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ANTICANCER ACTIVITY OF *CLERODENDRUM VISCOSUM* V LEAVES AND *MACROTYLOMA UNIFLORUM* L SEEDS AGAINST HUMAN COLORECTAL CARCINOMA CELL LINE

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ABSTRACT

Cancer is the second leading cause of death, and is responsible for an estimated 9.6 million deaths in 2018. Globally, about 1 in 6 death is due to cancer. Colorectal cancer is the fourth most commonly diagnosed cancer in American men as well as women and is the third leading cause of cancer deaths among them. Current methods for treatment such as chemotherapy have their limitations due to their toxic effects on non-targeted tissues furthering human health problems. Therefore, there is a demand for alternative treatments with naturally-derived anticancer agents with plants being the desired source. The *Clerodendron viscosum* V. leaves & *Macrotyloma uniflorum* L seeds are the traditionally important medicinal plants but still there is no scientific evidence for anticancer activity. So, present research is aimed to exploration of anticancer activity of *Clerodendron viscosum* V. leaves & *Macrotyloma uniflorum* L seeds. The anticancer activity was evaluated by using MTT cytotoxicity assay against Human colorectal carcinoma cell line. The hydro-ethanol extracts of *Clerodendron viscosum* V. leaves & *Macrotyloma uniflorum* L were studied at different concentrations against HCT-116 cell line using MTT cytotoxicity assay. Results indicated that, the both plant extracts were given good anticancer activity against HCT-116 cell line. Between both the plant extracts, hydro ethanolic extract of *Macrotyloma uniflorum* L seeds showed higher cytotoxicity potential in human colon cancer cell line, the activity of this extract might due to the presence of active constituents such as flavonoids especially Quercetin and alkaloids.

Keywords: HCT-116, Antitumor, Cytotoxicity, MTT, Quercetin

INTRODUCTION

Cancer is a group of diseases, where body cells divide abnormally i.e. without control and invade nearby tissues. Based on the origin of cancer, it is classified as Carcinomas, Sarcomas, Leukaemias, Lymphomas, multiple myeloma and Central nervous system cancers. Colon cancer is a carcinoma that develops in the colon **[1]**. Globally cancer is the second leading cause of death and disability after heart diseases (cdc.gov 2015) **[2]**. American Association for Cancer Research (AACR 2014) stated that in the United States, it is predicted that 1.7 million new cases of cancer will be diagnosed in 2019 and that this number will rise, reaching more than 2.3 million in 2040 (AACR 2019) **[3]**. The total cancer cases are likely to go up from 979,786 cases in the year 2010 to 1,148,757 cases in the year 2020 in India. Based on the 979,786 cancer cases reported in the year 2010 by National Cancer registry Programme (NCRP) recent studies stated that the cancer cases are likely to go up to 1,148,757 by the year 2020 in India **[1]**. Colorectal cancer is the fourth most commonly diagnosed cancer in American men as well as women and is the third leading cause of cancer deaths among them.

Cancer is the costliest among all the diseases **[4]**. National Institute of Health (NIH) estimated that the overall economic costs of cancer in USA and worldwide were $216.6 billion ($86.6 billion in direct medical costs and $130.0 billion for indirect costs) and $286 billion respectively in 2009. As per the current rate of cancer incidence, it has also estimated that by the end of 2030 the cost incurred in the management will shoot up to $458 billion annually. In India on an average each cancer patient is spending Rs. 36,812 for treatment which includes Rs. 14,597 spent before coming to the hospital, Rs. 14,031 at the hospital, and Rs. 8,184 during the prolonged period of radiotherapy course **[5]**.

New entities will be developed majorly either from the natural sources i.e. plants or from chemical synthesis. Natural products are being explored extensively as alternatives to conventional therapies partly due to their multiple beneficial effects, less cost and minimal adverse effects. 60% of FDA approved anticancer drugs are of natural origin (Newman *et al*., 2003). In late 90's WHO also stated that majority percentage of world's population depends on plant based therapies for their primary health needs **[6]**. Hence we want to consider the plant sources to identify new entities for the treatment of colon cancer. The *Clerodendron viscosum* V. leaves & *Macrotyloma uniflorum* L seeds are the traditionally important medicinal plants;

but still there is no scientific evidence for anticancer activity. So the, present research is aimed to explore the anticancer activity of *Clerodendron viscosum* V. leaves & *Macrotyloma uniflorum* L seeds.

MATERIALS AND METHODS

Collection and authentication of the plant

The *Clerodendron viscosum* V. leaves & *Macrotyloma uniflorum* L. seeds were collected from the natural population growing in the area of Erattayal, Palakkad dist., Kerala, India. The leaves were identified and authenticated by Dr. Harsha Hegde, Scientist 'B', Regional Medical Research Centre (RMRC), Indian Council of Medical Research (ICMR), Belgaum. A voucher specimen of the plant has been deposited in the herbarium of RMRC with accession number RMRC-541.

Preparation of the plant extract

The collected *Clerodendrum vicosum* V leaves were shade dried (28±2ºC) for 7 days followed by drying in hot air oven (50±2ºC), ground and sieved to get fine powder. The *Macrotyloma uniflorum* seeds were also processed in the same way. The dried powdered leaves of *Clerodendrum vicosum* V and the seeds of *Macrotyloma uniflorum* were subjected to cold maceration with hydroalcohol (1:1 Water: Ethanol) separately in a shaker system at room temperature to obtain Hydro alcoholic extracts. Then each extracts were filtered. The filtrate was subjected to evaporation under reduced pressure to obtain dry extract. The obtained extracts were labelled as Hydro-ethanol extract of *Clerodendrum vicosum* V leaves (HECL) and Hydro-ethanol extract of *Macrotyloma uniflorum* L seeds (HEMS) **[7, 8]**.

In vitro anticancer activity using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) cytotoxicity assay

Cell line and culture medium

HCT-116 (Human colorectal carcinoma cell line) cell line was procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM supplemented with 10 % inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in a humidified atmosphere of 5 % CO_2 at 37^0C until confluent. The cells were dissociated with (TPVG) solution (0.2 % trypsin, 0.02 % Ethylenediaminetetraacetic acid (EDTA), 0.05 % glucose in Phosphate Buffered Saline (PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates.

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/ml using DMEM containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension was

added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100 µl of different test concentrations (100-1000µg/ml) of HEMS and HECL were added on to the partial monolayer in microtitre plates.100µl of treated cells were incubated with 50µl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) at 37^0C for 3 hours. After incubation, 200 µl of PBS was added to all the samples and aspirated carefully to remove excess MTT. 200 µl of acid-propanol was added and left overnight in the dark for solubilization. The absorbance was read at 650 nm in a microtitre plate reader (Bio RAD U.S.A). The optical density of the control cells were fixed to be 100 % viable and the percent viability of the cells in the other treatment groups were calculated using the formula, and concentration of test drug needed to inhibit cell growth by 50 % (LD_{50}) (Lethal Dose, 50%) values is generated from the dose-response curves of cell line **[9]**.

$$\text{Percentage Lethality} = \frac{Control\ OD - Sample\ OD}{Control\ OD} X\ 100$$

RESULTS AND DISCUSSION

The percentage yield of hydro alcoholic extracts of *Clerodendron viscosum* V leaves was found to be 3% and *Macrotyloma uniflorum* L seeds was 7%. The results of the cytotoxicity study of different extracts of leaves of *Clerodendrum vicosum* V and the seeds of *Macrotyloma uniflorum* L using MTT assay against colon cancer cell line are shown in **Table 1**, and diagrammatically it is mentioned in **Figure 1**.

The LD_{50} values of different extracts were calculated from the percentage lethality of different doses of plant extracts. From the results of cytotoxicity study HEMS shows higher cytotoxicity potential in human colon cancer cell line, supporting that it possesses potent anticancer activity when compared with HECL. The preliminary studies conducted by our study revealed that the presence of carbohydrates, tannins, flavonoids and saponins were present in all the extracts **[10]**. The activity of this extract might due to the presence of active constituents such as flavonoids and alkaloids.

Table 1: Cytotoxicity assay of plant extracts against HCT-116

Concentration (µg/ml)	Percentage lethality of HECL	Percentage lethality of HEMS
100	18.12±1.23	21.33±0.88
200	27.23±0.78	35.41±0.15
300	35.21±1.45	45.53±1.01
400	42.51±1.52	51.18±0.28
500	56.53±1.23	66.75±1.27
1000	64.25±1.41	79.21±0.48
LD_{50} values	604.24 µg/ml	454.55 µg/ml

Values were expressed as Mean±SEM

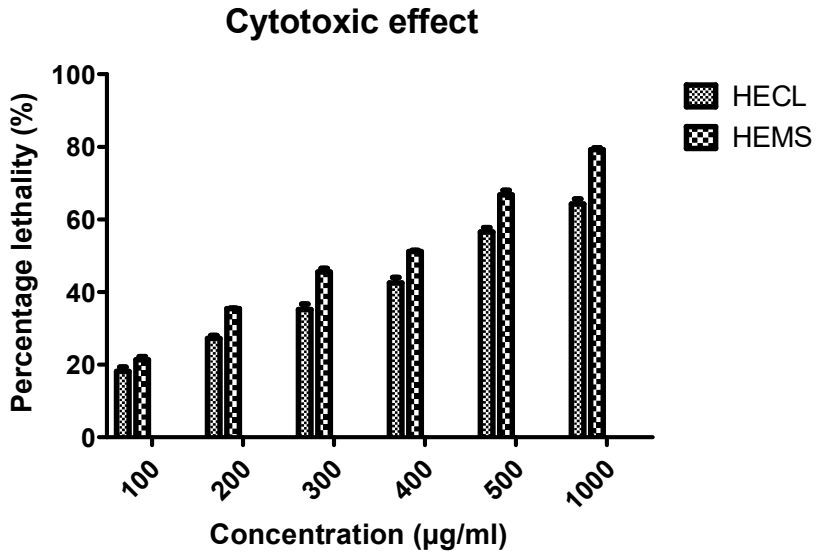

Figure 2: Cytotoxicity assay of plant extracts against HCT-116

CONCLUSION

In conclusion, HEMS has more activity than HECL. The activity might due to the presence of higher quantity of active constituent such as Quercetinin crude extract of HEMS.

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