Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2025, Volume 13, Issue 02
ISSN: 2321–3647(online)

Evaluation of *In-vitro* Anticancer Screening Using SRB Assay on Human Cell Lines of Various Extracts of Globally Endangered Ethnomedicinal Tree Species *Adansonia digitata* L.

Shama Nazrin, Rajinikanth Marka and Rama Swamy Nanna*
Plant Biotechnology Research Laboratory, Department of Biotechnology, Kakatiya University, Warangal –506009 (TS), India

ABSTRACT

The species *Adansonia digitata* L is commonly called as baobab belongs to family Bombacaceae. In the present report, we have evaluated the *in vitro* anticancer activity based on its reported phytoconstituents. The extracts of callus, stem, root, leaf and fruit pulp were obtained using solvents like methanol, hexane and ethyl acetate by Soxhlet extraction method (organic). The *in-vitro* anticancer activity was evaluated against four human cancer cell lines Colo-205 (Colon cancer cell line), DU-125 (Prostate cancer cell line), MCF-7 (Breast cancer cell line) and HeLa (Cervix cancer cell line) using Sulforhodamine B (SRB) assay. The cytotoxic potential of DU-125 cell lines was examined, and the results showed that the GI_{50} value of the methanolic callus, methanolic leaf, hexane leaf, and ethyl acetate leaf extracts was >80 µg/ml. The GI_{50} values of the leaf extracts in hexane and ethyl acetate were found to be >80 µg/ml on the Colo-205 cell line. For the MCF-7 cell line, methanolic callus extracts GI_{50} values were noted >80 µg/ml and hexane and ethyl acetate leaf extracts were recorded <80 µg/ml (54.6 µg/ml and 52.6 µg/ml). Methanolic root and leaf extracts were found to have GI_{50} values >80 µg/ml on the HeLa cell lines, while hexane and ethyl acetate leaf extracts had GI_{50} values of 71.2 µg/ml and 76.2 µg/ml, respectively. These findings introduce *A. digitata* as potentially useful as anti-cancer agent.

Keywords: *Adansonia digitata,* Sulforhodamine B, callus, stem, root, leaf, fruit pulp extracts, human cell lines

*Corresponding Author Email: swamynr.dr@gmail.com
Received 02 February 2025, Accepted 03 March 2025

Please cite this article as: Nanna RS *et al*., Evaluation of In-vitro Anticancer Screening Using SRB Assay on Human Cell Lines of Various Extracts of Globally Endangered Ethnomedicinal Tree Species Adansonia digitata L . American Journal of Pharmacy & Health Research 2025.

INTRODUCTION

Since ancient times, people have considered cancer to be the most dreaded sickness. Cancer affects almost every household in the world, including those throughout India. In a September 2019 report the World Health Organization (1), noted that deaths from cancer are more common in low- and middle-income countries, accounting for about 70% of all cancer deaths. The WHO also stated that cancer deaths in developing nations will continue to rise, reaching roughly 10 million by 2020 and 11.5 million by 2030.

The entire treatment is still a mystery despite numerous advancements in the medical sciences. The incidence of cancer is constantly increasing, and treating it is a significant issue in both developed and developing nations (2, 3)

In the last decade of the 20th century, a variety of techniques were developed to treat cancer, including radiation therapy, surgery, and the use of chemotherapeutic medications. According to Rajpal et al. (4), modern cancer treatments include anti-angiogenic chemotherapy, targeted therapies, RNA expression proliferation techniques, and proteomics. Chemotherapy agents that are widely or often used have more harmful effects on the body than on cancer cells. For example, 5-fluorouracil is a common chemotherapeutic agent that causes myotoxicity and cardiotoxicity (5).

In contrast to the present chemotherapy treatment, the research is mostly focused on naturally occurring or derived compounds because they are readily available, less expensive, and have fewer side effects (6). Higher medicinal plants have a significant impact on cancer treatment. Plant secondary metabolites (phytochemicals/phytomedicines) and their derivatives have been employed to fight against cancer. Currently, plant-derived antitumor and anticancer agents/drugs in regular clinical use, to kill or inactivate tumor cells without damaging healthy and normal body cells/ tissues.

The National Cancer Institute collected about 35,000 plant samples from around 20 countries and screened around 114,000 extracts for anticancer activity (7). According to a study, 324 compounds exhibited cytotoxicity against two cancer cell lines in an assay using ten cancer cell lines (A-549, BEL-7402, BGC823, B-16, DU-145, HT-29, MCF-7, MDA-MB-231, SGC-7901, and U-251) (8).

In view of the importance of phytomedicine to cure cancer, we made an attempt to explore the screening of baobab tree extracts on cancer cell lines.

The baobab is a widely used plant species that can be used for both food and medicine. The tree has a number of pharmacological qualities, including as anti-inflammatory, antipyretic, antioxidant, prebiotic-like, anti-diarrheal, and anti-dysenteric effects. Therefore, the presence of active chemical elements such as vitamin C, flavonoids, phytosterols, amino acids, fatty acids, and minerals may give it anticancer properties (9, 10). According to Al-Qawari et al. (11), the fruit of *A. digitata* also contains the triterpenoids beta-sitosterol, ursolic acid, and betaamyrin palmitate. As a result, it is important to consider whether these extracts have the potential to be cytotoxic or anticancer. Therefore, based on its stated phytoconstituents (12), *A. digitata* is chosen for investigation on anticancer activity.

While conservation efforts have been attempted for *A. digitata* (12-14), and as well as phytoconstituents but no report on the anticancer properties of plant extracts obtained from this globally endangered species.

The current study is the first to use the Sulforhodamine B (SRB) assay to examine the potential anticancer potential of *A. digitata* extracts against four human cancer cell lines: DU-125 (prostate), Colo-205 (colon), MCF-7 (breast), and HeLa (cervix).

MATERIALS AND METHOD

Collection and authentication of research plant material

The plant material (root, stem, leaves, fruits) of *A. digitata* was collected from *Tukkuguda* area located at 17°12′35.1″N 78°30′48.0″E, service road, Hyderabad, Telangana state, India. Authentication was done by Prof. Rama Swamy Nanna, Department of Biotechnology, Kakatiya University, Warangal, Telangana State. Fruits were broken to collect fruit pulp surrounding the seeds.

Powder preparation and sample extraction

The roots, stem and leaves of *A. digitata* were washed thoroughly with water and dried at room temperature in laboratory for 2-3 weeks. The pulp surrounding the seeds was collected by breaking the fruits, later the coir and seeds were separated from the pulp. The callus developed on MS+ 4.0 mg/L 2,4 D from cotyledonary leaf explants was used for the present investigation. The callus was dried in an incubator for 2 days and was used for powder preparation. All the above mentioned plant parts were coarsely powdered separately using electric grinder. Methanol is reported to be the most effective solvent for isolation of bioactive compounds (15, 16). Hence, in the present investigation we have used methanol as solvent and also used Hexane and Ethyl acetate (EA) for leaf extracts. Five grams (5 gm) of powder is extracted with 100 ml of solvents, separately using Soxhlet apparatus for eight hours. The extract was filtered using Whatman No. 1 filter paper at ambient temperature. Excess solvent was removed and concentrated to 5 ml using rotary evaporator. The extract of 5 ml of each sample was collected for analysis and stored at 4°C for further use.

***In vitro* anticancer screening using SRB assay:**

The *in vitro* anticancer screening using SRB (Sulforhodamine B) assay of the prepared extracts were performed on various human cell lines at Tata Memorial Centre-Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Navi Mumbai (Maharashtra State), India. Sulforhodamine B (SRB) assay is the most reliable, rapid method for analyzing the cellular protein content of adherent and suspension cultures in 96-well microtiter plates. SRB assay is more reliable and sensitive than tetrazolium (MTT) assay where the former provides better linearity with cell number (17). The cell lines were collected from the National Cancer Institute (NCI), USA and the National Center for Cell Science (NCCS), Pune. The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. The anticancer screening of extracts was studied by the following method of Philip et al. (18). The cell lines used in the present studies are shown in Table 1

Reagents used

10% Fetal bovine serum (FBS), Dimethyl sulfoxide (DMSO), Trichloroacetic acid (TCA), Sulforhodamine B (SRB), 1% acetic acid, 10 mM trizma base.

Experimental procedure

For the present screening experiments, cells were inoculated into 96 well microtiter plates 990µL/well at appropriate plating densities, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C in 5% CO_2, 95% air and 100% relative humidity for 24 hr prior to addition of experimental drugs. After 24 hrs, cells from one plate of each cell line were fixed *in-situ* with TCA (Trichloro acetic acid), to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental extracts were solubilized in appropriate solvent at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to 10 times the desired final maximum test concentration with complete medium containing test sample at a concentration of 100, 200, 400 and 800µg/ml. Aliquots of 10 µl of these different dilutions were added to the appropriate microtiter wells already containing 90µl of cell suspension, resulting the required final drug concentrations of 10, 20, 40 and 80 µg/ml. For each experiment, the known anticancer drug Adriamycin was used as a positive control.

Cell fixation and staining

After compound addition, plates were incubated at standard conditions for 48 hrs and assay was terminated by the addition of cold TCA. Cells were fixed *in-situ* by the gentle addition of 50 µl of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed 5 times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing 5 times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10mM Trizma base.

OD measurement and calculation

Absorbance was read on an Elisa Plate Reader at 540nm with 690nm reference wavelength. Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. It is calculated as the ratio of average absorbance of the test well to the average absorbance of the control wells X100. Using the 6 absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the 4 concentration levels (Ti)]; the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti ≥ Tz (Ti-Tz) positive or zero.

[(Ti-Tz)/Tz] x 100 for concentrations for which Ti< Tz (Ti-Tz) negative.

The dose-response parameters were calculated for each test article. Growth inhibition (GI) of 50% (GI_{50}) was calculated from [(Ti-Tz)/(C-Tz)] x 100=50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The experiment data were estimated using the linear regression method of plots of the cell viability against the molar drug concentration of tested compounds.

Data analysis

The screening of anticancer experiments was repeated thrice with four different concentrations of extracts (10, 20, 40, and 80 µg/ml) to test the cytotoxic effects and average values were calculated. The average values of 3 experiments were used in calculating GI_{50} values (Concentration of drug causing 50% of inhibition of cell growth). As per Advanced Centre for Treatment, Research and Education in Cancer (ACTREC, India) anticancer drug screening in a panel of human cancer cell lines, extracts with GI_{50} value less than 20 µg/ml ($GI_{50} \leq 20$) are considered to having anticancer activity.

RESULTS AND DISCUSSION

The present studies used the Sulforhodamine B (SRB) assay to test the anticancer properties of methanolic extracts (leaf, stem, root, fruit pulp, and callus) as well as hexane and ethyl acetate leaf extracts of *A. digitata* against four distinct human cancer cell lines: DU-145 (prostate), Colo-205 (colon), MCF-7 (breast), and HeLa (cervix) (Table 1).

Table 1: List of cell lines used for sample screening

Sl. No	Species	Tissue of origin	Cell line
1	Human	Colon	Colo-205
2	Human	Prostate	DU 125
3	Human	Breast	MCF-7
4	Human	Cervix	HeLa

The cytotoxic effects of anticancer screening in *A. digitata* were tested using four different extract concentrations (10, 20, 40, and 80 µg/ml) three times, and average values were computed. Results on growth control percentage for all the four cell lines are presented in Tables 2-5. A comparative profile of GI_{50} values for samples for human prostate cancer cells (DU-145), colon cancer cells (Colo-205), breast cancer cells (MCF-7), and cervix cancer cells (HeLa) are shown in Table 6.

According to our findings on DU-145 cell lines, methanolic stem and root extracts shown cytotoxic potential of up to 94.3/89.3 µg/ml at a concentration of 10 µg/ml. The GI_{50} value of every other extract, however, was higher than 80 µg/ml. Non-evaluable extracts were found to be the rest (Table 2; Figure 1).

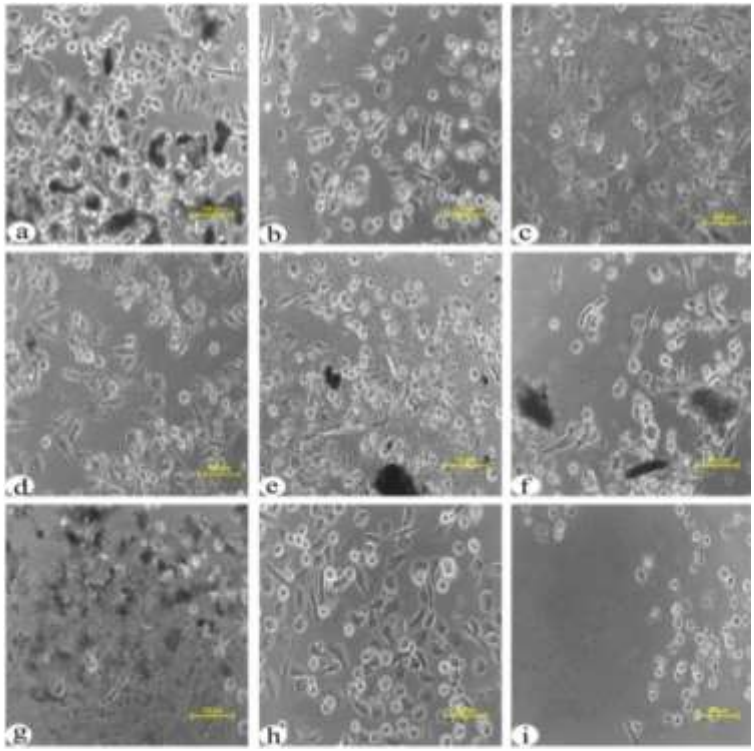

Figure 1a- i: *In vitro* cytotoxic effects of different solvent extracts of *A. digitata* on DU-145 cell line. Methanolic extracts: (a) fruit pulp, (b) callus, (c) stem, (d) root, (e) leaf, (f) Hexane-leaf extract, (g) Ethyl acetate-leaf extract, (h) Control, (i) Adriamycin (+ve control).

Table 2: Effect of methanolic/hexane/ ethyl acetate extracts of *A. digitata* on human prostate cancer cell line, DU-145.

Extracts	Sample concentration (µg/ml)															
	Experiment-1				Experiment-2				Experiment-3				Average values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
Methanolic-Fruit Pulp	132.6	187.4	206.8	140.0	122.7	126.3	130.7	129.0	104.8	111.2	120.9	121.4	120.0	141.6	152.8	130.1
Methanolic- Callus	160.6	193.1	193.6	155.1	120.4	137.4	163.9	123.0	109.2	114.4	110.7	105.5	130.1	148.3	156.1	127.9
Methanolic- Stem	89.4	96.4	100.1	134.8	93.4	76.2	114.5	157.6	100.0	104.4	110.0	176.4	94.3	92.4	108.2	156.3
Methanolic-Root	86.3	94.7	87.8	100.1	85.9	79.4	98.9	117.8	95.6	97.8	91.1	119.7	89.3	90.6	92.6	112.5
Methanolic- Leaf	159.0	178.6	198.9	133.8	115.2	138.3	159.5	135.6	109.2	113.6	110.8	114.0	127.8	143.5	156.4	127.8
Hexane-leaf	157.4	175.4	166.9	71.5	108.1	128.2	121.7	59.9	102.0	100.7	83.6	42.6	122.5	134.8	124.1	58.0
Ethyl acetate-leaf	146.2	166.9	141.0	74.1	94.6	112.8	104.2	64.9	90.3	83.6	65.7	36.5	110.3	121.1	103.6	58.5
Adriamycin	-20.3	-21.1	-28.7	-32.1	-24.5	-25.2	-33.0	-31.1	-30.7	-34.7	-35.7	-37.1	-25.2	-27.0	-32.5	-33.5

Adriamycin (ADR): Positive control compound.

Table 3: Effect of methanolic/hexane/ethyl acetate extracts of *A. digitata* on human colon cancer cell line, Colo-205

Extracts	Sample concentration (µg/ml)															
	Experiment-1				Experiment-2				Experiment-3				Average values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
Methanolic-Fruit Pulp	96.3	100.8	109.5	107.2	76.5	73.8	74.2	86.8	68.4	61.2	65.9	90.7	80.4	78.6	83.2	94.9
Methanolic- Callus	85.8	92.1	111.6	109.9	72.9	79.3	91.7	109.8	71.7	71.3	81.9	103.8	76.8	80.9	95.1	107.8
Methanolic- Stem	51.5	85.2	127.6	119.1	78.1	86.3	107.9	186.1	72.8	95.8	127.9	176.7	67.5	89.1	121.1	160.6
Methanolic-Root	54.1	75.0	87.4	110.9	61.5	75.3	94.8	133.1	57.2	80.2	105.4	146.0	57.6	76.8	95.9	130.0
Methanolic- Leaf	109.8	119.0	124.1	127.5	97.9	105.9	113.1	114.7	64.5	66.3	82.9	93.5	90.8	97.1	106.7	111.9
Hexane-leaf	121.9	119.2	118.2	81.8	108.9	105.2	103.0	72.9	69.6	65.4	67.0	68.5	100.2	96.6	96.1	74.4
Ethyl acetate-leaf	101.1	114.6	101.1	75.9	108.1	115.6	102.6	83.2	72.5	73.7	83.7	64.4	93.9	101.3	95.8	74.5
Adriamycin	3.4	-22.8	-10.5	-9.2	7.7	13.3	6.8	19.0	-45.6	-44.3	-33.9	-25.0	-11.5	-17.9	-12.5	-5.1

Adriamycin (ADR): Positive control compound.

Table 4: Effect of methanolic/hexane/ethyl acetate extracts of *A. digitata* on human breast cancer cell line, MCF-7

Extracts	Sample concentration (µg/ml)															
	Experiment-1				Experiment-2				Experiment-3				Average values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
Methanolic-Fruit Pulp	91.6	90.2	94.2	97.0	91.6	92.6	91.0	99.5	96.3	109.5	117.7	109.4	93.2	97.4	100.9	101.9
Methanolic- Callus	84.5	84.8	88.3	84.9	83.2	84.7	84.3	85.8	95.7	108.4	110.6	87.5	87.8	92.6	94.4	86.1
Methanolic- Stem	74.4	68.7	104.8	173.2	80.4	82.6	101.6	125.5	86.3	90.6	112.4	122.3	80.4	80.6	106.3	140.4
Methanolic-Root	59.2	65.1	84.3	117.7	83.4	72.9	87.5	96.9	83.1	82.1	88.7	96.4	75.2	73.4	86.8	103.7
Methanolic- Leaf	85.7	83.0	92.0	96.6	86.4	83.7	92.9	98.5	99.6	107.3	114.6	95.0	90.6	91.3	99.8	96.7
Hexane-leaf	81.4	66.7	52.0	26.7	76.4	65.2	51.5	25.3	91.3	94.1	82.8	30.5	83.1	75.3	62.1	27.5
Ethyl acetate-leaf	75.8	66.9	50.6	28.8	68.7	63.0	52.5	35.7	84.1	87.9	72.0	38.9	76.2	72.6	58.4	34.5
Adriamycin	-73.6	-70.9	-35.9	-47.2	-75.8	-75.3	-38.2	-63.6	-73.6	-75.9	-75.0	-68.0	-74.3	-74.0	-49.7	-59.6

Adriamycin (ADR): positive control compound.

Table 5: Effect of methanolic/hexane/ethyl acetate extracts of *A. digitata* on human cervical cancer cell line, HeLa

Extracts	Sample concentration (µg/ml)															
	Experiment-1				Experiment-2				Experiment-3				Average values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
Methanolic-Fruit Pulp	112.4	126.9	136.5	138.6	103.1	108.1	111.9	128.5	101.3	107.5	106.7	117.0	105.6	114.2	118.4	128.0
Methanolic- Callus	122.7	140.2	151.6	153.1	104.7	108.6	120.6	134.7	102.9	106.0	114.7	126.1	110.1	118.3	128.9	138.0
Methanolic- Stem	98.4	109.6	120.4	117.9	103.4	115.2	127.8	104.7	105.5	111.8	121.0	119.6	102.4	112.2	123.1	114.1
Methanolic-Root	99.4	105.6	105.7	81.4	105.1	112.0	108.4	83.9	103.9	106.5	106.1	74.9	102.8	108.0	106.7	80.1
Methanolic- Leaf	112.9	134.0	137.9	130.5	99.4	105.0	105.9	112.1	99.2	105.4	106.3	109.6	103.8	114.8	116.7	117.4
Hexane-leaf	117.3	122.5	105.9	47.7	96.5	97.8	85.1	29.0	98.8	104.1	84.1	31.7	104.2	108.2	91.7	36.1
Ethyl acetate-leaf	103.1	112.6	94.6	57.3	84.1	86.3	68.4	40.1	89.4	92.4	76.1	37.8	92.2	97.1	79.7	45.1
Adriamycin	-68.9	-73.0	-75.3	-63.2	-77.7	-76.7	-77.4	-67.5	-72.1	-73.6	-80.5	-65.4	-72.9	-74.4	-77.8	-65.4

Adriamycin (ADR): Positive control compound.

The cytotoxic potential of methanolic stem and root extracts on Colo-205 cell lines was less than 80 µg/ml at a dose of 10 µg/ml (Table 3; Figure 2). Hexane-leaf and ethyl acetate-leaf extracts, on the other hand, had GI_{50} values more than 80 µg/ml. All the remaining extracts were recorded non-evaluable (Table 6).

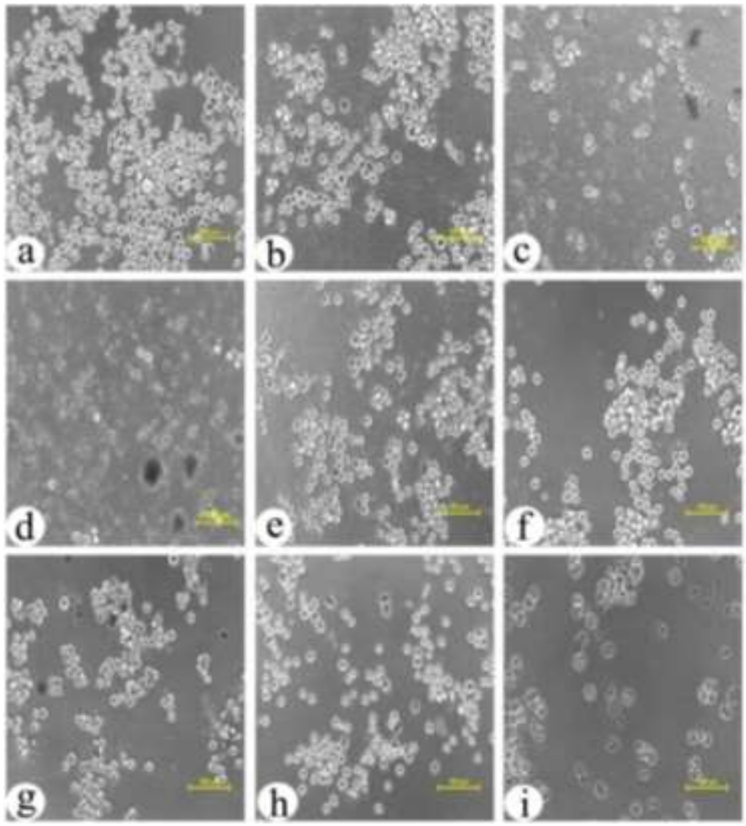

Figure 2a- i: *In vitro* cytotoxic effects of different solvent extracts of *A. digitata* on Colo-205 cell line. Methanolic extracts: (a) fruit pulp, (b) callus, (c) stem, (d) root, (e) leaf, (f) Hexane-leaf extract, (g) Ethyl acetate-leaf extract, (h) Control, (i) Adriamycin (+ve control).

When tested on the MCF-7 cell lines, the cytotoxic potential of methanolic stem and root extracts showed 80.4/75.2 µg/ml, respectively at a dose of 10 µg/ml (Table 4; Figure 3). Out of every sample that was evaluated, the hexane and ethyl acetate leaf extracts had GI_{50} values that were less than 80 µg/ml, or 52.6 µg/ml and 54.6 µg/ml, respectively (Table 6).

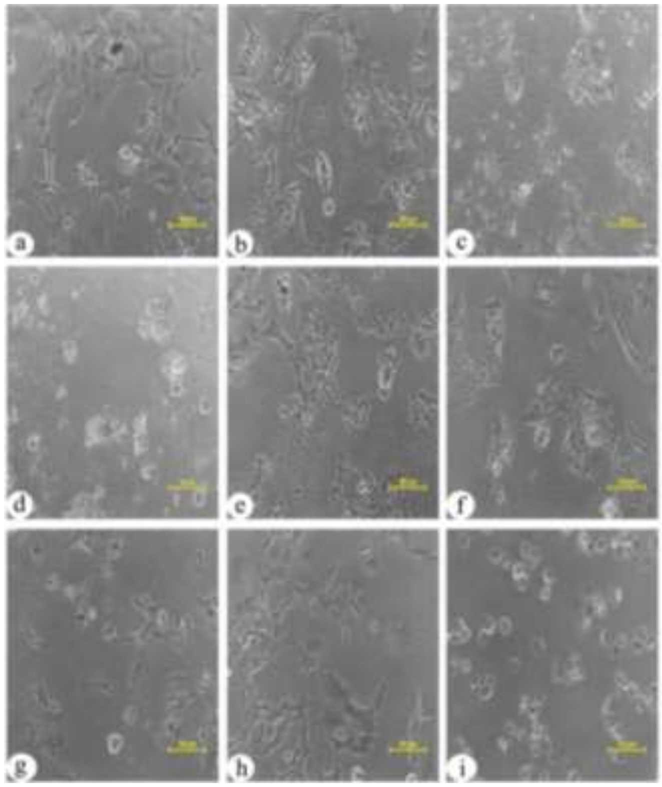

Figure 3 a- i: *In vitro* cytotoxic effects of different solvent extracts of A. digitata on MCF-7 cell line. Methanolic extracts: (a) fruit pulp, (b) callus, (c) stem, (d) root, (e) leaf, (f) Hexane-leaf extract, (g) Ethyl acetate-leaf extract, (h) Control, (i) Adriamycin (+ve control).

Cytotoxic potential of all extracts on HeLa cell lines was higher than 80 µg/ml at a dose of 10 µg/ml (Table 5; Figure 4). The GI_{50} values for the hexane-leaf and EA-leaf extracts were 71.2 µg/ml and 76.2 µg/ml, respectively. Non-evaluable samples and remaining ones showed >80 µg/ml. The GI_{50} value of Adriamycin (ADR), the positive control drug, was <10 (Table 6).

It was noted that neither the extracts nor the usual control showed any signs of cell death. The standard drug, Adriamycin, caused cell lines to exhibit cell death. The results of our experiments clearly reveal that all of the *A. digitata* extracts examined had very low cytotoxic effect on all of the human cancer cell lines.

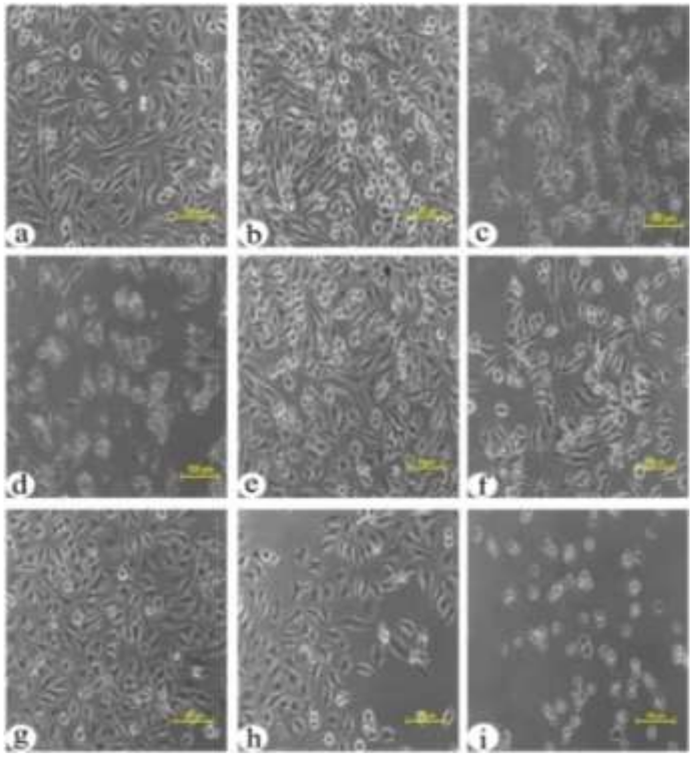

Figure 4 a- i: *In vitro* cytotoxic effects of different solvent extracts of *A. digitata* on HeLa cell line. Methanolic extracts: (a) fruit pulp, (b) callus, (c) stem, (d) root, (e) leaf, (f) Hexane-leaf extract, (g) Ethyl acetate-leaf extract, (h) Control, (i) Adriamycin (+ve control).

According to our findings, none of the *A. digitata* extracts had any cytotoxic effects at all on the cell lines that were used in the study (Table 6). Extracts of hexane and ethyl acetate leaves have demonstrated growth inhibition (GI_{50}) against HeLa and MCF-7 cell lines of less than 80 µg/ml. All other examined extracts, however, were either >80 or non-evaluable.

Table 6: GI_{50} values of samples of different solvent extracts of *A. digitata*.

Extracts	GI_{50} Concentration (µg/ml)			
	DU-145	**Colo-205**	**MCF-7**	**HeLa**
Methanolic- Fruit Pulp	NE	NE	NE	NE
Methanolic- Callus	>80 (786.7)	NE	>80 (853.36)	NE
Methanolic- Stem	NE	NE	NE	NE
Methanolic-Root	NE	NE	NE	>80 (173.2)
Methanolic- Leaf	>80 (1305.4)	NE	NE	>80 (744.7)
Hexane-leaf	>80 (95.7)	>80 (152.8)	52.6	71.2
Ethyl acetate-leaf	>80 (95.2)	>80 (162.8)	54.6	76.2
Adriamycin	<10	<10	<10	<10

GI_{50} – Concentration of drug causing 50% of inhibition of cell growth, **NE** – Non-evaluable data, Adriamycin (ADR): Positive control compound.

Mageed and Abd alaziz (19) have used the SRB assay to measure the cytotoxicity of *A. digitata* seed extracts at 100, 250, 500, and 1000 µg/ml dilutions against three cancer cell lines: liver (Hep-G2), breast (MCF 7), and lung (A549). As positive drug, paclitaxel (5µg/ml), cisplatin (10µg/ml), actinomycin-D (10µg/ml), and Kojec acid (100µg/ml) were employed. In contrast to our work, Ateya et al. (20) have found that, out of 8 extracts tested for *in vitro* cytotoxicity against the Hep G2 cell lines using the MTT assay, the methanolic leaf extract of *A. digitata* was more effective. They have used the reference drug doxorubicin.

Very little research work has been done to assess the anti-carcinogenic qualities of *A. digitata*. The anti-tumor effect of methanolic seed and fruit pulp extracts of *A. digitata* was studied using Ehrlich Ascites Carcinoma (EAC) cells in 3 months old female BALB/c mice. For 14 days, the tumor-mice were given different oral doses of methanolic seed and fruit pulp extracts (300 mg/Kg body weight) separately (21). Antioxidant qualities and anticarcinogenic activity have been reported for this species by Zahra'u et al. (22).

Although there are a number of safe and effective cancer treatments in the market, none of them are 100% effective. Furthermore, established medications make cancer chemotherapy extremely toxic (23). Natural substances derived from plants have been employed to cure human illnesses in recent years due to their safety and lack of adverse effects, which has drawn the interest of numerous scientific experts. Drugs based on natural products (NPs) have been steadily entering the market.

Thus, this study provides a new hope for further investigation on cytotoxic effects of different solvent extracts of *A. digitata* by using high concentrations of samples.

CONCLUSION

Cancer is one of the most pressing health concerns worldwide. Modern cancer treatment includes chemotherapy as a key alternative, and plant-derived chemotherapeutic drugs have significantly advanced oncology chemotherapy. For many types of cancer, plants have been a major source of very effective conventional medications. As new technologies are created, some of the compounds that did not work in previous clinical trials are now generating renewed attention.

However, further research is required to evaluate the various plant extracts of *A. digitata* against different types of cancer cell lines.

ACKNOWLEDGMENT

The author Shama thanks to University Grants Commission, New Delhi, India for providing the financial assistance under UGC-MANF (F1-17.1/2014-15/MANF-2014-15-MUS-TEL-40612).

Funding

This work was financially supported by UGC-MANF (F1-17.1/2014-15/MANF-2014-15-MUS-TEL-40612).

Author's contribution

SN and RM, were carried out experiments, evaluated the data and wrote the manuscript. The research was planned by RN, who also oversaw, edited, and reviewed the paper. The manuscript has been viewed and approved final version by all authors and is now ready for publishing.

Conflict of Interest

All the authors declare there is no conflict of interest concerning the publication of the manuscript.

REFERENCES

1. World Health Organisation. New toolkit to improve collection and use of data in cervical cancer programmes. (2019). www.who.int/ncds/surveillance/new-toolkitimprove-collection-use-data-cervical-cancer/en/.
2. Parkin DM, Bray F, Ferlay J. Pisani P. Global cancer statistics, 2002. CA Cancer J Clin (2005); 55:74-108.
3. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Inter J Cancer (2010); 127(12): 2893-2917.
4. Rajpal S, Kumar A, William J. Economic burden of cancer in India: Evidence from cross-sectional nationally representative household survey, 2014. PloS One (2018); 13(2): 1-17.
5. Sudhakar A. History of cancer, ancient and modern treatment methods. J cancer Sci Therapy (2009); 1(2):1-4.
6. Desai AG, Qazi GN, Ganju RK, Mahmoud ET, Singh J, Saxena AK, Bedi YS, Taneja SC, Bhat HK. Medicinal plants and cancer chemoprevention current drug metabolism. NIH Public Access Author Manuscript, (2008); 9(7): 581-591.
7. Shoeb M. Anti-cancer agents from medicinal plants. Bangladesh J Pharmacog (2006); 1(2): 35-41.
8. Zeng GZ, Tan NH, Hao XJ, Mu QZ, Li RT. Natural inhibitors targeting osteoclast-mediated bone resorption. Bioorg Med Chem Lett (2006): 16(24): 6178-6180.
9. Greenwell M, Rahman PKSM. Medicinal plants: Their use in anticancer treatment. Int J Pharm Sci Res (2015); 6(10): 4103-4112.

10. Sundarambal M, Muthusamy P, Radha R, Jerad SA. A review on *Adansonia digitata* Linn. J Pharmacogn Photochem (2015); 4(4): 12-16.
11. Al Qarawi AA, Al Damegh MA, El Mougy SA. Hepatoprotective influence of *Adansonia digitata* pulp. J Herbs Spices Med Plants (2003); 10(3): 1-6.
12. Shama Nazrin. *In vitro* conservation, screening of bioactive compounds and assessment of genetic diversity in globally endangered species-*Adansonia digitata* L. (2021); Ph. D thesis, Kakatiya University, Waragnal, Telangana.
13. N'doye AL, Sambe MAN, Sy MO. Propagation of african baobab (*Adansonia digitata* L., Bombacoideae, Malvaceae) germplasm through *in vitro* cloning. Adv Environ Biol (2012); 6(10): 2749-2757.
14. Sugandha S. Callus induction, anti-microbial screening and *in vitro* plantlet regeneration of *Adansonia digitata* L.: An endangered medicinal tree. Int J Sci Res Biol Sci (2015); 2(5):10-16.
15. Al Hazzani AA, Shehata AI, Moubayed NMS, Al Houri HJ. Antimicrobial and biochemical properties of selected edible brown and red marine macroalgae. J Pure Appl Microbiol (2014); 8: 1275-1282.
16. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants (Basel) (2017); 6(4):42.
17. Keepers YP, Pizao PE, Peters GJ, Van Ark-Otte J, Winograd B, Pinedo HM. Comparison of the sulforhodamine B protein and tetrazolium (MTT) assays for *in vitro* chemosensitivity testing. European J Cancer Clin Oncol (1991); 27(7): 897-900.
18. Philip S, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening. J Nat Cancer Inst (1990); 82(13): 1107-1112.
19. Mageed MA, AbdAlaziz HSEI. Photochemical analysis, antioxidant activity and cytotoxicity of *Adansonia digitata* L. seeds ethanol extract. Excellence J Medical Sci (2019); 1(1): 24-32.
20. Ateya A, Ammar N, El-Eraky W, El-Senousy W, El Awdan S, Amer A. Antiviral, cytotoxicity, antioxidant and chemical constituents of *Adansonia digitata* grown in Egypt. Inter J Pharmac Phytochem Res (2016); 8(3): 499-504.
21. Elsaid FG. The effect of seeds and fruit pulp of *Adansonia digitata* L. (Baobab) on ehrlich ascites carcinoma. Food and Nutr Sci (2013); 4: 38-46.

22. Zahra'u B, Mohammed AS, Ghazali HM, Karim R. Baobab tree (*Adansonia digitata* L) parts: nutrition, applications in food and uses in ethno-medicine–a review. Ann Nutr Disord Ther (2014); 1(3): 01-09.
23. Gladys B, Patterson B, Subar A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutr Cancer (1992); 18(1): 1-29.