ANTICANCER AND CYTOTOXIC POTENTIAL OF ETHANOLIC EXTRACT OF *CEIBA PENTANDRA* L. LEAF AND BARK ON HCT-116 CELL LINES.

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Abstract.
The aim of this study is to investigate the cytotoxic effect the ethanolic leaf and bark extracts of *Ceiba pentandra*. In this study phytochemical extraction has been done by cold maceration method using four different types of solvents of increasing polarity (petroleum ether, ethyl acetate, acetone, and ethanol). In the phytochemical analysis the ethanolic leaf and bark extracts of *Ceiba pentandra*, showed the presence of more phytochemicals when compared to other solvent extracts. MTT assay was used to determine the anticancer potential of the selected plant extracts. The ethanol bark extract possessed a higher inhibitory effect against colon cancer cell line, compared to ethanol leaf extract and the IC50 values were 74.22% and 194.52% respectively. In our study observation, ethanol bark extract showed the highest inhibitory effect on colon cancer cell line when compared to the ethanol leaf extract.

***Keywords*: Ceiba pentandra; Cytotoxicity; MTT assay; HCT-116 cancer cells**

1. Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy and the fourth leading cause of cancer related deaths in the world (Bopanna *et al.*, 2013). In India, the estimated statistics for colon cancer the year 2015 for males 12,483 and India females 15,205 which by the year 2020 will ascend to for 13,420 and 19,013 cases respectively (Takiar *et al.*, 2010). The limited success of the currently used clinical therapies including radiation, chemotherapy, immunomodulation, and surgery in treating cancer, as evident by the high morbidity and mortality rates, indicates that there is an imperative need of new cancer management. Due to lack of effective drugs, cost of chemotherapeutic agents, and the side effects such as giddiness, skin discoloration, hypoesthesia, audio-visual impairment, nausea, diarrhea, hair loss, decreased appetite, malnutrition, decreased libido, permanent organ damage, organ failure and internal hemorrhage (Desai *et al.*, 2008) can be a cause of death. On top of that poor diagnostic strategy and unsatisfactory therapy instigate higher mortality rate among CRC patients. This entails the investigation of other opportunities for the treatment of CRC (Hawk and Levin, 2005; Aggarwal *et al.*, 2013) Therefore, efforts should be made to search and formulate chemo-drug with effective naturally occurring anti-carcinogens that would kill the highest number of cancerous cells with maximum therapeutic index to the cancer patients with less adverse effect prevent or even reverse the cancer development (Lee *et al.*, 2006). Medicinal plants have a special place in the management of cancer since it is estimated that plant derived compounds in one or the other way constitute more than 50% of anticancer agents (Veeresham , 2012).

Ceiba pentandra L. Gaertn. belongs to the family Bombacaceae (Jaya and Anilkumar; 2018) and preliminary research has demonstrated its *in vitro* antiviral and antimicrobial properties (Peter and Lateef; 2012).It has also been suggested that it may prove to have potential activity against some cancers (Abouelela *et al.*, 2018). Experimental evidence both *in vitro* and *in vivo* showed that its bark have shown potential efficacy against cancer (Kumar *et al.*, 2016). Besides that, the plant also has anti-hyperglycemic, antioxidant and anti-diabetics activities too (Fofie *et al.*, 2019; Christian *et al.*, 2014).
The present study was carried out to evaluate the anticancer activities of ethanolic extracts of leaves and bark of *C.pentandra* using HCT-116 colon cancer cell lines.

2. Materials and methods

2.1. Collection and identification of plant material

Leaves and bark of *Ceiba pentandra* (L.) Gaertn was collected from Ellispettai Erode, Tamil Nadu, India and the specimen were identified and authenticated by Botanical Survey of India, Coimbatore (BSI/SRC/5/23/2018/Tech/2733). The specimens were stored in Department of Biochemistry, Bharathidasan College of Arts and Science, Erode, Tamil Nadu, India.

2.2. Preparation of plant extracts

Leaves and bark of *Ceiba pentandra* sample were collected, cleaned, shade dried and made into a coarse powder. 10 grams of each plant sample was immersed in a 200 ml of different type of solvents of increasing polarity (petroleum ether, ethyl acetate, acetone, and ethanol). All samples were left at room temperature for three days. Then, samples were filtered using filter paper and concentrated using vacuum rotary evaporator at 80°C prior to drying process. Crude extracts were collected until thick and viscous paste or powder of extract is visible and stored at -20 ^{o}C. Dhar *et al.,* 1968).

2.3. ANALYSIS OF PHYTOCHEMICAL ASSAY IN LEAF AND BARK OF *Ceiba pentandra*

The phytochemical analysis of various solvent extracts of Leaf and bark of *Ceiba pentandra* were tested for the presence of various photochemical such as alkaloids, anthroquinones, flavonoids, phenol, tannins, saponins, terpinoids, and steroids, saponins alkaloids, carbohydrates, flavonoids, amino acids and carbohydrates and oils &resins were done as per standard methods described by [Brain and Turner 1975 ; Evans 1996].

2.4 Anticancer Activity:

Cell Culture:

HCT-116 Colon cancer cell line was purchased from National Centre for Cell Sciences Pune. HCT-116 Cells was cultured and maintained in essential medium (Foetal bovine serum).

Cytotoxicity by MTT Assay:

To determine the cytotoxicity of the ethanolic extract of leaf and bark of *Ceiba pentandra*, cell viability study was done using MTT assay following the procedure of Mosmann *et al.,* 1983. HCT-116 cells in the concentration of 1 ×106 cells/ml were taken into 96 well plates. Then, the cells were treated with different concentration of the ethanolic extract of leaf and bark of *Ceiba pentandra* with different concentration (10 to 300µg/mL) and incubated in the presence of 5% CO2 and 95% humidity at 37 ◦C for 24 h. MTT (5 mg/ml) was added to the incubated-cells, then further incubated for another 4 h. After incubation, DMSO were added in the wells as control and read at 570 nm using photometer. Cytotoxicity and cell viability were calculated by below formula.

Cytotoxicity = Control – Treated/ Control x 100

Cell viability= Treated / Control x 100

Results and Discussion

Phytochemical assay in *Ceiba pentandra* (L.) leaf and bark

The medicinal plants possess natural compounds such as phytochemicals which have chemopreventive activities and preclinical antitumor effects hence leading to the further exploration of the phytochemicals Rao *et al.,* 2008.

Phytochemical screening of various solvents extracts in *Ceiba pentandra* L. leaf and bark showed the presence of various phytochemical compounds. In the leaf ethanol extract, alkaloids, flavonoids, steroids, terpenoids, phenols, tannis and carbohydrates were tested positive. Alkaloids, flavonoids, steroids, terpenoids, phenols, tannis and carbohydrates were tested positive in acetone leaf extracts. In ethyl acetate extract terpenoids, saponins, carbohydrates and oils &resins showed positive results and others showed negative results. In petroleum ether extract terpenoids, saponins, carbohydrates and oils &resins showed positive results and others showed negative results.

In the bark ethanol extract, alkaloids, flavonoids, steroids, terpenoids, phenols and anthroquinones were tested positive. Flavonoids, steroids, terpenoids, phenols and saponins were tested positive in acetone bark extracts. In ethyl acetate extract flavonoids, saponins, carbohydrates and oils &resins showed positive results and others showed negative results. In petroleum ether bark extract saponins, carbohydrates and oils &resins showed positive results and others showed negative results (Table -1).

The need to identify new compounds with anticancer properties that are more effective and less toxic than conventional drugs has motivated research on natural products isolated from plant species. These substances predominantly consist of alkaloids, terpenes, flavonoids, phenolics; have been identified in plant species with documented anticancer activity. According to Gali *et al* (2011) the anticancer effects of methanol extract of *Argemone mexicana* Linn. leaves may be related to their content of flavonoids. According to Pradhan (2014), flavonoids may exert their chemopreventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis. Among four solvent extractions, ethanol is high polarity solvent and leaf and bark ethanolic extract showed the presence of high quantity of phytochemicals. Hence, the leaf and bark ethanolic extract were preferred for further analysis.

Table 1: Phytochemical assay in *Ceiba pentandra* (L.) leaf and bark

	leaf extract				Bark extract			
Name of the Phytochemical	**E**	**A**	**E.A**	**P.E**	**E**	**A**	**E.A**	**P.E**
Alkaloids	++	+	-	-	+	-	-	-
Flavonoids	+	+	-	-	++	+	+	-
Steroids	++	+	-	-	+	+	-	-
Terpenoids	++	+	+	-	+	+	-	-
Anthroquinones	-	-	-	-	+		-	-
Phenols	+	+	-	-	+	+	-	-
Saponins	-	-	+	+	-	+	+	+
Tannins	+	+	-	-	-	-	-	-
Carbohydrates	++	+	+	+	-	-	+	+
Oils& Resins	-	-	+	+	-	-	+	+
'+' indicates Presence ; '_' Indicates absence,E:Ethanol, A: Aceton E.A: ethyl acetate P.E: Petroleum Ether								

Cytotoxicity by MTT assay:

MTT assay is based on the reduction of MTT (3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide) by mitochondrial dehydrogenase to purple formazan product. The growing healthy cell shows a high rate of MTT reduction in formazan, while dead cell fail to do so. The study of the anticancer activity of *Ceiba pentandra* (L.) against colon cancer cells was carried out with the ethanol extract of both *C.pentandra* leaf and bark.

The results showed the inhibition of in ethanolic extract in leaf and bark of *Ceiba pentandra* and were found to be cytotoxic towards HCT-116 cells at different concentrations of 10, 20, 40, 60, 80,100,150,200 and 300µg. The IC50 values of the ethanolic leaf and bark extracts were 194.52% and 74.22% respectively. The lower IC50 value indicates higher anticancer properties. (Fig.1). Previous studies concerning the antitumor activity of the *Ceiba pentandra* extracts, on EAC cells, (MCF-7) and melanoma cell line (B16F10), Kumar *et al.*, 2016, stated that the hemopoietic system along with elevation of the endogenous antioxidant defense system might play an important role in the antitumor activity of the extracts.

Hence, the results obtained from MTT assay highlighted that the ethanolic *C.pentandra* bark extract was the most active amongst the two extracts. Figure 2 displayed the morphological differences between

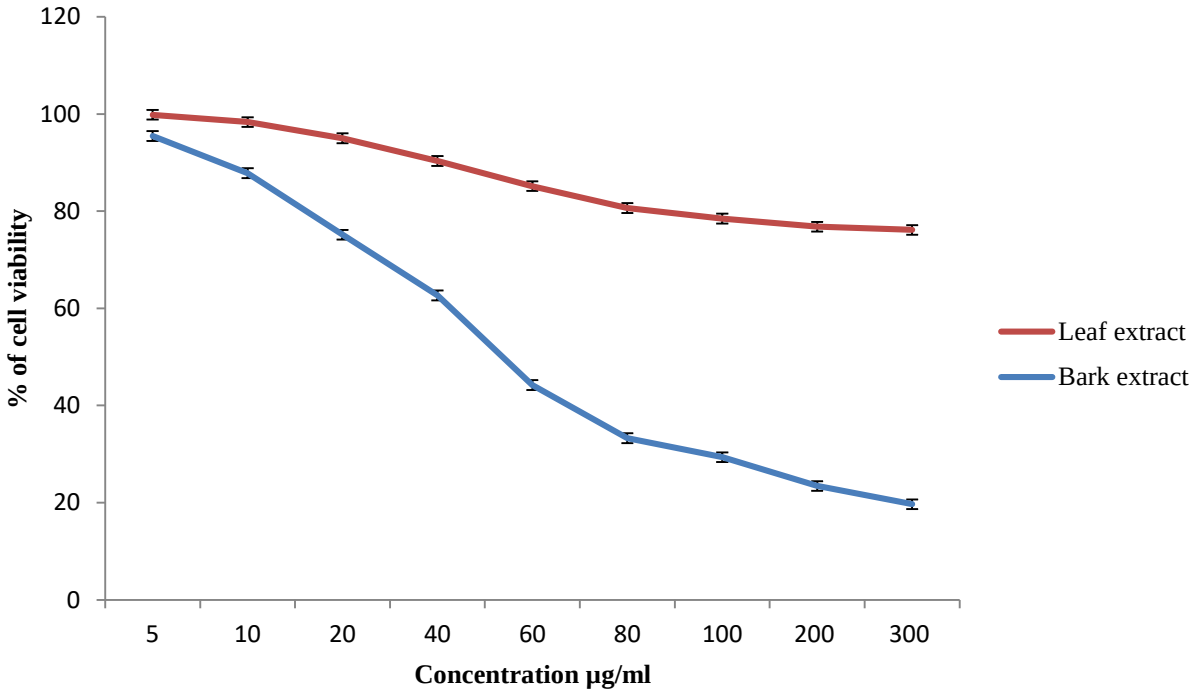

Figure 1: Cytotoxicity effect of *Ceiba pentandra* ethanolic leaf and bark extract.

The results of this present works suggest the presence of bioactive compounds present in *Ceiba pentandra* ethanolic bark extract had showed decrease cytotoxic activity against HCT-116 colon cancer cell line, when compared to *Ceiba pentandra* ethanolic leaf extract.

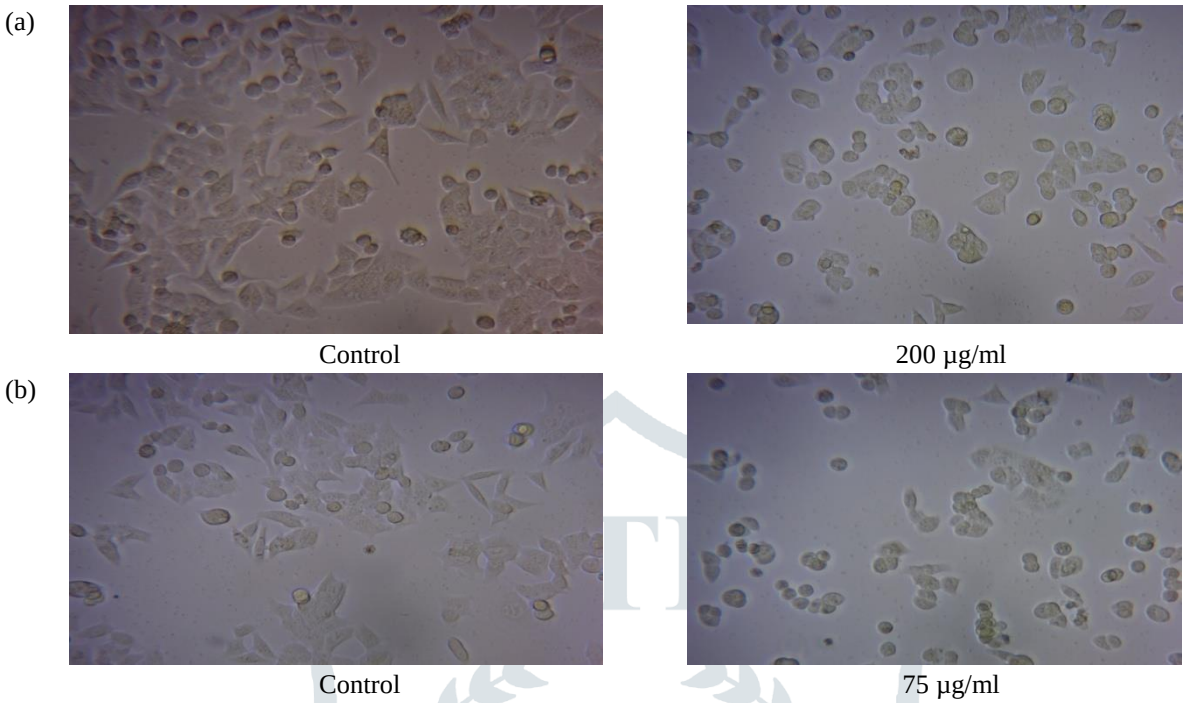

Figure 2: Cytotoxicity MTT assays morphology of a) *Ceiba pentandra* ethanolic leaf extract, b) *Ceiba pentandra* ethanolic bark extract.

CONCLUSION

Studies in the recent past indicate the potential of *Ceiba pentandra* in cancer treatment and prevention. However, gaps in the studies conducted are apparent which need to be bridged in order to exploit the full medicinal potential of *Ceiba pentandra*. Hence further studies are needed to synthesis nanoparticles out of this plant. It is possible to find the better therapies for many cancerous diseases from this plant. The *Ceiba pentandra* would be helpful in the treatment of cancerous diseases.

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