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ANTIMICROBIAL ACTIVITY AND IN VITRO ANTICARCINOGENIC PROPERTIES OF 'PORTULACA QUADRIFIDA LINN'ON COLON CANCER USING DIFFERENT EXTRACTS

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ABSTRACT

We are living in the country where we finds a tremendous plant sources used for various treatment of diseases. Portulaca quadrifida is one among the Indian plant found in tropical parts. Portulaca quardifida Linn (portulacaceae) is traditionally used for the treatment against various ailments which is scientifically not revealed. In the present study chloroform extract (CHCL3), methanol extract (CH3OH) and aqueous extract of Portulaca quadrifida on human colon cancer cell lines HT-29 is investigated by MTT assay and also plant was screened for its antimicrobial potential by agar well diffusion method. By comparing all the three extracts, aqueous extract showed the highest inhibition with the lowest concentration of 15.87 µg/ml. and it is comparatively safer when compare to other extracts as it is easily eliminated from the body. The antibacterial activity was measured by nutrient agar method and antifungal activity by potato dextrose agar method was noticed with all the three extracts. Among all the three extracts aqueous extract showed significant activity by inhibiting Gram negative bacteria Escherichia coli, Gram positive bacteria Staphylococcus aureus, and fungal organism like Aspergillus flavus and Aspergillus niger at MIC of 4.3, 4.2, 4.4 and 3.7 at 25mcg/ml, respectively. The anti cancer and antimicrobial effects of Portulaca quadrifida may be related to their content like flavonoids, saponins and tannins.

KEYWORDS: Portulaca quadrifida, Colon cancer, Antimicrobial activity, In vitro.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in men (663,000 cases, 10.0% of the total cancers) and the second in women (570,000 cases, 9.4% of the total cases) worldwide.[1] It occurs mainly due to various reasons such as diet, lifestyle, older age and inherited genetic disorder etc., A change in bowel habit including diarrhoea or constipation this leads to change in the consistency of stool, rectal bleeding or blood in stool, resistant abdominal discomfort such as gas or pain, weakness as fatigue or weight loss are following types of sign and symptoms of colon cancer. Colon cancer may be curable with surgery if it has spread widely they are usually not curable in such cases it is placed in improving quality of life prognosis depends upon how advance the colon cancer is diagnosed.

Colon cancer is an important health concern worldwide and requires considerable attention in terms of disease management. Serious Chemotherapy and non steroidal Anti-inflammatory drugs (NSAIDs), improves the disease but they possesses many serious adverse effect so natural herbs are gaining importance.[2] In this search directed to identify a plant material which will be useful and posses less side effect will be beneficial in the treatment of colon cancer as we found a plant which is extensively grown in tropical parts.

The bacterial density in the large intestine (~10^{12} cells per ml) is much greater than that in the small intestine (~10^{2} cells per ml), and this is paralleled by an approximately 12-fold increase in cancer risk for the large intestine compared with the small intestine. These two observations combined point towards the hypothesis that colon cancer may be induced by bacteria.

Since natural herbs are gaining more importance because of lesser side effects when compared to synthetic medicines, therefore, there is a need of discovering potentially useful active ingredients of plants that can serve as antimicrobial drug as well as useful in colorectal cancer.

Portulaca quadrifida Linn which belongs to the family Portulacaceae is the small diffused herb found in the tropical parts of India and commonly grown at river banks. It is obtained from both wild and cultivated plants. It is also used as vegetable.[3] According to epidemiological studies consumption of fruits and vegetables reduces the risk of developing several types of cancer.[4] Inspite of possessing rich activities against various ailments the

literature survey has not revealed, Portulaca quardifida Linn towards colon cancer using aqueous extract.

MATERIALS AND METHODS

Collection of Plant Material

Fresh whole plant material of Portulaca quadrifida Linn was collected from the local fields of Hyderabad. The plant specimen was identified and authenticated by Prof. Dr. (Mrs) Pratibha Devi, Department of Botany, Osmania University, Hyderabad. A voucher specimen is preserved in the herbarium of Department of Botany (Voucher No.024), Osmania University Hyderabad.

EXTRACTION PROCEDURE.[5]

Preparation of Chloroform and Methanolic Extracts

1. The whole plant was dried under shade, powdered and passed through 40 meshes and stored in closed vessel for further use.
2. The dried powder material (2000 gms) was subjected to soxhlet extraction with chloroform and methanol for continuous hot extraction respectively. The marc was pressed after the extraction and the filters were pooled and concentrated under reduced pressure to obtained dried solid mass. The percentage yield was calculated and tabulated. (Table 1)

PREPARATION OF AQUEOUS EXTRACT

1. About 1000 gms of dried marc was taken in a 2000 ml of beaker and macerated with 1000ml of distilled water to which 10ml of chloroform was added as a preservative and kept it for seven days with occasional shaking daily in a closed vessel.
2. The supernatant was decanted and the marc was pressed then the pooled extract was concentrated on water bath at 50°c to get a dry solid mass. The percentage yield was calculated and tabulated. (Table 1)

CELL LINES

Human colony cancer cell line, HT-29 cell lines were procured from National Centre for Cell Sciences (NCCS), India. HT-29 is a human colorectal adenocarcinoma cell line with epithelial morphology. These cells are sensitive to the chemotherapeutic drugs 5-fluorouracil and oxaliplatin, which are standard treatment options for colorectal cancer. In addition to being a xenograft tumor model for colorectal cancer, the HT-29 cell line is also used as an in-

vitro model to study absorption, transport, and secretion by intestinal cells. Under standard culture conditions, these cells grow as a non polarized, undifferentiated multilayer. Altering culture conditions or treating the cells with various inducers, however, results in a differentiated and polarized morphology, characterized by the redistribution of membrane antigens and development of an apical brush-border membrane.

IN VITRO CYTOTOXICITY ASSAY

Determination of Mitochondrial Synthesis by MTT Assay

PRINCIPLE

The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The cleavage of MTT to a blue formazan derivative by living cells is clearly a very effective principle on which the assay is based. The principle involved is the cleavage of tetrazolium salt MTT (3-(4,5 dimethyl thiazole-2 yl)- 2,5-diphenyl tetrazolium bromide) into a blue coloured product (formazan) by mitochondrial enzyme succinate dehydrogenase. The numbers of cells were found to be proportional to the extent of formazan production by the cells used.[6]

PROCEDURE

1. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0x105 cells/ml using DMEM medium containing 10% FBS.
2. To each well of a 96 well microtitre plate, 100µl of the diluted cell suspension (approximately 10,000 cells/well) was added.
3. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, the monolayer was washed once with medium and 100ml of different sample/extract concentrations prepared in maintenance media were added per well to the partial monolayer in microtitre plates. The plates were then incubated at 37oC for 3 days in 5% CO2 atmosphere, and microscopic examination was carried out and observations recorded every 24 hours.
4. After 72 hours, the sample solutions in the wells were discarded and 20 ml of MTT (2mg/ml) in MEM-PR (MEM without phenol red) was added to each well.
5. The plates were gently shaken and incubated for 3 hours at 37oC in 5% CO2 atmosphere.

6. The supernatant was removed and 50ml of iso-propanol was added and the plates were gently shaken to solubilize the formed formazan.
7. The absorbance was measured using a microplate reader at a wavelength of 540nm.

The percentage growth inhibition was calculated using the following formula and concentration of drug or test samples needed to inhibit cell growth by 50% values were generated from the dose-response curves for each cell line (Table-2).

$$\% \text{ Growth Inhibition} = \left[100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \right] \times 100$$

Results were tabulated in table 2

ANTI MICROBIAL ACTIVTY

Media Preparation and Its Sterilization

For agar well diffusion method antimicrobial susceptibility was tested on solid (Agar-agar) media in petri plates. For bacterial assay nutrient agar (NA) (40 gm/L) and for fungus Potato Dextrose Agar (39 gm/L) was used for developing surface colony growth. All the media prepared was then sterilized by autoclaving the media at (121°C) for 20 min.

AGAR WELL DIFFUSION METHOD

Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria and fungi. Wells (10mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Stock solution of plant extracts was prepared at a concentration of 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml in Ethanol and Water. About 100 µl of different concentrations of plant solvent extracts were added sterile syringe into the wells and allowed to diffuse at room temperature for 2hrs. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 18-24 h for bacterial pathogens and 28°C for 48 hours fungal pathogens. The diameter of the inhibition zone (mm) was measured. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.[7] Results were tabulated in table 3, 4 and 5.

RESULT

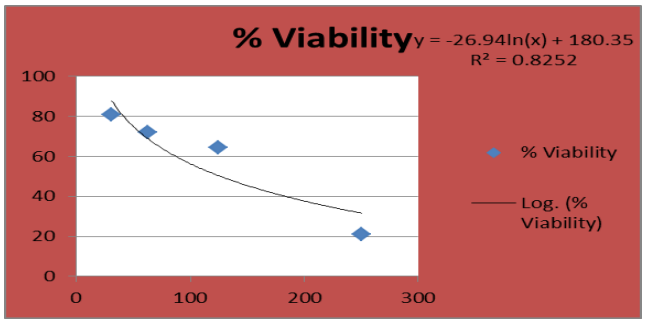
Table -1: Percentage Yield of Extracts

S. No.	Extract	Nature of Extract	Colour	Weight (gm)	% Yield (w/w)
1.	Chloroform	Semi Solid	Dark Green	25.49	2.5
2.	Methanol		Dark Green	32.03	3.2
3.	Aqueous		Dark Brown	73.07	7.3

Table 2: Cytotoxicity Studies

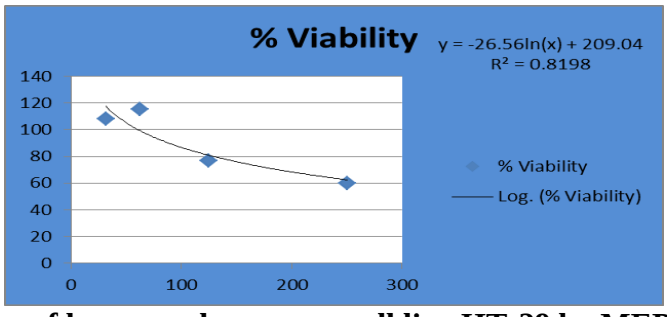
Sl. No.	Sample	CTC50 µg/ml (HT- 29)
1	CHCL3	126.28
2	CH3OH	398.59
3	AQ	15.87

SAMPLE - I



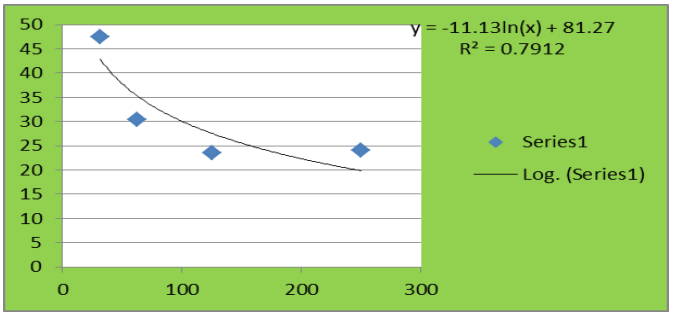
Growth inhibition of human colon cancer cell line HT-29 by CEPQ in MTT assay

SAMPLE - II



Growth inhibition of human colon cancer cell line HT-29 by MEPQ in MTT assay

SAMPLE - III



Growth inhibition of human colon cancer cell line HT-29 by AEPQ in MTT assay

Table 3: Antimicrobial activity of chloroform and methanol extract.

Extract	**Zone of inhibition measured in 'mm' for bacteria and fungi compared with Antimicrobial agents**										
Microorganism	**Chloroform (TRIPLICATE)**						**Methanol (TRIPLICATE)**				
Bacteria		1(mm)	2(mm)	3(mm)	AVG	STD	1(mm)	2(mm)	3(mm)	AVG	STD
E. Coli	Concentration mcg/ml										
	25	4.1	3.6	3.7	3.8	0.2	3.2	3	3.4	3.20	0.16
	50	9.0	10.0	9.6	9.5	0.4	7	8	8.2	7.73	0.52
	75	11.7	12.3	12.5	12.2	0.3	9	11	12.5	10.83	1.43
	100	17.4	16.9	17.1	17.1	0.2	13.4	13.9	15.1	14.13	0.71
S. Aureus	Concentration mcg/ml										
	25	3.4	3.7	4.0	3.7	0.3	3	3.2	3.3	3.17	0.12
	50	9.9	9.6	10.2	9.9	0.3	6.8	7.2	8.1	7.37	0.54
	75	12.6	12.4	12.9	12.6	0.2	8.2	9	9.7	8.97	0.61
	100	16.9	16.9	16.8	16.9	0.0	10.2	10.5	10.8	10.50	0.24
Fungi											
A. niger	Concentration mcg/ml										
	25	3.4	3.2	3.4	3.3	0.1	3.4	3.2	3.4	3.33	0.09
	50	7.9	8.0	8.2	8.0	0.1	7.9	8	8.2	8.03	0.12
	75	11.7	12.3	12.5	12.2	0.3	11.7	12.3	12.5	12.17	0.34
	100	16.8	16.0	16.6	16.5	0.3	13.8	13	13.6	13.47	0.34
A.flavus	Concentration mcg/ml										
	25	4.3	4.0	3.6	3.9	0.3	3.2	3	3.5	3.23	0.21
	50	8.1	8.5	8.8	8.5	0.3	8.1	8.5	8.8	8.47	0.29
	75	13.4	13.7	13.5	13.6	0.1	10.4	10.7	10.5	10.53	0.12
	100	17.1	17.3	17.4	17.2	0.1	12.1	12.3	12.4	12.27	0.12

Table 4: Antimicrobial activity of aqueous extract.

Extract	**Zone of inhibition measured in 'mm' for bacteria and fungi compared with Antimicrobial agents**					
Microorganism			**Aqueous(TRIPLICATE)**			
Bacteria		1(mm)	2(mm)	3(mm)	AVG.	STD.
E. coli	Concentration mcg/ml					
	25	4.6	4.1	4.2	4.3	0.2
	50	10.1	11.2	10.8	10.7	0.5
	75	13.2	13.8	14	13.7	0.3
	100	19.5	19	19.2	19.2	0.2
S. aureus	Concentration mcg/ml					
	25	3.8	4.2	4.5	4.2	0.3
	50	11.1	10.8	11.5	11.1	0.3
	75	14.2	13.9	14.5	14.2	0.2
	100	19	19	18.9	19.0	0.0
Fungi						
A. niger	Concentration mcg/ml					
	25	3.8	3.6	3.8	3.7	0.1
	50	8.9	9	9.2	9.0	0.1
	75	13.2	13.8	14	13.7	0.3
	100	18.9	18	18.6	18.5	0.4

A.flavus	Concentration mcg/ml					
	25	4.8	4.5	4	4.4	0.3
	50	9.1	9.6	9.9	9.5	0.3
	75	15.1	15.4	15.2	15.2	0.1
	100	19.2	19.4	19.5	19.4	0.1

Table 5: Antimicrobial activity of standard drug.

STANDARD						
		Chloroform, Methanol and Aqueous(TRIPLICATE)				
AMPICILLIN						
E. Coli	Concentration mcg/ml					
	25	12.5	12.6	12.9	12.7	0.2
	50	21	21.9	20.5	21.1	0.6
	75	32	30.2	33.1	31.8	1.2
	100	40.5	40.9	41.2	40.9	0.3
S. Aureus	Concentration mcg/ml					
	25	19	19.5	18.3	18.9	0.5
	50	31.5	30.6	31.2	31.1	0.4
	75	40.2	40.9	39.8	40.3	0.5
	100	46.9	46.8	47.1	46.9	0.1
FLUCONAZOLE						
A. niger	Concentration mcg/ml					
	25	15.3	15.7	14.7	15.2	0.4
	50	25.4	24.6	25.1	25.0	0.3
	75	32.4	32.9	32.0	32.4	0.4
	100	37.8	37.7	37.9	37.8	0.1
A.flavus	Concentration mcg/ml					
	25	11.6	11.7	11.9	11.7	0.2
	50	19.4	20.3	19.0	19.5	0.5
	75	29.6	27.9	30.6	29.4	1.1
	100	37.5	37.8	38.1	37.8	0.3

DISCUSSION

Epidemiological studies have suggested that individuals consuming diets high in fruits and vegetables have a reduced risk of developing several cancers.[8] The identification of active constituents and pharmacological studies showed Portulaca quadrifida can be used as potent anti cancer and antimicrobial drug. The result of present study indicated that aqueous extract of Portulaca quadrifida was significant among all the extracts used.

CONCLUSION

The cheap herbal drug treatment with less toxic effects may highly be recommended to the rural and poor people for any disease. The above study reveals Portulaca quadrifida and its active constituents is treated effectively for colon cancer and antimicrobial activity.

In conclusion, our study indicates that among all the extracts the aqueous extract of Portulaca quadrifida exerts its antiproliferative effects by in vitro inducing HT-29 cells and it has showed significant antibacterial activity against Escherichia coli and Staphylococcus aureus and antifungal activity against Aspergillus flavus, Aspergillus niger when compared to standard.

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