Anticancer Effect of *Myriostachya Wightiana* Methanolic Extract on Human Cancer Cell Lines: An *Invitro* Experimental Study

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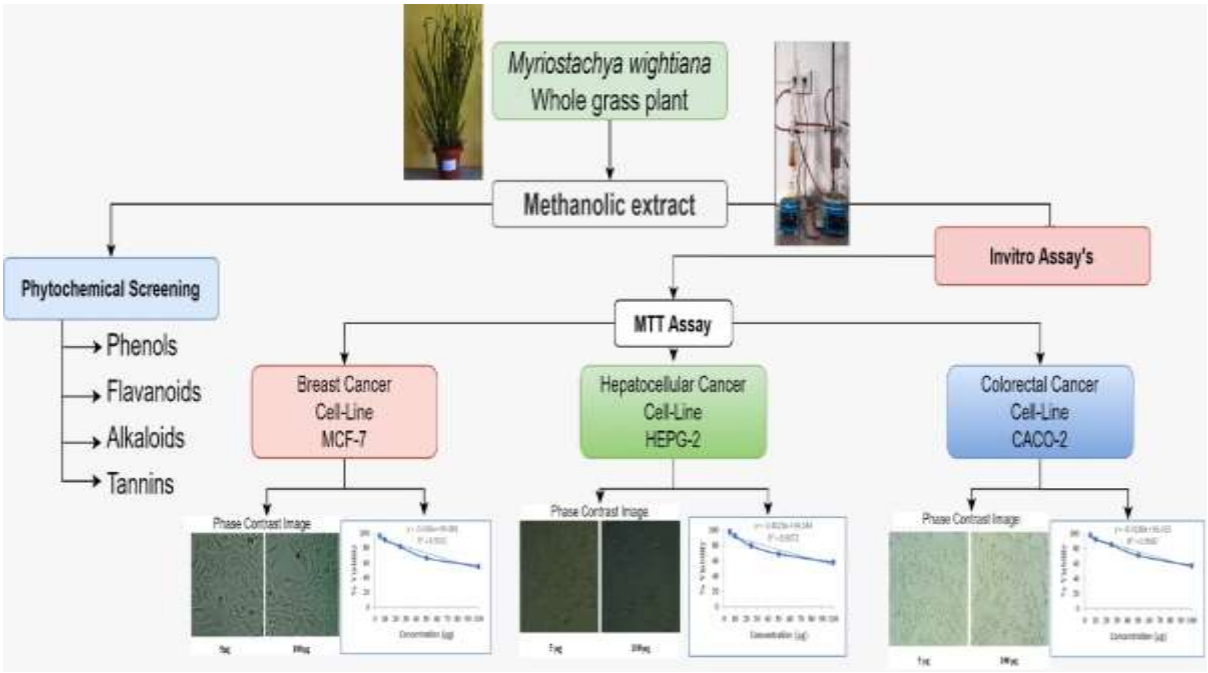
GRAPHICAL ABSTRACT



ABSTRACT

Background: *Myriostachya wightiana* (Nees ex Steud) Hook.f., (Poaceae) a grass plant with profound medicinal properties native of India. Cancer is an evil spirit that causes alarming symptoms of death owing to the disease's global growth. There is now a growing issue and burden for the creation of novel anticancer medications in the treatment of cancer, as well as for the relief of cancer-related illnesses. Mangroves are of tremendous ecological importance since they are rich in potential bioactive chemicals as well as bioactive components that have a wide range of applications in cancer eradication. The majority of the medications used and licenced for treatment for cancer are of natural origin, paving the route for the creation of novel chemical compounds from natural sources over time. **Aim of the study:** The present

work aimed to evaluate methanolic extract of *Myriostachya wightiana* whole plant for its anti-cancer activity using human cancer cell lines. **Materials and Methods:** The crude extract from the entire plant was prepared. The extract was screened for flavonoids, alkaloids, carbohydrates, tannins, and other phytochemicals. In-vitro cytotoxic study was performed on the Human Breast Cancer cell-line (MCF-7), Human Colorectal Cancer cell line (CACO-2) and Human Hepatocellular Cancer cell line (HEPG-2) using 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The MTT Assay was used to assess cell viability in three independent trials with five concentrations of the extract in triplicate. Cells were seeded on 24-well then incubated either DMSO as well as extract (with IC_{50} concentration) for 24 hrs. Images were captured by phase contrast microscopy during the treatment period. **Results:** The Anti-cancer activity was tested with different concentrations of the methanolic extract of *Myriostachya wightiana* like 5, 10, 20, 50, 100 µg/ml on MCF-7, HEPG-2, CACO-2 where 100 µg/ml was found to be effective with IC_{50} values 103.47 µg/ml, 110.22 µg/ml, 110.85 µg/ml respectively. MCF-7, HEPG-2 and CACO-2 exhibited remarkable percentage of cell inhibition in a dose dependant manner. The susceptibility of the cells to the extract was characterised by IC_{50} values. The methanolic extract of *Myriostachya wightiana* was capable of anticancer activity with increasing dose. **Conclusion:** The study indicated that methanolic extract of *Myriostachya wightiana* was active with IC50 against Human Breast Cancer cell-line (MCF-7), Human Colorectal Cancer cell line (CACO-2) and Human Hepatocellular Cancer cell line (HEPG-2). There is an urgent need for more research into this plant in order to uncover and isolate its active anticancer components.

Key words: *Myriostachya wightiana, methanoloic extract, in vitro cytotoxicity, cell lines*

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INTRODUCTION

Cancer is an evil spirit that causes alarming symptoms of death owing to the disease's global growth. The estimated number of incident cases of cancer in India for the year 2022 was found to be 14,61,427 (crude rate:100.4 per 100,000). In India, one in nine people are likely to develop cancer in his/her lifetime. Lung and breast cancers were the leading sites of cancer in males and females, respectively. The incidence of cancer cases is estimated to increase by 12.8 per cent in 2025 as compared to 2020. [1] According to a recent assessment, there would be approximately 10.9 million new cases of incidence, 6.7 million deaths, and over 24.6 million cancer patients worldwide by 2022.[2] In 2022, 1,918,030 (1.9 million) new cancer

cases and 609,360 cancer deaths are projected to occur in the United States,[2] suggesting an increase of 8.8% in cases and 0.41% in death when compared to 2019.

As per GLOBOCAN 2020, approximately expected 19.3 million additional cancer diagnoses and about 10 million died from the disease occurred worldwide. Breast Carcinoma has overtaken lung cancer as the most often identified cancer, with an anticipated 2.3 million new diagnoses (11.7%), followed by lungs (11.4%), colorectal (10%), prostatic (7.3%), and gastric (5.6%) cancers. The GLOBOCAN data with region-wise statistics indicate that Eastern Asia reported the most cases, 6.0 million (31.1% of the total), with 3.6 million deaths (36.3%). North America reported 2.6 million cases (13.3%) with a 7% share of cancer deaths, while South-Central Asia recorded 1.95 million cases (10%) and 1.3 million (12.6%) deaths. Europe reported 4.4 million incidences, with 1.9 million (20%) deaths. [3]

Due to declined favourable outcomes of conventional therapies like radiation, chemotherapy, immune modulation, and surgery in treating cancer, as evidenced by high morbidity and mortality rates, underscores the urgent need for novel approaches to cancer management. In view of this raising prevalence there is now a growing issue and burden for the creation of novel anticancer medications in the treatment of cancer, as well as for the relief of cancer-related illnesses.

Mangroves are of tremendous ecological importance as they are rich in potential bioactive chemicals as well as bioactive components that have different facets of applications in cancer eradication. Majority of the medications used and licenced for treatment for cancer are of natural origin, paving the route for the creation of novel chemical compounds from natural sources over time. These plants include anticancer chemicals that are effective against breast, stomach, lung, colon, prostate, and leukaemia cancers. [5]

The Poaceae family of mangroves has a wide range of different actions in the medication cure of many maladies like cardiovascular disease, Parkinsonism, auto immune diseases, and other activities such as antibacterial,[6] anti-inflammatory, antifungal, antioxidant etc . The Poaceae family is a key hub of nutritional qualities, with a core of chemical components that have various functions such as antibacterial, anti-inflammatory, antifungal, antioxidant, and many more. [7] It paves the door for substantial research into the development of novel chemical entities in order to discover newer medications.

Myriostachya wightiana (Poaceae) is indeed a perennial tuft grass that grows up to 100-150 cm in height and is widespread across India in tropical mangrove forests. The leaves are

primarily basal, longitudinal, 1-1.5m long, 10-20 mm broad, accuminate, smooth, sheaths are quite long, and the ligule has been reduced to a thin narrow rim. The inflorescence comprises its elongated spikelets 15-cm diameter with 4-10 cm width, a smooth rachis, and multi packed, closed cluster, and verticiled branches. Evened pedicel spikelet, 6-8 mm in length, 4-8 flowering caryopsis with attached pericarp, ovoid, laterally compressed.[8] This mangrove grass, Myriostachya wightiana, was employed for more than just fodder and thatching.[9]

MATERIALS AND METHODS

Plant collection

The medicinal plant *Myriostachya wightiana* (Nees ex Steud) Hook.f. of the Poaceae family was collected in October, 2019 from several locations in the Koringa Mangrove woods of Kakinada, Andhra Pradesh. Dr.V.S.Gopal Rao Naidu, Principal Scientist, Central Tobacco Research Institute (CTRI), Rajahmundry, India, verified and authenticated the plant. After removal of adherent particles of *Myriostachya wightiana* (Nees ex Steud) Hook.f. (Poaceae) it has been carefully cleaned, shade dried for a week, and powered. The powdered sample was stored in an airtight container for further analysis.

Chemicals

DMEM (Dulbeuos modified eagles medium), MTT (3-(4,5- dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide), trypsin, EDTA, phosphate buffered saline (PBS) and foetal bovine serum (FBS) from Sigma Research Laboratories Pvt. Ltd, Mumbai, India. Eppendorf, India, provided cell culture flasks of 25 cm^2 and 75 cm^2, as well as 96 well culture plates. The other chemical components and reagents were bought from a nearby supplier.

Extraction

To obtain the extract, 25 gm of dried powdered sample was subjected to Soxhlet extraction with 200 ml of methanol for a period of ten hours. Conventional Soxhlet extraction is still considered one of the most widely used techniques for this purpose, and it plays an important role in the overall analytical process.[9] The resulting extract was then condensed using a rotary evaporator, and the sample was reconstituted in its respective solvent to prepare a stock solution of 100 mg/ml, which was stored in a refrigerator. The extract was then used for conducting studies on antimicrobial activity, qualitative phytochemical analysis, and cell viability. The extract's dry weight was estimated, and thus the dry extract was stored at 200°C for future use.

Phytochemical Screening

For the identification of phytoconstituents, a preliminary phytochemical analysis of the entire plant methanolic was performed. Secondary metabolite identification included alkaloids (Mayer's and Draggendorff's tests), flavonoid contents (Shinoda test), terpenes (Salkowski test), tannins (Ferric chloride test), saponins (Frothing test), Cardiac glycosides (Keller-Killani test), and phenols.[10-11]

Both ash and extract values were obtained using the standard methods with in Indian Pharmacopeia and the World Health Organization recommendations. Total phenolic content (Folin-Ciocalteu method), total flavonoid content (Aluminium Chloride Calorimetric assay) were quantified.[12]

Cytotoxicity Screening

Tumour Cell Lines

For this invitro study the cell lines viz., human breast cancer cell line (MCF-7), human hepatocellular cancer cell line (HepG-2), and human colorectal cancer cell line (CACO-2) are procured from National Centre for Cell Science (NCCS), Pune, India. MCF-7, HepG-2 and CACO-2 cells were cultured in Eagles Minimal Essential Medium supplemented with 10% Foetal bovine serum (FBS) and the antibiotics penicillin / streptomycin (0.5m/lit) under a 5% CO_2 or 95% air environment at 37^0C.

Determination of cytotoxicity using Microculture tetrazolium (MTT) Assay

MTT Asaay [13]

The MTT assay, based on the conversion of the yellow tetrazolium salt-MTT, to purple-formazan crystals by metabolically active cells, provides a quantitative determination of viable cells. Cells are plated on to 96 well plates at a cell density of 2×10^5 mL-1 per well in 100 µL of RPMI 1640 and allowed to grow in CO2 incubator for 24 h (37 ˚C, 5 % CO2). The medium is then removed and replaced by fresh medium containing different concentrations of sample for 48 h. The cells are incubated for 24-48 h (37 ˚C, 5 % CO2). Then, 20 µL MTT ([3- (4, 5-dimethylthiazol-yl)-2, 5- diphenyltetrazolium bromide]) stock solution (5 mg/mL in PBS) is added to each well and incubated for 5 h. The medium is removed and 200 µL DMSO is added to each well to dissolve the MTT metabolic product. Then the plate is shaken at 150 rpm for 5 min and the optical density is measured at 560nm. Untreated cells (basal) are

used as a control of viability (100 %) and the results are expressed as % viability (log) relative to the control. Total optical density of dissolved crystallites in DMSO was determined at 560 nm using a microplate reader. The percentage growth inhibition was calculated using the following formula.

%Inhibition =100 (Control-Treatment) / Control

By using the linear regression equation, y = mx +c the IC_{50} values. The viability graph was used to get y = 50, m, and c values.

Morphology study

Cells were seeded on 24-well then incubated either DMSO as well as extract (with IC_{50} concentration) for 24 hrs. Images were captured by phase contrast microscopy during the treatment period.

Statistical analysis

The study obtained data for cell viability. The values were tabulated to analyze the anticarcinogenic activity of *Myriostachya wightiana* on MCF- 7, HEPG- 2 and CACO-2 cancer cell lines. Statistical analysis was done using SPSS software version 22.0. where the t-value and p-value were calculated to determine the mean absorbance of a sample significantly different from a control value. Significant *p* values (significant probability value) at less than 0.05.

RESULTS

Qualitative phytochemical analysis of extract

Based on therapeutic potential of secondary metabolites, the phytochemical characters of the *Myriostachya wightiana* methanol extract was investigated. The detailed phytochemical analysis of *Myriostachya wightiana* indicated the existence of Carbohydrates, Phenols, tannins, glycosides, flavonoids, and proteins.Table 1 depicts the different physiological data for *Myriostachya wightiana* methanolic extract. Table 2 displays the presence of the above said phytochemicals.

The extract was used to determine the entire phenolic content and total flavonoid content, which revealed 58.91$\pm$0.352%w/w equivalent gallic acid and 46.62 $\pm$ 0.337mg/g equivalent quercetin with 1000µg/ml concentration of *Myriostachya wightiana* as shown in table 4 and 6 respectively. By utilising the calibration data with the total phenolic content in the table 3 a

calibration curve was constructed with the data expressed as mean ± SD as depicted in Figure 1. Similarily, the entire phenolic content in the methanolic extract of *Myriostachya wightiana* was calculated using the calibration data in table 5 and a calibration curve was constructed with the data expressed as mean ±SEM as shown in Figure 2.

Table 1 shows the different physical parameters for *Myriostachya wightiana* methanolic extract.

Parameters	***Extract***
Loss on drying	5.19
Total ash	19.15
Acid insoluble Ash	6.46
Water soluble Ash	10.34
%CFC	15.7
Swelling Index	3
Foaming index	9

Table 2 Phytochemical screening *Myriostachya wightiana Whole plant Methanolic Extract*

Test	**Extract Results**
Alkaloids	+
Carbohydrates	+
Tannins	+
Flavonoids	+
Steroids & Terpenoids	-
Glycosides	+
Saponins	-
Phenols	+
Proteins	-
Fixed oils & Fats	+

+indicates Presence -- indicates Absence

Table 3: Calibration data for total phenolic content of *Myriostachya wightiana* methanolic extract	
Gallic acid concentration (µg/mL)	Absorbance (Mean ± SD)
20	0.873±0.004
40	0.961±0.006
60	1.255±0.001
80	1.418±0.009
100	1.643±0.003

Table 4: Total phenolic content estimation of *Myriostachya wightiana* extract		
Treatment	Concentration (µg/mL)	Total phenolic content (mg/g of gallic acid equivalent) (Mean ± SD)
Extract	1000	58.91±0.352

Results are mean of three values ± SD

Table 5: Calibration data for total flavonoid content of *Myriostachya wightiana* extract	
Quercetin concentration (µg/mL)	Absorbance (Mean ± SD)
20	1.352±0.02
40	1.464±0.04
60	1.568±0.01
80	1.639±0.07
100	1.745±0.02

Table 6: Total flavonoid content estimation of *Myriostachya wightiana* extract		
Treatment	Concentration (µg/ml)	Total flavonoid content (mg/g of quercetin equivalent) (Mean ±S.D)
Extract	1000	46.62±0.337

Results are mean of three values ± SD

Table 7: Determination of cytotoxicity of the Methanolic extract of *Myriostachya wightiana* by MTT assay

Plant Extract	**Conc (µg)**	**% Cell Viability**		
		MCF-7	**HEPG 2**	**CACO 2**
Methanolic Extract of *Myriostachya wightiana*	5	96.37	97.55	98.11
	10	90.49	92.16	91.96
	25	81.49	79.09	84.71
	50	66.09	68.8	70.67
	100	55.02	57.68	57.1
	Control	96.37	97.55	98.11

Table 8: Determination of cytotoxicity of the Methanolic extract of *Myriostachya wightiana* by MTT assay on MCF-7

Concentration µg/ml	**Absorbance at wavelength 570nm**	**% Inhibition**	**% Viability**	**IC_{50} µg**
5	0.557	3.63	96.37	
10	0.523	9.51	90.49	
25	0.471	18.51	81.49	
50	0.382	33.91	66.09	
100	0.318	44.98	55.02	
Untreated	0.578	0	100	103.47
Blank	0	0	0	

Table 9: Determination of cytotoxicity of the Methanolic extract of *Myriostachya wightiana* by MTT assay on HEPG-2

Concentration µg/ml	**Absorbance at wavelength 570nm**	**% Inhibition**	**% Viability**	**IC_{50} µg**
5	0.597	2.45	97.55	
10	0.564	7.84	92.16	
25	0.484	20.91	79.09	
50	0.421	31.2	68.8	
100	0.353	42.32	57.68	
Untreated	0.612	0	100	110.22
Blank	0	0	0	

Table 10: Determination of cytotoxicity of the Methanolic extract of *Myriostachya wightiana* by MTT assay on CACO-2

Concentration µg/ml	Absorbance at wavelength 570nm	% Inhibition	% Viability	IC_{50} µg
5	0.622	1.89	98.11	
10	0.583	8.04	91.96	
25	0.537	15.29	84.71	
50	0.448	29.33	70.67	
100	0.362	42.9	57.1	
Untreated	0.634	0	100	110.85
Blank	0	0	0	

Table 11: Determination of cytotoxicity of the Methanolic extract of *Myriostachya wightiana* IC_{50} values

S. No	Sample Name	IC_{50} (µg/ml)		
		MCF 7	HepG2	$CaCO_2$
1	Methanol Extract	103.47	110.22	110.85
2	Vinblastine	4.82	4.41	31.29

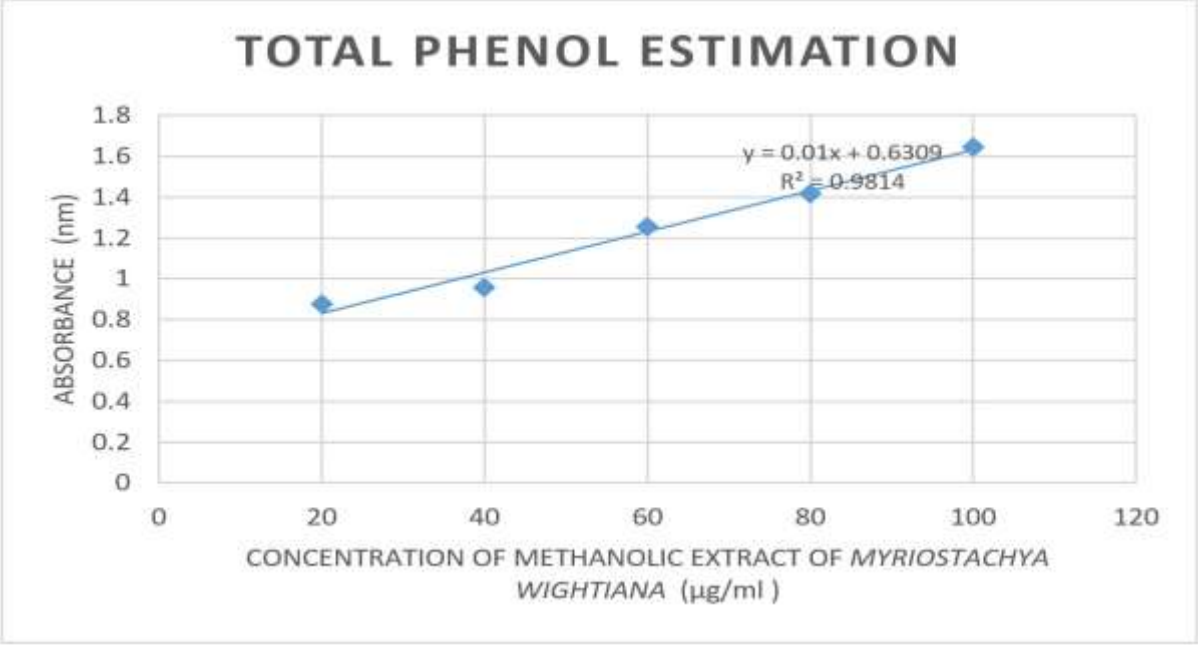


Figure 1: Total phenolic content estimation of *Myriostachya wightiana* extract

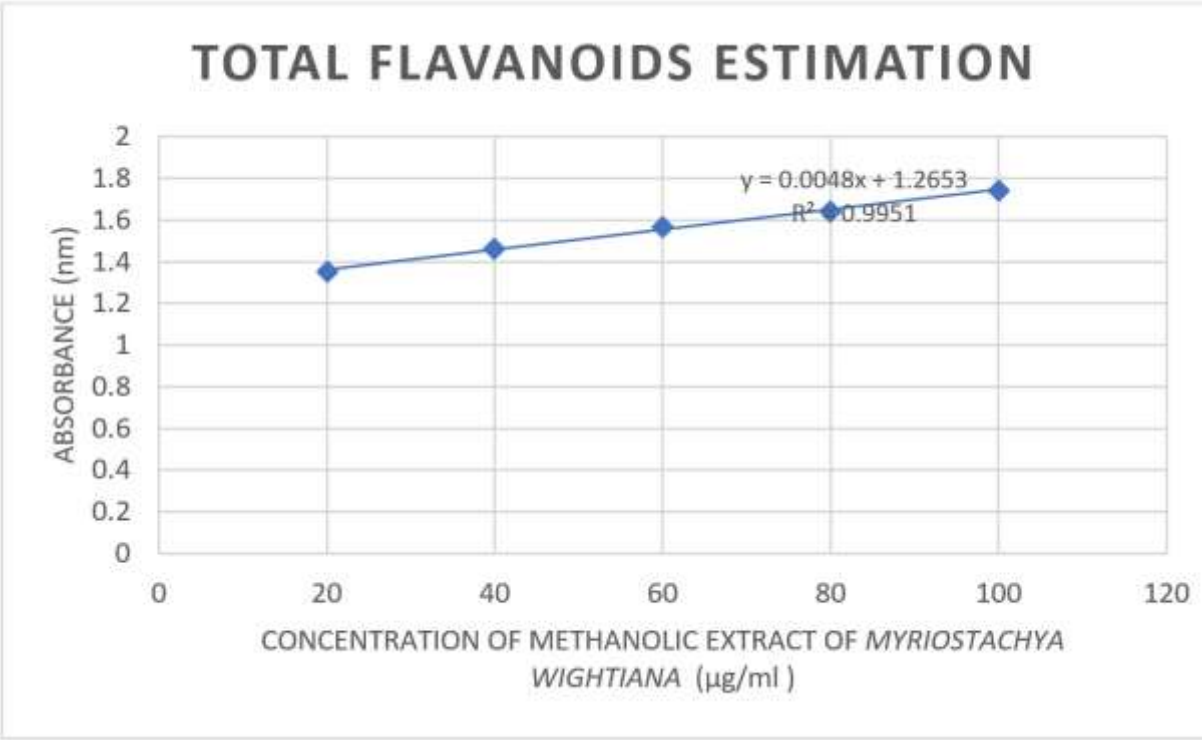
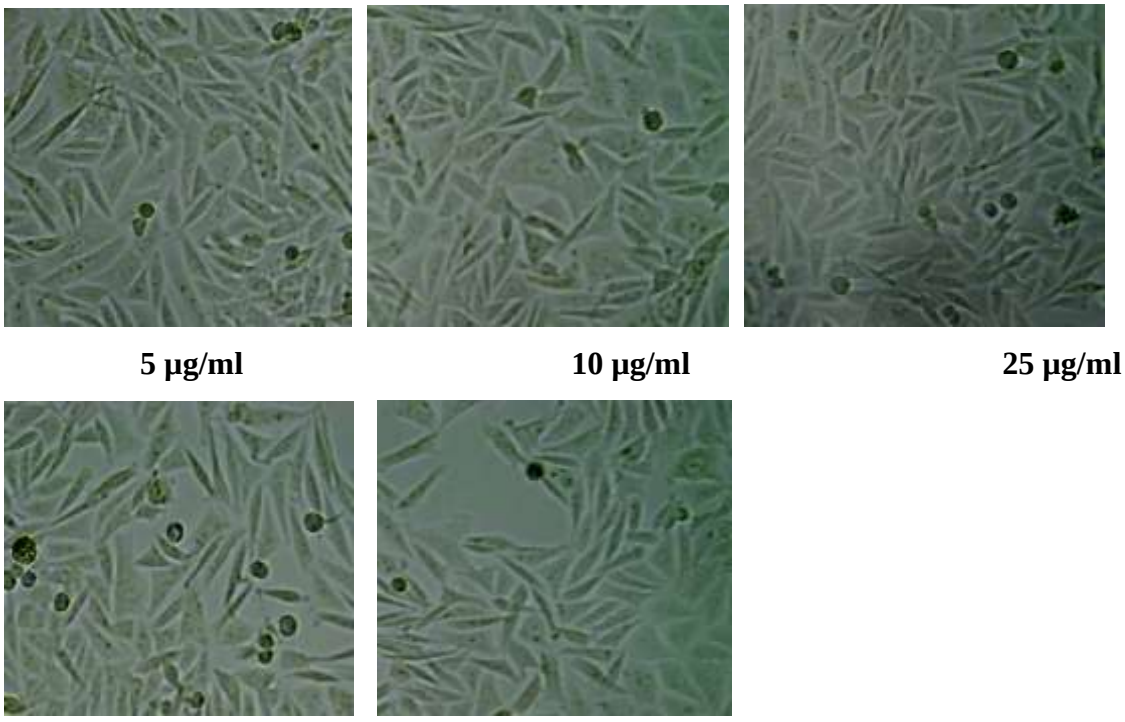


Figure 2: Total flavonoid content estimation of *Myriostachya wightiana* extrac

Figure 3: Phase contrast images with 5, 10, 25, 50, 100 µg/ml on MCF-7



5 µg/ml **10 µg/ml** **25 µg/ml**

50 µg/ml **100 µg/ml**

Figure 4: Phase contrast images with 5, 10, 25, 50, 100 µg/ml on HEPG-2

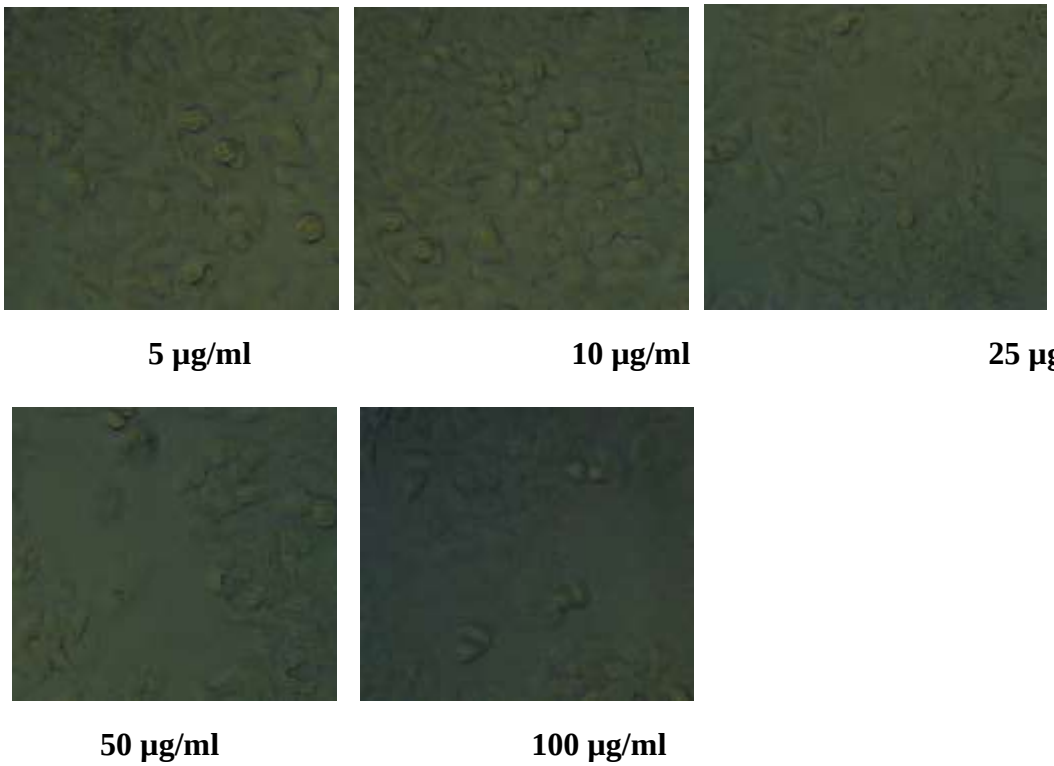
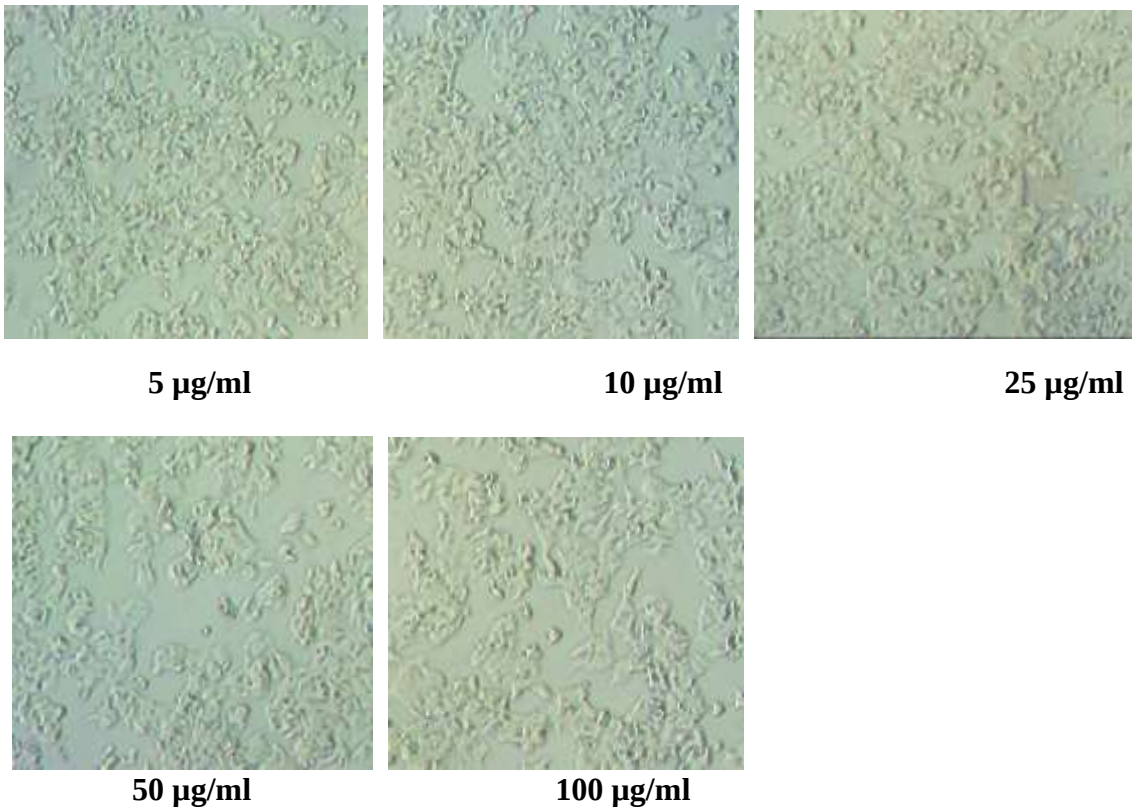


Figure 5: Phase contrast images with 5, 10, 25, 50, 100 µg/ml on CACO-2



Cytotoxic potential of Methanolic extract of *Myriostachya wightiana*

The percentage cell viability with varying concentrations such as 5,10,25,50,100 µg of methanolic extract of *Myriostachya wightiana* was performed to determine the cytotoxicity on different cell lines MCF- 7, HEPG- 2 and CACO2 with the control drug vinblastine by MTT assay as depicted in table 7.

The cytotoxic potential of the methanolic extract of *Myriostachya wightiana* was performed with different concentrations as percentage inhibition, percentage viability and IC_{50} values as the major parameters with the absorbance at 570nm wavelength on cell lines MCF- 7, HEPG-2 and CACO2 with the data given in the tables 8,9,10. The IC_{50} values of methanolic extract of *Myriostachya wightiana* were 103.47, 110.22 and 110.85 µg/ml respectively for incubation period of 24hr as shown in table 10.On X-axis concentration in µg and on Y-axis percentage viability has been taken respectively, the MTT assay findings revealed that *Myriostachya wightiana* methanolic extracts inhibited proliferation in breast cancer cell lines MCF-7 as depicted in Figure 6, hepatocellular carcinoma cells HepG-2 as depicted in Figure 7, and colon cancer cells CACO-2 as depicted in Figure 8. Methanolic extract of *Myriostachya wightiana* treated with MCF- 7, HEPG- 2 and CACO-2 cells showing the IC_{50} values with reference to the standard drug Vinblastine were provided in comparison to each type of cell line that is depicted in the Figure 9 clearly indicated the percentage of inhibition with rise in the concentration of the extract. The half maximal inhibitory concentration (IC_{50}) of methanolic extract of *Myriostachya wightiana* determined the appropriate concentration to kill the 50% of the cells with reference to the standard drug Vinblastine was clearly shown in the Figure 10.

The percentage of cell death was observed with increase in the concentration of the methanolic extract of *Myriostachya wightiana* with indication of necrotic cells by decrease in the percentage of viable cells. The *Myriostachya wightiana* methanolic extract showed the anticancer activity against MCF- 7, HEPG- 2 and CACO-2 cell lines in a dose dependant manner. A similar observation was done in the cell lines MCF- 7, HEPG- 2 and CACO-2 treated with methanolic extract of *Myriostachya wightiana* with the rise in concentration as shown in the Figure 3, 4 and 5 respectively.

DISCUSSION

Cancer incidence has been steadily increasing, with the majority of cases being related to numerous lifestyle factors such as smoking tobacco, dietary habits that include minimal or no vegetables and fruits, lack of exercise, heavy drinking, sun exposure, and environmental

pollutants.[14] Even early detection and diagnosis of cancer can increase the survival rate with reduction in the mortality rate in contrast to that there is moderate rise in the incidence of cancer worldwide. Henceforth, there is obviously emergence need for the evolution of new drugs or chemical entities which will specifically act on cancer targeted cells.

Here the present investigation explored the anticancer activity of *Myriostachya wightiana* methanol extract against different cell lines like MCF-7, CACO-2 and HEPG-2. Based on their sensitivities to the extract MCF-7, CACO-2, HEPG-2 cells were then chosen for further studies.MCF-7 cells were more sensitive compared to HEPG-2 and CACO-2 with the inhibitory concentrations.

MCF-7, HepG-2 and CACO-2 exhibited remarkable percentage of cell inhibition in a dose dependant manner. The susceptibility of the cells to the extract was characterised by IC_{50} values.The IC_{50} values of MCF-7, HepG-2 and CACO-2 were found to be 103.47 $\pm$ 0.78, 110.22 $\pm$ 1.02, 110.85 $\pm$ 1.01 respectively with the methanolic extract of *Myriostachya wightiana* which indicated that inhibitory proliferation of cells with gradual increase in the dose. The magnitude of these effects are greater with gradual increase in the dose of the extract (100 µg). *Myriostachya wightiana* methanolic extract showed least IC_{50} value as 103.47 µg/ml for MCF-7 cell lines. Myriostachya wightiana contains a high concentration of important phytochemicals, including phenols at 1035.9 mg/gm and flavonoids at 4168.2 mg/gm. The methanolic extract contained 171.36.11 mg/gm of tannins as well as antioxidants, indicating its therapeutic efficacy.[10]

CONCLUSION

The current study found that such methanolic extract of *Myriostachya wightiana* exhibits exceptional anticancer properties. As a result, the plant could serve as a source of potential of targets for cancer therapy efforts. There is an urgent need for more research into this plant so order to uncover and isolated its active anticancer components. The study findings must also be validated being used in vivo models. Furthermore, additional research is required to define intermediary implicated in the mechanism of growth inhibition activity.

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would also like to thank Vaahu's solutions, Hyderabad for carrying out the invitro cell line study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

NCCS: National Centre for Cell Science; **DMEM:** Dulbecco's Modified Eagle Media; **MTT:** 3-(4,5- dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide; **FBS:** Foetal Bovine Serum; **PBS**: Phosphate Buffered Saline**;** $\mathbf{IC_{50}}$: Half maximal inhibitory concentration; **SD**: Standard deviation; **SEM**: Standard error of the mean

SUMMARY

Mangroves are of vital ecological importance as they are of high in medicinal properties with the unique property of salt resistant that can withstand high salinity conditions with wide application in the treatment of many ailments. *Myriostachya wightiana* a mangrove grass plant belonging to Poaceace family has shown their efficacy in inhibiting the cancer cells with percentage viability and IC_{50} values. Methanolic extract of varying concentrations were studied on different cell lines MCF-7, HEPG-2 and CACO-2 with the percentage cell viability and IC_{50} values indicating their role in inhibiting the growth of cancer cells.Present study investigated the crucial role of *Myriostachya wightiana* with its invitro anticancer activity on cancer cell lines MCF-7, HEPG-2 and CACO-2.The investigations also imparted the presence of phytochemicals in it.

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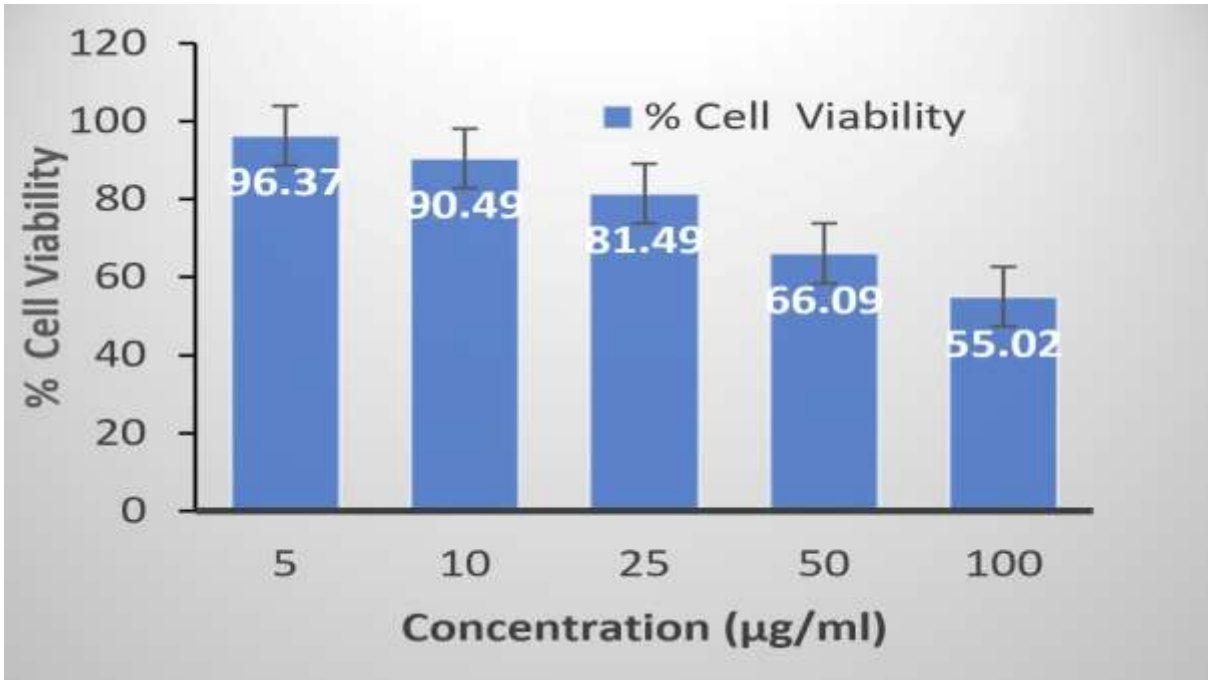

Figure 6: Cell viability assay on MCF-7

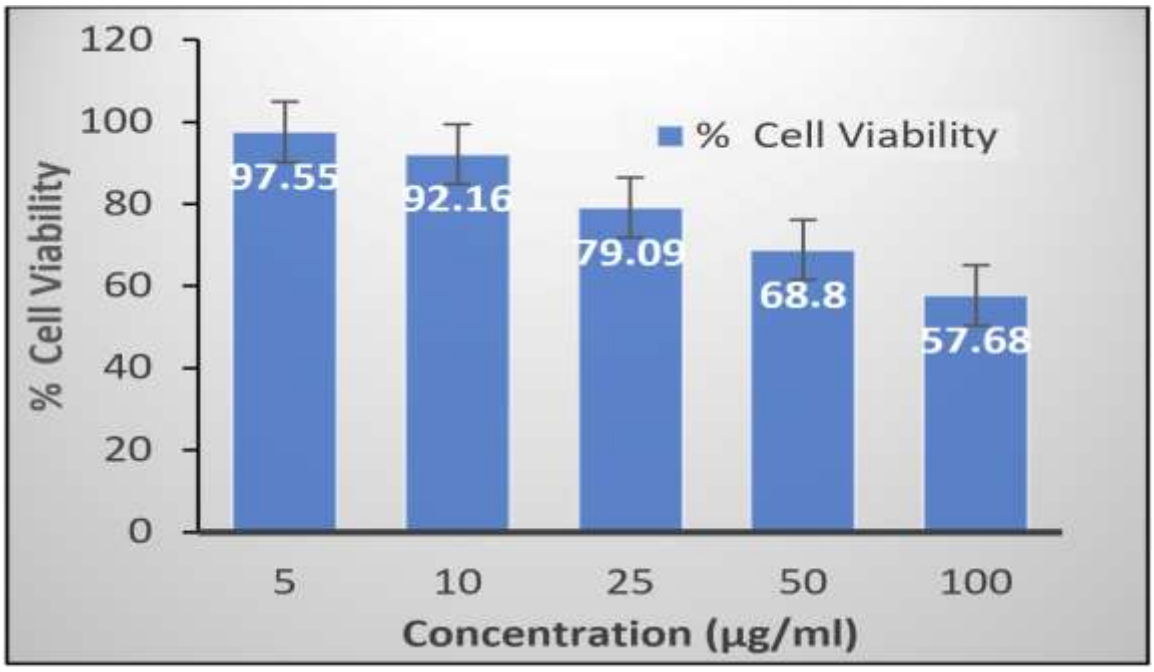

Figure 7: Cell viability assay on HEPG-2

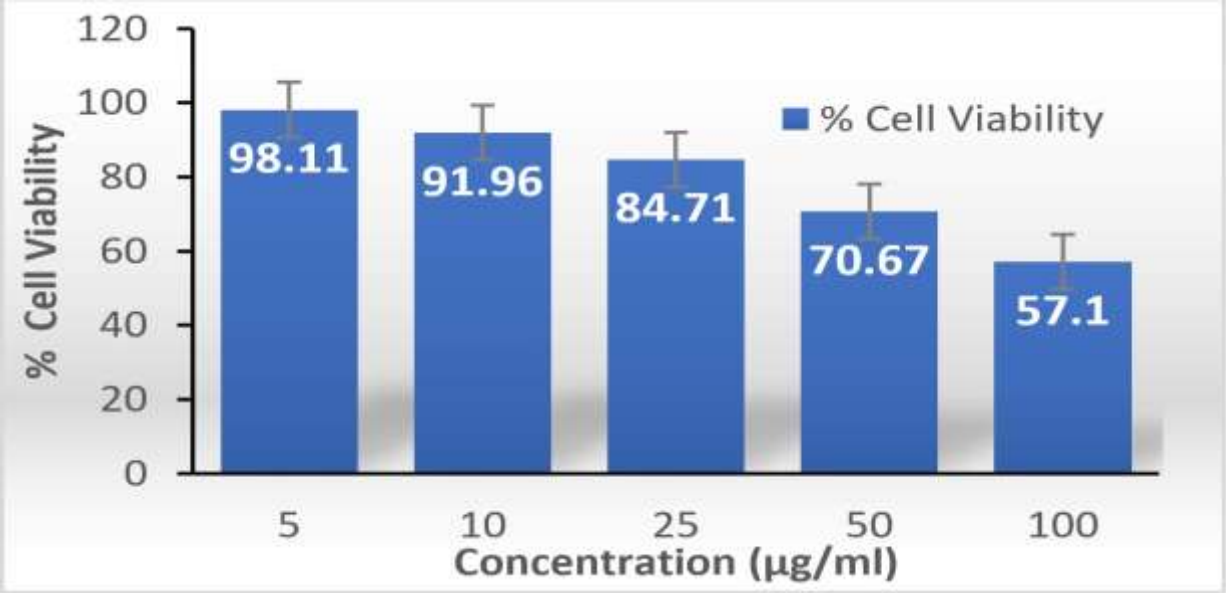

Figure 8: Cell viability assay on CACO-2

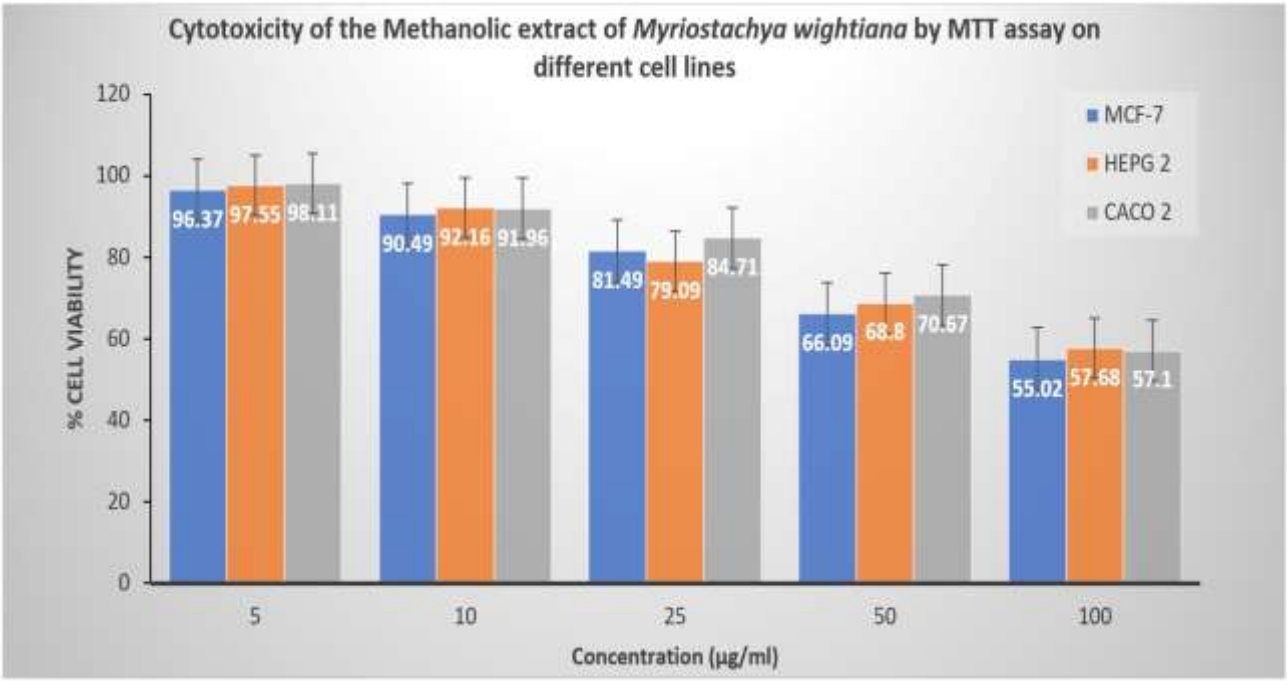

Figure 9: Cytotoxicity of the Methanolic extract of *Myriostachya wightiana* by MTT assay on different cell lines

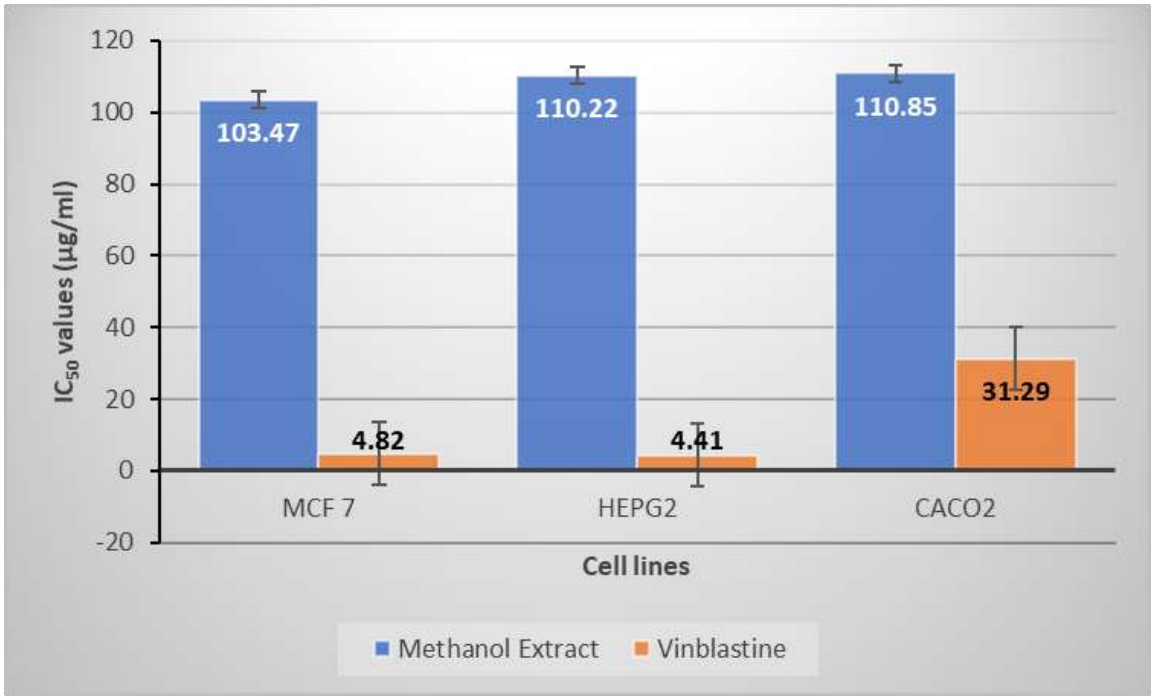

Figure 10: IC 50 values of methanolic extract of *Myriostachya wightiana* on different cell lines with reference to the standard drug Vinblastine