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In-vitro Cytotoxicity of Root of Gendarussa Vulgaris on MCF 7 and Vero Cell

Jay Dasadia^a, Vivek Vyas^a, Sonal Patel^a, Maitreyi Zaveri^a

a. Department of Pharmacognosy, K.B. Institute of Pharmaceutical Education and Research, Sector-23, GH-6, Gandhinagar-382023, Gujarat, India.

ABSTRACT:

Breast cancer remains the second most common cause of cancer death in women. Eighty per cent of women diagnosed are alive at five years. It is the leading cause of death in women aged 40-55 years. Potential herb *Gendarussa vulgaris* is a bioactive plant that has been used as an ethanomedicine to treat various diseases. This experiment is aimed to evaluate the cytotoxic effect of the root of *Gendarussa vulgaris* on MCF7 cell line and vero cell line. The effect of defatted methanolic extract of root of *Gendarussa vulgaris* at 100, 500, 1000 µg/ml concentration and compared with standard Vinblastine on MCF7 cell line using MTT cell viability assay and Trypan blue assay was evaluated. MTT cell viability assay of methanolic extract of root shows 7.3% cytotoxicity at 1000µg/ml concentration in MCF 7 cell line. MTT cell viability assay of methanolic extract of root shows 24.9 % cytotoxicity at 1000µg/ml concentration in vero cell line. The methanolic root extract of *Gendarussa vulgaris* does not shows cytotoxic activity in VERO &MCF 7 cell line

KEY WORDS: Gendarussa vulgaris, VERO & MCF 7 cell line, cytotoxicity, MTT cell viability assay, Trypan blue assay

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*For Correspondence:

Maitreyi Zaveri

Head & Assistant Professor,
Department of Pharmacognosy &
Phytochemistry,
K.B. Institute of Pharmaceutical
Education and Research,
Sector-23, GH-6, Gandhinagar-382023,
Gujarat, India.

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INTRODUCTION:

Breast cancer remains the second most common cause of cancer death in women. In 2013, breast cancer continued to be the most common female cancer and incidence is still rising. However, mortality from breast cancer is falling. Eighty per cent of women diagnosed are alive at five years. This may be a result of earlier diagnosis through screening, improved surgical and radiotherapy techniques, and more advanced adjuvant medical therapies. It is the leading cause of death in women aged 40-55 years. Breast cancer has a complicated etiology that includes genetic, biological, behavioral, environmental and social factors. Factors known to increase a woman's chance of developing it include age (risk increases with age), family history (inherited genetic predisposition), previous radiotherapy to the chest wall, certain breast conditions (such as lobular neoplasia and multiple papillomatosis), early menarche, late menopause, later age at first full-term pregnancy and nulliparity (all related to exposure of higher levels of hormones for longer).

Risk factors include use of alcohol and tobacco, prolonged use of the oral contraceptive pill, HRT6 and obesity in postmenopausal women.1 MCF-7 is a breast cancer cell line isolated in 1970 from a 69-year-old Caucasian woman. MCF-7 is the acronym of Michigan Cancer Foundation-7, referring to the institute in Detroit where the cell line was established in 1973 by Herbert

Soule and co-workers². The Michigan Cancer Foundation is now known as the Barbara Ann Karmanos Cancer Institute. Prior to MCF-7, it was not possible for cancer researchers to obtain a mammary cell line that was capable of living longer than a few months³. MCF-7 and two other breast cancer cell lines, named T-47D and MDA-MB-231, account for more than two-thirds of all abstracts reporting studies on mentioned breast cancer cell lines, as concluded from a Medline-based survey. HTB-22 is other name of MCF 7 cell line given by ATCC⁴.



One such plant *Gendarussa vulgaris*, is widely used in Indian and Chinese traditional medicines but that has been not studied in a systematic way

till date⁵. *Gendarussa vulgaris* belonging to the family Acanthaceae, is a shade-loving, quick-growing, evergreen plant mostly found in moist areas. It is believed to be native to China and is distributed widely across India, Sri Lanka, and Malaysia. In India at seashore area of Gujarat like Valsad, Surat and Hills like Pavagarh⁶. The leaf of *Gendarussa vulgaris* are simple, opposite, lanceolate or linear-lanceolate, acute at base, tapering into rounded apex and glabrous and shining leaves (8.0-12.5 cm long, 1.2-2.0 cm broad) with prominent purple veins beneath⁷. The stem is quadrangular, thickened at and above the nodes and internodes measure 2-7 cm long⁸. The flowers are in terminal or axillary spikes and are irregular, bisexual, sessile, and white with pink or purple spots inside and red in the throat and lip⁹

Root of *Gendarussa vulgaris* contains alkaloids, flavonoids, saturated steroidal saponins or triterpenoidal saponins, amino acids, aromatic amines, and also rich in potassium salts. Root of *J. gendarussa* contains 2-amino benzyl alcohol, 2(2'-amino benzyl amino) benzyl alcohol¹⁰, and their respective O-methyl ethers, friedelin, lupeol, and β -sitosterol^{11,12}.

Gendarussa vulgaris plant exhibits antioxidant, hepatoprotective, anthelmintic, anti-inflammatory,

antiarthritic, antiangiogenic, antimicrobial, analgesic, anti anxiety and anticancer activities. The goal of screening medicinal plant is to search for excellent anticancer agent avertable to human malignancies. Based on this, our main aim is to evaluate the cytotoxic effect of the root of *Gendarussa vulgaris* on MCF7 cell line and Vero cell line.

MATERIALS AND METHODS

Collection of plant material: The plant *Gendarussa vulgaris* was collected from Anand nursery farm near, Raisan petrol pump, Gandhingar, Gujarat. The root was separated with care from the other parts of plant, dried it and then crushed into fine powder.

Preparation of defatted methanolic extract of *Gendarussa vulgaris* root:

100gm crushed powder of *Gendarussa vulgaris* root was weighed and 500ml of petroleum ether was added. Reflux it for 1hr and then macerate for 24 hrs. This was continued until get exhausted defatted marc. Obtained defatted marc again weighed and 500ml of methanol was added. Reflux the mark for 1hr and macerate it for 24 hrs. The exhaustive extraction was done for 3 successive days. At the end of three days all the filtrate were combined and concentrate it. Weight the yield of methanolic extract of *Gendarussa vulgaris* root.

Cytotoxicity study using Vero and MCF 7 cell line:

African green monkey kidney Normal cell line (Vero) and Human breast cancer cells MCF 7 cell line were purchased from NCCS, Pune^{13,14}.

Details of cell line as mentioned below:

Cell line	Morphology	Origin	Species	supplier
Vero	Epithelial	Kidney	Monkey	NCCS, Pune
MCF 7	Epithelial	breast cancer cells	Human	NCCS, Pune

Reagents: Minimal Essential Media (MEM), foetal bovine serum (FBS), trypsin, Methyl thiazolyldiphenyl-tetrazolium bromide (MTT), Dimethyl sulfoxide (DMSO) and Cisplatin and vinblastin are used as a standard

anticancer drug, were purchased from Hi-Media & Sigma Aldrich, Mumbai, India.

Treatment

Drug treatment	Dose($\mu\text{g/ml}$)
Methanolic extract of root	1000, 500, 100
Cisplatin	1000,500,100
Vinblastine	500,250,100

Solvent used: Ethanol, distilled water, iso propyl alcohol, DMSO, trypsin, MTT

Complete Growth Medium: Eagle's minimum essential medium, 10% foetal bovine serum, penicillin streptomycin solution, amphotericin B.

Equipment required: Inverted microscope, sterile tubes (5.0 mL), 37°C incubator, and Laminar flow hood.

Sub culturing Procedure: Volume is given for a 25cm² flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes. Corning T-25 flasks are recommended for sub culturing this cell line. Remove and discard culture medium. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 1 to 5 minutes). Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C in CO₂ incubator.

Trypan Blue assay:

Make a 1:1 mixture of the cell suspension and the 0.4% trypan blue solution. 100 μL of the cell suspension and dye was taken and gently mixed. Dilution factor is 2. Prior to use, wash the hemocytometer with 70% (v/v) ethanol and allow drying. Wash a coverslip with 70% (v/v) ethanol, allow it to dry. Place the coverslip on top of the hemocytometer counting chamber. Apply small volume of cell suspension to the edge of the chamber between the cover slip and the V-shaped groove in the chamber. Allow the cell suspension to be drawn into the chamber by capillary action. Let sit for 1–2 min and then count

cells. The viable cells were counted using the following formula: %viable cells= (no. of viable cells/ total no. of cells)*100

MTT ASSAY: Cells were seeded in a flat-bottomed 96-well plate and incubated for 24 hour at 37°C and in 5% CO₂. Vero and MCF7 cells were exposed to different extracts at various concentrations (1000 $\mu\text{g/ml}$, 500 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$) for 48 hours. The solvent DMSO treated cells served as control and cisplatin and Vinblastine were used as a Positive Standard. Cells were then treated with MTT reagent (0.5 mg/ml as final concentration, i.e. 20 $\mu\text{l/well}$ of stock) for 4 h at 37°C. Then all the media and MTT reagent was removed from the wells and DMSO (200 μl) was added to each well to dissolve the formazan crystals. The optical density (OD) was recorded at 570 nm in Microplate (ELISA) reader. Percentage of cell viability was determined as:

$$= (\text{Average Optical density of treated cells} / \text{Average Optical density of control cells}) \times 100$$

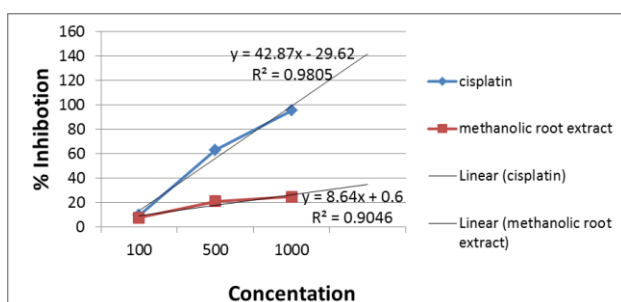
RESULT

The % yield of methanolic extract of root of *Gendarussa vulgaris* was 18% w/w. Seeding density of cells in each well was, VERO cell line is 5000 cells/well and MCF7 cell line is 10000 cells/well counted. Cytotoxicity of *Gendarussa vulgaris* root extract was assessed in the growth of MCF-7 cells (Human breast cancer cells) by MTT (3,4,5-dimethyl thiazole-2-yl)-5-5diphenyltetrazolium bromide) assay, which is based on the reduction of MTT. The cytotoxic activity was investigated using MTT assay, and the human breast cancer cell line MCF-7 cells are treated at different concentrations. The viability of the control was designated as 100% and the others were expressed as percent compared to the control.

In the present work, the cytotoxicity studies on the root of *Gendarussa vulgaris* using Vero cell line. The methanolic extract of root of *Gendarussa vulgaris* showed non cytotoxic activity, as shown in Table 1 and graph 1.

Table-1: In-vitro cytotoxicity of methanolic extract of root of *Gendarussa vulgaris* on Vero cell line

Sr. No	Sample	Concentration $\mu\text{g/ml}$	% Viable cells	% Inhibition
1	Media Control	NA	100%	00.00%
2	Cisplatin	100	90.14%	09.76%
		500	36.90%	63.10%
		1000	04.50%	95.50%
3	Methanolic extract of <i>Gendarussa vulgaris</i> root	100	92.38%	07.62%
		500	78.88%	21.12%
		1000	75.1%	24.9%



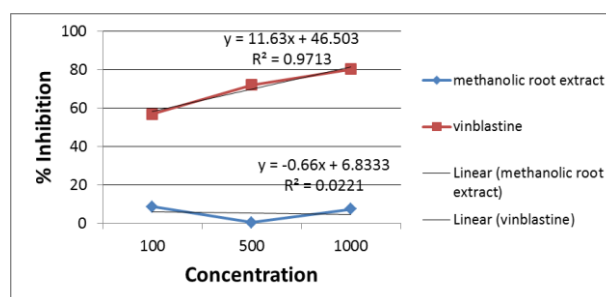
Graph 1:

The cytotoxicity studies on the root of *Gendarussa vulgaris* using MCF7 cell line. The methanolic extract of the *Gendarussa vulgaris* root showed non cytotoxic activity as shown in Table 2 and graph 2.

Table-2: In-vitro cytotoxicity of methanolic extract of root of *Gendarussa vulgaris* on MCF 7 cell line

Sr. No.	Sample	Concentration $\mu\text{g/ml}$	% Viabile cells	% Inhibition
1	Media Control	NA	100%	0
2	Vinblastine	100	43.02%	56.98%
		500	27.93%	72.07%
		1000	19.76%	80.24%
3	Methanolic rootextract	100	91.28%	8.71%
		500	99.5%	0.44%
		1000	92.54%	7.39%

Graph 2:



DISCUSSION:

Agents capable of inhibiting cell proliferation, inducing apoptosis or modulating signal transduction are currently used for the treatment of cancer¹⁵. The use of multiple chemo preventive agents or agents with multiple targets on cancer cells are considered to be more effective in cancer treatment¹⁶. Breast cancer is the most common malignancy among women. MCF-7 cell has become a prominent model system for the study of breast cancer as it relates to the susceptibility of the cells to apoptosis¹⁷. Despite the fact that many tumors initially respond to chemotherapy, breast cancer cells can subsequently survive and gain resistance to the treatment¹⁸.

Gendarussa vulgaris is a potent plant whose empirical use in the cancer treatment has spread in recent years. Some studies have proposed the potential anticancer activity of extracts from *Gendarussa vulgaris*. Root extracts of the *Gendarussa vulgaris* with various solvents like hexane, methanol and water were investigated for cytotoxicity of the plant using a brine shrimp lethality test. Methanolic extract of the *Gendarussa vulgaris* root was studied for cytotoxicity in human cancer cell lines HT29, HeLa and BxPC-3) by using MTT assay. The present study shows the non-cytotoxicity of methanolic root extract of *Gendarussa vulgaris*, using MCF 7 and vero cell line.

Based on our results, the cytotoxicity of methanolic extract of *Gendarussa vulgaris* root on MCF 7 cell line is 7.3 % and on vero cell line is 24.9 %.

This shows non cytotoxic of methanolic extract of *Gendarussa vulgaris* root to breast cancer cells [MCF 7] as well as to normal cells [Vero]. The outcome of the present study encourages to carrying out further studies to determine the cytostatic activity of root extract of *Gendarussa valgaris* is non-cytotoxic

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