International Journal of Preventive Medicine

Original Article Open Access

Isolation and Characterization of the Anticancer Compound Piceatannol from *Sophora Interrupta* Bedd

Pardhasaradhi Mathi, Snehasish Das[1], Kumar Nikhil[1], Partha Roy[1], Srikanth Yerra[2], Suryachandra Rao Ravada[3], Venkata Raman Bokka[4], Mahendran Botlagunta

Department of Biotechnology, Biomedical Research Laboratory, KLEF University, Guntur, Andhra Pradesh, India, [1]Department of Biotechnology, Molecular Endocrinology Laboratory, Indian Institute of Technology Roorkee, Roorkee, Uttarakhand, India, [2]Analytical R&D, Laxai Avanti Life Sciences Pvt. Ltd., Hyderabad, Telangana, India, [3]R&D Center, Laila Neutraceuticals, Vijayawada, Andhra Pradesh, India, [4]Department of Basic Sciences-chemistry, Madanapalle Institute of Technology and Science, Chittoor, Andhra Pradesh, India

Correspondence to:
Dr. Mahendran Botlagunta, Department of Biotechnology, Centre for Biomedical Research, KLEF University, Guntur, Andhra Pradesh, India.
E-mail: bmnchowdary@gmail.com

How to cite this article: Mathi P, Das S, Nikhil K, Roy P, Yerra S, Ravada SR, Bokka VR, Botlagunta M. Isolation and characterization of the anticancer compound piceatannol from *sophora interrupta* bedd. Int J Prev Med 2015;6:101.

ABSTRACT

Background: *Sophora* belongs to the family of *Fabaceae* and the species in this genus are currently used as a folklore medicine for preventing a variety of ailments including cancer. Our aim was to identify and validate an anticancer compound from *Sophora interrupta* using multi-spectroscopic, anticancer screening, and molecular docking approach.

Methods: The cytotoxicity of the various solvent extracts, petroleum ether, *n*-butanol, and ethyl acetate (EtOAc) of the *S. interrupta* root powder was evaluated in a breast cancer cell lines (MCF-7). The extract that had anticancer activity was subjected to column chromatography based on the polarity of the solvents. The anticancer activity of the elution fractions was validated using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The isolated metabolite fraction with anticancer activity was run through a C18 column isocratic and gradient high-performance liquid chromatography (HPLC). The structure of the isolated compound was characterized using ^{1}H nuclear magnetic resonance (NMR), ^{13}C-NMR, Fourier transform infrared spectroscopy, and liquid chromatography-mass spectrometer methods.

Results: The crude EtAOc extract effectively inhibited the proliferation of MCF-7 cells. The column eluted chloroform and EtOAc (4:6) fraction of the EtOAc extract showed significant anticancer activity in the MCF-7 cells compared with normal mesenchymal stem cells. This fraction showed three major peaks in the HPLC chromatogram and the first major peak with a retention time (RT) of 7.153 was purified using preparative-HPLC. The structure of the compound is a piceatannol, which is a metabolic product of resveratrol. Piceatannol formed direct two hydrogen bond interactions between Cys912 (2H), and Glu878 of vascular endothelial growth factor receptor 1 (VEGFR1) with a glide-score (G-score) of −10.193, and two hydrogen bond interactions between Cys919, and Asp1046 of VEGFR2, with a G-score of −8.359. The structure is similar to that of the crystallized protein for VEGFR1 and R2.

Conclusions: Piceatannol is a secondary metabolite of *S. interrupta* that has anticancer activity. Moreover, piceatannol has been isolated for the first time from *S. interrupta*.

Keywords: Active fraction, cancer cell lines, characterization, phenol, piceatannol, *Sophora interrupta* roots

Access this article online	
Quick Response Code:	**Website:** www.ijpvmjournal.net/www.ijpm.ir
	DOI: 10.4103/2008-7802.167181

INTRODUCTION

Sophora interrupta belongs to the family of *Fabaceae*, and there are approximately 51 species in this genus. Plants in this genus are distributed across the world, ranging from South-East Europe, Southern Asia, Australasia, the Pacific Islands, Western South America, the Western United States, Florida, and Puerto Rico. *S. interrupta* is endemic to India, situated at Tirumala Hills (Latitude 13.667790 and Longitude 79.345880) and Nallamalla Forests (Latitude 15.987266 and Longitude 78.954707) of Andhra Pradesh, India.[1] This plant parts such as leaves, roots, and flowers are enriched with numerous secondary metabolites such as flavonoids, alkaloids, and terpenoids, which are proven to have antioxidant, anti-inflammatory, and antiproliferative activities.[2] Phytochemical evaluations of the roots revealed the presence of flavonoids, alkaloids, saponins, glycosides, and carbohydrates. Thin-layer chromatography (TLC) and high-performance (TLC) profiling of the benzene extract was performed for flavonoids.[3] Root ethyl acetate (EtOAc) extract acting as an anti-inflammatory was confirmed by the inhibition of BSA denaturation assay,[4] antiproliferation was confirmed using an *in vitro* (MCF-7) cell culture model; in cellular systems, the extract has been shown to enhance the cell death and DNA fragmentation in actively proliferating breast cancer cells[5] and as well as *in vivo* (Dalton's ascitic lymphoma mouse model).[6] Furthermore, the methanol extract of *S. interrupta* leaves has been reported to protect and increase the life expectancy of mice suffering from leukemia.[7] It suggests that this plant originally has a lot of medicinal importance, and it much be used to be explained for the identification and characterization of active principles existing in the various region of this plant. However, four of the compounds were recently documented by Munikishore *et al*.[8] (1) O-prenylated flavonol, that is, 3′, 4′-dimethoxy-7-(γ, γ-dimethylallyloxy) flavonol was isolated from the root methanol extract and its activity was not studied. (2) 2′-hydroxy-3,4-dimethoxychalcone showed significant antitrichomonal activity against *Trichomonas gallina* and also inhibited the intracellular survival of *Leishmania donovani* parasites.[9] (3) Biochanin A, another major well-known compound belonging to the flavonoid class of phytochemicals, and was shown to inhibit fatty acid amide hydrolase.[10] (4) Kaempferol-3-O-β-D-glucopyranoside inhibited the proliferation of cells, such as PC-3, Bcap-37, BGC-823; NIT3T3 cell lines[11] and also significantly appeared to be anti-HIV.[12] Kaempferol has turned out to induce apoptosis via a mitochondria-dependent pathway in human osteosarcoma U-2 OS cells.[13] In our previous study, we found that *S. interrupta* root EtOAc extract has inhibited the proliferation of breast cancer cell line (MCF-7) in a dose-dependent manner. Moreover, this extract has not shown any toxicity in Brine Shrimp Model. Overall, it suggests that *S. interrupta* plant needs an extensive investigation and the active compounds present in this medicinal plant need to be explored using bioanalytical techniques like, liquid chromatography-mass spectrometer (LC-MS) Fourier transform infrared spectroscopy (FT-IR), ^{1}H and ^{13}C-nuclear magnetic resonance (^{13}C-NMR) methods. LC coupled with MC was the most common method used for determining the molecular mass of the analytes. FT-IR was utilized to determine the major functional groups such as alkyl, phenyl, hydroxyl, saturated and unsaturated hydrocarbons.[14] ^{1}H and ^{13}C-NMR tools used for elucidating the structures present in the active fraction. Results showed that the molecular weight of the purified compound is 244 m/z, and the name of the compound is piceatannol, which is a metabolic derivative of well-known antiangiogenic compound resveratrol. Resveratrol was known for its action against vascular endothelial growth factor receptors (VEGFRs) and being its metabolic derivative piceatannol can also have the similar type of activity against VEGFR's. In this study our aim was to identify and validate an anticancer compound from *Sophora* interrupta using multi-spectroscopic, anticancer screening, and molecular docking approach.

METHODS

Preparation of crude extracts of *Sophora interrupta* roots

Sophora interrupta roots were collected from Tirumala forest, Tirupati (Chittoor District, Andhra Pradesh, India) identified by taxonomist a voucher specimen deposited, in K L E F University, Guntur, India (voucher number KLU 1211). Shade dried and powdered roots were successively extracted from nonpolar to polar solvents such as 100% petroleum ether, *n*-butanol, and EtOAc and *Aqueous* is mixed individually with 2.5 kg of dry root powder and macerated for 48 h at room temperature. The extracts were filtered with Whatman filter paper (type 4), and the filtrate was concentrated under reduced pressure on rotavapor under vacuum (BÜCHI, R-3000, Switzerland) at 40°C temperature. The complexity of EtOAc extract on purification over a silica gel column chromatography (60–120 mesh) with step gradient systems that is, *n*-hexane, chloroform, EtOAc, ethanol, and methanol.

Cell viability assay

To study the action of the *S. interrupta* root EtOAc extract on cancer cell viability, we seeded approximately

5×10^3 MCF-7 cells/well in 96-well plate and were maintained in Dulbecco's modified Eagle's medium, respectively. The cells were supplemented with 10% fetal bovine serum (heat inactivated) and 1% antibiotic (100 U/mL of penicillin and 100 μg/mL streptomycin) gently mixed and placed in a 5% CO_2-humidified incubator at 37°C. Cells were treated with increasing concentrations (1, 10, 50, 100, 250, 300, and 500 μg/mL) of *S. interrupta* root EtOAc extract in dimethyl sulfoxide (DMSO) for 24 h. Following treatment, 15 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/mL) reagent was added to the culture media and further incubated for 4 h at 37°C in a CO_2 incubator.[15] After an incubation period MTT containing medium was aspirated, 200 μL of DMSO and 25 μL of Sorenson glycine buffer (0.1 M glycine and 0.1 M NaCl, pH 10.5) were added to lyse the cells and solubilize the water-insoluble formazan crystals. Absorbance values of the lysates were determined on a Fluostar optima microplate reader (BMG Labtech., Germany) at 570 nm. The percentage inhibition was calculated as:

$$\text{Mean OD of vehical treated cells-} = \frac{\text{Mean OD of drug treated cells}}{\text{Mean OD of vehical treated cells}} \times 100$$

The IC_{50} values were calculated using Microsoft Excel version 2010 software. Negative controls were maintained with DMSO.

Column chromatography

The dried and powdered root material (500 g) was extracted with EtOAc by maceration. The resulting extract was dried under vacuum yielded 150 g of crude extract. A portion of the extract (10 g) was subjected to bioactivity-directed silica column chromatography through a column (58 cm × 2 cm) of silica gel (60–120 mesh) eluted gradient with Hex (100%, v/v), Hex:$CHCl_3$ (90:10% to 10:90%, v/v), $CHCl_3$ (100%, v/v), $CHCl_3$:EtOAc (90:10% to 10:90%, v/v), EtOAc (100%, v/v), EtOAc:EtOH (90:10% to 10:90% v/v), EtOH (100%, v/v), EtOH:MeOH (90:10% to 10:90%, v/v) and finally MeOH (100%, v/v) as mobile phase, yielding 15 fractions.[16] The cytotoxic actions of fractions were analyzed by MTT assay on MCF-7 cancer cell line.

Antibacterial activity

The following microorganisms were used as test organisms in the screening: Two Gram-positive strains namely, *Staphylococcus aureus* (ATCC 29213), *Bacillus subtilis* (ATCC 6051), four Gram-negative strains namely, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 6380), and *Klebsiella pneumoniae* (ATCC 700603). The cultures were diluted to achieve optical densities corresponding to 2.0×10^6 CFU/mL. *Streptomycin* standard drug used for *S. aureus*,[17] rest all strains used tetracycline as standard drug.[18]

Preparation of plates

Pour an equal amount of nutrient agar medium and Mc Conkey agar into petri dishes, which were then dried. After solidification, a loopful of culture was spread onto the media. Controls contained only DMSO. Autoclaved paper discs containing plant extracts with different concentrations were placed on the media and incubated overnight (18 h) at 37°C. Streptomycin and tetracycline are used as positive control. Negative controls were performed with discs loaded with DMSO. After the incubation period, the antibacterial activity was determined by recording the inhibition zones.[19]

High-performance liquid chromatography analysis of the active fraction

The number of compounds present in the $CHCl_3$:EtOAc (4:6) active fraction was confirmed by high-performance liquid chromatography (HPLC). The HPLC was run in a Waters Alliance 2695 apparatus with Empower software and column is XBridge RP-C18 column (150 mm × 4.6 mm, 5 μm). The samples were eluted with a linear gradient of 0.01M NH_4OAc containing water and acetonitrile. The gradient program was 0.01M NH_4OAc in water (A) and acetonitrile (B), starting with 5% acetonitrile and installing a gradient to obtain 90% B at 16 min, up to 19 min and 5% B at 22 min up to 25 min at a flow rate constantly kept at 1 mL/min.[20] Detection was achieved with a 2996 PDA detector. The chromatogram was constantly measured at 220 nm.

Preparative-high-performance liquid chromatography

Following determination, the number of compounds preparative HPLC was performed to isolate relatively pure components from the active fraction. The preparative HPLC was run in an SHIMADZU apparatus, using a pump LC-8A and SPD-20A UV-Visible detector, with a column XBridge C18 (250 mm × 50 mm, 10 μm) was used for the purification of relatively pure compounds obtained from fractions eluted by column chromatography. Mobile phases include 0.01M NH_4OAc in water (solvent A) and acetonitrile (solvent B). A gradient elution program was as follows: 85% A (5 min), 70% A (25 min), and 50% A (30 min). Each injection was in a concentration of 500 mg of the dried fraction dissolved in 1 mL of the solvent system.[21] The injection volume up to 5 mL was injected into the column, and the flow rate of the mobile phase was at 50 mL/min. The compound peaks were detected by UV detector and collected manually. Approximately 500 mL of the sample was collected and concentrated to get 50 mg of the compound.

Nuclear magnetic resonance spectroscopy

The 1D, ^1H, and ^{13}C-NMR spectra were recorded at 300° K on Avance III Bruker 400 MHz NMR spectrometer. The samples were dissolved in deuterated solvents (DMSO-d6) and tetramethylsilane as an internal standard; the choice

of the solvent depends mostly on the solubility of the compound. Residual solvent signals of (DMSO-d6 at 2.49 ppm and 39.5 ppm) were considered as an internal reference signal for calibration. The observed chemical shift values (δ) were given in ppm and the coupling constant (*J*) in Hz. The ^{1}H and ^{13}C-NMR spectra were recorded under the following conditions: ^{1}H-NMR: Acquisition time, 3.9846387 s; pulse width, 13.54 μs; pulse delay, 2 s; number of scans, 24, sweep width: 8223.685 Hz, and ^{13}C-NMR: Acquisition time, 1.3631988 s; pulse width, 9.85 μs; pulse delay, 2 s; number of scans, 32000, sweep width: 24038.461 Hz, respectively.

IR spectra of the samples

PerkinElmer FT-IR spectrometer equipped with (Lithium tantalate detector). The Sample was introduced into Hygroscopic KBr glass windows where exactly 100 μL sample. FT-IR spectra were obtained by collecting 100 scans with spectra was collected using a resolution of 64 cm^{-1}. A pure vehicle solvent was analyzed before each sample analysis as a background. The FT-IR spectra corrected and baseline ranging in 4000–450 cm^{-1} recorded using Spectrum Two™ Spectroscopy software (Perkin Elmer Corporation, Lambda).[22]

Liquid chromatography-mass spectrometer

Analysis was performed using an Agilent 6120 single quadrupole liquid chromatograph (Agilent Technologies, USA) consisting of a binary pump and an automatic sampler. The extracts were filtered through a Millex HA 0.45 μm filter (Millipore Corp., USA) before injection (50 μL). Chromatographic separation was performed on a Phenomenex C18 column (25 cm × 4.6 mm i.d., 5 μm), was equipped with a 5 μm C18 guard column and the column temperature was 25°C. The mobile phases were (A) 0.01 M NH_4OAc in water and (B) Acetonitrile. The gradient used was 0 min, 2% B; 7 min, 90% B; 10 min, 90% B; 12 min, 2%. The solvent flow rate was 0.8 mL/min. Analysis was achieved by positive ion mode using a mass spectrometer (6120 single quadrupole) in ESI mode. Some important parameters of the mass spectrometer are nebulizer gas flow 60 psi, dry gas flow 5 L/min, and dry gas temp. 275°C, vaporization temp, 200°C, charging voltage 2000 V, capillary voltage 2000 V, carona 1 μA. Nitrogen was used as nebulizing and collision gas. The data was collected and processed by Analyst chemstation software (Agilent Technologies, Santa Clara, U.S.A).[23]

Docking studies

Protein and ligand preparation

The three-dimensional (3D) coordinate structures of VEGF-R1 (PDB ID: 3HNG), and VEGF-R2 (PDB ID: 3U6J) with resolutions of 2.7 Å, and 2.15 Å, respectively, were retrieved from a protein data bank.[24] A protein preparation wizard in the Maestro module, (version 9.6 Schrödinger, LLC, version 9.6, New York, U.S.A) was used in the preparation of protein by adding hydrogens. Finally, the potential energy of the protein was minimized using an OPLS 2005 force field. The ligand molecules

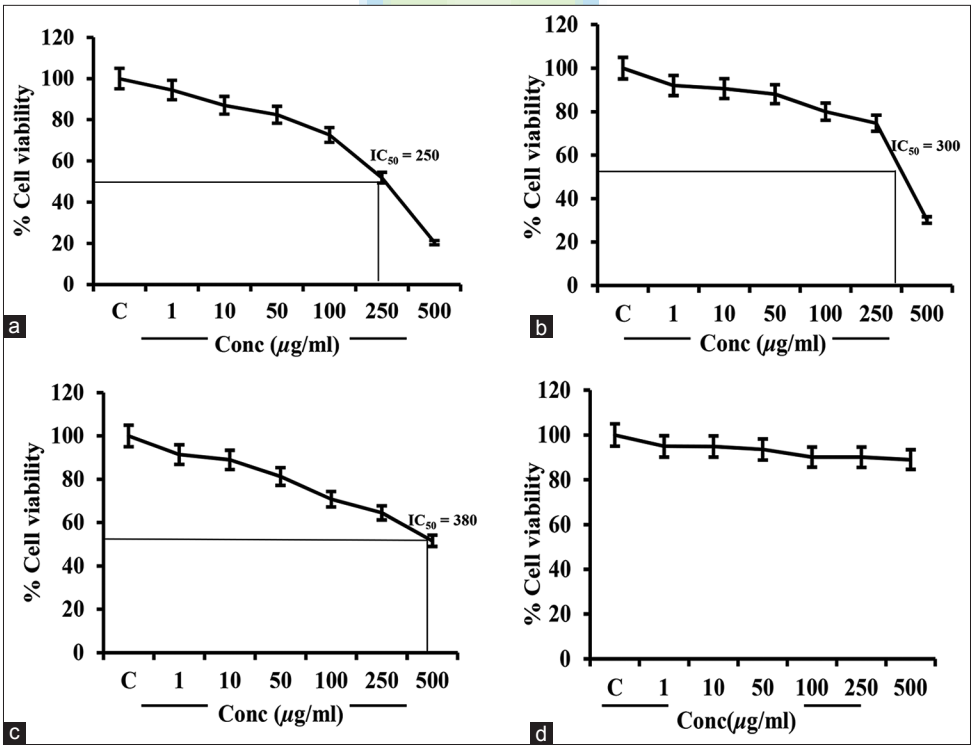


Figure 1: The graphs showing the data of cancer cell viability against root extracts of Sophora interrupta. (a) Ethyl-acetate (b) n-butanol (c) Petroleum ether (d) Aqueous extracts. The experiments were conducted three times, and the results are represented in mean ± standard deviation

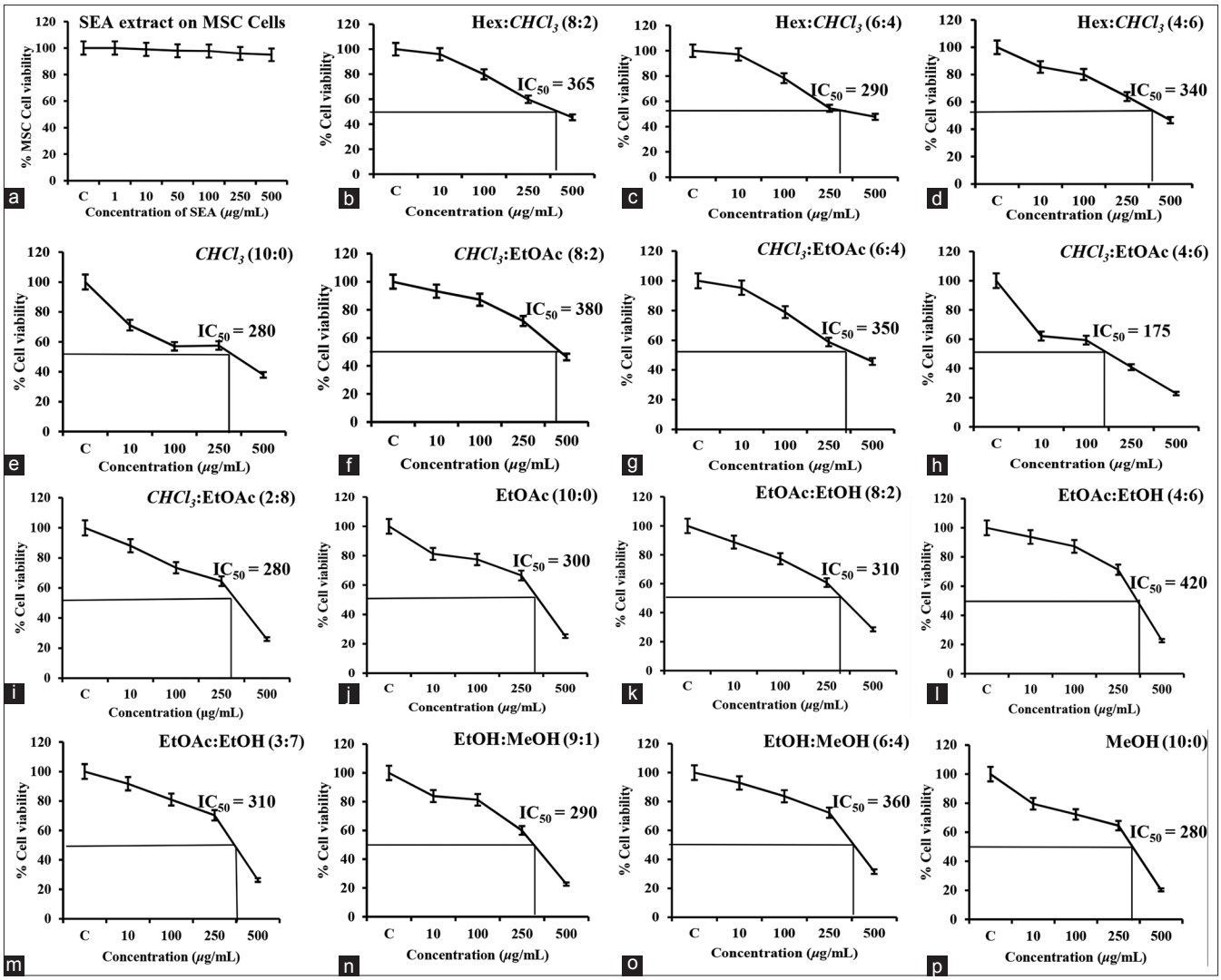


Figure 2:The graphs showing the cancer cell viability against the column eluted fractions of root ethyl-acetate extract (a) SEA extract on mesenchymal stem cells (b) Hex:CHCl3 (8:2) (c) Hex:CHCl3 (6:4) (d) Hex:CHCl3 (4:6) (e) CHCl3 (10:0) (f) CHCl3:ethyl acetate (EtOAc) (8:2) (g) CHCl3:EtOAc (6:4) (h) CHCl3:EtOAc (4:6) (i) CHCl3:EtOAc (2:8) (j) EtOAc (10:0) (k) EtOAc:EtOH (8:2) (l) EtOAc:EtOH (4:6) (m) EtOAc:EtOH (3:7) (n) EtOH:MeOH (9:1) (o) EtOH:MeOH (6:4) (p) MeOH (10:0) The results are represented in mean ± standard deviation of three independent experiments

were retrieved from the Chemspider database and imported into the Schrödinger Maestro suite, Lig Prep module, (version 2.6, LigPrep, version 2.6, Schrödinger, LLC, New York, U.S.A) was used to neutralize, desalt, correct the Lewis structure, and check for any metal binding sites as well as to generate tautomers and 32 stereoisomers generation per ligand at physiological pH 7.0 ± 2.0. All the ligand 3D geometries were optimized using an OPLS 2005 force field.

Ligand docking

The GLIDE module (Glide, version 6.1, Schrödinger, LLC, New York, U.S.A) in the Schrödinger suite was implemented for ligand docking, which is a grid-based ligand docking method with energetics, was used. A grid box with a size of 72 Å × 72 Å × 72 Å and coordinates of X = 12.188, Y = 20.152, and Z = 48.922 was generated at the centroid of the ligand present in the PDB.[25] Subsequently, a prepared ligand was docked using extra precision (XP) docking according to the protocols.[26] The receptor-ligand complex interactions were calculated based on their energy and the quality of the geometric contacts. The binding modes of the receptor-ligand are represented as a glide-score (G-score).

Statistical analysis

Data were analyzed using GraphPad Prism® 5 (Version 5.01, GraphPad Software, Inc., USA). Results are expressed as the mean ± Standard deviation of three independent experiments. The data were analyzed for statistical significance by one-way ANOVA test; $P < 0.05$ was considered to be significant.

RESULTS

Cell viability

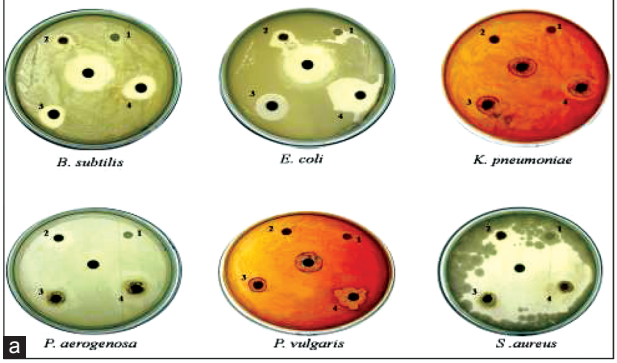
To measure the anticancer activity of *S. interrupta* EtOAc roots, we treated the MCF-7 breast cancer cell line with increasing concentrations of different solvent extracts (1, 50, 100, 250, and 500 μg/mL) for 24 h. The standard MTT assay results are depicted in Figure 1. As shown in the top left panel of Figure 1, use of EtOAc root extracts resulted in a higher percentage of cell death as compared to *n*-butanol, petroleum ether, and aqueous extracts. The EtOAc extract also led to a lower IC_{50} value for the MCF-7 cell line (250 μg/mL) as compared with other solvent extracts. These findings suggest that growth inhibitory principles are present in all organic fractions, and we rank them as EA> BU>PE>AQ. On the other hand, samples treated with DMSO did not have any impact on cell viability.

Cell viability for column-eluted fractions

In order to identify the active metabolites against cancer growth, we subjected the *S. interrupta* EtOAc root extract to column chromatography based on the gradient polarity. Elution fractions were incubated with increasing concentrations (10, 100, 250, and 500 μg/mL) to determine the anticancer activity using MCF-7 cell lines. Cell viability was determined using a standard MTT assay and the results are depicted in Figure 2. Use of EtOAc: EtOH (4:6); $CHCl_3$: EtOAc (8:2); Hex: $CHCl_3$ (8:2); and EtOH: MeOH (6:4) of root fractions led to a higher percentage of viable cells in the MCF-7 cell line as compared to the $CHCl_3$: EtOAc (4:6) fraction which showed a sharp decline in the cancer cell growth at 10 μg/mL and significant (68%) inhibition at 500 μg/mL. Apparently, it was evident from the calculated dose of 50% inhibition of cell viability (IC_{50}). The $CHCl_3$ and EtOAc fraction led to lower IC_{50} values for the MCF-7 (175 μg/mL) cell line as compared to other solvent fractions. These findings suggest that growth inhibitory principles are present in all the solvent ratio fractions. On the basis of this study, these fractions can be ranked in order from highest to lowest degree of inhibition as follows: $CHCl_3$:EtOAc (4:6) > $CHCl_3$ (10:0) > $CHCl_3$:EtOAc (2:8); MeOH (10:0) > Hex:$CHCl_3$ (6:4) > EtOH:MeOH (9:1) > EtOAc (10:0) > EtOAc:EtOH (8:2) > EtOAc:EtOH (3:7) > Hex:$CHCl_3$ (4:6) > $CHCl_3$:EtOAc (6:4) > EtOH:MeOH (6:4) > Hex:$CHCl_3$ (8:2) > $CHCl_3$:EtOAc (8:2) > EtOAc:EtOH (4:6).

Antibacterial activity

The active $CHCl_3$:EtOAc (4:6) fraction against bacterial systems was chosen the bacterial system as an anticancer model, due to its proliferative ability.[27] Six pathogenic bacterial strains were treated with different concentrations (100, 500, and 1000 μg/mL) of this fraction. The antibacterial activity of all the strains is presented in Figure 3a. Results showed the presence of antibacterial activity for the fraction in a dose-dependent manner (μg/mL). As compared with standard drugs, *S. aureus*, *P. aeruginosa* and *E. coli* were more sensitive to the fraction as compared with *K. pneumoniae*, *P. vulgaris*, and *B. subtilis*. The growth inhibition zone was measured and is ranged from 0 to 1.8 mm for all of the measured bacteria that were sensitive to the fraction [Figure 3b].



S.No	Strains	100 μg/mL	250 μg/mL	1000 μg/mL	Standard (10μg)
1	*B. subtilis*	0.2	0.5	1	1.2
2	*E. coli*	0.6	1.7	1.8	1.2
3	*P. vulgaris*	0	0.3	0.7	0.8
4	*S. aureus*	0.4	1	1.8	1
5	*K. pneumoniae*	0	0.3	1	0.8
6	*P. aerogenosa*	0.3	0.9	1.5	0.7

b

Figure 3: Antibacterial activity of active fraction CHCl3:ethyl acetate(4:6) (a) Photographic view showing the bacterial inhibitory zones on different bacteriological plates (b) Table representing zone of inhibitions in mm, values are mean ± standard deviation of three parallel experiments

Isolation and purification of the active metabolite of the *Sophora interrupta* ethyl acetate extract

To determine whether the anti-cancer activity of the $CHCl_3$/EtOAc (4:6) active fraction of the *S. interrupta* EtOAc root extract metabolites is due to the presence of a single metabolite or a complex mixture, the fraction was subjected to HPLC analysis. Results showed that this fraction consists of three major metabolites with different retention times (RTs) [Figure 4a]. Among several lower peaks, three peaks can be readily detected in the chromatogram, due to their maximum absorbance units at different RT points [Table 1]. In order to isolate the single metabolite, we have subjected the active fraction to preparative HPLC analysis. Based on the HPLC spectrum profile, a compound with early RT and major percentage area from spectrum was collected using preparative HPLC, pooled and concentrated. The HPLC profile of the purified single compound is depicted in Figure 4b and the RT and % area is summarized in Table 2.

Spectroscopy

Structure elucidation of phytoconstituents by (^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR, Fourier transform infrared spectroscopy and liquid chromatography-mass spectrometer) spectroscopy

The ^{1}H-NMR spectra (400 MHz) and ^{13}C-NMR (100 MHz) DMSO-d_6 spectra of the purified active fraction showed characteristic signals for flavones. The calculated proton (^{1}H-NMR) spectrum of the compound showed two distinguishable signals at δ at 6.08 (1H, d, J = 2.4 Hz), 6.34 (2H, d, J = 2.4 Hz), with the aromatic ring having a plane of symmetry with containing three protons that possibly constitutes a 3, 5 disubstituted aromatic system; 6.67 (1H, d, J = 7.6 Hz); 6.79 (1H, dd, J = 7.6, 2.0 Hz), 6.92 (1H, d, J = 2.0 Hz) indicates a ring containing ABX systems and two double bond protons. 6.68 (1H, d, J = 15.6 Hz), 6.81 (1H, d, J = 15.6), which have trans coupling with all of the above correlations identified as piceatannol (3, 4, 3′, 5′-tetrahydroxystilbene), were compared with calculated ^{13}C-NMR, with δ at 102.0, 104.6, 113.54, 116.1, 118.9, 125.7, 128.6, 128.8, 139.5, 145.7, 145.9, and 158.7; and data related to this were previously reported for this compound.[28] Taken together, compound is identified as Piceatannol (trans-3, 4, 3′,5′-tetrahydroxystilbene) [Figure 5a and b].

Table 1: HPLC analysis of active fraction with RT, area and area %

Signal peak	RT (min)	Area	Area %
4	7.153	6,565,998	32.08
29	15.750	3,632,130	17.74
38	19.248	1,983,358	9.69

HPLC= High-performance liquid chromatography, RT= Retention time

Table 2: HPLC analysis of eluted sample from preparative-HPLC

Signal peak	RT (min)	Area	Area %
1	12.427	43,368	0.80
2	13.697	114,359	2.10
3	13.849	5,292,026	97.11

HPLC= High-performance liquid chromatography, RT= Retention time

In order to identify the functional groups present in the purified compound, FT-IR analysis was performed. Results showed that the compound has a maximum percentage of transmittance in the 3500–1500 cm^{-1} region [Table 3]. This region exhibited three major peaks. The peak at 2265.78 cm^{-1} and 3722.73 cm^{-1} is attributable to the hydroxyl (OH) group, while the peak at 1991.39 cm^{-1} and 2156.77 cm^{-1} is due to the characteristic transmittance of aromatic stretching C=C [Figure 6a]. The 2265.78 cm^{-1} shows an 18.36% transmittance (% T) signal indicating a strong hydroxyl group.

The LC-MS findings for the compound revealed that the molecular mass was M$^+$, m/z 244 (100%). Taken together, it can be speculated from our results that the purified metabolite with a yellow color may belong to a phenolic compound [Figure 6b and c].

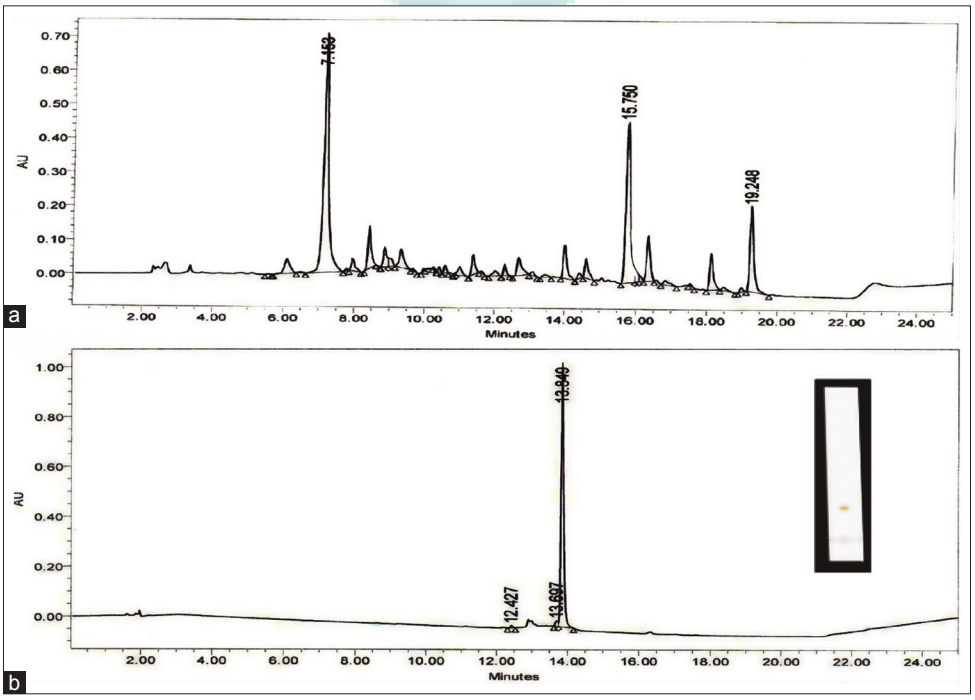


Figure 4: High-performance liquid chromatography (HPLC) spectrum analysis: (a) HPLC chromatogram of the active fraction reporting three major peaks (b) HPLC chromatogram representing the purity of the sample after the sample collected from the Prep-HPLC, inset reporting a thin-layer chromatography plate with single band

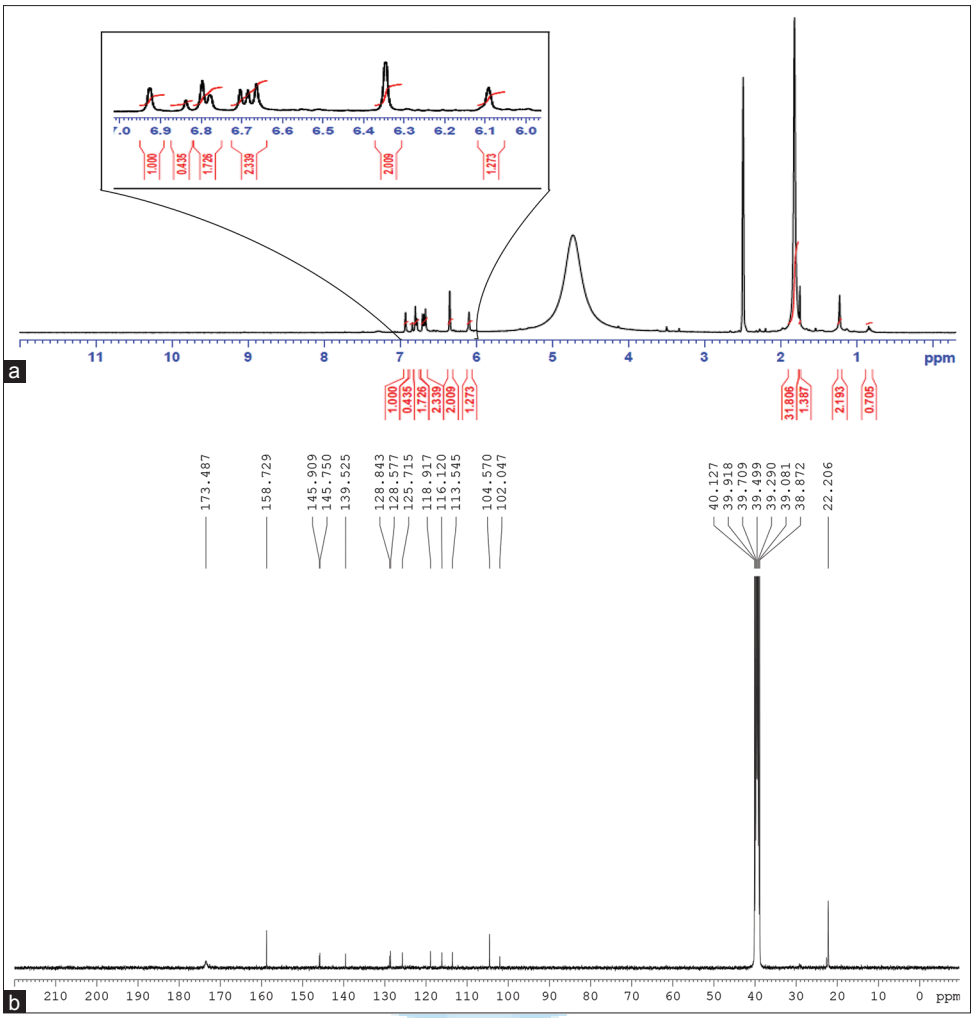


Figure 5: Nuclear magnetic resonance (NMR) spectroscopic analysis (a) ^{1}H-NMR representing the protons with signals, selective region is stretch of the specific region in ^{1}H-NMR (b) ^{13}C-NMR depicting the carbon signals

Table 3: FT-IR functional groups of piceatannol

Percentage of transmittance	Wave number (cm^{-1})	Major functional group
17.63	895.90	-C-H
16.16	960.72	-C-H
14.85	1207.94	-C-O
13.39	1752.50	Phenyl ring substitutes overtones
13.22	1991.39	-C=C
12.37	2156.77	-C=C
18.36	2265.78	-O-H
16.55	2911.56	-C-H
14.80	3722.73	-O-H

FT-IR= Fourier transform infrared spectroscopy

Docking studies

Identification of active site amino acid contacts with piceatannol in vascular endothelial growth factor receptors

Vascular endothelial growth factors R1 and R2 receptors were both subjected to the XP docking with piceatannol. G scores and hydrogen bond interactions were obtained for piceatannol and as comparator for the standard inhibitor resveratrol [Table 4]. As shown in Figure 7a and b, piceatannol was found deep into the narrow pocket formed by the inner lobe cleft as reported to X-ray crystallographic structures.

Piceatannol had strong hydrogen bond interactions with VEGFR1 (3HNG) in various amino-acids Cys912 (two hydrogen bonds), Glu878, Lys861 (π-cation), and Phe 1041 (π-π stacking), with a G-score of–10.193. Piceatannol inside the active site of VEGFR2 (3U6J) was maintained with the help of single hydrogen bond at Cys919, Asp1046, and Lys868 (π-cation), with a G-score of –8.359. Resveratrol inside the active site of VEGFR1 engaged in single hydrogen bond interactions with Cys912 and Asp1040, with a G-score of −8.787; VEGFR2 docking interactions showed Cys919 (two hydrogen bonds) and Lys868, with a G-score of −8.226. Due to the presence of an extra hydroxyl group in piceatannol,

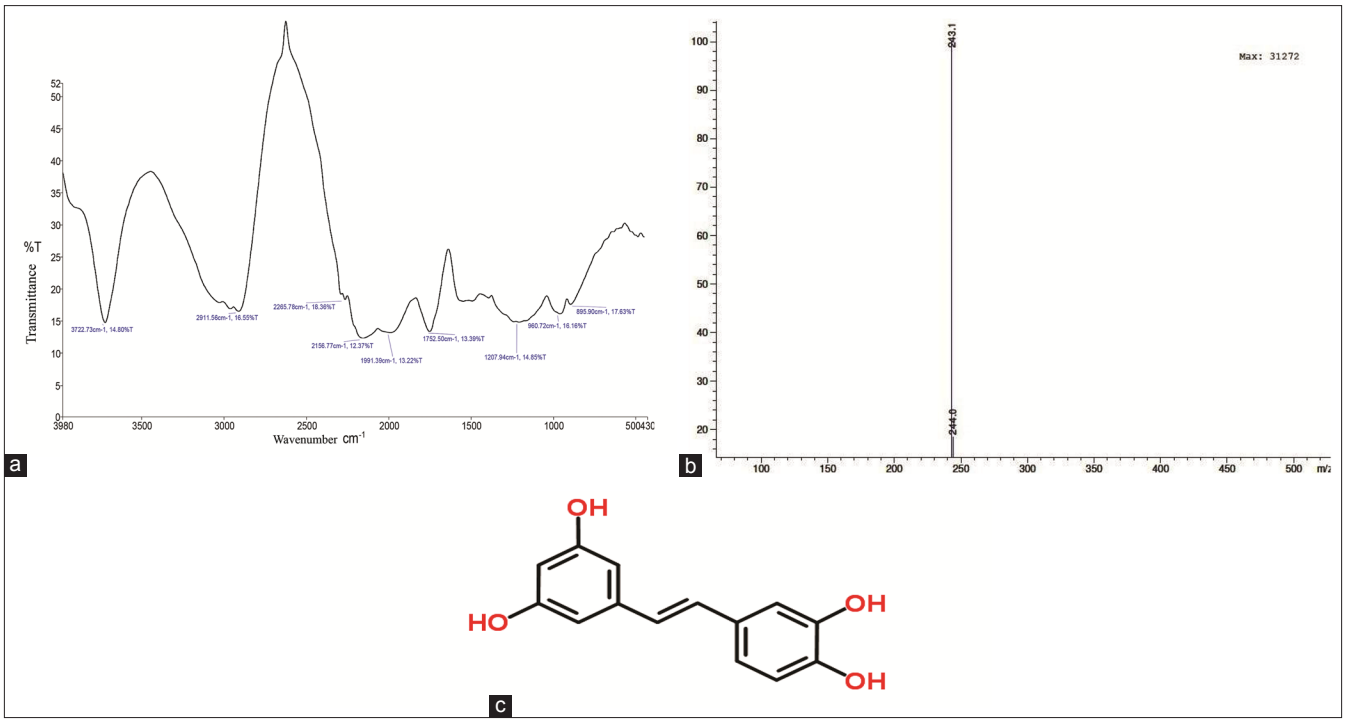


Figure 6: Spectroscopy analysis (a) calculated infrared spectrum of the speculated compound from Fourier transform infrared spectroscopy (b) MS profile of the Prep-high-performance liquid chromatography eluted purified compound (c) Piceatannol molecular structure

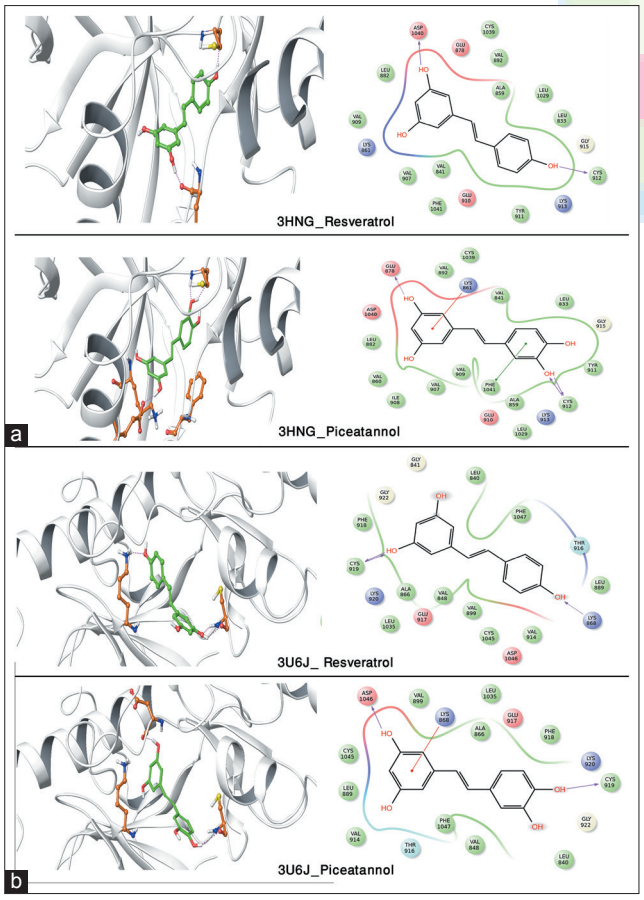


Figure 7: *In silico* docking (a) Vascular endothelial growth factor receptor 1 (VEGFR1) interaction with piceatannol (b) VEGFR2 interaction with piceatannol (red color arrows indicate hydrogen bond interaction with receptor)

Table 4: Glide scores with predicted interactions of resveratrol and piceatannol with VEGFR1 and VEGFR2 proteins

Ligands	Glide scores	Proteins	Interactions
Resveratrol	−8.747	VEGFR1	Cys912, Asp1040
	−8.226	VEGFR2	Cys919, Lys868
Piceatannol	−10.193	VEGFR1	Cys912, Glu878, Lys 861, Phe1041
	−8.359	VEGFR2	Cys919, Asp1046, Lys868

VEGFR's= Vascular endothelial growth factor receptors

it has shown high docking score when compared with resveratrol. This is because, the hydroxyl group is in good orientation with acidic part of the binding pocket which is absent in resveratrol.

DISCUSSION

Previously, we have reported the anticancer activity of *S. interrupta* root EtOAc extract in breast and prostate cancer cell lines. To identify the compounds with anticancer activity, the root EtOAc extract was subjected to column chromatography based on the polarity of the solvents. A total of 15 fractions were collected and tested for anticancer activity based on an MTT assay. MTT is a well-known assay for measuring the cytotoxicity of phytochemical compounds based on the reduction of MTT by mitochondrial dehydrogenase activity.[29] All the tested fractions reduced the cancer cell growth in a dose-dependent manner, supporting the importance of the *S. interrupta* EtOAc root extract in anticancer

activity. However, the $CHCl_3$: EtOAc (4:6) ratio, fraction showed higher cell death in the MCF-7 cancer cell line compared with the other fractions, with an average IC_{50} value of 150 μg/mL. Indeed, this fraction did not display cytotoxicity in mesenchymal stem cells. HPLC analysis showed that this fraction consists of three major peaks with different RTs and arbitrary units, suggesting the presence of multiple secondary metabolites. To isolate individual compounds, preparative HPLC was performed, and the compound was successfully separated based on gradient elution program. It is interesting to note that the RT and arbitrary units (13.849 min and 1.0) of the purified compound differed from RT and arbitrary units (7.153 min and 0.7) of the crude fraction. This finding is due to the amount of sample loaded into the preparative HPLC column and the flow rate, which is different for normal HPLC as opposed to preparative HPLC.[30] Nevertheless, we could not able to remove some minor impurities. The molecular mass of the purified compound was found to be 244 m/z. FT-IR analysis identified analogs with phenol derivatives reported earlier in the *Sophora* genus.[31] A hydroxylation pattern on the benzene ring at 3722.73 cm^{-1} and an alkene functional group at 1991.39 cm^{-1} and 2156.77 cm^{-1} was observed. We performed NMR spectroscopic analysis to identify and elucidate the exact structure of the compound we performed NMR spectroscopic analysis. Thus, the structure of compound assigned as piceatannol (3, 3', 4, 5'-trans-trihydroxystilbene). To the best of our knowledge, no report was found in the literature showing that piceatannol was a secondary metabolite of *S. interrupta*. Piceatannol is a type of phenolic compound and belongs to the class of stilbenes.[32] Stilbenes (C6–C2–C6) are derived from the common phenylpropene (C6–C3) skeleton building block.[33] Piceatannol has been found in various plants, including grapes, passion fruit, white tea, and Japanese knotweed.[34] Piceatannol is a metabolite of resveratrol,[35] and it has shown to possess the antitumor, antioxidant, and anti-inflammatory activities. The only difference between the resveratrol and piceatannol is the presence of an extra hydroxyl group at the C3 position of the aromatic rings.[36] Identical compound (s) were found in *S. yunnanensis*[37] and led to arrest cell cycle and inhibit angiogenesis by down-regulating the expression of VEGF in ECV304 cell line,[38] It suggests that compounds from these family members possibly inhibit angiogenesis. Angiogenesis is the action of contemporary blood vessels growing from preceding capillaries and postcapillary venules.[39] These steps occur in several physiological and pathological circumstances, such as during embryonic development and wound healing, as well as in patients with chronic inflammatory diseases and various metastatic tumor growths.[40] To validate the antiangiogenic activity of piceatannol, a metabolic product of resveratrol was performed molecular docking analysis. The molecular docking study shows that both resveratrol and piceatannol have shown interactions with Cys912 of VEGFR1 and Cys919, Lys868 with VEGFR2. Piceatannol has showed extra interactions with VEGFR1 (Glu878, Phe1041, and Cys861) when compared with resveratrol (Asp1040) other than common interactions.

In the similar way, piceatannol has showed additional interaction (Asp1046) with VEGFR2 compared to resveratrol. This may be due to the presence of an extra hydroxyl group in piceatannol, it has shown high docking score as compared with resveratrol. This is because, the hydroxyl group is in good orientation with acidic part of the binding pocket which is absent in resveratrol. Overall, it suggests that piceatannol is the antiangiogenic compound present in *S. interrupta* root EtOAc extract, and this can be used as nutritional and pharmacological applications to treat a variety of cancer models. However, further studies are needed to unravel the molecular mechanism of piceatannol.

CONCLUSIONS

Overall, our findings suggest that piceatannol is an antiangiogenic compound that is present in the *S. interrupta* root EtOAc active fraction and that it can be used to treat a variety of cancers. However, further studies are needed to elucidate the molecular mechanism of piceatannol.

Received: 07 Aug 14 **Accepted:** 11 May 15
Published: 12 Oct 15

REFERENCES

1. Dalith MD, Lakshmi SM, Kumar AS, Kumar GA, Basha DJ, Naganjaneyulu R. Antioxidant activity of *Sophora interrupta* bedd. Int J Phytopharmacol 2011;2:43-7.
2. Matkowski A, Jamiolkowska-Kozlowska W, Nawrot I. Chinese medicinal herbs as source of antioxidant compounds – Where tradition meets the future. Curr Med Chem 2013;20:984-1004.
3. Kumar PS, Sandhya S, Rao K, Banji D, Krishna PM, Rajeshwar T. Pharmacognostical studies and preliminary phytochemical investigations on roots of *Sophora interrupta* Bedd., *Fabaceae*. J Phytol 2011;3:42-7.
4. Mathi P, Nikhil K, Ambatipudi N, Roy P, Bokka VR, Botlagunta M. *In-vitro* and *in-silico* characterization of *Sophora interrupta* plant extract as an anticancer activity. Bioinformation 2014;10:144-51.
5. Manjula C, Ammani K. Phytochemical analysis and pharmacological importance of *Sophora interrupta* leaves. Int J Res Pharm Biomed Sci 2012;3:1798-804.
6. Hemamalini K, Bhargav A. Evaluation of Phytochemical and pharmacological activity of methanolic extract of *Sophora interrupta*. Indo Am J Pharm Res 2013;3:6381-90.
7. Vithya T. Anticancer activity of *Gyrocarpus asiaticus* and *Sophora interrupta* on dalton's lymphoma ascites (DLA) induced mice. World J Pharm Pharmacol Sci 2013;2:6613-8.
8. Munikishore R, Rammohan A, Padmaja A, Gunasekar D, Deville A, Bodo B. A new O-prenylated flavonol from the roots of *Sophora interrupta*. Nat Prod Res 2013;27:1823-6.
9. Oyedapo A, Makanju V, Adewunmi C, Iwalewa E, Adenowo T. Antitrichomonal activity of 1, 3-diaryl-2-propen-1-ones on *Trichomonas gallinae*. Afr J Tradit Complement Altern Med 2004;1:55-2.
10. Thors L, Burston JJ, Alter BJ, McKinney MK, Cravatt BF, Ross RA, *et al.*

Biochanin A, a naturally occurring inhibitor of fatty acid amide hydrolase. Br J Pharmacol 2010;160:549-60.
11. Xu X, Gao X, Jin L, Bhadury PS, Yuan K, Hu D, *et al.* Antiproliferation and cell apoptosis inducing bioactivities of constituents from *Dysosma versipellis* in PC3 and Bcap-37 cell lines. Cell Div 2011;6:14.
12. Wang HK, Xia Y, Yang ZY, Natschke SL, Lee KH. Recent advances in the discovery and development of flavonoids and their analogues as antitumor and anti-HIV agents. Flavour Living Syst 1998;439:191-25.
13. Rao YK, Geethangili M, Fang SH, Tzeng YM. Antioxidant and cytotoxic activities of naturally occurring phenolic and related compounds: A comparative study. Food Chem Toxicol 2007;45:1770-6.
14. Ofner J, Krüger HU, Grothe H, Schmitt-Kopplin P, Whitmore K, Zetzsch C. Physico-chemical characterization of SOA derived from catechol and guaiacol – A model substance for the aromatic fraction of atmospheric HULIS. Atmos Chem Phys 2011;11:1-15.
15. Nikhil K, Sharan S, Chakraborty A, Bodipati N, Krishna Peddinti R, Roy P. Role of isothiocyanate conjugate of pterostilbene on the inhibition of MCF-7 cell proliferation and tumor growth in Ehrlich ascitic cell induced tumor bearing mice. Exp Cell Res 2014;320:311-28.
16. Hossain MA, Islam A, Jolly YN, Kabir MJ. A new flavanol glycoside from the seeds of Zea mays. Indian J Chem 2006;45:1319-21.
17. Palaksha MN, Ahmed M, Das S. Antibacterial activity of garlic extract on streptomycin-resistant *Staphylococcus aureus* and *Escherichia coli* solely and in synergism with streptomycin. J Nat Sci Biol Med 2010;1:12-5.
18. Sajjan S, Chetana SH, Paarakh PM, Vedamurthy AB. Antimicrobial activity *Momordica cymbalaria* Fenzl aerial parts extracts. Indian J Nat Prod Res 2010;1:296-300.
19. Ekwenye UN, Elegalam NN. Antibacterial activity of ginger (*Zingiber officinale* Roscoe) and garlic (*Allium sativum* L.) extracts on *Escherichia coli* and *Salmonella typhi*. J Mol Med Adv Sci 2005;1:411-6.
20. Hela W, Brandtner M, Widek R, Schuh R. Determination of sulfonamides in animal tissues using cation exchange reversed phase sorbent for sample cleanup and HPLC – DAD for detection. Food Chem 2003;83:601-8.
21. Aimoto S, Takao T, Shimonishi Y, Hara S, Takeda T, Takeda Y, *et al.* Amino-acid sequence of a heat-stable enterotoxin produced by human enterotoxigenic *Escherichia coli*. Eur J Biochem 1982;129:257-63.
22. Dogan A, Ergen K, Budak F, Severcan F. Evaluation of disseminated candidiasis on an experimental animal model: A Fourier transform infrared study. Appl Spectrosc 2007;61:199-203.
23. Hodson MP, Dear GJ, Roberts AD, Haylock CL, Ball RJ, Plumb RS, *et al.* A gender-specific discriminator in Sprague-Dawley rat urine: The deployment of a metabolic profiling strategy for biomarker discovery and identification. Anal Biochem 2007;362:182-92.
24. A. http://www.rcsb.org/pdb/explore/explore.do?structureId=3U6J [Last accessed on 2014 Jun 17]. B. http://www.rcsb.org/pdb/explore/explore.do?structureId=3HNG. [Last accessed on 2014 Jun 17].
25. Friesner RA, Banks JL, Murphy RB, Halgren TA, Klicic JJ, Mainz DT, *et al.* Glide: A new approach for rapid, accurate docking and scoring 1. Method and assessment of docking accuracy. J Med Chem 2004;47:1739-49.
26. Assudani G, Tyagi MG, Vimalanathan AB. Pathophysiology of angiogenesis and novel angiomodulators from plant sources for therapeutic purpose. J Phytol 2010;2:13-4.
27. Byrne RT, Klingele AJ, Cabot EL, Schackwitz WS, Martin JA, Martin J, *et al.* Evolution of extreme resistance to ionizing radiation via genetic adaptation of DNA repair. Elife 2014;3:e01322.
28. Han SY, Bang HB, Lee HS, Hwang JW, Choi DH, Yang DM, *et al.* A new synthesis of stilbene natural product piceatannol (V). Bull Korean Chem Soc 2008;29:1800-2.
29. Chinthala Y, Chinde S, Kumar AN, Srinivas KV, Kumar JK, Sastry KP, *et al.* Anticancer active homoisoflavone from the underground bulbs of ledebouria hyderabadensis. Pharmacognosy Res 2014;6:303-5.
30. Garg S, Talwar GP, Upadhyay SN. Immunocontraceptive activity guided fractionation and characterization of active constituents of neem (*Azadirachta indica*) seed extracts. J Ethnopharmacol 1998;60:235-46.
31. Magalhães AF, Tozzi AM, Magalhães EG, Sannomiya M, Soriano MD, Perez MA. Flavonoids of *Lonchocarpus montanus* AMG Azevedo and biological activity. An Acad Bras Ciênc 2007;79:351-67.
32. Cai YZ, Mei Sun, Jie Xing, Luo Q, Corke H. Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. Life Sci 2006;78:2872-88.
33. Jain P, Jain S, Pareek A, Sharma S. A comprehensive study on the natural plant phenols: Perception to current scenario. Bull Pharm Res 2013;3:90-06.
34. Pageni P. Synthesis of Ester Derivatives of Resveratrol as Potential Anti-cancer Drugs. Electronic Theses and Dissertations; 2013. p. 1181.
35. Piotrowska H, Kucinska M, Murias M. Biological activity of piceatannol: Leaving the shadow of resveratrol. Mutat Res 2012;750:60-82.
36. Wesolowska O. Interaction of phenothiazines, stilbenes and flavonoids with multidrug resistance-associated transporters, P-glycoprotein and MRP1. Acta Biochim Pol 2011;58:433-48.
37. Daikonya A, Kitanaka S. Polyphenols from *Sophora yunnanensis*, and their inhibitory effects on nitric oxide production. Chem Pharm Bull (Tokyo) 2011;59:1567-9.
38. Zhang XL, Cao MA, Pu LP, Huang SS, Gao QX, Yuan CS, *et al.* A novel flavonoid isolated from *Sophora flavescens* exhibited anti-angiogenesis activity, decreased VEGF expression and caused G0/G1 cell cycle arrest *in vitro*. Pharmazie 2013;68:369-75.
39. Ribatti D, Crivellato E. "Sprouting angiogenesis", A reappraisal. Dev Biol 2012;372:157-65.
40. Ono M. Molecular links between tumor angiogenesis and inflammation: Inflammatory stimuli of macrophages and cancer cells as targets for therapeutic strategy. Cancer Sci 2008;99:1501-6.

Source of Support: Our studies have been supported by funds from the K L E F University management and also University Grants Commission (UGC) to Mahendran Botlagunta.
Conflict of Interest: None declared.