercetin at the highest concentration with 96.94%. These findings suggest that BSWP-M, despite being less effective than Quercetin and Ascorbic acid at lower concentrations, shows significant promise as a natural antioxidant at higher concentrations. The substantial HRSA of BSWP-M at higher doses highlights its potential for applications where higher concentrations are viable. Additionally, the possibility of synergistic use with Quercetin or Ascorbic acid could be explored to enhance overall antioxidant efficacy, leveraging the strong activity of the standards at lower concentrations and the increasing activity of BSWP-M at higher concentrations.

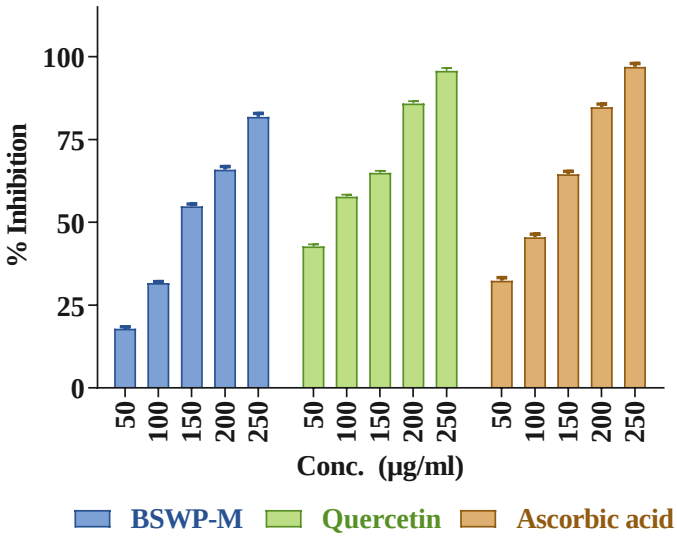

Figure 2. Hydroxyl Radical Scavenging Activity (HRSA) of the extract (BSWP-M)

The IC50 values from both assays suggest that BSWP-M possesses significant antioxidant properties, though not as potent as the standard antioxidants ascorbic acid and quercetin. In the ABTS radical decolorization assay, BSWP-M's higher IC50 value implies that it is less effective in scavenging ABTS radicals compared to ascorbic acid and quercetin. This may be due to the concentration or the nature of the active compounds within BSWP-M that contribute to its antioxidant activity.In the hydroxyl radical scavenging activity assay, BSWP-M also shows a higher IC50 value than quercetin and ascorbic acid, indicating lower efficacy in scavenging hydroxyl radicals. Quercetin, with the lowest IC50 value in both assays, stands out as the most potent antioxidant, likely due to its strong free radical scavenging capabilities attributed to its polyphenolic structure.The findings highlight that while BSWP-M is a capable antioxidant, it is relatively less potent compared to the pure standard compounds. However, its natural extract composition might offer additional synergistic health benefits that single-compound antioxidants do not provide. These results underscore the potential therapeutic applications of BSWP-M, particularly in formulations where a broad spectrum of antioxidant activities is desirable. Further studies could investigate the specific active constituents within BSWP-M to better understand and potentially enhance its antioxidant properties.

Table 2. IC50 values of the extract and standards

Assay	**Substance**	**IC50 Value (µg/mL)**
ABTS Radical Decolorizing	BSWP-M	133.87

	Ascorbic Acid	109.07
	Quercetin	99.76
Hydroxyl Radical Scavenging Activity (HRSA)	BSWP-M	122.87
	Quercetin	67.46
	Ascorbic Acid	88.27

Neuroprotective activity

Intracellular reactive oxygen species levels are lowered by BSWP-M

The study evaluated the effect of BSWP-M on intracellular reactive oxygen species (ROS) levels in cells exposed to oxidative stress induced by hydrogen peroxide (H_2O_2). The data clearly demonstrated that H_2O_2 significantly increases ROS levels in the cells, with a recorded level of 304 ± 6.16 compared to the control value of 100 µg/ml. This substantial rise in ROS indicates the oxidative stress condition created by H2O2 exposure.When cells were treated with BSWP-M at various concentrations, a dose-dependent reduction in ROS levels was observed. At the lowest concentration of 5 µg/ml, BSWP-M did not significantly mitigate the ROS levels, which remained virtually unchanged at 303 ± 5.77. However, as the concentration of BSWP-M increased, a notable decline in ROS levels was recorded. At 10 µg/ml, the ROS level decreased to 263 ± 5.87, indicating the beginning of antioxidant activity by BSWP-M.The most substantial reductions in ROS levels were observed at higher concentrations of BSWP-M. At 20 µg/ml, the ROS levels further dropped to 245 ± 5.23, suggesting a more pronounced antioxidant effect. This trend continued with 40 µg/ml and 80 µg/ml concentrations of BSWP-M, where the ROS levels were significantly reduced to 185 ± 2.78 and 181 ± 2.64, respectively. These findings highlight the potent antioxidant capacity of BSWP-M, especially at higher concentrations, effectively counteracting the oxidative stress induced by H2O2.The data showed the efficacy of BSWP-M in reducing intracellular ROS levels, which is crucial for protecting cells from oxidative damage. Oxidative stress is a major contributor to cellular damage and is implicated in a wide range of diseases, including neurodegenerative disorders. By lowering ROS levels, BSWP-M helps in mitigating the harmful effects of oxidative stress, thereby potentially offering neuroprotective benefits.In a nutshell, the study confirmed that BSWP-M possesses strong antioxidant properties, particularly evident at higher

concentrations. This suggested that BSWP-M could be a valuable natural therapeutic agent for managing oxidative stress-related conditions. Further research should focus on elucidating the specific mechanisms through which BSWP-M exerts its antioxidant effects and explore its potential clinical applications. These findings provide a promising foundation for developing BSWP-M-based interventions aimed at reducing oxidative damage and promoting cellular health

Table 3. Impact of BSWP-Min intracellular reactive oxygen species level

Control	**H_2O_2**	**BSWP-MConcentration (µg/ml)**				
	500 µm	**5**	**10**	**20**	**40**	**80**
100	304±6.16	303±5.77	263±5.87	245±5.23	185±2.78	181±2.64

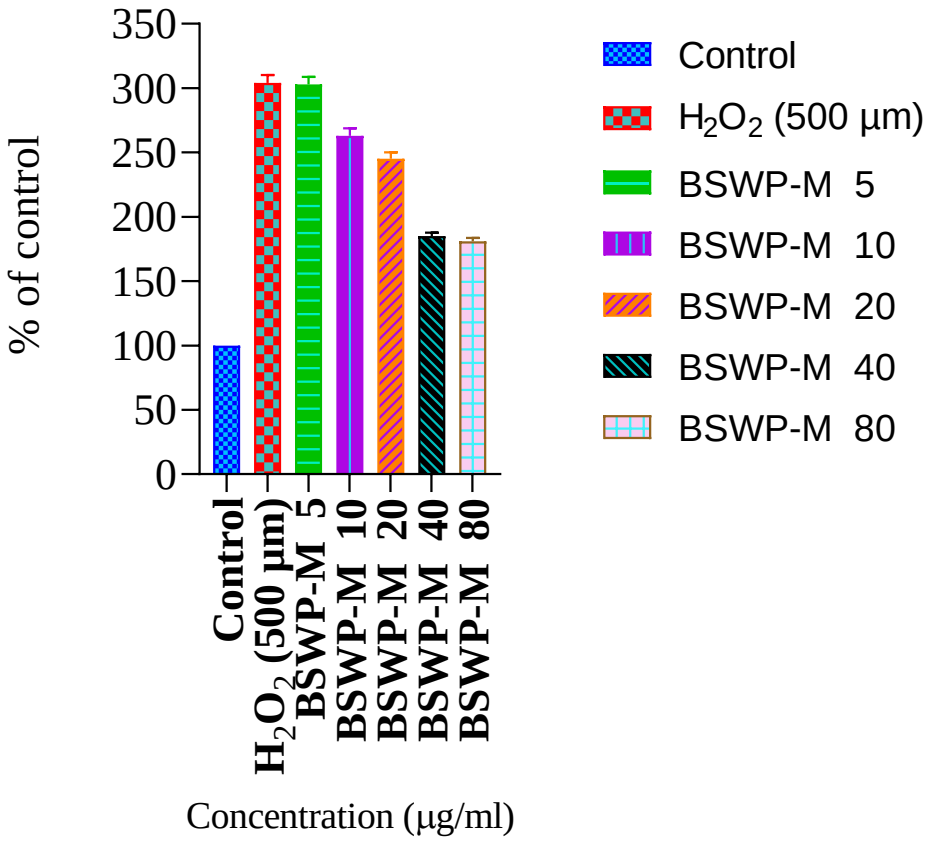

Figure 3. Intracellular reactive oxygen species levels were reduced by BSWP-M.

Cytokines profiling (IL-1β, IL-6 and TNF-α)

The results from this study demonstrate that BSWP-M has a substantial inhibitory effect on the production of key pro-inflammatory cytokines in RAW264.7 cells (Table 4 &

Figure 4). Specifically, BSWP-M inhibited IL-6 production by approximately 69.87%, IL-1 by 55.93%, and TNF-α by 61.84%. These findings suggest that BSWP-M has strong anti-inflammatory properties, potentially making it a valuable therapeutic agent for conditions characterized by excessive inflammation.IL-6, IL-1, and TNF-α are crucial mediators in the inflammatory response. Elevated levels of these cytokines are often associated with chronic inflammatory diseases and conditions such as rheumatoid arthritis, inflammatory bowel disease, and other autoimmune disorders. The significant reduction in cytokine production observed with BSWP-M treatment implies that it could help mitigate the inflammatory processes underlying these diseases.The pronounced inhibition of IL-6 suggests that BSWP-M could be particularly effective in diseases where IL-6 plays a pivotal role, such as rheumatoid arthritis and systemic lupus erythematosus. Similarly, the reduction of TNF-α production points to its potential in treating conditions like psoriasis and ankylosing spondylitis, where TNF-α is a critical factor.These results align with the broader understanding of the anti-inflammatory potential of plant extracts, which often contain a variety of bioactive compounds that work synergistically to produce therapeutic effects. The significant p-value ($p < 0.001$) further strengthens the reliability of these findings, indicating that the observed effects are not due to random chance but are a result of the treatment with BSWP-M.Future research could focus on isolating and characterizing the specific compounds within BSWP-M responsible for these anti-inflammatory effects. Additionally, in vivo studies and clinical trials would be essential to confirm these findings and to assess the safety and efficacy of BSWP-M in human subjects. This could pave the way for the development of new anti-inflammatory therapies derived from natural sources, offering an alternative to conventional synthetic drugs with fewer side effects

Table 4. Effect of 100 mg/ml BSWP-M on RAW264.7 cells' production of pro-inflammatory cytokines.

	Inhibition (%)		
Fraction	**IL-6**	**IL-1**	**TNF-α**
BSWP-M	69.87 ± 1.24**	55.93± 1.31**	61.84 ± 1.11**

** $p < 0.001$

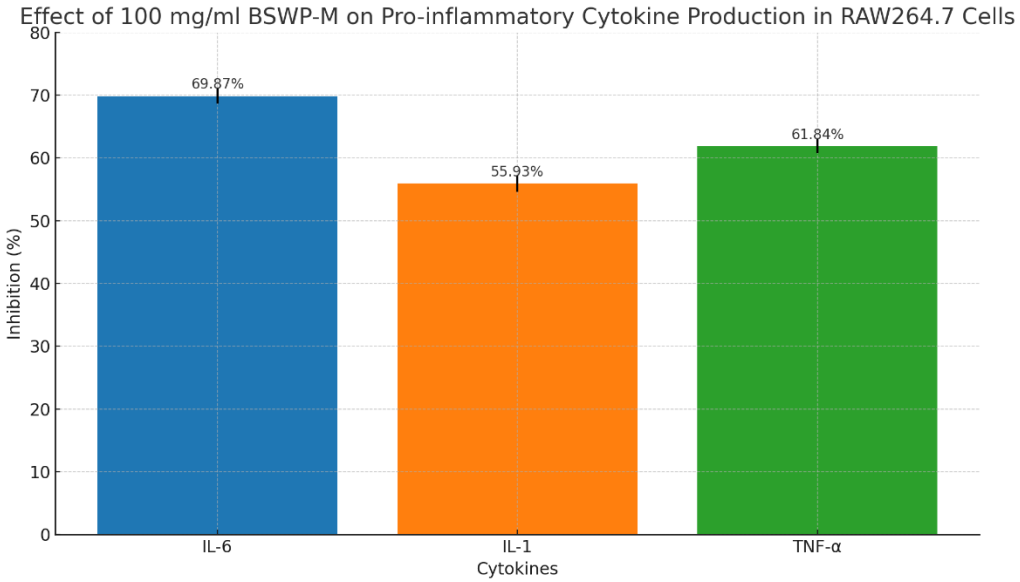

Figure 4. Pro-inflammatory cytokines profiling

CONCLUSIONS

The study demonstrated that the methanolic extract, BSWP-M possesses significant antioxidant, and neuroprotectiveproperties. The ABTS radical decolorization assay revealed that BSWP-M exhibits a notable antioxidant capacity, although slightly less potent than standard antioxidants such as ascorbic acid and quercetin. Additionally, the hydroxyl radical scavenging activity (HRSA) assay further confirmed the extract's ability to neutralize reactive oxygen species (ROS), highlighting its potential to mitigate oxidative stress.Furthermore, BSWP-M significantly inhibited the production of key pro-inflammatory cytokines IL-6, IL-1, and TNF-α in RAW264.7 cells. The observed inhibition rates of 69.87%, 55.93%, and 61.84%, respectively, emphasized the extract's strong anti-inflammatory effects, which could be beneficial in managing conditions characterized by chronic inflammation associate with various neuroinflammatory conditions. These findings suggested that BSWP-M could be a valuable natural therapeutic agent for conditions involving oxidative stress and inflammation. Future research should focus on identifying the specific bioactive compounds responsible for these effects and evaluating the extract's efficacy in vivo. The promising results from this study pave the way for potential applications of BSWP-M in developing novel treatments for oxidative stress-related and inflammatory diseases

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