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Research Article

CYTOTOXICITY OF STEM EXTRACTS OF SELECTED *CASSIA* SPECIES AGAINST HELA AND BREAST CANCER CELL LINES *IN VITRO*

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ABSTRACT

Objective: The use of plants for medicinal remedies is an integral part of the Indian cultural life, and the traditional background of Indian medicine shows widespread use of plant products in cancer treatment. In this study, stem extracts of some selected *Cassia* species have been evaluated for their cytotoxic activities under *in vitro* conditions.

Methods: The stems were shade dried at room temperature. The dried and coarsely powdered plant material were extracted with petroleum ether (60-80°C), chloroform, and ethanol using soxhlet apparatus. The cytotoxicity was evaluated by [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.

Results: Stem extracts of three *Cassia* species, *viz*., *Cassia glauca, Cassia Obtusifolia,* and *Cassia sophera* have been evaluated for their cytotoxic activities with chloroform, ethanol and pet ether against HeLa and breast cancer cell lines. Among the three different solvents used at different concentrations, the chloroform extracts of all *Cassia* species exhibited maximum cytotoxicity (%) against both cell lines. The CTC_{50} values are revealed the cytotoxic potential of *C. glauca* chloroform extracts against HeLa cell line and breast cancer cell lines with CTC_{50} values 180.00±3.0 and 146.67±0.5, respectively. Chloroform extracts of *C. Obtusifolia* and *C. sophera* showed maximum activity against HeLa (380.00±1.1 and 800.00±1.7, respectively) and breast cancer cell lines (310.00±1.1 and 633.33±0.6).

Conclusion: The results of this study demonstrate the potent cytotoxic activity of chloroform extracts of stems of *Cassia* species against HeLa and Breast cancer cell lines.

Keywords: *Cassia,* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Cytotoxicity, HeLa, Breast cancer.

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INTRODUCTION

Plant and plant products both as extracts and derived compounds are known to be effective and versatile chemopreventive agents against a variety of types of cancers [1]. About 60% of currently used anticancer agents are derived from natural sources directly or indirectly [2]. Development of several promising new agents in anticancer drug therapy such as vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, etoposide along with flavopiridol and combretastain A4 phosphate from natural sources stimulated renewed interest among the biologists in screening the medicinal plants in anticancer drug therapy [3,4]. The use of plants for medicinal remedies is an integral part of the Indian cultural life, and the traditional background of Indian medicine shows widespread use of plant products in cancer [5,6]. The members of *Cassia* species are rich sources of polyphenols, anthraquinone derivatives, flavonoids, and polysaccharides [7,8], and they have been found to exhibit anti-inflammatory, antioxidant, hypoglycemic, antiplasmodial, larvicidal, antimutagenic, and anticancer activities [9]. In this study, stem extracts of some selected *Cassia* species have been evaluated for their cytotoxic activities under *in vitro* conditions.

METHODS

Plant material and stem extraction

The stems of *Cassia glauca, Cassia obtusifolia,* and *Cassia sophera* were collected from in and around Bagalkot District of North Karnataka region of Southern India. The stems were shade dried at room temperature. The dried and coarsely powdered plant material was extracted with petroleum ether (60-80°C), chloroform and ethanol using soxhlet apparatus. The extracts wee dried under reduced pressure at temperature at 30°C to dryness to yield dried extract residue.

Cell lines and culture medium

HeLa and MDA MB 231 (breast cancer) cell lines were procured from National Centre for Cell Sciences, Pune, India. Stock cells were cultured in DMEM supplemented with 10% inactivated fetal bovine serum (FBS), penicillin (100 IU/ml), streptomycin (100 mg/ml), and amphotericin B (5 mg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with trypsin phosphate versene glucose solution (0.2% trypsin, 0.02% ethylenediaminetetraacetic acid, 0.05% glucose in phosphate-buffered saline [PBS]). The stock cultures were grown in 25 cm^2 culture flasks, and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

Preparation of test solutions

For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled dimethyl sulfoxide and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two-fold dilutions were prepared from this for carrying out cytotoxic studies.

Determination of cell viability by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The monolayer cell culture was trypsinized, and the cell count was adjusted to 1.0×10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell

suspension (approximately 10,000 cells) was added. After 24 hrs, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 ml of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 hrs interval. After 72 hrs, the drug solutions in the wells were discarded and 50 ml of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 hrs at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100 ml of propanol was added, and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

$$\% \text{ Growth Inhibition} = 100 - \left(\frac{\text{Mean OD of individual test group} - \text{Mean OD of individual group}}{\text{Mean OD of control group}} \times 100 \right)$$

RESULTS

MTT assay revealed the cytotoxic activities of stem extracts in different solvents against HeLa and MDA MB 231 cell lines. Among the three different solvents used at different concentrations, the chloroform extracts of all three *Cassia* species exhibited maximum cytotoxicity (%) against both HeLa and MDA MB 231 cell lines. *C. glauca* chloroform extracts showed maximum inhibition against HeLa (85.17%) and MDA MB cell lines (78.03%) followed by pet ether extracts (66.15% and 60.64%) at 1000 mcg/ml (Fig. 1).

C. obtusifolia chloroform extracts showed the highest cytotoxicity against HeLa (59.98% and 51.46% at 1000 and 500 mcg/ml, respectively) and breast cancer cell lines (66.00% and 55.17% at 1000 and 500 mcg/ml, respectively). The pet ether extracts *C. Obtusifolia* did not show significant cytotoxicity against both HeLa and breast cancer cell lines at 1000 mcg/ml.

C. sophera chloroform extracts exhibited high cytotoxicity against HeLa (70.78%) and breast cancer cell lines (69.27%) at 1000 mcg/ml concentration. The pet ether extracts of *C. sophera* showed moderate cytotoxicity against HeLa (39.65%) and breast cancer cell lines (44.08%) (Fig. 1).

The CTC_{50} values revealed the cytotoxic potential of *C. glauca* chloroform extracts against HeLa cell line and breast cancer cell lines with CTC_{50} values 180.00±3.0 and 146.67±0.5, respectively. The pet ether extracts of *C. glauca* also exhibited the cytotoxicity against HeLa breast cancer cell lines with values 750.00±1.2 and 646.67±0.5, respectively. Only chloroform extracts of *C. Obtusifolia* and *C. sophera* showed maximum activity against HeLa (380.00±1.1 and 800.00±1.7, respectively) and breast cancer cell lines (310.00±1.1 and 633.33±0.6, respectively (Table 1 and Fig. 2).

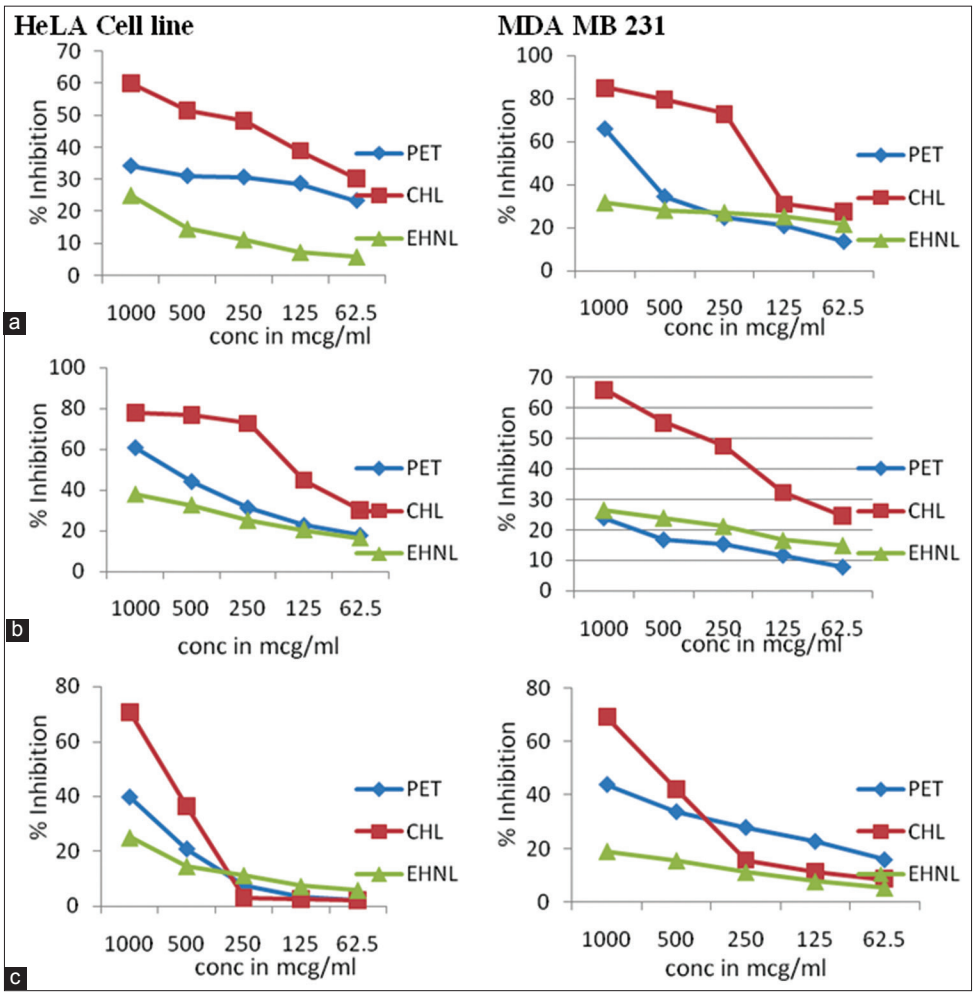

Fig. 1: Cytotoxicity (%) of plant extracts against HeLa and MDA MB 231 cell lines at different concentrations. (a) *Cassia glauca, (b) Cassia obtusifolia, (c) Cassia sophera.* *P < 0.05 The results represents the mean±SEM (n=3)

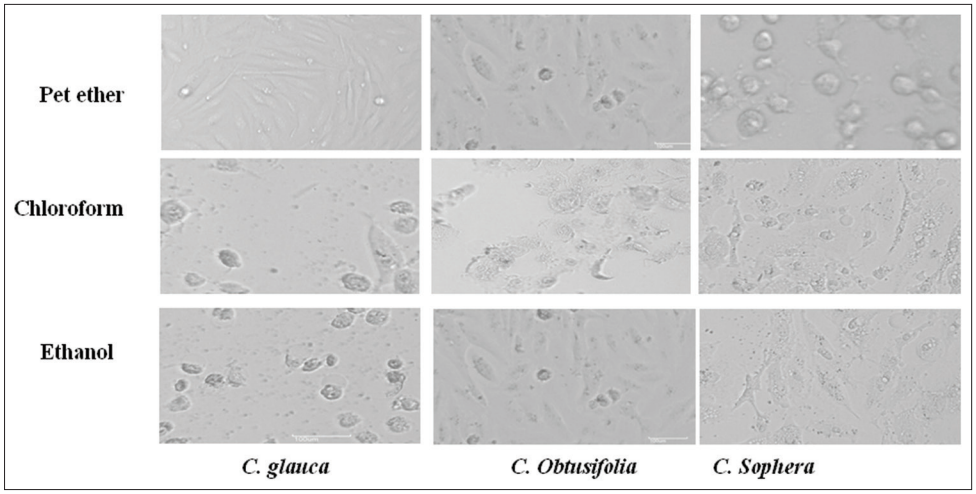

Fig. 2: Photographs showing activity against MDA MB 231 cell lines at 1000 concentration in mcg/ml

Table 1: The CTC_{50} (µg/ml) values of Cassia species against HeLa and MDA MB 123 cell lines

Plant	Extract	CTC_{50} (µg/ml)	
		HeLa	MDA
Cassia glauca	Pet ether	=750.00 ± 1.2	646.67 ± 0.5
	Chloroform	=180.00 ± 3.0	146.67 ± 0.5
	Ethanol	>1000 ± 0.00	>1000
Cassia obtusifolia	Pet ether	>1000 ± 0.00	>1000
	Chloroform	=380.00 ± 1.1	310.00 ± 1.1
	Ethanol	>1000 ± 0.00	>1000
Cassia sophera	Pet ether	>1000 ± 0.00	>1000
	Chloroform	=800.00 ± 1.7	633.33 ± 0.6
	Ethanol	>1000 ± 0.00	>1000 ± 0.00

*$P < 0.05$ Results are means of ± SD of three independent experiments.
SD: Standard deviation

DISCUSSION

The MTT assay revealed the cytotoxic potential of extracts against HeLa and breast cancer cell lines. A number of studies on *Cassia* species revealed the presence of phenols, flavonoids in the plant material [10]. The phytochemical studies of *Cassia* species have provided their ethnopharmtment of various diseases due to their contents of hydroxyl anthraquinone [11]. Pharmacognostical study on *C. obtusifolia* revealed the presence of tannins, flavonoids, steroids, and phylobatannins [12]. Similarly, ethanolic extracts of *C. sophera* proved to be hepatoprotective against carbon tetrachloride induced hepatic damage in rats [13]. In a recent study, the hexane extracts of the *Cassia alata* showed remarkable cytotoxicity against breast and lung carcinoma cells and the elucidation of hexane extracts revealed the presence of polyunsaturated fatty acids esters [14]. In our study, the chloroform extracts exhibited maximum cytotoxicity against the cell lines in all the plant studied. Different extracts of the plant exhibit different activity on different cell lines. This selectivity could be due to the sensitivity of the cell lines to the active compounds in the extract or to tissue-specific response [15]. However, further work is needed in the form of phytochemical screening and pharmacological activity of some more extracts about the therapeutic potential of these extracts.

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