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DETERMINATION OF *IN VITRO* ANTIPROLIFERATIVE EFFECT OF THREE IMPORTANT *CEROPEGIA* SPECIES ETHANOLIC EXTRACTS ON CULTURED HCT-118 CELL LINES

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ABSTRACT

Bio prospecting of plants with anti-proliferative activity has been a major focus on the search of plants based cure. The pharmacological importance of *Ceropegia* species (Apocynaceae) is mainly due to the presence of a pyridine alkaloid '*Ceropegin'* a potential antipyretic, analgesic, local anesthetic, antiulcer, mast-cell stabilizing, hepato-protective, tranquilizing, and hypotensive. Use of plant derived compounds as potent anticancer agents is of recent research interest. In the continuing effort to screen *Ceropegia* species (*Ceropegia spiralis, Ceropegia juncea,* and *Ceropegia candelabrum)* for anticancer activity, plants were collected, micro propagated and hardening. *In-vitro* propagated plants are collected, extracts tested for the activity using cell lines namely HCT-118 (Colon cancer cell) of the extracts from three species of same families tested (10,50,and100µg/ml). 10ug of*Ceropegia spiralis* showed 47.15% of cell death, which can be considered significant. Apoptosis was confirmed by acridine orange (AO) ethidium bromide (EB) fluorescent double staining which confirms the potent anticancer effect of ethyl acetate fraction of *Ceropegia spiralis*.

KEYWORDS: ***Ceropegia spiralis, Ceropegia juncea, Ceropegia candelabrum*, Anti-proliferative effect, HCT-118 Colon lines.**

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INTRODUCTION

Cancer is the hyerproliferative disorder that involves abnormal cell growth and uncontrolled activities disregarding the normal rules of cell divisions. These proliferating cells from a mass of undifferentiated cells diminished ,in version of local tissues and spread of etastasis to other parts of the body[1]. Cancer is one of the most severe health problem in developing countries. Colon cancer, accounting for nearly 608 thousand mortalities across the globe is accounted on the fourth dead list cancer. Ancient times, there was no cure for cancer, later surgery, radiation therapy, hyperthermia, hormone therapy, chemotherapy etc, was developed. Chemotherapy is widely practiced to treat disease with or without radiation, although it was accompanied by severe side effects due to toxicity of anti-cancer drugs[2]. Several anti-cancer drugs are derived from plants.*Ceropegia* L. is one of the largest and diversified genuses of Apocynaceae cosmopolitan of tropical to Sub- tropical at eastern side of the continent[3]. In India there are about 50 species are present[4]. Out of which 28 species are endemic to the peninsular region [5-6]. The tubers of Ceropegia are starchy and edible.In the fields of Ayurvedic drug preparation tubers are used as nutritive tonic and as a blood purifier. The pharmacological importance of *Ceropegia* species is mainly due to, the presence of a pyridine alkaloid "*Ceropegin*" a potential antipyretic local anesthetic, antiulcer mast cell stabilizing, hepato – protective tranquilizing and hypotensire activity of whole plant[7] .*Ceropegia spiralis* L. is an annual herb, it is an endangered category. The root tubers contain starch, sugars, gum, fats, crude fiber and that are active against many diseases especially diarrhea, dysentery and the starchy tubers are useful as a nutritive tonic[8].*Ceropegia juncea* Roxb. is an important medicinal plant Since Vedic period[9].This herb is used as a source of "soma", a plant drug of the ayurvedic medicine with a wide variety of uses[10,11].*Ceropegia candelabrum* L., The 'glabrous goglet flower' is an endangered, herb. Root tubers contain the alkaloid *Ceropegine* which is used in Indian ayurvedic drug preparation[12].In this study C*. spiralis* wight, *C.juncea* and *C.candelabrum* showed potent anticancer agents and the effect of HCT-118 colon cancer cell lines. Viability of each species has been carried out to evaluate the claims regarding its anticancer potential.

Plant material

Whole plant (*C. spiralis, C. juncea,* and *C. Candelabrum)* was collected from the Western Ghats of Kanyakumari district, Tamilnadu, India. The plant was identified and authenticated at Nesamony Memorial Christian College, Marthandam of Kanyakumari District, India .Then these plants were micro propagated and hardened.

Preparation of the plant extract

In-vitro propagated plant parts were collected air-dried and ground into powder. Each Powdered sample was soaked in 70% ethanol for 24hours and then the mixture was filtered. The filtrates were pooled and evaporated in the air at room temperature, to obtain a final residue (500 mg) for further experiment.

Cell culture

HCT-118 colon cancer cell lines were purchased from NCCS Pune was maintained in Dulbecco's modified eagles media (HIMEDIA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°c in 5 % CO2 (NBS, EPPENDORF, GERMANY) in a humidified atmosphere in a CO2 incubator. The cells were trypsinized (500µl of 0.025% Trypsin in PBS/ 0.5Mm EDTA solution (Himedia)) for 2 minutes and passaged to T flasks in complete aseptic conditions. Extracts were added to grown cells at a concentration of 10 µg, 50µg and 100µg from a stock of 10mg/ml and incubated for 24 hours. The % difference in viability was determined by standard MTT assay after 24 hours of incubation

Cell viability assay

Cell viability was determined by a micro culture tetrazolium technique (MTT) assay performed according to the method described by[13]. MTT is a colorimetric assay that measures the measures the reduction of yellow 3- (4, 5 dimethy thiazol- 2-yl) -2, 5- diqhenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, colored (dark purple) formazan product. The cells are then sollubilised with an organic solvent Dimethyl sulfoxide (Himedia) and the released, solubilised formazan product was measured at 540nm. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.The cell culture suspension was washed with 1 x PBS and then added 30µl of MTT solution to the culture (MTT-5mg/ml dissolved in PBS).It was then incubated at 37^0C for 3 hours.MTT was removed by washing with 1xPBS and 200µlof DMSO was added to the culture. Incubation was done at room temperature for 0 minutes until the cell got lysed and color was obtained. The solution was transferred to centrifuge tubes and centrifuged at top speed for 2minutes to precipitate cell debris. Optical density was read at 540 nm using DMSO as blank.

% viability = (OD of Test/ OD of Control) X 100

Determination of Apoptosis

DNA-binding dyes AO and EB (Sigma, USA) were used for the morphological detection of apoptotic and necrotic cells[14]. AO is taken up by both viable and non-viable cells and emits green fluorescence if intercalated into double stranded nucleic acid (DNA). EB is taken up only by non-viable cells and emits red fluorescence by intercalation into DNA. After treatment with samples at a concentration of 100 µg /ml for 24 hours. The cells were washed cold PBS and then stained with a mixture of AO (100 µg /ml) and EB (100 µg/ml) at room temperature for 10min. The stained cells were washed twice with IX PBS and observed by a fluorescence microscope in blue filter of fluorescent microscope (Olympus CKX41 with Optika pro5 camera). The cells were divided into four categories as follows: living cells (normal green nucleus), early apoptotic (bright green nucleus with condensed or fragmented Chromatin), late apoptotic (orange-stained nuclei with chromatin condensation or fragmentation) and necrotic cells (uniformly orange-stained cell nuclei).

RESULTS AND DISCUSSION

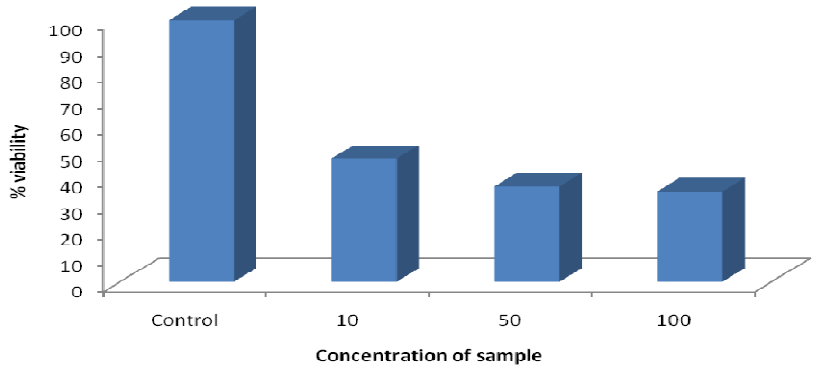
MTT ASSAY

Table-1

Sample Concentration (µg/ml)	OD at 540nm	% viability
Control	0.6609	100
Ceropegia spiralis		
10 µg/ml	0.2975	47.15
50 µg/ml	0.2300	36.45
100 µg/ml	0.2182	34.58
Ceropegia juncea		
10 µg/ml	0.4645	73.62
50 µg/ml	0.3743	59.32
100 µg/ml	0.1908	30.24
Ceropegia candelabrum		
10 µg/ml	0.5869	93.02
50 µg/ml	0.4548	72.08
100 µg/ml	0.4200	48.40

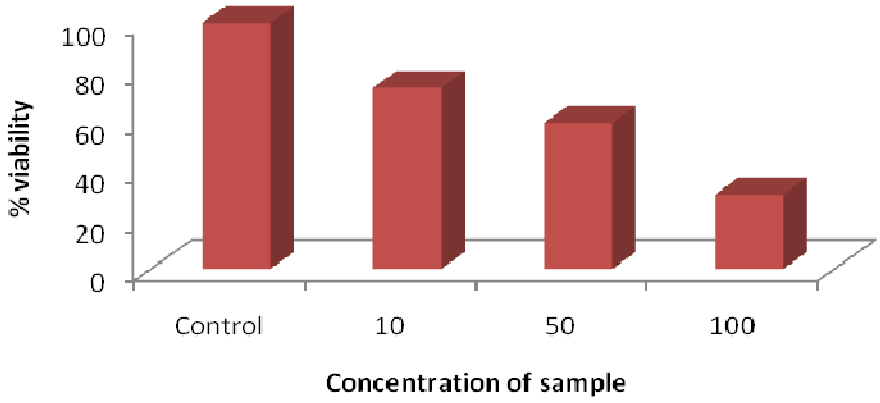
Graph 1

Effect of C.spiralis on HCT-118 colon cancer cell lines



(Decreased MTT suggests decreased cell viability)

Graph 2



Graph 3

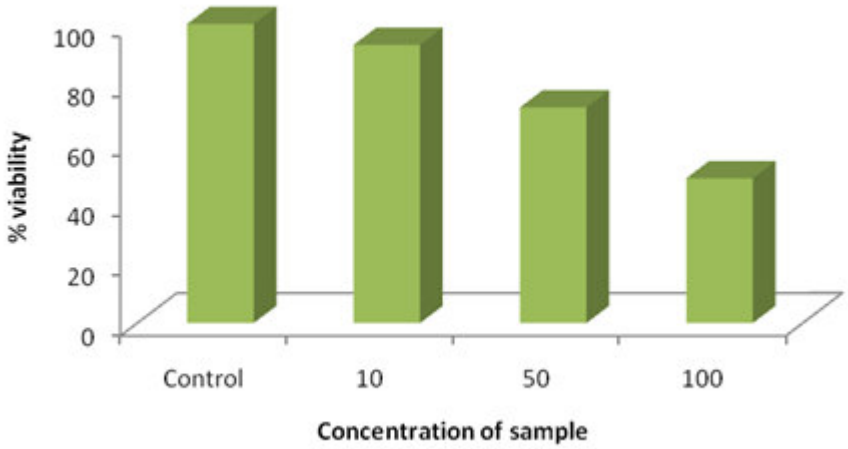
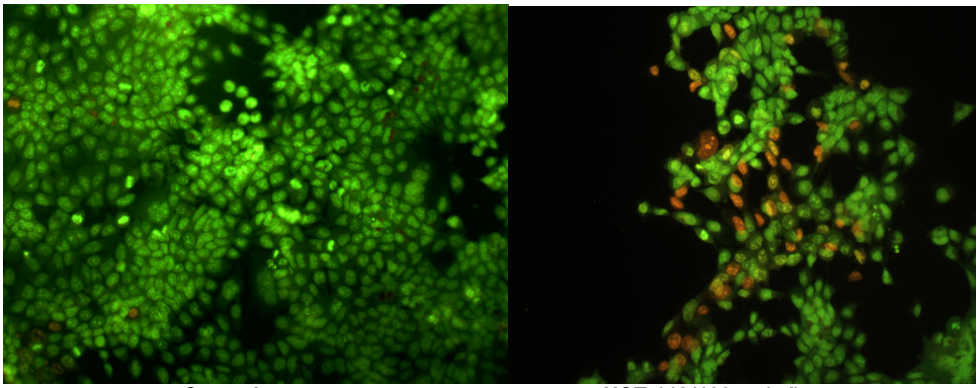


Figure 1
Apoptosis-Fluorescence microscopy (Ceropegia spiralis)



Control *HCT-118(100 µg/ml)*

Graph-1 and table 1 shows the actively profile extract tested on HCT-118 cell lines.When the survival fraction (% of control) OD value 0.6609 at 450nm is compared among the 3 initially active plant extract on *Ceropegia spiralis* 10,50,and 100 µg/ml concentration .Extracts of *Ceropegia spiralis* 10 µg/ml showed 0.2975 nm with a viability of 47.15%,50 µg/ml showed 0.2300nm with a viability of 36.45% and 100 µg/ml showed 0.2182nm with a viability of 34.58% Graph-2 shows the actively profile of 3 most active extracts tested on HCT-118 cell lines. When survival fraction (% of control) *Ceropegia juncea* 10 µg/ml of OD value 0.4645nm with a viability of 73.62%,50 µg/ml showed 0.3743nm with a viability of 59.32% and 100 µg/ml showed 0.1908nm with a viability of 30.24%. Graph-3 shows the actively profile of 3 most active extracts tested on HCT-118.When the survival fraction (% of control) of *Ceropegia candelabrum* 10µg/ml OD value 0.5869nm with a viability of 93.02%,50..g/ml OD value 0.4548nm with a viability of 72.08% and 100µg/ml OD value 0.4200nm with a viability of 48.40%. The result indicated that ethanolic extract of *Ceropegia spiralis* effectively reduced the cell viability.10µg of *C. spiralis* showed 47.15% of cell death which can be considered significantly. Fig.1 Apoptosis was confirmed by acridine orange ethidium bromide fluorescent double staining which suggests potent anticancer effect of *Ceropegia spiralis*. Isolation and purification of potent bioactive principle of *Ceropegia spiralis* can aid in cancer treatment.

CONCLUSION

Although the cytotoxicity demonstrated by the three extracts is potentially useful, further tests on other cancer cell lines need to be done to determine their tumor specificity. Subsequently, the active compounds can further be subjected to structure modifications to reduce the active cell proliferation or enhance activity. Study suggests potent anticancer effect of *Ceropegia* species (*C.spiralis, C.juncea,* and *C.candelabrum*) on colon cancer cell lines which can find its application in therapeutics.

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