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Research Article

Anticancer efficacy of ethyl acetate extract of *Senna uniflora* and its potential selective cytotoxicity on triple-negative breast cancer cells (MDA-MB-231)

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Abstract

Cell-based cytotoxicity assays have been utilized as the most common screening method for discovering cytotoxic anticancer drugs from natural products. The current research work aimed to investigate the anticancer efficacy of ethyl acetate extract of *Senna uniflora* (SUEA) against lung, liver and breast cancer cell lines, A549, HepG2, MDAMB-231 and MCF-7, with one non-tumorigenic cell line, MCF-10A. Cytotoxicity was assessed using the MTT assay on these cell lines at concentrations ranging from 12.5-200 µg/mL. SUEA exhibited dose-dependent minimal toxicity toward MCF-10A cells (>80% viability) and HepG2 cells (>87% viability), moderate toxicity in A549 cells (~70% viability) and MCF-7 cells (~68% viability) at 200 µg/mL, and potent selective cytotoxicity in the triple-negative breast cancer (TNBC) cell line model, MDA-MB-231 (~24% viability at 200 µg/mL), with an IC_{50} value of 56.63 µg/mL. Phytochemical profiling using GC–MS revealed six major natural constituents, particularly griseoxanthone C and a polyoxygenated xanthone compound which are widely recognized for inducing ROS-mediated mitochondrial dysfunction and apoptosis in TNBC models. The selective cytotoxicity of SUEA, supported by both biological assays and phytochemical characterization, suggests that *S. uniflora* is a promising source of anticancer molecules. These findings suggest further studies involving compound isolation, molecular characterization and mechanistic pathway analysis to enable the development of targeted anti-TNBC compounds.

Keywords: *Senna uniflora*, Ethyl acetate extract, Cell lines, MTT assay, Cytotoxicity, GC-MS analysis, Xanthones

Introduction

Cancer is considered as the most prevalent and leading factors of death in the world (Shokrzadeh *et al.,* 2010; Tagne *et al.,* 2014). It is a heterogeneous disease marked by abnormal cell proliferation, invasion and metastasis which often leads to significant tissue dysfunction (Martin *et al.,* 2013). Regardless of advancements in screening, molecular characterization and treatment, it is still a serious public health concern, especially in economically developing regions where healthcare access is limited (Bray *et al.,* 2024). In cancer research, *in vitro* cell line models are fundamental for studying tumour biology, discovering new therapeutic agents, and evaluating their anticancer activity. A standard, complete assessment of anticancer activity often requires a panel of cell lines representing different cancer types and a normal counterpart to determine safety and selectivity (Desai *et al.,* 2011). In the current investigation, the efficacy of potential chemotherapeutic drugs was assessed using five cell lines: MCF-10A, HepG2, A549, MCF-7, and MDA-MB-231.

Naturally derived substances continue to be an essential source of bioactive compounds with potential anticancer properties (Cragg & Newman, 2013). Several phytochemicals derived from medicinal plants have demonstrated cytotoxicity on diverse cancer cell lines, paving way for drug development. The species of the *Senna* genus belong to the family Fabaceae and are known to contain many therapeutically active secondary metabolites such as tannins, flavonoids, saponins and anthraquinones (Oladeji *et al.,* 2021). *Senna uniflora* (syn. *Cassia uniflora*) is an herbaceous plant and is traditionally employed in the management of ailments like febrile, hepatic disorders and inflammatory conditions. It has been shown to exhibit antioxidant and antimicrobial efficacies (Kirtikar & Basu, 2001; Khare, 2007). However, the cytotoxic and antiproliferative potentials of this herb has not been comprehensively studied.

In the current study, the cytotoxic efficacy of the ethyl acetate extract of *S. uniflora* was assessed on the above mentioned cell lines. The findings are intended to contribute to the identification of plant based compounds with specific anticancer activity, supporting further pharmacological development.

Materials and methods

Collection and authentication of plant material

The whole plant specimens of *Senna uniflora* were collected during August, 2024 from Thadangam Village (Lat: 12.107188, Long: 78.128471), Dharmapuri District, Tamil Nadu, India. The collected plant material was taxonomically validated by the Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu, India. A voucher specimen (GACDPISU1) was retained in the laboratory for future reference.

Collected plants were manually inspected to remove any contaminants or foreign plant material. To eliminate

the surface impurities, the samples were pre-cleaned with tap water and then washed with distilled water. The cleaned material was naturally dried under shade at ambient conditions and was coarsely powdered using an electric grinder and preserved in sealed containers at room temperature until further use (WHO, 2018).

Extraction of plant material

The obtained plant powder was subjected to Soxhlet extraction using ethyl acetate as the solvent, in a 1:6 (w/v) ratio. The extraction was carried out until the solvent in the siphon tube appeared colourless. The resulting extract was rotovapped to obtain a semisolid mass. The crude extract was dried completely and maintained in a vacuum desiccator at ambient temperature for further analysis.

Maintenance of cell lines

The cancer cell lines HepG2, A549, MCF-7, MDA-MB-231 and the non-tumorigenic human mammary epithelial cell line MCF-10A, were procured from NCCS, Pune, India. The cell lines HepG2 and A549 were cultured in McCoy's 5A Media (#AL057A, Himedia) and DMEM/F12 Media (#AL139A, Himedia), respectively. Dulbecco's Modified Eagle Medium (DMEM) High Glucose (#AL066A, HiMedia) was used to culture MDA-MB-231 and MCF-7 cells, while MCF-10A cells were maintained in DMEM High Glucose (#AL007A, HiMedia) media. All media were supplemented with 10% foetal bovine serum (FBS, #RM10432, HiMedia) and 1% antibiotic-antimycotic solution containing penicillin and streptomycin (#A001A, HiMedia). Cells were incubated at 37 °C in a humidified atmosphere with 5% CO_2. Sub-culturing was performed routinely to maintain cells in their logarithmic phase of growth, and experiments were conducted when cultures reached approximately 70% confluency.

Cytotoxicity assay

The cytotoxic activity of the *S. uniflora* ethyl acetate extract was assessed using the MTT assay, a colorimetric method based on the reduction of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] to insoluble formazan crystals by mitochondrial enzymes in metabolically active cells (Mosmann, 1983; Alley, 1988).

Seeding of the cells and treatment

The 96-well culture plate was seeded with 200 µL of cell suspension (HepG2, A549, MCF-7, MDA-MB-231 and MCF-10A cells) and allowed to adhere to the substrate overnight under standard culture conditions (37 °C, 5% CO_2, humidified).

After the preliminary 24-hour incubation, the experimental setup was established as follows: negative control wells were replenished with fresh complete medium without any treatment (untreated cells); test wells were exposed to different concentrations of the *S. uniflora* ethyl acetate extract (12.5, 25, 50, 100, and 200 µg/mL); positive control (standard control) wells received known cytotoxic agents, doxorubicin (#D1515, Sigma) at varying concentrations for HepG2, A549, MCF-7 and MDA-MB-231 cells, and cisplatin (12 µM, #PHR1624, Sigma) for MCF-10A cells. To account for background absorbance, blank control wells solely contained medium devoid of cells. After that, each plate was incubated for a further 24 hours at the same temperature (37 °C, 5% CO_2).

Following treatment and incubation, the existing culture medium was gently aspirated from each well and substituted with 200 µL of fresh medium containing MTT reagent (#4060, HiMedia) at a final concentration of 0.5 mg/mL. To enable formazan crystals to form in viable cells, the plates were subsequently incubated for 3 hours.

Upon completion of the incubation period, the MTT containing media was replenished with 100 µL of dimethyl sulfoxide (DMSO, #PHR1309, Sigma-Aldrich) to solubilize the formed formazan crystals. The optical density was then recorded at 570 nm using a microplate reader (ELX-800, BioTek, USA), with a reference wave length of 630 nm. The following formula was used to determine cell viability:

$$\% \text{ Viability} = \frac{OD_{\text{Treated}} - OD_{\text{Blank}}}{OD_{\text{Control}} - OD_{\text{Blank}}} \times 100$$

where
$OD_{Treated}$ = absorbance of extract or drug treated cells
$OD_{Control}$ = absorbance of untreated viable cells
OD_{Blank} = absorbance of wells containing only medium (no cells)

The half-maximal inhibitory concentration (IC_{50}) was calculated by plotting cell viability (%) against the logarithm of extract concentration. The data were then fitted to a logarithmic regression equation:

$$Y = M \ln(x) + C$$

where
Y = 50 (representing 50% viability)
M and C are constants derived from the regression analysis

Each experiment was conducted in triplicate, and the mean±standard deviation (SD) was used to express the results.

GC-MS analysis

The ethyl acetate extract of *S. uniflora* (SUEA) was examined by Gas Chromatography-Mass Spectrometry (GC-MS) using a PerkinElmer Clarus SQ 2400 system fitted with an Elite-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). A steady flow rate of 1.0 mL/min, helium (99.999% purity) served as a carrier gas. One microlitre (1 µL) of the derivatised sample (10 µL of extract dissolved in 1 mL ethyl acetate and silylated with BSTFA-TMCS) was injected in split mode (split ratio 20:1) with the

injector kept at 250 °C. The GC oven temperature program was initially sent at 50 °C held for 2 min, increased to 250 °C at 10 °C/min and finally held for 5 min at 250 °C.

The Mass Spectrometer was run in Electron Ionization (EI) mode at 70 eV. The ion source temperature was set at 220 °C, and the transfer line temperature was kept at 260 °C. No solvent delay was applied. The range m/z 40-600 was used to get full-scan mass spectra. Data acquisition and processing were performed using PerkinElmer Simplicity Chrome software, and compounds were determined by correlating their mass spectra with the NIST MS Search v3.0 library (2023 Edition) and retention indices relative to n-alkane standards (C_8-C_{28}). Only compounds with spectral similarity ≥85% and retention index deviation ≤ ±15 units were reported.

Statistical analysis

To ensure data dependability, all tests were conducted in triplicates, and the results were presented as mean±standard deviation (SD). One-way ANOVA was used to compare treatment groups statistically. Statistical significance was defined as a p-value of less than 0.05. Furthermore, the IC_{50} values, which indicate the extract concentration necessary to inhibit 50% of the cell viability, were computed using logarithmic regression analysis of the dose-response curve.

Results and discussion

Extraction is a decisive step in the isolation of bioactive compounds from the plant materials, as the use of appropriate solvents enables the selective separation of desired constituents (Dhanani *et al.,* 2017). In the present study, *S. uniflora* upon extraction with ethyl acetate resulted in a yield of 4.07 g (percentage extract yield: 8.14% of dry weight) of crude extract. The ethyl acetate extract of *S. uniflora* exhibited prominent and dose-dependent cytotoxicity against the treated cell lines. Treatment of MCF-10A, HepG2, A549, MCF-7 and MDA-MB-231 cells with SUEA (12.5-200 µg/mL) for 24 h revealed marked differences in sensitivity (Table 1). The non-tumorigenic MCF-10A cells and HepG2, the human hepatoblastoma cells showed >80% and >87% viability respectively even at the highest concentration, indicating negligible toxicity towards normal mammary epithelium and liver cancer cells. In contrast, ER-positive breast cancer cells, MCF-7 and lung adenocarcinoma cells, A549 showed moderate susceptibility, with viability reduced to approximately ~68% and ~70% respectively at 200 µg/mL. The most significant impact was observed in the triple-negative breast cancer (TNBC) cells, MDA-MB-231 where viability declined steeply from ~87% to ~24% across the tested range. Logarithmic regression of the dose-response curve (Figure 1) of this cell line yielded an IC_{50} value of 56.63±2.1 µg/mL (R^2=0.987).

The cytotoxicity standards for crude extracts and pure compounds have been established by the National Cancer Institute (NCI), USA as an IC_{50}<20 µg/mL or 10 µM respectively following 48 or 72 hours of incubation. Additionally, a crude extract with an IC_{50} of 30 µg/mL is regarded by the NCI as a promising anticancer agent (Belete *et al.,* 2025). However, certain researchers (Cos *et al.,* 2006) have defined the threshold for acceptable bioactivity as an IC_{50}<100 µg/mL for crude extracts and < 25 µM for pure compounds, acknowledging that crude extracts typically exhibit lower potency due to the presence of inactive constituents (Monagas *et al.,* 2006).

Although the IC_{50} value of the crude SUEA extract does not fall within the highly potent range, the extract exhibits clear, dose-dependent cytotoxicity (R^2=0.987) against the aggressive MDA-MB-231 triple-negative breast cancer cell line. The calculated selectivity index (>3.5 relative to MCF-10A) further emphasizes SUEA as a candidate with favourable therapeutic potential (Welsh, 2013).

Late-stage breast cancer is frequently modelled using the MDA-MB-231 cell line. This cell line is considered as a suitable model of triple-negative breast cancer since they lack the ER (oestrogen receptor), PR (progesterone receptor), and HER2 (human epidermal growth factor receptor 2). Additionally, the cells express mutant p53 and lack E-cadherin. Hence, the preferential cytotoxicity toward MDA-MB-231 cells imitates with the aggressive molecular phenotype of TNBC, which frequently exhibits heightened oxidative stress tolerance, hyperactivated PI3K/Akt/mTOR signalling, and defective apoptosis regulation (Lehmann *et al.,* 2011; Welsh, 2013; Waks & Winer, 2019).

The extracts of various *Senna* species are recognized to be rich in bioactive xanthone and anthraquinone compounds which are widely associated with anticancer potential (Oladeji *et al.,* 2021). In this perspective, the ethanolic leaf extract of *Senna rugosa* exhibits measurable cytotoxicity against MDA-MB-231 TNBC cells, confirming the relevance of *Senna* metabolites in breast cancer models (Cunha *et al.,* 2020). Additionally, several studies have reported that anthraquinone and xanthone based phytoconstituents exert their anticancer effects through mitochondrial membrane depolarization, ROS-induced cell-

Table 1: Cytotoxic effects of SUEA extract on cancer and normal epithelial cell lines after 24 hours of treatment

Concentration (µg/mL)	MCF-10A*	HepG2*	A549*	MCF-7*	MDA-MB-231*
12.5	100.0±0.006	97.44±0.002	92.26±0.003	89.01±0.009	79.21±0.002
25	99.9±0.004	95.05±0.006	87.18±0.006	84.24±0.001	67.29±0.025
50	96.97±0.025	90.96±0.002	80.63±0.002	79.6±0.001	54.21±0.012
100	92.58±0.012	87.13±0.009	74.56±0.003	74.58±0.001	38.34±0.001
200	80.04±0.002	82.62±0.024	70.40±0.003	67.61±0.009	23.58±0.003
IC_{50} (approx) *	>200	>200	>200	>200	56.63

* Values are expressed as mean±standard deviation (n=3). IC_{50} determined by logarithmic regression analysis

Table 2: Major compounds detected by GC-MS in the ethyl acetate extract of *S. uniflora* (SUEA)

Peak	RT (min)	Compound Name	Key m/z	Area
1	13.41	1-(Trimethylsilyloxy) heptadecane	207	75,803.1
2	14.86	9-Ethyl-9-heptyl- octadecane	57	43,027.4
3	20.55	Griseoxanthone C	401	45,502.9
4	21.62	Polyoxygenated xanthone (unidentified isomer)	73	35,571.0
5	23.09	Unidentified di-TMS derivative	73	98,719.0
6	23.85	Phenyl-pentamethyl-disiloxane (artifact)	193	201,830.9

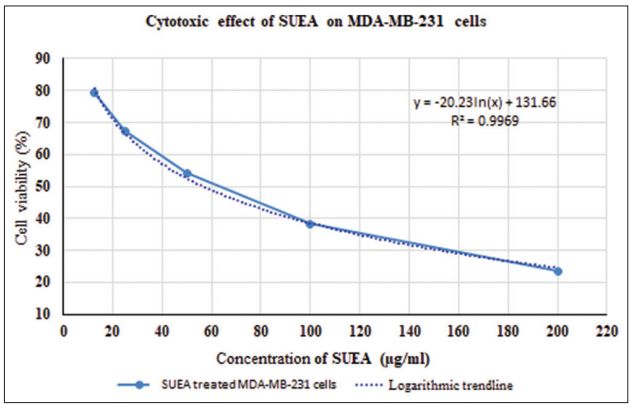

Figure 1: Logarithmic dose response curve of SUEA on MDA-MB-231 cells showing a concentration-dependent decrease in cell viability. The IC_{50} value was determined to be approximately 56.63 µg/mL

cycle arrest and apoptosis particularly in TNBC cell lines (Tu *et al.,* 2011). Together, these results offer a mechanistic basis for the selective cytotoxicity observed in the present study, especially toward MDA-MB-231 cells.

To establish the phytochemical basis of the observed activity, the extract was subjected to GC-MS analysis (Table 2). The total ion chromatogram displayed five distinct, well-resolved peaks corresponding to natural constituents, and a broad, tailing signal after 25 min (phenyl-pentamethyl-disiloxane, 40.33% of total area) which was identified as a common silylation artefact and excluded from biological interpretation.

The five major natural compounds were: 1) 1-(trimethylsilyloxy)heptadecane (RT 13.41 min, 15.15%), a long-chain fatty alcohol derivative, 2) 9-ethyl-9-heptyl-octadecane (RT 14.86 min, 8.60%), a branched hydrocarbon typical of plant waxes, 3) griseoxanthone C TMS derivative (RT 20.55 min, 9.09%), 4) a polyoxygenated xanthone (montixanthone or isomer) tri-TMS derivative (RT 21.62 min, 7.11%) and 5) an unidentified polar di-TMS compound ($C_{15}H_{28}O_2Si_2$, RT 23.09 min, 19.73%).

The identification of griseoxanthone C and a structurally related polyhydroxy xanthone (peaks 3 and 4) is of particular significance. Prenylated and polyoxygenated xanthones are well known for their potent and selective anticancer properties, especially against TNBC cell lines. According to reports, xanthone derivatives exhibit anticancer action by binding to various protein receptors, including cyclooxygenase, protein kinase, and topoisomerase (Shagufta & Ahmad, 2016). Several studies have shown that this class of compounds triggers reactive oxygen species (ROS) accumulation, disrupts mitochondrial membrane potential, inhibits Akt/mTOR signalling, and activates intrinsic apoptotic pathways involving caspase-9 and caspase-3/7 (Nguyen *et al.,* 2022; El Gaafary *et al.,* 2024). Thus the biological activities observed with SUEA strongly implicate the xanthone rich fraction as the primary contributor to the selective cytotoxicity against MDA-MB-231 cells, while leaving non-tumorigenic MCF-10A cells largely unaffected. The similarity of observed cytotoxicity in HepG2 cells to that seen in MCF-10A may be attributed to the retention of several key characteristics of hepatocyte in HepG2 cells and their partial resemblance to primary hepatocytes (Arzumanian *et al.,* 2021).

The long-chain alkanes (peak 1) and fatty alcohol derivatives (peak 2) are ubiquitous plant wax constituents and do not contribute significantly to the anticancer effect. The unidentified di-TMS compound (peak 5) may denote a degraded flavonoid or hydroxylated fatty acid fragment and suggests further investigation.

In conclusion, SUEA's high concentration of bioactive xanthones, especially griseoxanthone C and its polyoxygenated counterpart, seems to be convincingly linked to its strong and specific anti-TNBC activity. These results robustly support the separation and characterisation of bioactive components from SUEA with distinct anti-TNBC capabilities and offer a solid phytochemical background for the reported biological benefits. *S. uniflora* may therefore be a promising source for the development of anticancer drugs based on the demonstrated activity.

Author contributions

All the authors contributed equally to this work. All authors have read and approved the final manuscript.

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