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In Vitro Anticancer Activity Of Methanolic Extract Of Porteresia Coarctata With Special Emphasis On Human Cancer Cell Lines

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Abstract
Porteresia coarctata (Roxb.) The Tateoka is an Indian native wild rice grass, a species of Poaceae, with important medicinal properties. Cancer is an evil being that causes the painful signs of death because the disease has spread globally. There is mounting demand in the production of novel anticancer therapies to treat cancer and relieve cancer-related symptoms. Mangroves are ecologically important because they contain a high number of potential bioactive chemicals and compounds that can be used in the treatment of cancer. The development of new chemical compounds that have been approved to treat cancer over the years is aided by natural sources. This aim was to evaluate the anticancer activity of the methanolic extract of the whole plant of Porteresia coarctata in in vitro human cancer models. The extract of Porteresia coarctata was obtained as the crude methanol extract of the entire plant and examined in terms of phytochemicals (flavonoids, alkaloids, carbohydrates, tannins and other secondary metabolites). Its cytotoxic activity was in vitro tested against three human cancer cell lines, MCF-7 (breast cancer), CACO-2 (colorectal cancer) and HEPG-2 (hepatocellular carcinoma) by the MTT assay . Three independent experiments were performed to evaluate cell viability, evaluating five different concentrations of the extract (5, 10, 20, 50 and 100 µg/ml) in triplicate. The seeded cells were placed in 24 well plates and treated with either DMSO (vehicle control) or extract in concentrations necessary to kill 50 percent of cell viability over the 24 hours. Morphological changes were observed during treatment by phase contrast microscopy. All of the three cell lines revealed dose-dependent cytotoxicity in the extract. At the maximum concentration assayed (100 µg/ml), the concentrations of MCF-7, HEPG-2, and CACO-2 cells needed to inhibit 50 percent of cell viability were 103.47 µg/ml, 110.22 µg/ml, and 110.85 µg/ml, respectively. The anti-cancer effect of the methanolic extract of P. coarctata was confirmed by a high level of cell inhibition in a concentration-dependent manner. The concentration needed to cause an inhibition of 50 percent of cell viability was used to determine the sensitivity of the cells to the extract. Porteresia coarctata methanolic extract was dose-dependent in antitumor activity. It also worked on human breast cancer cell line (MCF-7), human colorectal cancer cell line (CACO-2) and human hepatocellular carcinoma cell line (HEPG-2). This indicates that more research should be done to determine and isolate the active anticancer components of the plant.
Keywords: *Porteresia coarctata, methanol-derived extract, in vitro cytotoxicity, human cancer cell lines.*

OVERVIEW

Cancer is a cancerous object that causes critical signs of death because the disease spreads globally. Cancer is an important and increasing population health issue in India. No data is available for trends in cancer cases in 2022 but the estimated cancer incidence was 1,461,427 new cases including a crude incidence of 100.4 per 100,000 population. According to epidemiological estimates, the cancer incidence rate is estimated to be one per nine persons in India. The situation seems to be that lung cancer is most common among men, and breast cancer is most common in women, hence these cases of the disease require special preventive, early diagnostic, and treatment measures. Lung cancer is the most common type of cancer diagnosed in men in India but breast cancer is the most common cancer in women. By 2025, the occurrence of cancer in the country is likely to increase by about 12.8% relative to 2020. It is projected that there will be nearly 10.9 million new cancer cases, 6.7 million cancer deaths and over 24.6 million cancer survivors globally in 2022 [1]. In the United States, it is projected that approximately 1.92 million new cancer cases will occur and approximately 609,000 deaths will happen in the same year, representing a 8.8% growth in cancer cases and a 0.41% growth in cancer deaths over 2019 [2]. It is estimated that there were 19.3 million new cancer cases globally in 2020 that caused about 10 million deaths. The most commonly diagnosed type was breast cancer with an estimated 2.3 million new cases (11.7 per cent), followed by lung cancer (11.4 per cent), colorectal cancer (10 per cent), prostate cancer (7.3 per cent), and

stomach cancer (5.6 per cent). Eastern Asia also registered the most cases (6.0 million new cases 31.1 per cent of all cases as well as 3.6 million deaths 36.3 per cent) in the region. North America had 2.6 million cases (13.3) and 7 percent of worldwide cancer deaths, whereas South-Central Asia had 1.95 million cases (10), and 1.3 million deaths (12.6). In Europe alone, it recorded 4.4 million new cases (equivalent to 20% of all cases) and 1.9 million fatalities, and the high morbidity and mortality rates underpin the urgent need to find new approaches to cancer treatment. Considering the rate of rise, there is growing need and task to come up with new anticancer treatments to curb and treat cancer and to ameliorate cancer related symptoms.

Mangroves are also ecologically important because they are rich in bioactive compounds and may contain molecules that could be used in a variety of ways in cancer therapy. A good number of the clinically approved anti-cancer agents are naturally obtained, and thus, there is a great opportunity to identify new therapeutic agents among these plants. The bioactive constituents of mangroves have proven to be effective against a number of different cancers such as breast, stomach, lung, colon, prostate and leukemia [5].

Mangroves family Poaceae is highest in variety and range of therapeutic activity, and may have some therapeutic use in cardiovascular disease and in Parkinson disease, as well as in autoimmune disease. This family has also been noted to have antibacterial, antiviral, anti-inflammatory, anti-fungal and antioxidant properties. Besides their medicinal importance, Poaceae species are a highly promising source of crucial bioactive compounds, which explains their significant focus as the subject of investigation in finding and synthesizing new therapeutic agents [7].

Porteresia coarctata (Poaceae) is a perennial tutfted grass with a height of 121 to 160 cm, which is commonly found in the tropical mangrove forest in India. The leaves are mainly basal-elongated and 1-1.4 m long and 1220 mm wide, acuminate, glabrous, and smooth. The sheaths are stout and the ligule is thinned out to a small slender rim. It has long spikelets of about 15 cm in diameter and 5-10 cm in width, a smooth rachis, thick bundles, and verticillate branches. The pedicel spikelets are homogenous, 5-7 mm in length and contain 4-8 flowering caryopses with attached pericarps which are ovoid and laterally pressed [8]. P. coarctata has been tried in a variety of other uses in addition to its conventional use as fodder and thatching [9].

METHODOLOGY

Botanical collection

One of the medicinal plants that we gathered in October in the Koringa Mangrove woodlands, in a few places near Kakinada in Andhra Pradesh was Porteresia coarctata (Roxb). It is a member of Poaceae. Ms. K.N.V.S.N. Eswari, Head of the Botany department of A.S.D. Govt. Degree College for Women (A), Kakinada, India confirmed and authenticated the plant. After removing adhering particles of Porteresia coarctata (Roxb.) Tateoka (Poaceae), this has been thoroughly washed, dried in shade one week, and then ground. The powdered specimen was stored in a hermetically sealed unit to be later analysed. The chemicals used are DMEM (Dulbecco Modified Eagle Medium), MTT (3 (45 dimethylthiazol-2) 2 5 diphenyl tetrazolium bromide), trypsin, EDTA, phosphate-buffered saline (PBS), and fetal bovine serum (FBS) that were purchased by Sigma Research Laboratories Pvt. Ltd., Mumbai, India. Cell culture flasks of 25 cm 2 and 75 cm 2 as well as the 96 well culture plates were supplied by Eppendorf, India. All the other chemical components and reagents were sourced locally.

Extraction

Powdered plant material (25 g) was prepared to extraction by a Soxhlet unit in which a solvent (methanol, 200 ml) was placed, and the extraction was conducted during 10 hours. The Soxhlet method may also be said to be a standard yet a good technique of phytochemical research, and has been included in the category of analytical research [9]. Once the extraction had taken place, the solvent was removed through the use of a rotary evaporator and the concentrated residue was re-dissolved in Methanol to form a stock solution of 100 mg/ml. This stock was stored in refrigeration waiting to be used. Methanol extract was then subjected to antibacterial testing, phytochemical profiling and cell viability testing. The extract was then weighed to determine the dry weight and the substance was kept at -20 C to be used in future experiments.

Phytochemical Analysis

In the first step of a phytochemical analysis of the methanolic extract, the most significant groups of secondary metabolites have been identified. The typical qualitative assays have been used, and they proved the presence of the alkaloids (Mayer/Dragendorff reagents) and flavonoids (Shinoda test), as well as the terpenoids (Salkowski assay), tannins (ferric chloride reaction), saponins (frothing test), cardiac glycosides (Keller-Kiliani test), and phenolic substances [10-11]. Extractive and also ash values were determined according to the guidelines provided in the Indian Pharmacopoeia and that developed by the World Health Organization. Under quantitative estimation, the determination of Folin-Ciocalteu reagent to estimate the total phenolic content and the determination of the total flavonoid concentration through the aluminum chloride colorimetric method were made [12].

Assessment of Cytotoxicity Neoplastic Cell Lines

The National Centre for Cell Science (NCCS), at Pune, India, India, was the source of the human breast cancer cell line (MCF-7), human hepatocellular cancer cell line (HepG-2) and human colorectal cell line (CACO-2) in this in vitro study. MCF-7, HepG-2, and CACO-2 cells were cultured in an Eagle Minimal Essential Medium with 10% fetal bovine serum (FBS) and penicillin / streptomycin antibiotic (0.5 mL/L) in 5% CO 2 and 95% air atmosphere at 37 C.

Evaluation of cytotoxicity by the Microculture Tetrazolium (MTT) Assay

MTT Assay [13]

MTT assay was used to determine cell viability quantitatively by the capacity of metabolically active cells to reduce the yellow tetrazolium salt (MTT) to insoluble purple formazan crystals. A density of 2 × 10-5 cells/mL was applied to cells seeded in 96-well microplates with 100 µL of RPMI-1640 medium and incubated 24 hours at 37 C in humidified medium with 5 per cent CO 2. The test sample incubated was removed and fresh medium with different concentrations of the test sample placed in the culture media kept further 2448 hours under the same conditions. After that, 20 µL of the MTT stock solution (5 mg/mL in PBS) was put in each well and allowed to incubate in 5 hours. The solvent, a supernatant, was slowly removed and 200 µL dimethyl sulfoxide (DMSO) was put into the solvent to dissolve formazan crystals. This plate was then incubated at 150 rpm with 5 minutes and the absorbance was measured at 560 nm in microplate reader. Unstimulated cells were used as control (taken to be 100 percent viable) and cell viability was reported as a percentage relative to group control. The percentage growth inhibition was determined by using the following formula:

$$\% \text{ Inhibition} = \text{Control } 100 \times (\text{Control} - \text{Treatment})$$

half-maximal inhibitory concentration (IC_{50}) was determined from the dose–response curve using the linear regression equation $y = mx + c$, where viability values corresponding to $y = 50$ were used to estimate the maximum inhibitory concentration.

Study of morphology

Cells were grown in 24 well plates and exposed to DMSO and extracts at the concentration it took to cause 50 percent cell growth inhibition over 24 hours. Phase contrast microscopy was used to acquire images throughout the duration of the treatment.

Statistical examination

The study obtained information on the viability of the cells. The data were summarized to test the anticarcinogenic effect of Porteresia coarctata on MCF-7, HEPG-2 and CACO-2 cancerous closests. Statistical analysis was performed using SPSS software version 22.0, whereby t -value and p -value were calculated to determine whether a particular sample was significantly different in its mean absorbance as compared to a control value. P-values under 0.05 are taken to be significant.

RESULTS

Qualitative phytochemical examination of the extract

The phytochemical composition of the methanol extract of Porteresia coarctata was studied because of the pharmacological relevance of the secondary metabolites. Qualitative assays proved the presence of carbohydrates, phenolic constituents, tannins, glycosides, flavonoids and proteins. Table 1 gives the physiological parameters of the extract and Table 2 gives the results of phytochemical screening. A quantitative analysis of sample presented the presence of an overall concentration of phenolic compound of 58.91 ± 0.352 w/w in terms of gallic acid and a flavonoid content of 46.62 ± 0.337 mg/g in terms of quercetin at 1000 ug/ml ratio of extract (Tables 4 and 6). The reference data that were used to construct

a standard curve of phenolic constituents are listed in Table 3, where the results were obtained as mean ± SD (Figure 1). The yield of phenolic was further assessed by a further re-evaluation of the methanolic fraction of P. coarctata based on the calibration data, as indicated in Table 5 and a corresponding calibration plot drawn with results reported as means and SEM (Figure 2).

Cytotoxic potential extract of Porteresia coarctata

Antiproliferative effects of an extract of Porteresia coarctata prepared in methanol were tested on breast, hepatic, and colorectal carcinoma cells (MCF-7, HEPG-2, and CACO-2 respectively) at the following concentrations of 5, 10, 25, 50, and 100 µg with vinblastine as the reference drug (Table 7). The cell viability was measured in these concentrations, and the measurements were given as a percentage of inhibition, percentage survival, and IC 50. The absorbance was measured at 570 nm and the values summarised in Tables 8-10.

Methanol fraction of P. coarctata that exhibited significant growth-inhibitory capacity with respective IC50s of 110.85, 109.85, and 108.36 of breast, hepatic and colorectal carcinoma cells after 24 hours of incubation (Table 10). These data suggest that the extract has a quantifiable inhibitory effect on the viability of cancer cells in a concentration-dependent fashion.

Horizontal axis illustrates the extract concentrations (µg) and vertical axis illustrates the cell viability (percentage). According to the MTT experiment outcomes, the growth of the breast cancer cell line MCF-7, the cells of hepatocellular carcinoma HEPG-2, and colon cancer CACO-2 were inhibited by methanolic extract of Porteresia coarctata as shown in Figures 6, 7, and 8, respectively. The Porteresia coarctata methanolic extract was tested on breast adenocarcinoma (MCF-7), hepatocellular carcinoma (HEPG-2), and colorectal carcinoma (CACO-2) cell lines and demonstrated IC50 values relative to the anti-tumor active drug, vinblastine. Figure 9 shows this comparison of each cell line and clearly shows the corresponding percentage of inhibition with the increasing concentration of the extract. The percentage of living cells reduced and the population of necrotic cells started growing, and the lower the percentage of live cells the lower was the value of the IC50 (Fig. 10). The methanolic extract of Porteresia coarctata showed anticancer activity against the breast adenocarcinoma (MCF-7), hepatocellular carcinoma (HEPG-2), and colorectal carcinoma (CACO-2) cells in an efficacy-dependent manner. A similar test was done on the breast adenocarcinoma (MCF-7), hepatocellular carcinoma (HEPG-2) and colon carcinoma (CACO-2) cells that were treated using a methanolic extract of Porteresia coarctata; the respective changes were correlated with increasing concentration as seen in Figures 3, 4 and 5 respectively.

DELIBERATION

Cases of cancer are on the increase and majority of these are associated with various lifestyle practices such as using tobacco, consuming small amounts of fruits and vegetables, inactivity, excessive consumption of alcohol, excessive sun exposure and exposure to environmental toxins [14]. Cancer can be detected and properly diagnosed at an early stage to provide better survival and lower death rates. Nevertheless, cancer rates in the world are ever-increasing, which makes it clear that next-generation therapeutic agents that will be able to selectively attack malignant cells are urgently needed. In this respect, natural products and plant-derived compounds have received significant interest with their various bioactive constituents, which show potentially anticancer properties with relatively low toxicity. Pactlitaxel, Taxus brevifolia extract has been successfully used to treat various cancer, such as ovarian and breast, non-small cell lung cancer, etc. [20]. Likewise, the alkaloids vinblastine and vincristine, which were obtained by isolating Catharanthus roseus, were widely used against cancer appearances including Hodgkin lymphoma and leukemia19. What is more, the active component of Curcuma longa called curcumin exhibits anticancer actions due to its ability to regulate multiple molecular pathways during cancer development [21]. These are just a few examples demonstrating the potential of phytochemicals as good chemotherapeutic agents. In this study, the author has studied the anticancer effects of the Porteresia coarctata methanol extract on several cell lines such as breast adenocarcinoma (MCF-7), hepatocellular carcinoma (HEPG-2), and colorectal carcinoma (CACO-2) cells. The following studies were further conducted on the three representative human cancer models on the basis of sensitivity to extract. The MCF-7 cells were more sensitive than the hepatocellular carcinoma (HEPG-2) and the colorectal carcinoma (CACO-2) cells with regard to the percentage of cell inhibition in a dose described manner. Half-maximal inhibitory concentration values were used as a measure of the sensitivity of the cells to the extract. The percentage of breast adenocarcinoma (MCF-7), hepatocellular carcinoma (HEPG-2) and colorectal carcinoma (CACO-2) cells were found to be 110.85 + 0.65, 109.85 + 0.67, and 108.36

+ 1.01, respectively, when incubated with the methanolic extract of Porteresia coarctata respectively the half-maximal concentration of the inhibitory concentrations of These effects get increasingly intense as the dosage of the extract (100 µg) increases. Porteresia coarctata methanolic extract reported the lowest value of half-maximal inhibitory concentration of 108.36 ug/ml on MCF-7 cell lines. Porteresia coarctata has a high content of key phytochemicals, which were 1035.9 mg/g in phenols and 4168.2 mg/g in flavonoids. Its therapeutic efficacy is demonstrated by the presence of 171.36 mg/g of tannins and antioxidants in the methanol-derived extract.

CONCLUSION

The findings of the present study indicate that the methanol-derived extract of *Porteresia coarctata* exhibits significant anticancer properties. Consequently, this plant may serve as a promising source of bioactive compounds for the development of novel cancer therapeutics. Further research is warranted to isolate and characterize its potent anticancer constituents. In addition, validation of these results using in vivo models is essential. Moreover, comprehensive studies are needed to elucidate the molecular intermediates and mechanisms underlying its growth-inhibitory activity.

ACKNOWLEDGEMENTS: We would also want to express our gratitude to Inception Solutions, Hyderabad, for conducting the in vitro cell line study.

CONFLICT OF INTEREST: The authors assert that no conflict of interest exists.

ACRONYMS

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

OUTLINE

Exhibiting remarkable salt tolerance and bioactive properties, mangroves support ecosystem stability and medicinal applications. Porteresia coarctata, a mangrove grass species within the Poaceae family, has demonstrated its effectiveness in decreasing cancer cell viability, as evidenced by % viability and IC50 values. The methanol-derived extract at various concentrations was examined on distinct cell lines of breast adenocarcinoma (MCF-7), hepatocellular carcinoma (HEPG-2), and colorectal carcinoma (CACO-2) cells, with % cell viability and half maximal concentration values reflecting its capacity to inhibit cancer cell proliferation. This study examined the significant function of Porteresia coarctata and its in vitro anticancer activities on the breast adenocarcinoma (MCF-7), hepatocellular carcinoma (HEPG-2), and colorectal carcinoma (CACO-2) cells. The investigations also revealed the existence of phytochemicals in it.

Table 1 shows the different physical parameters for *Porteresia coarctata* 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 *Porteresia coarctata 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 *Porteresia coarctata* 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 *Porteresia coarctata* extract		
Treatment	Concentration (µg/mL)	Total phenolic content (mg/g of gallic acid equivalent) (Mean ± SD)
Extract	1000	56.91±0.352

Results are mean of three values ± SD

Table 5: Calibration data for total flavonoid content of *Porteresia coarctata* 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 *Porteresia coarctata* extract		
Treatment	**Concentration (µg/ml)**	**Total flavonoid content (mg/g of quercetin equivalent) (Mean ±S.D)**
Extract	1000	47.54±0.435

Results are mean of three values ± SD

Table 7: Determination of cytotoxicity of the Methanolic extract of *Porteresia coarctata* by MTT assay.				
Plant Extract	**Conc (µg)**	**% Cell Viability**		
		MCF-7	**HEPG 2**	**CACO 2**
Methanolic Extract of *Myriostachya wightiana*	5	99.25	98.12	95.87
	10	91.22	91.87	90.22
	25	83.65	78.94	80.27
	50	75.21	69.1	68.95
	100	54.23	57.68	55.21
	Control	96.37	97.55	95.87

Table 8: Determination of cytotoxicity of the Methanolic extract of *Porteresia coarctata* by MTT assay on MCF-7				
Concentration µg/ml	**Absorbance at wavelength 570nm**	**% Inhibition**	**% Viability**	**IC_{50} µg**
5	0.622	1.89	99.25	
10	0.583	8.04	91.22	
25	0.537	15.29	83.65	
50	0.448	29.33	75.21	
100	0.362	42.9	54.23	
Untreated	0.634	0	100	110.85
Blank	0	0	0	

Table 9: Determination of cytotoxicity of the Methanolic extract of *Porteresia coarctata* by MTT assay on HEPG-2				
Concentration µg/ml	**Absorbance at wavelength 570nm**	**% Inhibition**	**% Viability**	**IC_{50} µg**
5	0.547	2.88	98.12	
10	0.525	7.96	91.87	
25	0.466	21.24	78.94	
50	0.42	33.78	69.1	
100	0.343	50.14	57.68	
Untreated	0.598	0	100	109.85
Blank	0	0	0	

Table 10: Determination of cytotoxicity of the Methanolic extract of *Porteresia coarctata* by MTT assay on CACO-2				
Concentration µg/ml	**Absorbance at wavelength 570nm**	**% Inhibition**	**% Viability**	**IC_{50} µg**
5	0.597	3.25	95.87	
10	0.568	9.64	90.22	
25	0.498	18.12	80.27	
50	0.422	33.24	68.95	
100	0.327	48.57	55.21	
Untreated	0.596	0	100	108.36
Blank	0	0	0	

Table 11: Determination of cytotoxicity of the Methanolic extract of *Porteresia coarctata* IC_{50} values				
S. No	**Sample Name**	**IC_{50} (µg/ml)**		
		MCF 7	**HepG2**	**$CaCO_2$**
1	Methanol Extract	110.85	109.85	108.36
2	Vinblastine	10.52	5.54	33.27

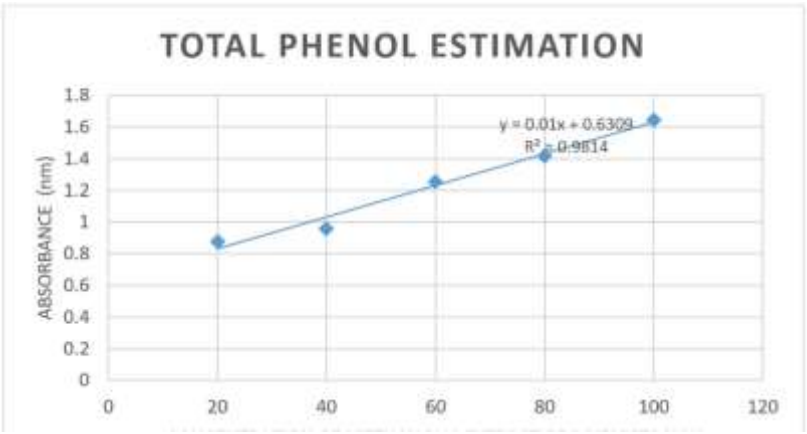


Figure 1: Total phenolic content estimation of *Porteresia coarctata* extract

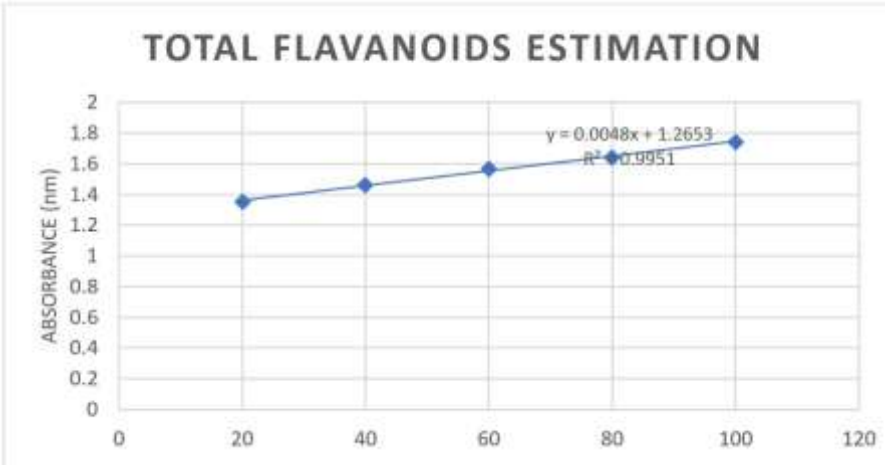
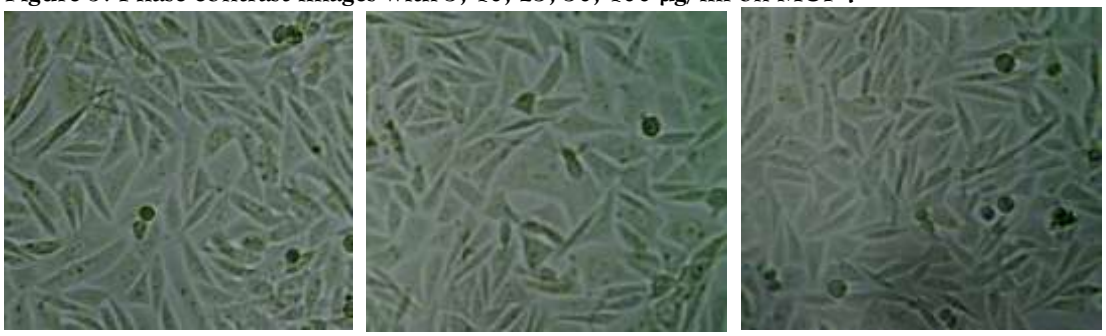
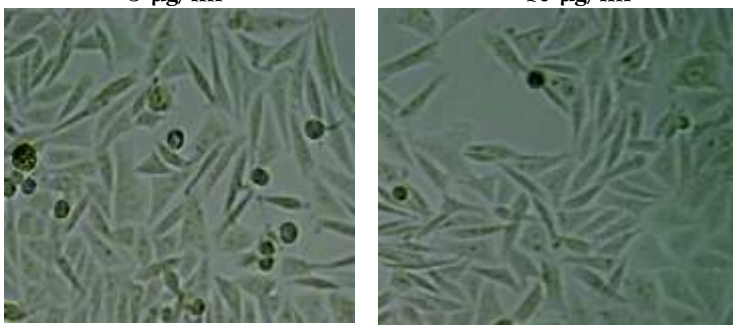


Figure 2: Total flavonoid content estimation of *Porteresia coarctata* extract.

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

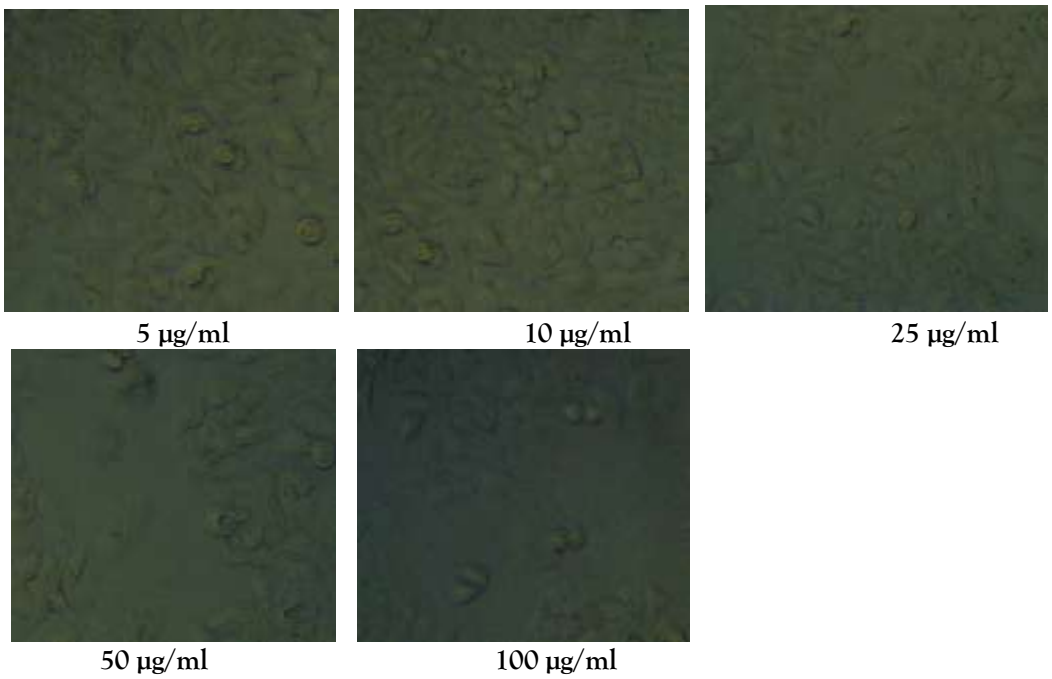
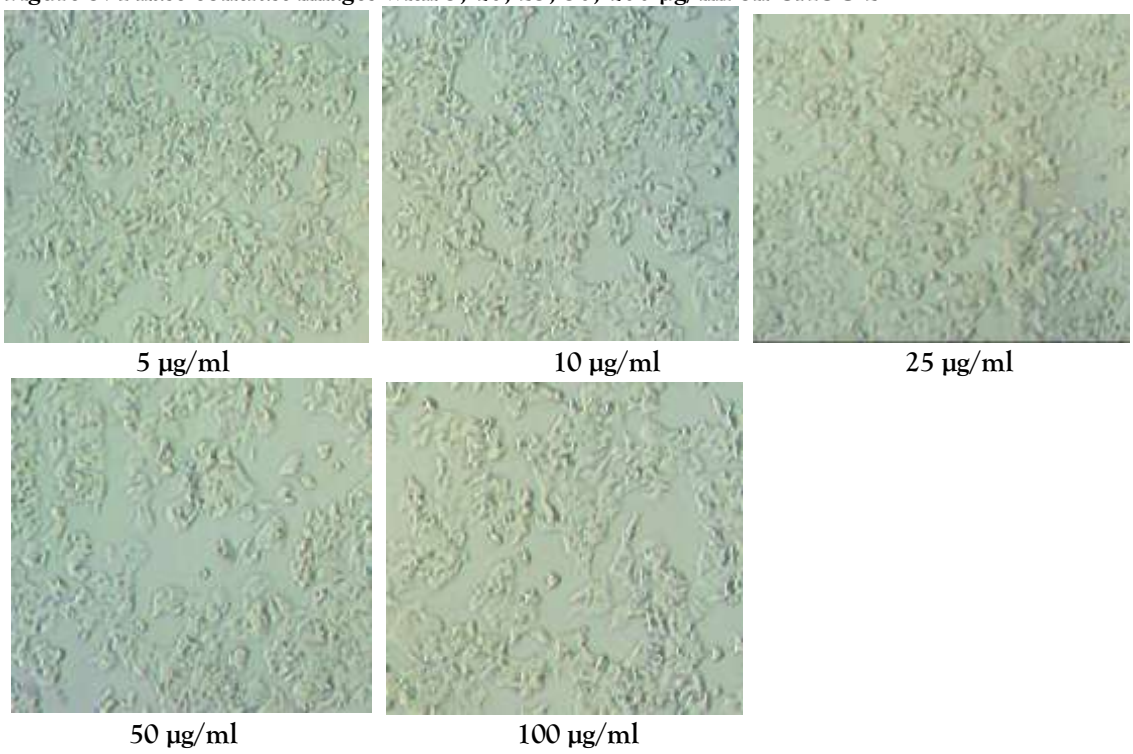


Figure 5: Phase contrast images with 5, 10, 25, 50, 100 µg/ml on CACO-2



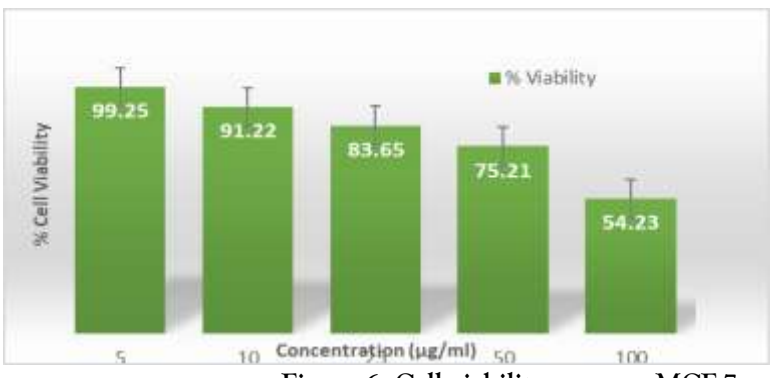


Figure 6: Cell viability assay on MCF-7

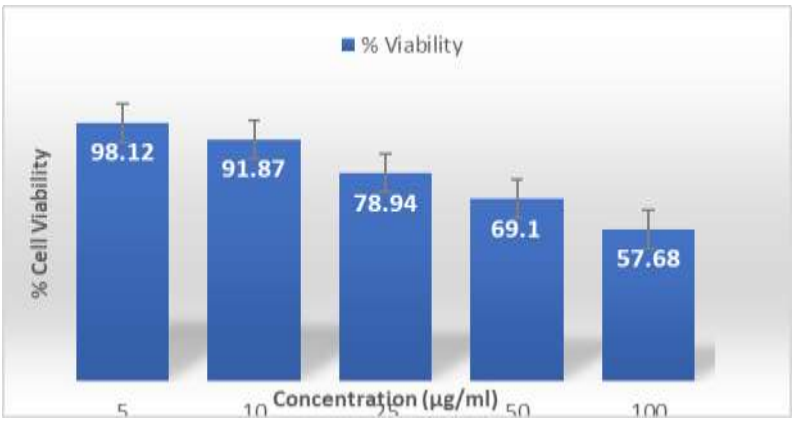


Figure 7: Cell viability assay on HEPG-2

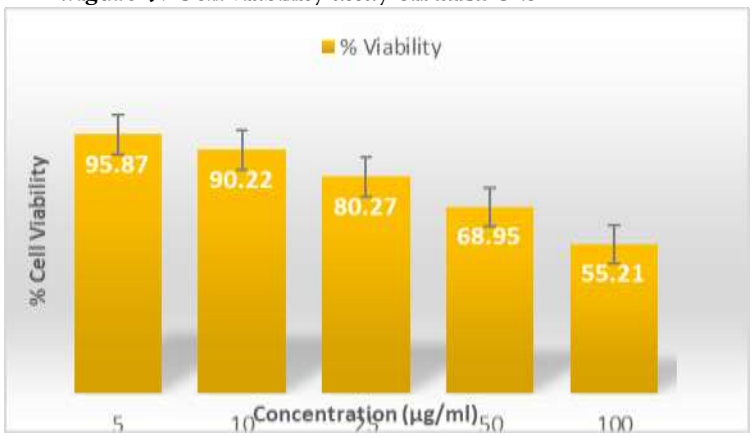


Figure 8: Cell viability assay on CACO-2

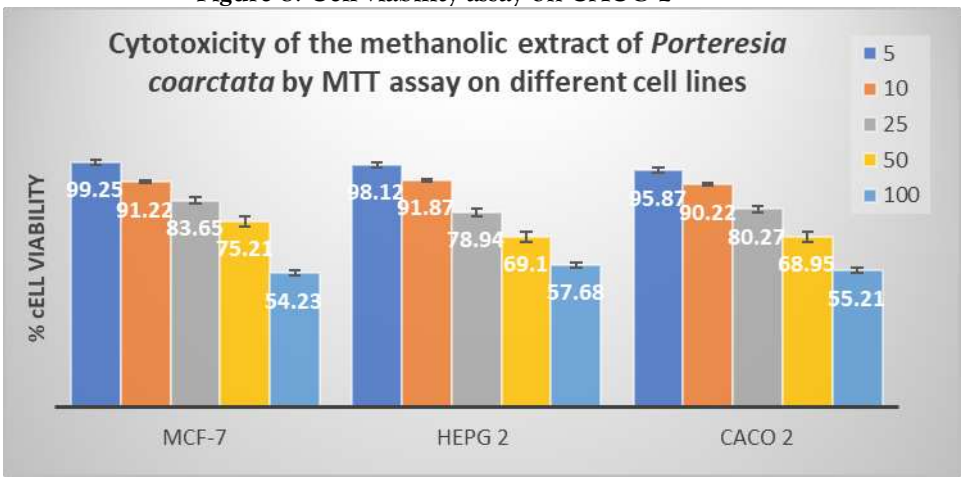


Figure 9: Cytotoxicity of the Methanolic extract of *Porteresia coarctata* by MTT assay on different cell lines

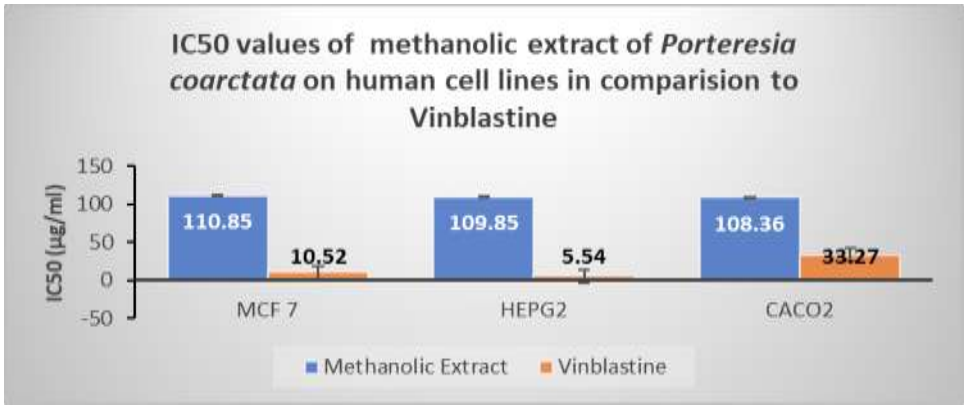


Figure 10: IC 50 values of methanolic extract of *Porteresia coarctata* on different cell lines with reference to the standard drug Vinblastine

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