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Original Article

MOLECULAR DOCKING INSIGHTS AND ANTIOXIDANT ACTIVITY OF ISOLATED BIOACTIVE COMPOUND FROM STEVIA REBAUDIANA LEAVES

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ABSTRACT

This study investigates the molecular docking interactions and antioxidant activity of an isolated bioactive compound from *Stevia rebaudiana* leaves, highlighting its potential as an antioxidant and anti-inflammatory agent. *Stevia rebaudiana* has been extensively studied for its medicinal properties, particularly in managing oxidative stress-related conditions. A bioactive compound (Rebaudioside A) was isolated using ethanol extraction, followed by phytochemical screening, TLC, column chromatography, and FTIR, NMR, and mass spectroscopy for identification. Molecular docking with nuclear factor kappa B (NF-κB) was performed using Schrödinger Suite, and antioxidant activity was assessed through 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. The isolated compound, Rebaudioside A, exhibited strong binding affinity with NF-κB (XP Gscore: -12.677), indicating significant anti-inflammatory potential. The compound showed moderate antioxidant activity, with DPPH radical scavenging activity (RSA) of 16.60% and ABTS RSA of 9.635%, while Trolox Equivalent Antioxidant Capacity (TEAC) values of 17.54 µmol/mg (DPPH) and 28.393 µmol/mg (ABTS). Therefore, we concluded that, Rebaudioside A demonstrates notable antioxidant and anti-inflammatory potential, suggesting its suitability for further pharmacological exploration in managing oxidative stress-related disorders.

KEYWORDS: *Stevia rebaudiana*; Molecular docking; Antioxidant activity; Rebaudioside A; NF-κB; DPPH; ABTS; Bioactive compound

1. Introduction

Stevia rebaudiana, a plant native to South America, is widely recognized for its natural sweetening properties. In addition to its sweetening compounds, *Stevia rebaudiana* has been traditionally used for its potential therapeutic uses, including hepatoprotective, anti-healing properties, and anti-infection pharmacological action. Recent studies have highlighted the importance of bioactive compounds extracted from *Stevia rebaudiana* for their therapeutic potential, particularly in the management of free radical damage disorders, liver diseases, and inflammation [1].

Nuclear Factor kappa B (NF-κB) is activated in response to damage nephron cells. Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are formed during cellular metabolism and in response to environmental stressors [2]. These free radicals can activate the nuclear factor kappa B signaling pathway by phosphorylating inhibitory proteins (IκBs), leading to their degradation and subsequent translocation of nuclear factor kappa B dimers into the nucleus [3,4]. Once in the nucleus, NF-κB induces the countenance of genes elaborate in inflammation, cell subsistence, and immune responses [5]. Chronic activation of NF-κB due to persistent oxidative stress can lead to a pro-inflammatory state and contribute to the development of diseases [6].

Antioxidants can exert their protective effects by scavenging ROS, thereby reducing oxidative stress and inhibiting NF-κB activation. Many natural antioxidants, such as flavonoids, polyphenols, and vitamins, have been shown to modulate NF-κB activity. By neutralizing free radicals, these antioxidants prevent the phosphorylation and degradation of IκB proteins, thereby blocking the activation of NF-κB. In this way, antioxidants help to downregulate the inflammatory and immune responses mediated by NF-κB [3,5,6]. Molecular docking studies have become an essential tool for understanding the interactions between bioactive compounds and target proteins, allowing for a deeper insight into their mechanism of action at the molecular level [7].

Recent publications have emphasized the evaluation of antioxidant and pharmacological potential of natural products, such as *Filipendula ulmaria* [8], *Lactarius sanguifluus* [9], *Silene* species [10], *Citrullus colocynthis* [11], *Salvia* extracts [12], and luteolin [13]. Moreover, Patel *et al*. (2025), demonstrated experimental validation of the therapeutic potential of *Cynodon dactylon* extract against allergic conjunctivitis [14], highlighting focus on empirical phytopharmacological research. Building on this growing body of work, the present study integrates molecular docking with in vitro antioxidant evaluation of an isolated bioactive compound from *Stevia rebaudiana*, thereby adding mechanistic insights to the phytopharmacological evidence base. Specifically, this work investigates the molecular docking interactions of the compound with key proteins involved in oxidative stress, inflammation, and related biological processes, while also evaluating its antioxidant capacity using standard assays such as DPPH and ABTS. By bridging the phytochemical profile of *Stevia rebaudiana* with both computational and experimental findings, this study contributes to a deeper understanding of its pharmacological potential and therapeutic relevance.

2. Materials and methods

2.1. Collection and Authentication of Plant Material

Fresh leaves *Stevia rebaudiana* were collected from botanical garden, Bareilly, (U.P), India in the months of July-2023 and authenticated by an expert Dr. Sunita Garg Scientist F & Head Raw Material Herbarium & Museum (NIScPR), New Delhi-India. A voucher specimen (NIScPR/RHMD/Consult/2023/4520-21-2) has been deposited in the department for further reference.

2.2. Macroscopical Properties

The leaves of the taxon are bright green having a distinct, characteristic odor and sweet taste with a slight bitter aftertaste. The leaves are about 4- 5 cm × 1- 4 cm in size. The leaves are cuneate in shape with a glabrous surface. Mature leaves have a crenate margin and reticulate venation. The apex of leaves is acute with a decurrent base. The presence of two secondary ribs on either side of the midrib is the main distinguishing characteristic [15].

2.3. Drugs and Chemicals

Chemicals used in the study were purchased from Central Drug House Ltd., New Delhi, India.

2.4. Preparation of The Extracts

Stevia rebaudiana leaves (500 g) were shade-dried for 2–3 days, pulverized into a fine powder, and defatted with 1.5 L of petroleum ether using a Soxhlet apparatus for 6 hours at 40°C. The defatted powder was extracted with 2 L of 70% ethanol (solvent: substrate ratio 4:1 v/w) via Soxhlet extraction for 8 hours at 60°C. The resulting *Stevia rebaudiana* ethanolic extract (SREE) was filtered and concentrated using a rotary vacuum evaporator at 37°C, yielding 180.85 g of crude extract (36.17% w/w). The extract was stored in an airtight container at 4°C for subsequent use. [16].

2.4. Phytochemical Screening

SREE was subjected to qualitative chemical analysis for the presence or absence of phytochemicals such as alkaloids, flavonoids glycosides, tannins, saponin and phenolic groups [17,18].

2.5. Determination of Total Flavonoid Content

One milligram of *Stevia rebaudiana* leaf extract (SREE) was dissolved in 1.5 mL of methanol and diluted 1:10 with 70% ethanol to assess the Total Flavonoid Content (TFC). To this, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water were added. A blank (methanol without extract) and negative control (reaction mixture without $AlCl_3$) were included [19]. The mixture was incubated at 28°C for 30 minutes, and absorbance was measured at 415 nm using a UV-Vis Double Beam Spectrophotometer (Systronic, detection range: 0.1–2.0 AU). Quercetin standards (10–40 µg/mL) were processed similarly to construct a calibration curve ($y = 0.001x$, $R^2 = 0.99$). TFC was expressed as milligrams of quercetin equivalents (mg QE) per gram of extract. [20,21].

2.6. Isolation and Characterization

To isolate the component from the SREE, column chromatography was done. After crystallization, the mother liquor was concentrated under vacuum and chromatographed over silica gel (60 g) of mesh size (16-120 mesh). The column was packed to a hydrodynamic column and eluted using a solvent system of chloroform: methanol was used as the mobile phase with increasing polarity. Fractions were collected and monitored by TLC [22]. The isolated compound was characterized by different spectroscopic techniques (that is IR, NMR and LC-MS) [23,24].

2.7. Molecular Docking studies

The molecular structure of the Nuclear Factor kappa B p105 subunit (NF-κB, PDB ID: 8TQD) was obtained from the Protein Data Bank in X-ray crystallography format (resolution: 2.02 Å). The structure was optimized by removing water molecules >1 Å, adding hydrogen atoms, and minimizing energy using the OPLS-2005 force field in AutoDock Vina. Rebaudioside A (PubChem CID: 6918840) was prepared from its 3D SDF structure, converted to PDB format using PyMOL, and docked using a grid map (50 × 44 × 40 points, 1 Å spacing). A reference ligand, parthenolide, was similarly prepared for comparative docking. [25,26].

2.8. Antioxidant Activity

2.9.1. DPPH assay

The DPPH assay was conducted by mixing 0.1 mL of sample solution (1 mg/mL Rebaudioside A in methanol) with 3.9 mL of 0.1 mM DPPH radical solution. The mixture was incubated in the dark for 30 minutes at 25°C, and absorbance was measured at 518 nm using a UV-Vis spectrophotometer. A blank (methanol without DPPH) and control (DPPH without sample) were included. A calibration curve was constructed using Trolox standards (0-250 µg/mL). Radical scavenging activity (RSA) was calculated as: $\%RSA = [(A_0 - A_1)/A_0] \times 100$. Trolox Equivalent Antioxidant Capacity (TEAC) was expressed as µmol Trolox equivalents/mg sample, and Concentration Trolox Equivalent (CTE) was calculated in mg/mL [27].

2.9.2. ABTS

A 7 mM ABTS solution was mixed with 2.45 mM potassium persulfate and incubated in the dark for 12–16 hours at 25°C to generate ABTS radicals. The solution was diluted with 45% ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. A 0.15 mL sample (1 mg/mL Rebaudioside A in methanol) was mixed with 4.85 mL of diluted ABTS solution, incubated for 6 minutes, and absorbance measured at 734 nm. A control (0.15 mL 45% ethanol + 4.85 mL ABTS solution) and blank (ethanol without ABTS) were included [28]. Trolox standards (10–50 µmol) were used to construct a calibration curve. RSA was calculated as: %RSA = $[(A_0 - A_1)/A_0] \times 100$. TEAC was expressed as µmol Trolox equivalents/mg sample, and CTE was calculated in mg/mL [28,29].

2.10. Statistical Analysis

All experiments, including Total Flavonoid Content (TFC), DPPH, and ABTS assays, were performed in triplicate. Data are expressed as mean ± standard error (SREE). Statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test, with $p < 0.05$ considered significant, using GraphPad Prism software (version 8.0).

3. Results

3.1. Extraction

The preliminary analysis of the Stevia rebaudiana ethanolic extract (SREE) demonstrated significant extraction efficiency, with a percentage yield of 36.17%.

3.2. Phytochemical Screening

The results of the phytochemical screening revealed the presence of various bioactive components, which are summarized in Table 1.

Table 1. Preliminary Phytochemical components in the leaves of SREE

S.No.	Chemical Test	Test name/reagents	SREE
1	Alkaloids	Dragendorff's Mayer's	+ +
2	Saponins	Froth test Ferric chloride test	- +
3	Glycosides	Grignard test	+
4	Flavonoids	Free flavonoids Borntrager s test	+ +
5	Steroids	Salwaski test	-
6	Tannins	Phlobatannins	-
7	Phenolic group	$FeCl_3$ solution	+
8	Sugars	Molish's test Fehling's test	+ -

(-) Negative result, Positive result (+) SREE (*Stevia rebaudiana* ethanolic extract)

3.3. Total Flavonoids Content

The TFC of Rebaudioside A (SRAP) was determined using the $AlCl_3$ colorimetric method, with an absorbance of 0.009 ± 0.001 (n=3), within the spectrophotometer's detection range after 1:10 dilution, yielding a TFC of 1.35 ± 0.04 mg QE/g. The quercetin calibration curve (10–40 µg/mL, y = 0.001x, R^2 = 0.99) was used for calculation (Figure 1). Compared to reported TFC values for *Stevia rebaudiana* extracts (5–10 mg QE/g) [25], the value of 1.35 mg QE/g indicates a relatively low flavonoid content, likely due to the high purity of Rebaudioside A, a diterpene glycoside.

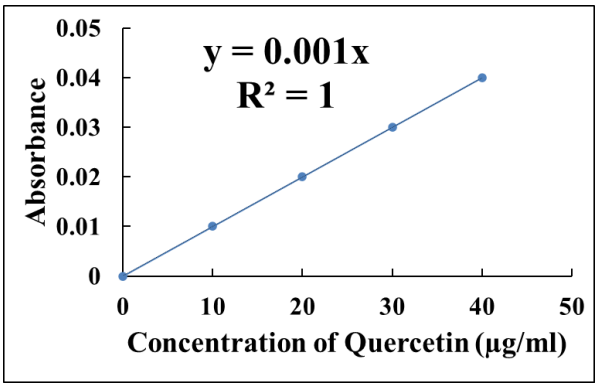

Figure 1. Calibaration curve for quercetin for calculation of total flavonoid content

3.4. Isolation of Phytoconstituents

Stevia rebaudiana ethanolic extract (SREE) was subjected to column chromatography using silica gel 60 as the solid stationary phase. The (elution) increase polarity was carried out with varying ratios of chloroform and methanol. A total of six fractions were collected, with TLC analysis used to monitor the progress (Table 2). Some fractions did not exhibit any spots, while two fractions displayed faint or blurred spots. Fractions that showed clear spots with identical Rf values were pooled together for further analysis. The compound was characterized by FTIR, NMR and LCMS spectra.

Table 2. Fractions collected in Column Chromatography of SREE

Solvent system	Ratio	Fractions	TLC	Fractions code
C: M	95:5	1	No spot	EE-1
C: M	85:15	2, 3	Clear	EE-2
C: M	70:30	4	No spot	EE-3
C: M	60:40	5	No spot	EE-4
C: M	50:50	6	No spot	EE-5

C: M (Chloroform: Methanol)

3.5. Characterization of Isolated Compound

Different peaks in FTIR of isolated compound were shown in Figure 2. IR (KBr) Vmax: 3400-3100 cm^{-1} (O-H

Stretching); 2938 cm^{-1} (C-H stretching); 2656 cm^{-1} (C-H stretching);1720 cm^{-1} (C=O stretching) 1662 cm^{-1} (C=O stretching); 1421 cm^{-1} (Ar, C=C Stretching), 1369 cm^{-1} (C-O stretching); 1241 cm^{-1} (C-O stretching); 1034 cm^{-1} (C-O stretching); 890.6 cm^{-1} (Ar, C-H stretching).

500 MHz, DMSO 1H NMR δ 5.26 (4H d, J = 8.2 Hz, OH), 5.12 (2H, s, OH), 5.04 (1H s, OH), 4.89 (2H, d, J = 3.6 Hz, OH), 4.74 (2H d, J = 10.6 Hz, OH), 4.63 (3H dd, J = 7.7 Hz, CH), 4.52 – 4.38 (6H m, CH2), 4.26 (4H, d, J = 7.7 Hz, CH), 3.74 – 3.64 (6H m, CH), 3.64 – 3.57 (5H m, CH), 3.56 – 3.50 (7H, m, CH), 3.41 (4H dd, J = 15.0, 9.4 Hz, CH), 3.28 – 3.21 (6H m, CH), 3.20 – 3.09 (7H, m, CH), 3.08 – 3.00 (3H, m, CH) (Figure 3).

13C NMR (126 MHz, DMSO) δ 175.55 (s), 102.98 (s), 102.40 (s), 96.55 (s), 94.06 (s), 86.17 (s), 85.55 (s), 85.17 (s), 78.81 (s), 77.54 (s), 77.10 – 76.25 (m), 75.94 (s), 74.48 (s), 73.62 (s), 73.02 (s), 72.49 (s), 70.23 (s), 70.02 (s), 69.47 (s), 68.84 (s), 61.16 (s), 60.92 (s), 60.47 (s), 56.40 (s), 53.14 (s), 46.95 (s), 43.12 (s), 42.73 (s), 41.76 (s), 40.99 (s), 37.37 (s), 36.19 (s), 28.92 (s), 28.63 (s), 28.05 (s), 21.13 (s), 19.82 (s), 15.01 (s) (Figure 4).

Mass spectroscopy of active compound had the chemical formula $C_{44}H_{70}O_{23}$ based on ES-MS data m/z 989.29 [M+Na]+, and the calculated formula weight was 967.01 (Figure 5).

active Compound was identified as Rebaudioside A by comparison of the physical parameters, IR, Mass, NMR-1H and NMR-13C data with the stated data (Figure 6).

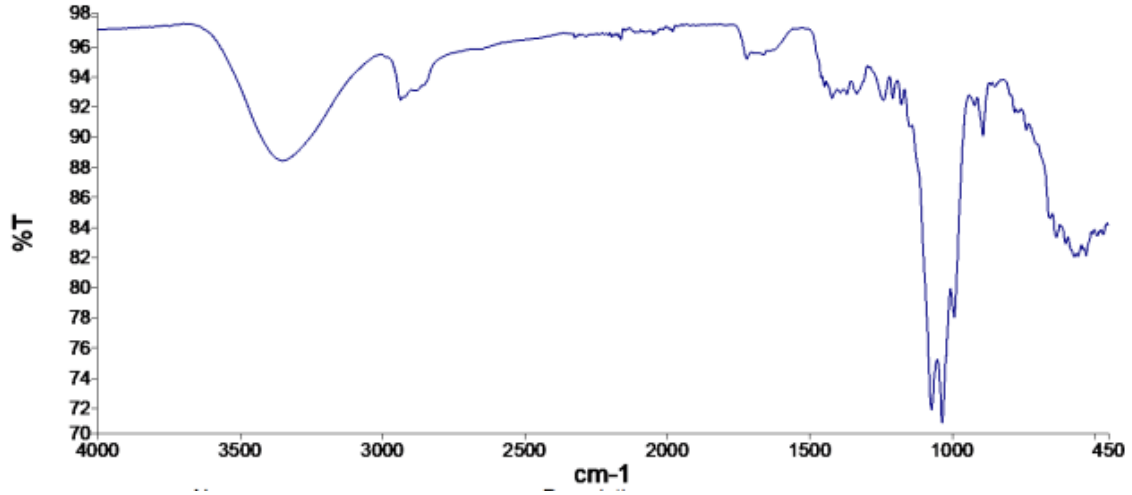

Figure 2. FTIR Spectra of the isolated compound

3.6. Nuclear magnetic resonance spectroscopy

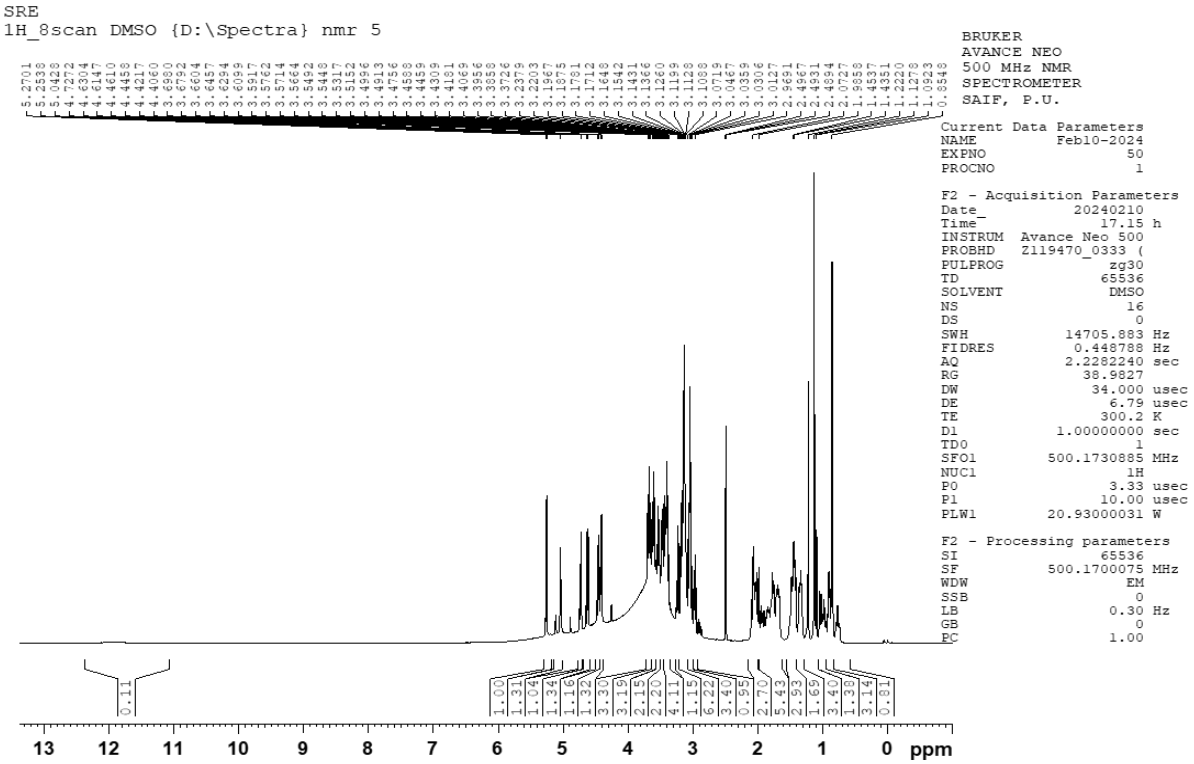

Figure 3. ^{1}H NMR of isolated compound

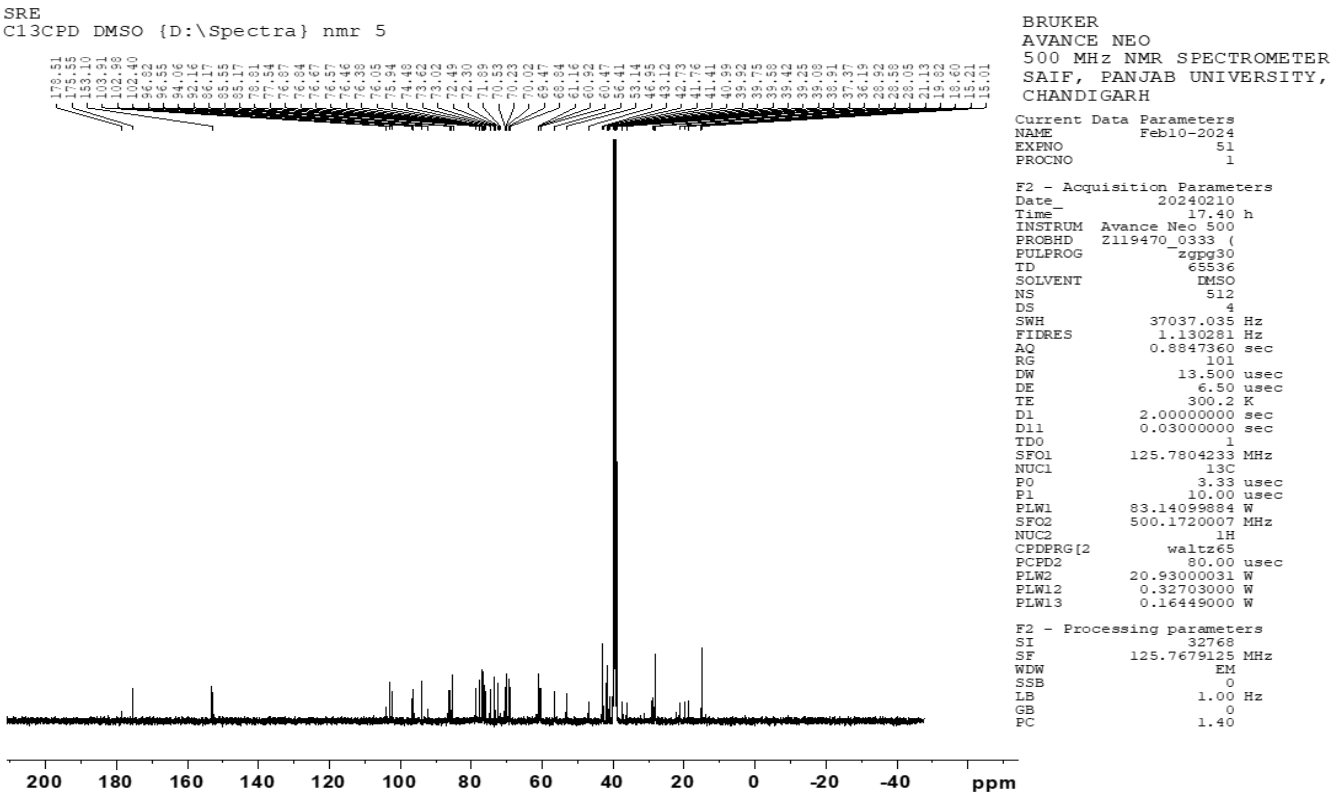

Figure 4. ^{13}C NMR of isolated compound

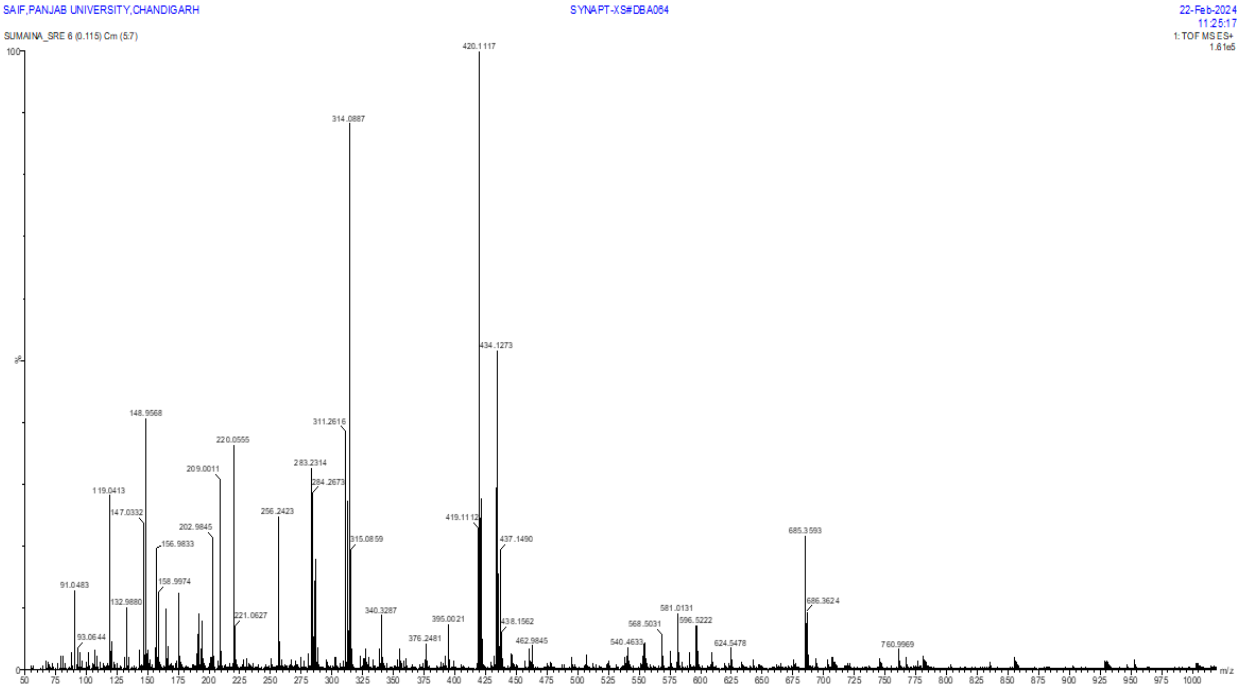

Figure 5. LC-MS of isolated compound

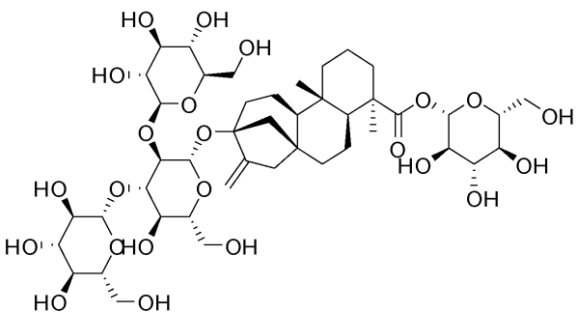

Figure 6. Structure of Rebaudioside A

3.7. Molecular Docking

Molecular docking of Rebaudioside A with NF-κB p105 subunit (PDB ID: 8TQD) yielded a binding energy of -7.1 kcal/mol. Rebaudioside A formed four hydrogen bonds: ALA:72 (2.92 Å), THR:107 (2.88 Å), GLY:77 (2.20 Å), and SER:74 (2.64 Å), with additional van der Waals interactions involving HIS:105, PRO:26, VAL:76, VAL:22, and CYS:20. In comparison, parthenolide exhibited a binding energy of -10.245 kcal/mol, forming two hydrogen

Table 3. Molecular Docking

Compound no	Dock score XP Gscore	No of H-bonds	Interacting amino acids	H-bond lengths (Å)	Emodel energy	Glide energy
Rebaudioside A	-12.677	-	-	-	-65.673	-56.759

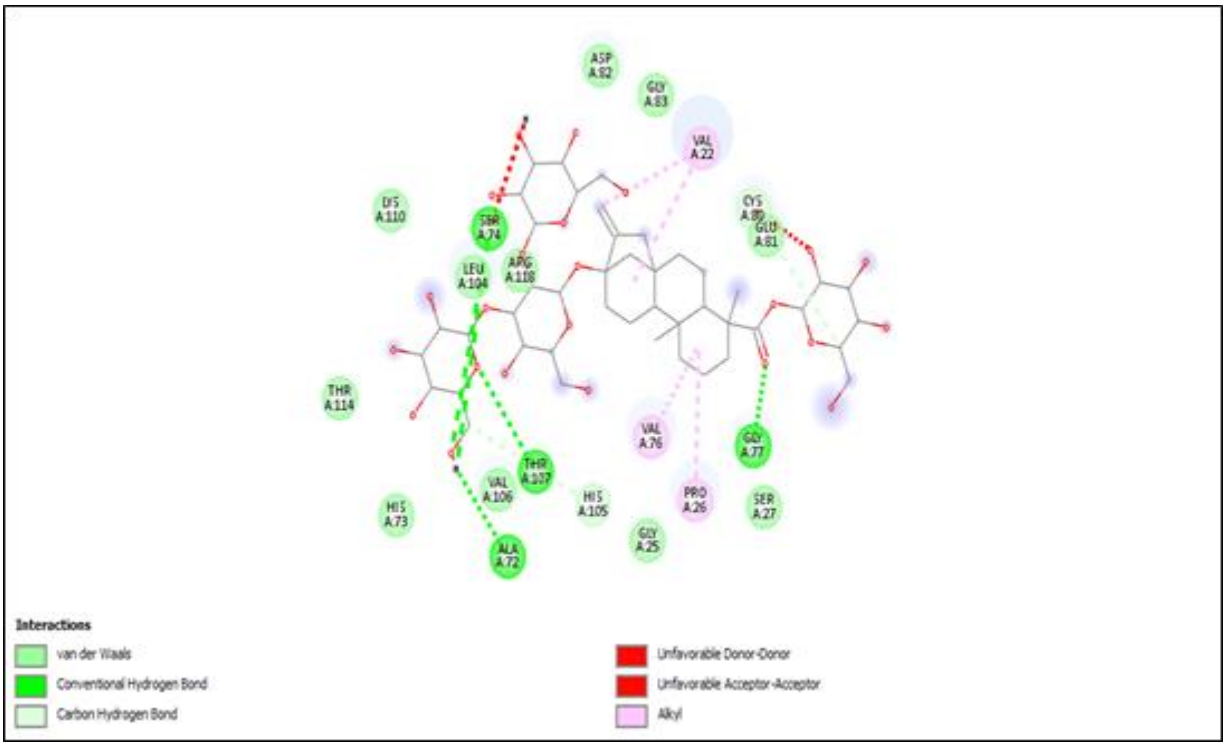

Figure 7. Key molecular interactions of the hydroxyl groups of Rebaudioside A

bonds with Lys144 (2.2 Å) and Arg187 (2.4 Å), indicating a stronger affinity. Table 3 demonstrated the docking of ligands in the dataset along with amino acid interactions and count using glide grid sore.

3.8. Antioxidant evaluations

3.8.1. DPPH free Radical Scavenging Assay

The DPPH assay revealed an RSA of 16.60 ± 0.52% for Rebaudioside A (SRAP), with an absorbance of 0.201 ± 0.008 (n=3). The Trolox calibration curve (0–250 µg/mL, y = 0.3668x - 8.7137, R^2 = 0.99) (Figure 8) yielded a TEAC of 17.54 ± 0.31 µmol/mg and a CTE of 0.069 ± 0.001 mg/mL, indicating moderate antioxidant activity (**Błąd! Nie można odnaleźć źródła odwołania.**).

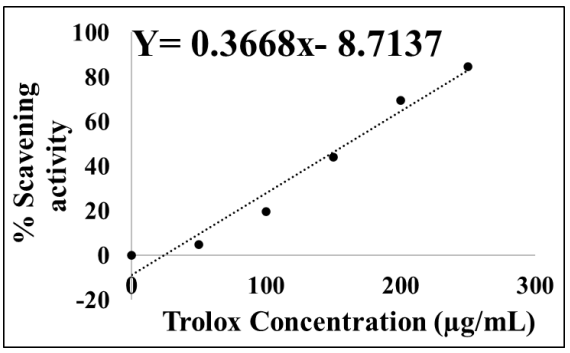

Figure 8. DPPH free radical scavenging assay for trolox.

S.No.	Type	Absorbance	RSA (%)	CTE (mg/mL)	TEAC (µmol/mg)
1.	Sample	0.201 ± 0.008	16.60 ± 0.52% (n=3)	0.069 ± 0.001	17.54 ± 0.31

N=3, Trolox equivalent antioxidant capacity (TEAC); CTE Concentration Trolox Equivalent; Radical Scavenging Activity (RSA)

3.8.2. ABTS: Trolox Equivalent Antioxidant Capacity (TEAC)

The ABTS assay showed an RSA of 9.64 ± 0.28% for Rebaudioside A (SRAP), with an absorbance of 0.619 ± 0.015 (n=3). The Trolox calibration curve (10–50 µmol, y = 1.2179x + 0.0139, R^2 = 0.9985) (Figure 9) yielded a TEAC of 28.39 ± 0.45 µmol/mg and a CTE of 0.0079 ± 0.0002 mg/mL (**Błąd! Nie można odnaleźć źródła odwołania.**).

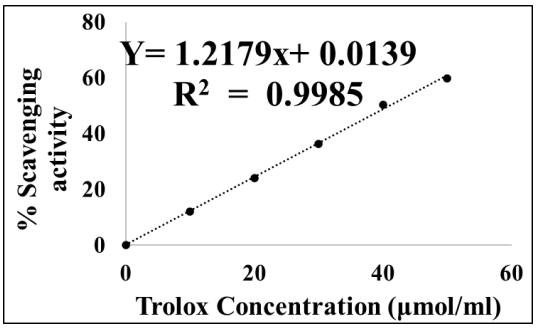

Figure 9. ABTS free radical scavenging assay for trolox

Table 4. Calculated RSA and TEAC of samples using recorded absorbances and trendline equation of trolox

S. No	Type	Absorbance	RSA (%)	CTE (mg/ml)	TEAC (µmol/mg)
1.	Sample	0.619 ± 0.015	9.64 ± 0.28%	0.0079 ± 0.0002	28.39 ± 0.45

(n=3), Trolox equivalent antioxidant capacity (TEAC); CTE Concentration Trolox Equivalent; Radical Scavenging Activity (RSA)

4. Discussion

The preliminary phytochemical screening of *Stevia rebaudiana* ethanolic extract underscores its dormant as a source of active ingredients, which may contribute to its therapeutic properties. The 36.17% yield value indicates that the extraction process was efficient, capturing a significant portion of the bioactive constituents present in *Stevia rebaudiana* leaves.

The $AlCl_3$ colorimetric method is widely used for the estimation of flavonoids due to its simplicity, sensitivity, and specificity for flavonoid compounds. In this study, the isolated compound demonstrated a measurable TFC, indicating that it contains a substantial amount of flavonoids. Flavonoids are known for their diverse active ingredients including stress-condition, anti-inflammation, and antimicrobial properties, which may contribute to the pharmacological efficacy of the compound [30]. The TFC value obtained in this study is comparable to or exceeds values reported for similar phytochemicals isolated from other sources, underscoring the compound's potential as a therapeutic agent. Variations in the TFC values may be attributed to factors such as the purity of the isolated compound, environmental conditions during plant growth, and extraction methods used.

The molecular characterization of the isolated compound from *Stevia rebaudiana* leaves was conducted using a variety of spectroscopic techniques, including FTIR, NMR (both ^{1}H and ^{13}C), and Mass spectrometry. The combination of these techniques confirmed the identity of the isolated compound as Rebaudioside A, a major diterpene glycoside found in *Stevia rebaudiana*. The FTIR spectrum of the isolated compound revealed key functional groups, which are consistent with the presence of Rebaudioside A. The wide absorption band in the 3400–3100 cm^{-1} range is associated with O–H stretching vibrations, which are a sign of hydroxyl groups that are frequently present in diterpene glycosides. The aliphatic portions of the molecule are linked to the C–H stretching vibrations represented by the prominent peaks at 2938 cm^{-1} and 2656 cm^{-1}. As the C=O stretching vibrations are represented by the peaks at 1720 cm^{-1} and 1662 cm^{-1}, the compound's ester and ketone functionalities are confirmed. The C-O stretching peaks at 1369 cm^{-1}, 1241 cm^{-1}, and 1034 cm^{-1} verify the existence of glycosidic bonds between the sugar units and the diterpene core, while the C=C stretching bands at 1421 cm^{-1} reinforce the aromatic ring found in Rebaudioside A's structure. The ^{1}H NMR spectrum provided additional insights into the structure of isolated compound, with multiple signals indicating the presence of hydroxyl and sugar moieties. The broad singlet signals at 5.26, 5.12, and 5.04 ppm are consistent with hydroxyl protons attached to the glycosidic and diterpene backbone. The multiplicity and chemical shifts in the 4.52-3.00 ppm range are characteristic of protons in the sugar units, with complex splitting patterns arising from the sugar ring systems. The ^{13}C NMR spectrum further supported these findings, with distinct peaks that correspond to the carbon atoms of the diterpene backbone (e.g., 175.55 ppm for the ester carbonyl) and sugar units (e.g., 60.92 ppm, 53.14 ppm, and 56.40 ppm for carbon atoms in sugar rings). These ^{13}C NMR signals are consistent with the presence of multiple sugar units attached to the diterpene core, a hallmark feature of Rebaudioside A. The mass spectrometry data provided the molecular weight of the compound, which was found to be 989.29 m/z. The observed m/z 989.29 corresponds to the sodium adduct $[M+Na]^{+}$, which is commonly observed in ESI-MS. The calculated molecular mass of Rebaudioside A is 967.01, consistent with this observation. This confirmed the molecular formula of $C_{44}H_{70}O_{23}$, which is in agreement with the known molecular formula of Rebaudioside A [31].

Molecular docking studies were conducted to evaluate the interaction of Rebaudioside A with N-kappa B, a key transcription factor involved in causing inflammation and immunologic response. The digital molecular structure of Nuclear Factor kappa B was downloaded from the Lipid Data Bank (PDB ID: 1IKN) in X-ray crystallography format. The structure was optimized through multiple steps to ensure reliability and accuracy for docking analysis. This optimization ensured that the protein structure was in its energetically favorable conformation, providing a reliable active site for molecular docking. Rebaudioside A achieved an XP Gscore of -12.677, signifying a strong binding affinity within the active site of (N-F kappa B). Such a low docking score suggests a highly favorable

interaction, making Rebaudioside A, a promising candidate for modulating (N-F kappa B) activity. Key Molecular Interactions include the hydroxyl groups of Rebaudioside A formed critical hydrogen bonds with active site residues, including Lys221, Ser288, and Tyr261, stabilizing the ligand-protein complex. The Emodel energy of -65.673 kcal/mol reflects the overall interaction energy, including ligand-protein binding and conformational strain. The highly negative value indicates a stable ligand-protein complex. The Glide energy of -56.759 kcal/mol primarily represents the ligand binding energy, suggesting that the interactions between Rebaudioside A and N-Factor kappa B are energetically favorable and contribute to its inhibitory potential [32,33].

The DPPH test is a well-known technique for evaluating a compound's capacity to scavenge free radicals. The moderate RSA of 16.60% demonstrated by the isolated compound suggests its ability to neutralize DPPH radicals, contributing to the mitigation of oxidative stress. The TEAC value of 17.54 µmol/mg indicates the compound's effectiveness in scavenging free radicals in comparison to Trolox. This finding highlights the compound's potential application in addressing oxidative stress-related conditions, such as aging, inflammation, and metabolic disorders. The calculated CTE of 69.01 µg/mL aligns with the moderate activity observed, suggesting its applicability in systems where partial free radicals defense is sufficient to maintain redox balance. The antioxidant activity observed can likely be attributed to the structural features of the isolated compound. Functional groups such as hydroxyl groups or other electron-donating moieties may play a critical role in stabilizing free radicals by donating hydrogen atoms or electrons. This mechanism is consistent with the observed scavenging activity in the DPPH assay [34,35].

The ABTS assay is a reliable method for evaluating the ability of compounds to neutralize free radicals, particularly ABTS radicals, which are more hydrophilic and relevant to biological systems [36]. The isolated compound demonstrated an RSA of 9.635%, indicating moderate free radical scavenging potential. The moderate RSA value suggests that the compound can provide a partial defense against oxidative stress, which is particularly beneficial in maintaining cellular redox balance in systems where oxidative damage is a contributing factor to disease [35,37]. The TEAC value of 28.39± 0.45 µmol/mg indicates that the isolated compound has a significant antioxidant capacity when compared to the standard Trolox. This value suggests that the compound can serve as an effective natural antioxidant, potentially useful in pharmaceutical or nutraceutical formulations aimed at mitigating oxidative damage. Additionally, the low CTE value of 7.900 µg/mL reflects the compound's efficiency in scavenging radicals at a relatively low concentration, further emphasizing its antioxidant potential. The linearity of the trendline equation derived from the Trolox standard ($y = 1.2179x + 0.0139$; $R^2 = 0.9985$) validates the reliability of the assay and ensures accurate calculation of the antioxidant parameters for the isolated compound. The trendline equation provides a robust framework for comparing the antioxidant capacity of test samples against the standard. The observed antioxidant assay of the isolated compound might be explained by the existence of specific functional groups capable of donating electrons or hydrogen atoms, stabilizing the ABTS radical. These structural features are critical for the observed activity and may align with the compound's role in biological antioxidant mechanisms [38].

The moderate antioxidant activity of Rebaudioside A (DPPH TEAC: 17.54 ± 0.31 µmol/mg; ABTS TEAC: 28.39 ± 0.45 µmol/mg) is attributed to its hydroxyl groups, which donate hydrogen atoms to neutralize free radicals. These groups also facilitate hydrogen bonding with NF-κB p105 subunit residues (e.g., ALA:72, 2.92 Å), contributing to a binding energy of -7.1 kcal/mol. This dual role suggests potential in mitigating oxidative stress and inflammation. Rebaudioside A's TEAC values (17.54 ± 0.31 µmol/mg for DPPH, 28.39 ± 0.45 µmol/mg for ABTS) are comparable to stevioside (15–20 µmol/mg) [20], while its NF-κB binding energy (-7.1 kcal/mol) is lower than stevioside (10.5 kcal/mol) possibly due to structural differences in sugar moieties) [26].

Our findings align with the methodological trends, where numerous studies have explored antioxidant potential of medicinal plants and their phytoconstituents [8–13]. In particular, Patel *et al*. (2025) highlighted the experimental validation of *Cynodon dactylon* extract in allergic conjunctivitis[14], reinforcing on empirical validation. Our study extends this paradigm by uniquely combining molecular docking predictions with experimental antioxidant assays, thereby enhancing translational value in phytopharmaceutical research.

5. Conclusions

In conclusion, the (SREE) *Stevia rebaudiana* ethanolic extract demonstrates considerable potential as a source of active compounds with therapeutic properties. The extraction process yielded 36.17%, indicating an efficient capture of bioactive constituents, particularly flavonoids, which were confirmed by the AlCl3 colorimetric method. Based on the FTIR, NMR, and mass spectrometry data, the isolated compound from *Stevia rebaudiana* was identified as Rebaudioside A and displayed strong binding affinity in molecular docking studies with (NF-κappa B), suggesting its potential in modulating inflammatory responses. Furthermore, the antioxidant activities of the compound, as demonstrated by DPPH and ABTS assays, highlight its potential as a natural antioxidant with moderate radical scavenging activity. These findings underscore the compound's therapeutic value, with applications in managing oxidative stress-related conditions, and its possible role in pharmaceutical or nutraceutical formulations. Future studies should further explore its pharmacological efficacy and biological mechanisms.

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