ORIGINAL ARTICLE

Isolation, *in vitro* Evaluation, and Molecular Docking Analysis of a Bioactive Phenylpropanoid from *Syzygium jambos* with Antioxidant and Anticancer Potential against IMR-32 Neuroblastoma Cells

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Abstract

Background: Neuroblastoma is a highly aggressive pediatric cancer with limited treatment options and a meager prognosis in advanced stages. Plant-derived natural products represent a valuable source of novel anticancer agents. *Syzygium jambos* (L.) Alston, traditionally used in folk medicine, has been reported to contain bioactive phenolics, but its anticancer potential remains underexplored. **Objective:** This study aimed to isolate and characterize bioactive compounds from *S. jambos* leaves and evaluate their cytotoxic potential against human neuroblastoma infant mortality rat (IMR)-32 cells. **Materials and Methods:** Leaves of *S. jambos* were sequentially extracted with solvents of increasing polarity, and extracts were screened for antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The ethanol extract, which showed the highest radical scavenging activity, was subjected to bioassay-guided fractionation by Thin-layer chromatography and silica gel column chromatography. Fractions were tested for antioxidant and cytotoxic activity, and the most active fraction was structurally characterized by spectroscopic and chromatographic methods. Cytotoxicity against IMR-32 cells was evaluated by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay, intracellular reactive oxygen species (ROS) levels were measured, and Western Blotting was used to assess apoptosis- and survival-related proteins. *In silico* molecular docking and ADME analysis were performed to explore the mechanism and drug-likeness. **Results:** The ethanol extract of *S. jambos* leaves showed strong antioxidant activity, with *Syzygium jambos* Ethanol fracion (SJEF-16) exhibiting 75.25% DPPH inhibition at 500 µg/mL and an IC_{50} of 40.93 µg/mL. SJEF-16 decreased IMR-32 neuroblastoma cell viability in a dose-dependent manner, with an IC_{50} of 28.92 µg/mL, reduced intracellular ROS levels, and moderately downregulated EGFR expression (66.2% of control). Molecular docking revealed a binding affinity of −6.2 kcal/mol with neuroblastoma-related targets, and ADME analysis predicted good oral bioavailability (bioavailability score = 0.55). These findings suggest SJEF-16 as a promising antioxidant and anticancer agent. **Conclusion:** This study reports, for the 1st time, the isolation of (2E)-3-(3-Methoxyphenyl)-2-propen-1-ol from *S. jambos* leaves with potent cytotoxic and antioxidant activity against neuroblastoma cells. These findings highlight its potential as a promising lead compound for the development of plant-derived therapeutics against pediatric neuroblastoma.

Key words: (2E)-3-(3-Methoxyphenyl)-2-propen-1-ol, antioxidant activity, cytotoxicity, IMR-32 cells, neuroblastoma, *Syzygium jambos*

INTRODUCTION

Neuroblastoma is the most common type of cancer in children that develops outside the brain.[1] It starts from nerve cells involved in the body's sympathetic nervous system. It starts from nerve cells involved in the body's sympathetic nervous system.[2] While treatment options have advanced, children with aggressive forms of neuroblastoma still have a

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low chance of recovery, and the treatments themselves can be very harsh.[3] Therefore, there is a strong need for new therapies that can fight cancer more effectively but with fewer side effects.[4]

Natural products from medicinal plants have historically served as a valuable source of anticancer agents.[5] Secondary metabolites, such as polyphenols and flavonoids, often exhibit potent antioxidant activity, modulate apoptotic pathways, and interfere with oncogenic signaling, making them promising candidates for drug development.[6] Among these, compounds that can simultaneously reduce oxidative stress and induce apoptosis in cancer cells are of particular interest.[7]

Syzygium jambos (L.) Alston, commonly known as rose apple, belongs to the family Myrtaceae and has been traditionally used to treat infections, inflammation, and metabolic disorders.[8] Phytochemical studies indicate the presence of phenolics, flavonoids, and other bioactive compounds, many of which are associated with antioxidant and cytoprotective properties.[9] However, there are limited reports on the isolation of pure compounds from *S. jambos* and their mechanistic evaluation against cancer cells, especially neuroblastoma.[10]

In the present study, we performed bioassay-guided fractionation of *S. jambos* leaf extracts to isolate antioxidant compounds with cytotoxic activity against IMR-32 neuroblastoma cells. The most active fraction was characterized spectroscopically to identify the bioactive compound (2E)-3-(3-Methoxyphenyl)-2-propen-1-ol. Its cytotoxic mechanism was investigated through reactive oxygen species (ROS) modulation and the expression of apoptosis- and survival-related proteins. In addition, molecular docking studies were performed to evaluate its binding affinity to neuroblastoma-associated protein targets and assess drug-likeness properties. This integrated approach highlights the potential of plant-derived compounds as promising leads for neuroblastoma therapy.

MATERIALS AND METHODS

Plant material collection and authentication

The experimental plant, *Syzygium jambos* (L.) Alston, was collected from the Anamalai Hills, Coimbatore district, South India, and authenticated at the Rapinat Herbarium (RHT). A voucher specimen (No. 3125) has been deposited in the herbarium for future reference. Fresh, healthy leaves were harvested with the assistance of local tribes, while damaged or diseased leaves were carefully excluded. The collected material was preserved at −25°C for molecular identification studies[11] and the obtained sequence was submitted to GenBank under the accession number PP754605.1. The leaves were subsequently air-dried, pulverized into powder, and stored for further analyses.

Plant extraction by cold maceration

The powdered leaves of *S. jambos* were extracted using the cold maceration method of extraction using solvents in increasing polarity–hexane, chloroform, ethyl acetate, and ethanol. The extracts were filtered, concentrated, and stored at 4°C until further use.[12,13]

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant potential of all extracts and fractions was evaluated using the DPPH radical scavenging assay. Ascorbic acid served as the reference standard, and IC_{50} values were calculated.[14]

Bioactive fraction isolation from ethanol extract

The ethanol extract was analyzed by Thin-layer chromatography (TLC) using silica gel 60 F_{254}plates. Mobile phase: chloroform: acetone:methanol (6:4:6, v/v/v). column chromatography was performed on silica gel (60–120 mesh) using a 45× 2.5 cm column. Elution started with 100% hexane and continued with gradually increasing polarity mixtures (hexane: chloroformto chloroform: ethyl acetate to ethyl acetate: methanol). Fractions (~20 mL each) were collected at ~1 mL/min and monitored by TLC (chloroform: methanol6:4). Similar fractions were pooled, and they were evaluated for radical scavenging activity using the DPPH assay at concentrations of 20–100 µg/mL. The fraction exhibiting the highest activity was selected for further structural characterization and biological evaluation.[15]

Compound characterization

The most bioactive fraction (SJEF-16) was subjected to comprehensive characterization to determine its chemical identity and purity. High-performance liquid chromatography (HPLC; Shimadzu UFLC) was used to assess purity and retention time.[16] Ultraviolet UV-Visible spectroscopy (PerkinElmer Lambda 365) confirmed the presence of conjugated systems and indicated a phenolic nature, while Fourier-transform infrared spectroscopy (FTIR; PerkinElmer Spectrum TWO) identified functional groups such as hydroxyl (–OH) and aromatic rings.[17] Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR; Bruker AVIII 500, solution-state in deuterated methanol) spectroscopy was employed to elucidate the atomic environment and molecular structure.[18] Liquid chromatography–quadrupole time-of-flight high-resolution mass spectrometry (LC-QTOF-HRMS; Waters ACQUITY H-Class UPLC/XevoG2XS QTOF) confirmed the molecular formula and accurate mass.[18,19]

In vitro cytotoxicity assay

MTT assay

The cytotoxicity of SJEF-16 was evaluated using the MTT assay on IMR-32 human neuroblastoma cells. Cells were cultured in DMEM supplemented with 10% FBS and antibiotics at 37 °C in 5% CO_2. They were plated in 96-well plates (1×10^5 cells/mL, 200 µL/well) and incubated for 24–48 h. After washing with PBS, cells were treated with various concentrations of SJEF-9 for 24 h. MTT solution (10 µL of 5 mg/mL) was added and incubated for 2–4 h, followed by dissolution of formazan crystals in 100 µL DMSO. Absorbance was measured at 570 nm, and cell viability (%) and IC_{50} values were calculated using GraphPad Prism 6.0.[20]

Total ROS assay

The generation of ROS in IMR-32 human neuroblastoma cells was evaluated using a Total ROS Detection Kit (Invitrogen, USA). Cells were cultured in DMEM supplemented with 10% FBS and antibiotics at 37°C in a humidified 5% CO_2 atmosphere. Cells were plated in 24-well plates at a density of 1×10^6 cells/mL and incubated for 24 h. After washing, cells were treated with the IC_{50} concentration (28.92 µg/mL) of SJEF-16 in serum-free DMEM for 24 h. Following treatment, 1 mL of ROS assay buffer and 100 µL of 1X ROS staining solution were added to each well and incubated for 60 min at 37°C. ROS production was then immediately visualized using a fluorescent imaging system (ZOE, Bio-Rad, USA).

Western blotting

Western blotting was performed to assess the effect of SJEF-16 on EGFR expression in IMR-32 cells. Proteins were extracted from treated cells using RIPA buffer, and concentrations were determined. SDS-PAGE was performed with a 10% separating gel and 4% stacking gel, and protein samples (50–100 µg/mL) were mixed with sample buffer containing 2-mercaptoethanol, glycerol, bromophenol blue, and SDS, then heated at 90°C for 5 min. Samples were loaded with a protein marker, and electrophoresis was carried out at 50–100 mA. Proteins were transferred onto activated PVDF membranes, blocked with 5% skimmed milk in 0.1% Tween-20 PBS for 1 h, and incubated overnight at 4°C with primary antibodies against EGFR or β-actin. Membranes were then incubated with HRP-conjugated secondary antibodies for 2–4 h, and protein bands were visualized using DAB and hydrogen peroxide. Densitometry analysis was performed using ImageJ software to quantify relative protein expression.[21]

Molecular docking and ADME analysis

Molecular docking of the isolated compound was performed against neuroblastoma-associated protein targets using AutoDock Vina. Binding affinity scores and interaction residues were analyzed.[22] *In silico* ADME and drug-likeness properties were assessed using SwissADME to evaluate pharmacokinetic potential.[23]

Statistical analysis

All experiments were performed in triplicate. Data are expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way analysis of variance with $P < 0.05$ considered statistically significant.

RESULTS

DPPH radical scavenging assay

The antioxidant potential of *S. jambos* leaf extracts was evaluated using the DPPH radical scavenging assay, with ascorbic acid as the reference standard [Table 1]. Among the crude extracts, the ethanol extract exhibited the strongest radical scavenging activity in a concentration-dependent manner, achieving 75.25% inhibition at 500 µg/mL and 38.56% at 10 µg/mL. The IC_{50} value for the ethanol extract was 40.93 µg/mL, indicating a higher antioxidant capacity compared to hexane (IC_{50} = 108.00 µg/mL), chloroform (IC_{50} = 64.17 µg/mL), and ethyl acetate extracts (IC_{50} = 87.45 µg/mL). These results suggest that the ethanol extract contains the most potent bioactive compounds responsible for free radical scavenging.

Table 1: DPPH radical scavenging activity of *Syzygium jambos* leaf extracts

Concentration (µg/mL)	Ascorbic acid (Std)	Hexane extract	Chloroform extract	Ethyl acetate extract	Ethanol extract
500	82.55	74.72	61.72	49.24	75.25
250	63.68	63.68	52.37	36.08	71.87
100	54.39	54.39	44.97	29.56	67.55
50	44.54	44.54	37.52	23.62	62.18
10	35.14	35.14	16.08	7.12	38.56
IC_{50} (µg/mL)	–	108.00	64.17	87.45	40.93

Bioactive fraction isolation and antioxidant activity

TLC of the ethanol extract revealed five distinct spots under UV light (254 nm) with Rf values of 6.1, 5.7, 5.2, 2.2, and 0.7. Column chromatography of the ethanol extract initially yielded 22 fractions, which were pooled into 19 combined fractions (SJEF-1 to SJEF-19) based on TLC profiles. These fractions were screened for antioxidant activity using the DPPH radical scavenging assay at concentrations of 20–100 µg/mL. Among the tested fractions, SJEF-16 exhibited the highest radical scavenging activity in a concentration-dependent manner, followed by moderate activity in SJEF-12, SJEF-14, and SJEF-17 [Figure 1]. Based on its superior antioxidant and cytotoxic activity, SJEF-16 was selected for subsequent structural characterization and biological evaluation.

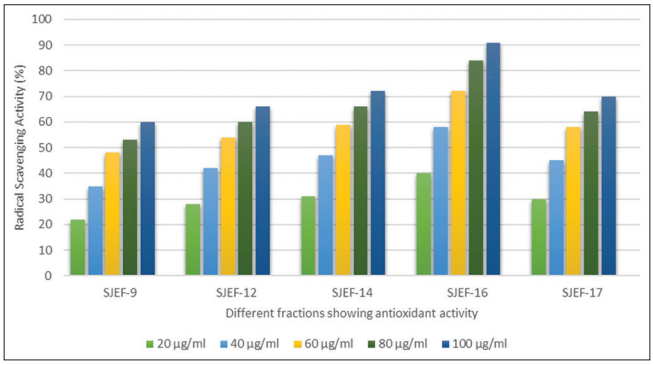

Figure 1: DPPH radical scavenging activity of selected ethanol extract fractions (SJEF-9, SJEF-12, SJEF-14, SJEF-16, SJEF-17) at concentrations ranging from 20 to 100 µg/mL. SJEF-16 shows the highest activity among all tested fractions

Figure 2: Physical appearance of SJEF-16 isolated from ethanolic leaf extract of *Syzygium jambos* showing golden-brown crystalline solid

Compound characterization

The bioactive fraction SJEF-16, isolated from the ethanolic leaf extract of *S. jambos*, was obtained as a golden-brown crystalline solid, odorless, and soluble in polar solvents [Table 2 and Figure 2]. HPLC analysis showed a major peak at 2.951 min with an area percentage of 97.96%, confirming high purity [Figure 3]. UV-Visible spectroscopy revealed absorbance maxima at 191.5 and 221.8 nm, consistent with conjugated aromatic systems typical of simple phenolics or flavonoid aglycones [Figure 4].

FTIR spectra indicated O-H stretching at 3369 cm^{-1}, C-H stretching at 2975–2900 cm^{-1}, C=O stretching at 1654 cm^{-1}, C-O stretching at 1273–1088 cm^{-1}, and out-of-plane =C-H bending at 880 and 803 cm^{-1}, confirming hydroxylated aromatic and carbonyl functionalities [Figure 5].

^{1}H NMR signals between δ 6.2 and 7.8 ppm corresponded to aromatic protons, with additional signals for hydroxyl and methoxy protons, while ^{13}C NMR confirmed aromatic, methoxy, and hydroxylated aliphatic carbons [Figures 6 and 7]. LC-QTOF-HRMS showed a molecular ion peak at m/z = 179.107 $[M+H]^{+}$, confirming the molecular formula $C_{11}H_{14}O_2$ and identifying the compound as (2E)-3-(3-Methoxyphenyl)-2-propen-1-ol [Figure 8]. Together, these analyses establish SJEF-16 as a highly pure phenolic compound with hydroxyl, methoxy, and aromatic functionalities, providing a structural basis for its observed bioactivity.

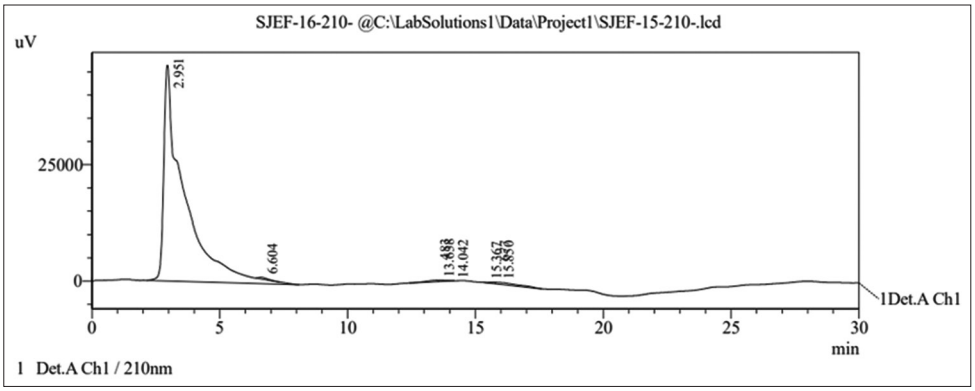

Figure 3: HPLC chromatogram of SJEF-16 indicating a major peak at 2.951 min, confirming high purity

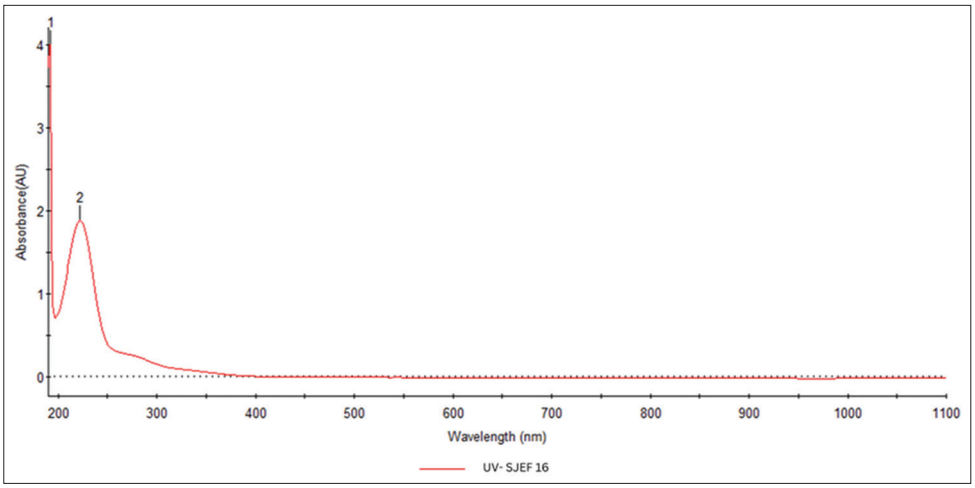

Figure 4: Ultraviolet-visible spectrum of SJEF-16 showing absorption maxima at 191.5 and 221.8 nm, characteristic of phenolic aromatic compounds

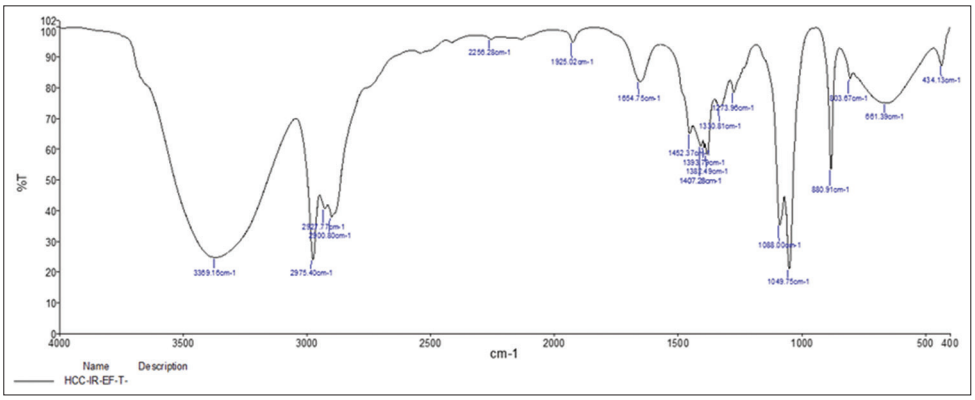

Figure 5: Fourier-transform infrared spectrum of SJEF-16 displaying functional group peaks including O-H, C-H, C=O, and C-O stretching vibrations

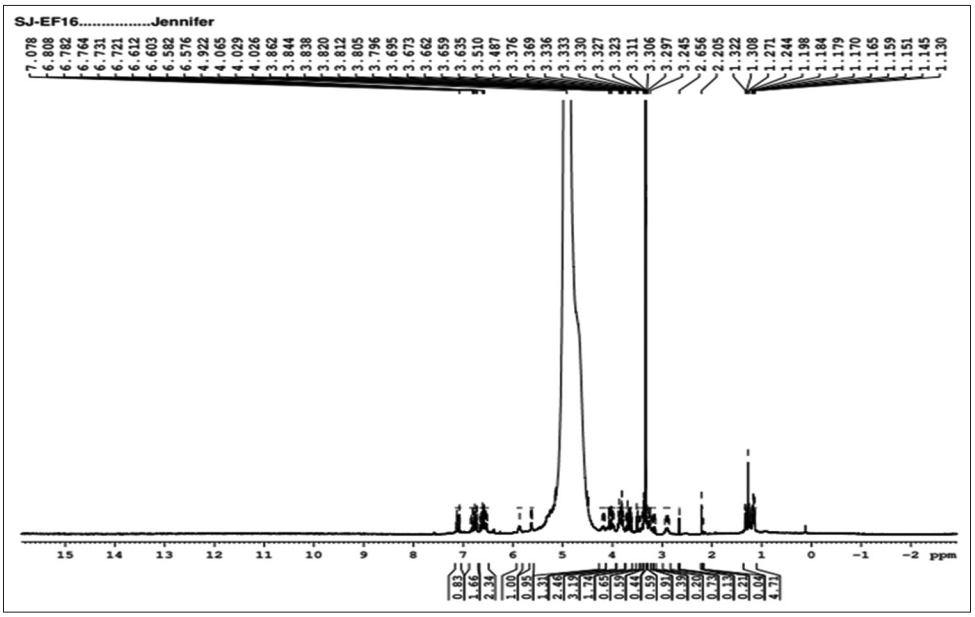

Figure 6: [1]H nuclear magnetic resonance spectra of SJEF 16 fraction isolated from ethanolic leaf extract of *Syzygium jambos*

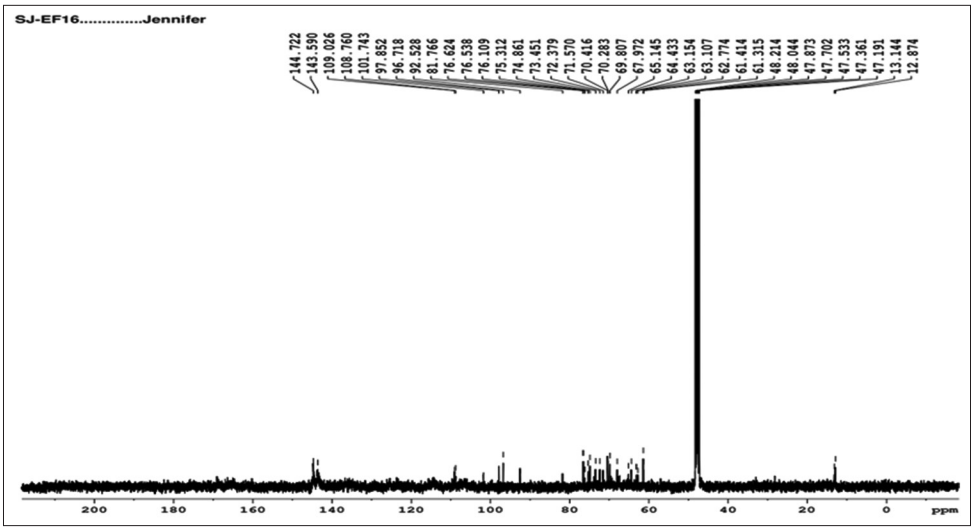

Figure 7: ^{13}C nuclear magnetic resonance spectra of SJEF 16 fraction isolated from ethanolic leaf extract of *Syzygium jambos*

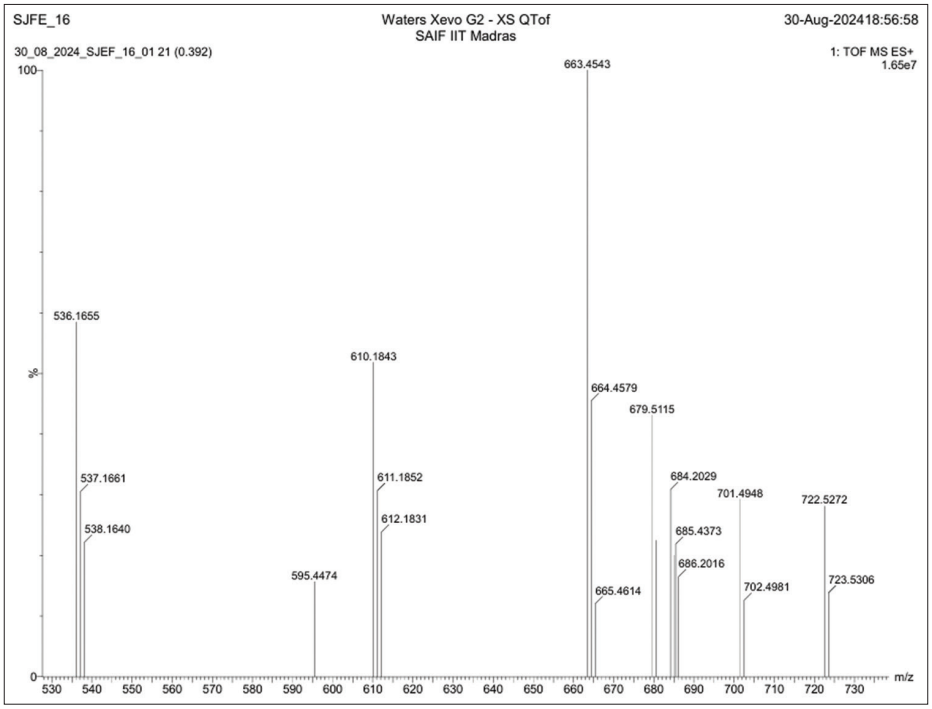

Figure 8: Liquid chromatography–quadrupole time-of-flight high-resolution mass spectrometry spectrum of SJEF-16 showing the molecular ion peak at m/z 179.107 $[M+H]^+$, confirming the molecular formula $C_{11}H_{14}O_2$

Table 2: Physical properties of SJEF 16

Property	Observation
Colour	Golden-brown
Odor	Odorless
Solubility	Soluble in polar solvents
Physical state	Crystalline solid

Table 3: Densitometry analysis of EGFR expression in SJEF-16-treated IMR-32 cells

Sample	EGFR area (ImageJ)	Relative expression (%)
Control	23042.39	73.94
SJEF-16	18955.35	66.17

In vitro cytotoxicity assay

MTT assay

The cytotoxic effect of SJEF-16 on IMR-32 neuroblastoma cells was evaluated using the MTT assay. Cell viability decreased in a dose-dependent manner with increasing concentrations of SJEF-16 (1–500 µg/mL). Control cells showed 100% viability with high OD values at 570 nm (mean OD ≈ 1.42). At the highest tested concentration (500 µg/mL), cell viability dropped markedly to ~46.8%, indicating strong

cytotoxicity. Moderate cytotoxicity was observed at 300–200 µg/ml with mean viabilities of ~47.5% and ~51.0%, respectively, while lower concentrations (25–5 µg/mL) maintained higher viability (65–85%). The least reduction in cell viability was noted at 1 µg/mL (90.3%) [Figure 9].

Dose–response curve analysis revealed that SJEF-16 exhibited an IC50 value of 28.92 µg/ml against IMR-32 cells, with a goodness-of-fit R^2 of 0.9617, indicating strong reliability of the model. Microscopic images confirmed morphological changes in treated cells, including cell shrinkage and loss of adherence, in contrast to the intact morphology of control cells [Figure 10].

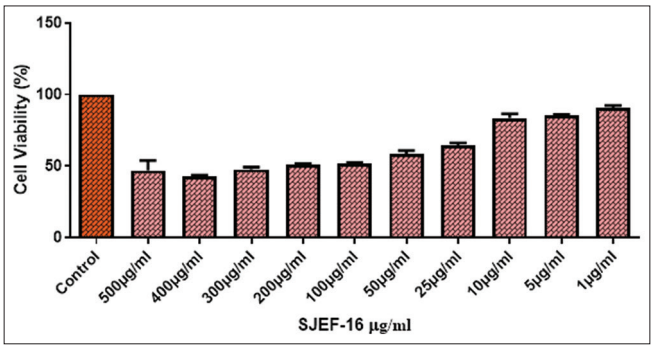

Figure 9: MTT assay showing the effect of SJEF-16 on cell viability of IMR-32 cells

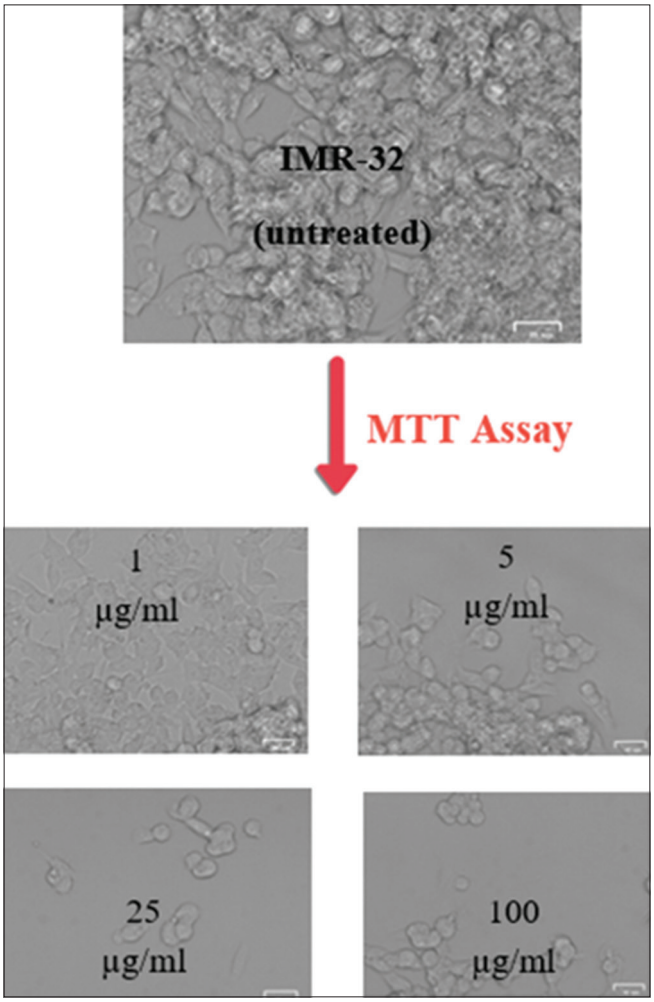

Figure 10: MTT assay results showing dose-dependent cytotoxicity of SJEF-16 on IMR-32 neuroblastoma cells

Total ROS assay

The intracellular ROS levels were evaluated in IMR-32 cells using the total ROS detection kit. Control cells displayed strong green fluorescence, indicating elevated basal ROS generation. In contrast, cells treated with SJEF-16 at its IC_{50} concentration (28.92 µg/mL) showed markedly reduced fluorescence intensity, demonstrating a significant decrease in ROS accumulation [Figure 11]. This suggests that SJEF-16 effectively attenuates intracellular oxidative stress in IMR-32 cells.

Western blotting

Western blot analysis of IMR-32 cells treated with SJEF-16 showed a moderate reduction in EGFR expression compared to control cells, with β-actin serving as the loading control. Densitometry analysis using ImageJ indicated that EGFR expression in SJEF-16-treated cells was 66.2%, compared to 73.9% in control cells [Table 3].

Molecular docking and ADME analysis

The isolated compound SJEF-16, identified as (2E)-3-(3-Methoxyphenyl)-2-propen-1-ol [Figure 12], was subjected to molecular docking analysis to evaluate its interaction with neuroblastoma-associated targets. Docking was performed using the crystal structures with PDB IDs 4CLI and 5G1X, both of which are implicated in neuroblastoma signaling pathways.

SJEF-16 exhibited a binding affinity of −6.2 kcal/mol, indicating a stable and energetically favorable interaction with the active sites of the target proteins. Docking visualization revealed the presence of hydrogen bonding and hydrophobic interactions with key amino acid residues, which may contribute to the compound's biological activity.

Furthermore, *in silico* ADME profiling demonstrated favorable pharmacokinetic properties. SJEF-16 showed good gastrointestinal absorption, absence of cytochrome

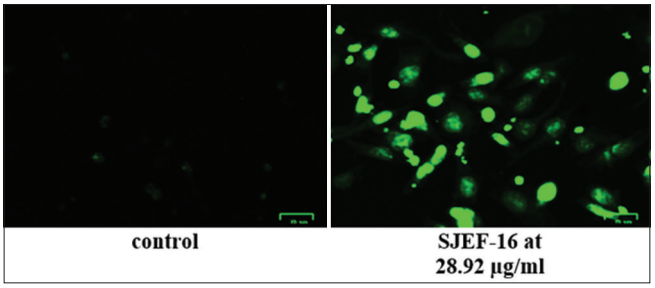

Figure 11: Effect of SJEF-16 on intracellular reactive oxygen species generation in IMR-32 cells

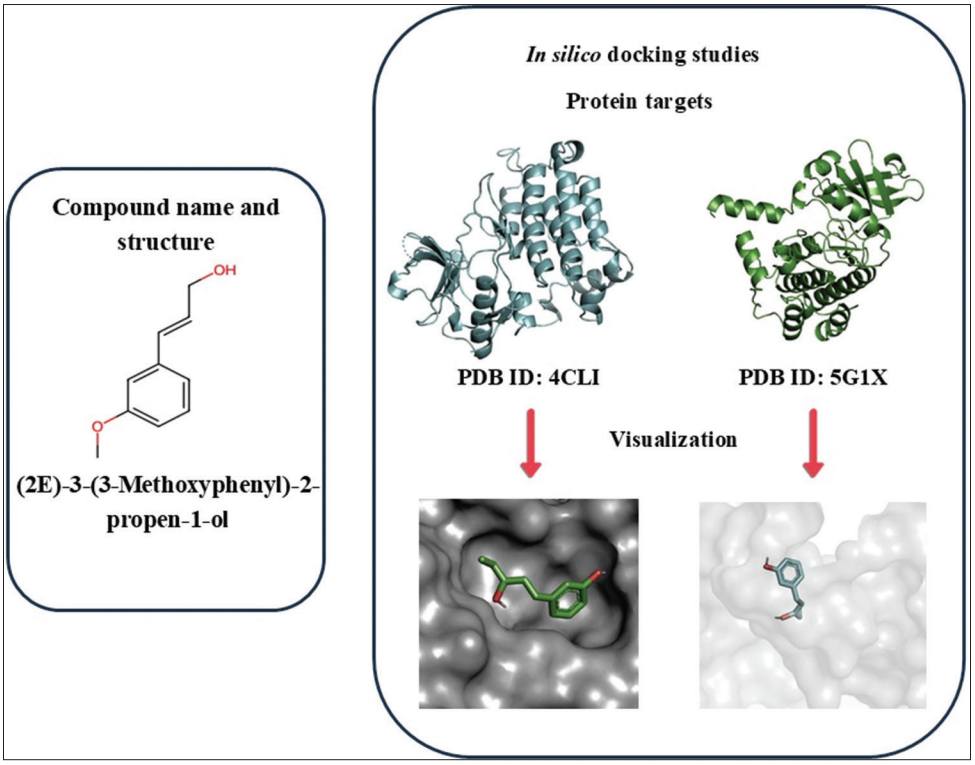

Figure 12: Molecular docking analysis of SJEF-16 with neuroblastoma-associated protein targets (PDB IDs: 4CLI and 5G1X)

Table 4: Predicted ADME Properties of (2E)-3-(3-Methoxyphenyl)-2-propen-1-ol

Parameter	Result
GI absorption	High
BBB permeant	Yes
P-gp substrate	No
CYP inhibition (1A2, 2C19, 2C9, 2D6, 3A4)	No
Log K (skin permeation)	–5.61 cm/s
Lipinski	Yes (0 violation)
Ghose/Veber/Egan	Yes
Muegge	No (MW<200)
Bioavailability score	0.55

BBB: Blood-brain barrier, CYP: Cytochrome P450

P450 enzyme inhibition, and no violations of Lipinski's Rule of Five, suggesting its potential as an orally bioavailable compound [Table 4]. The predicted bioavailability score of 0.55 further supports its suitability for oral drug development.

CONCLUSION

This study highlights the antioxidant and anticancer potential of *S. jambos* leaf extracts, particularly the bioactive fraction SJEF-16. Comprehensive structural analyses identified SJEF-16 as (2E)-3-(3-Methoxyphenyl)-2-propen-1-ol, a phenolic compound with hydroxyl and methoxy groups that likely contribute to its bioactivity. *In vitro* experiments demonstrated that SJEF-16 effectively reduced neuroblastoma (IMR-32) cell viability in a dose-dependent manner, decreased intracellular ROS levels, and moderately downregulated EGFR expression. Molecular docking confirmed stable interactions with key neuroblastoma-related targets, and ADME predictions suggested favorable pharmacokinetic properties and oral bioavailability. Taken together, these findings position SJEF-16 as a promising natural compound with potential for development as an antioxidant and anticancer agent against neuroblastoma.

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