ASSESSMENT OF VARIOUS SELECTED PLANT EXTRACTS FOR COLORECTAL ADENOCARCINOMA CELL LINE BY MTT ASSAY

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Abstract

Colorectal adenocarcinomas constitute approximately 90% of the malignant large bowel tumours. The absence of symptoms is a common characteristic of early-stage colorectal cancer; thus, early detection through screening programmes is critical. The objective of this investigation is to study the effects of selected plant extracts for colorectal adenocarcinoma cell line by MTT assay. Three plants have been selected for the MTT assay are *Vanda tessellata, Ipomoea aquatica, Semen armeniacae*. In methodology *Vanda tessellata* and *Ipomoea aquatica,* "leaves were extracted with PE followed by DCM and then MeOH. *Semen armeniacae* was extracted with PE followed by EA and then MeOH". Colorectal adenocarcinoma cell line used in MTT assay *viz* Colo205 were procured from "National Centre for Cell sciences, Pune, India". Results depicted that *Vanda tessellata* extracts were 100% proliferative against Colo205. Phytomedicines accomplish this by means of the additive or synergistic effects of multiple chemical compounds that target a single or multiple sites on carcinoma that are linked to a physiological process. The inhibition of various signal transduction pathways implicated in tumour development has been observed in phytochemicals.

Keywords: *Vanda tessellata, Ipomoea aquatica,, Semen armeniacae,* MTT assay

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1. Introduction

Cancer is the name given to a group of illnesses marked by the unchecked growth and spread of abnormal cells. Inheritable mutations, the body's hormone immune-regulating circumstances, and hepatic variations are examples of internal variables. External causes include nicotine, pathogenic microorganisms, substances and sunlight contribute to its development. Carcinogenesis may be initiated or promoted by the sequential or simultaneous action of these causal factors (Kitic et al., 2023). Among these are colorectal malignancies (Siegel et al., 2023).

In cancer therapy, the emergence of resistant by tumour cells to chemotherapy drugs is a significant obstacle. Finding products with cytotoxic mechanisms distinct from those of pharmaceuticals currently in clinical use is one method to combat this (Suntar, 2020). The exploration of novel classes of compounds is facilitated by the biochemical and chemical diversity observed in nature (Khan and Ahmad, 2019). Pharmaceutical compounds that are commercially significant have been derived from the vast array of secondary metabolites found in plants (Wang, 2023). Presently, there is an international, methodical endeavour to screen natural products, with a specific focus on herbal remedies, for diverse pharmacological activities, particularly those related to cancer. The ultimate goal is to identify novel compounds that possess distinct mechanisms for action (Singh et al., 2020). The aim of the research is to study the effects of selected plant extracts. for colorectal adenocarcinoma cell line by MTT assay.

2. Material and method

In a three-necked flask, The extracts from plants were created by heating the selected solvents with the dried, ground matter. The solvent was eliminated through the utilisation of a rotary evaporator. For future use, the crude extracts were freeze-dried.

CELL CULTURE

Colo205, a cell line for colorectal adenocarcinoma, was obtained from the “National Centre for Cell Sciences in Pune, India”. A gracious donation from “Dr. Ramakrishnan of BARC”, located in Mumbai, India, ENT407. “In Roswell Park Memorial Institute medium (RPMI)” 1640, Colo205 was preserved. “Complete medium, pH 7.4 (with 10% heat-inactivated foetal bovine serum added for supplementation), L-glutamine (2 mmol/l), 100 units/ml penicillin, and 100 ug/ml streptomycin were utilised to maintain the cell lines. The cells were cultivated in a humidified environment comprising 95% air and 5% CO2 at a temperature of 37°C”.

“SCREENING OF VARIOUS PLANT EXTRACTS FOR ANTICANCER ACTIVITY BY MTT ASSAY”

“The MTT assay is a colorimetric method utilised to quantify the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)” by the "succinate-tetrazolium" reductase system located within mitochondria. The viability of the cells (percentage proliferation) is determined by the level of activity (which is proportional to the intensity of the colour), as the reduction of MTT can only occur in metabolically active cells.

The examination's botanical specimens were gathered from a variety of districts. The plant components were ground into powder after being air dried in regulated heat and humidity environments.

PREPARATION OF EXTRACTS

A series of solvents were utilised in a sequential manner to extract the air-dried pulverised plant material.

A 1:10 ratio of solvent to plant material was utilised in each instance. Using a three-necked flask fitted with an overhead agitator, the substance was refluxed at 40°C for three hours while being continuously stirred. Following three repetitions of the aforementioned procedure with a single solvent to ensure complete extraction, the marc was desiccated entirely before being re-extracted with the subsequent solvent. Rotary evaporation was utilised to remove the solvent from the combined extracts. To preserve the unrefined extracts for subsequent use, they were freeze-dried.

i.Vanda tessellata was subjected to extraction using PE, DCM, and MeOH.

ii. Ipomoea aquatica was subjected to extraction using PE, DCM, and MeOH.

iii. Semen armeniacae was subjected to extraction using PE, EA, and MeOH,.

To conduct cytotoxicity experiments, aliquots of the desiccated extract were reconstituted in cell culture medium at concentrations equivalent to those required for subsequent analyses. l0 mg/ml stock of the extract was prepared in DMSO of tissue culture grade.

STATISTICAL ANALYSIS

Significant P values were defined as <0.05. The mean value with standard deviation is used to represent all data. Using the Graph Pad Prism 5 statistical software, every statistical analysis was conducted.

3. Result and Discussion

"Cell viability assay by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay"

Using the MTT assay, cellular proliferation of extracts from plants was determined. Plant extracts were utilised to treat Colo205 cells, with concentrations ranging from 0.5 to 50 ug/ml. Vanda tessellate was assessed for cell viability at a higher rate compared to the other two plant extracts. For each well, lxl04 cells of Colo205 were inoculated in a 96-well plate. The cells were then left to attach overnight in serum-starved condition.

The cytotoxicity of the selected plant extracts on the Colo205 colorectal adenocarcinoma cell line was evaluated utilising the MTT assay. A total of three quantities of the extracts—0.5, 5, and 50 ug/ml—were chosen for the screening process. All responses were presented as mean±standard deviation. The outcomes of the Colo205 MTT assay are illustrated in Figure (1) (a,b,c).

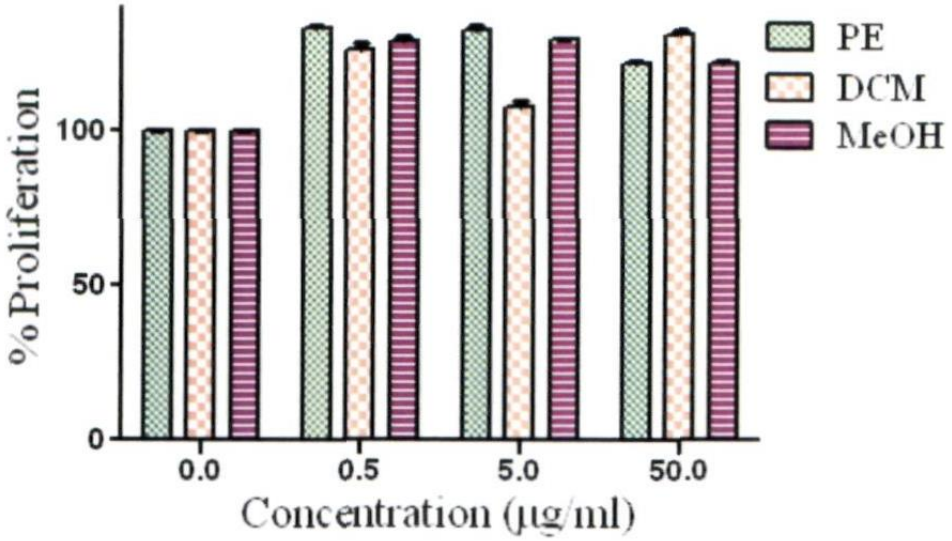

Figure 1a: "Screening of various plant extracts of *Vanda tessellata* on Colo205 by the MTT assay"

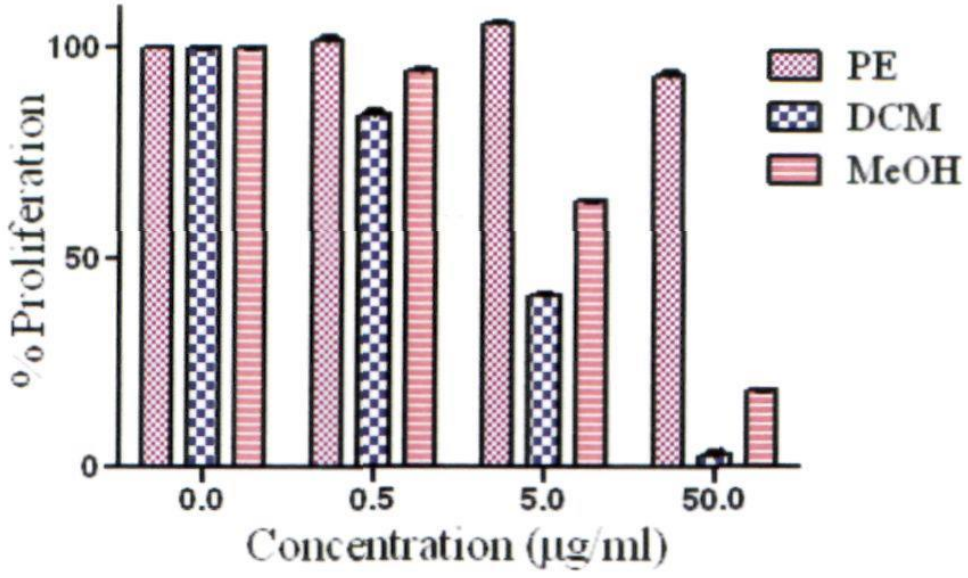

Figure 1b: "Screening of various plant extracts of *Ipomoea aquatica* on Colo205 by the MTT assay"

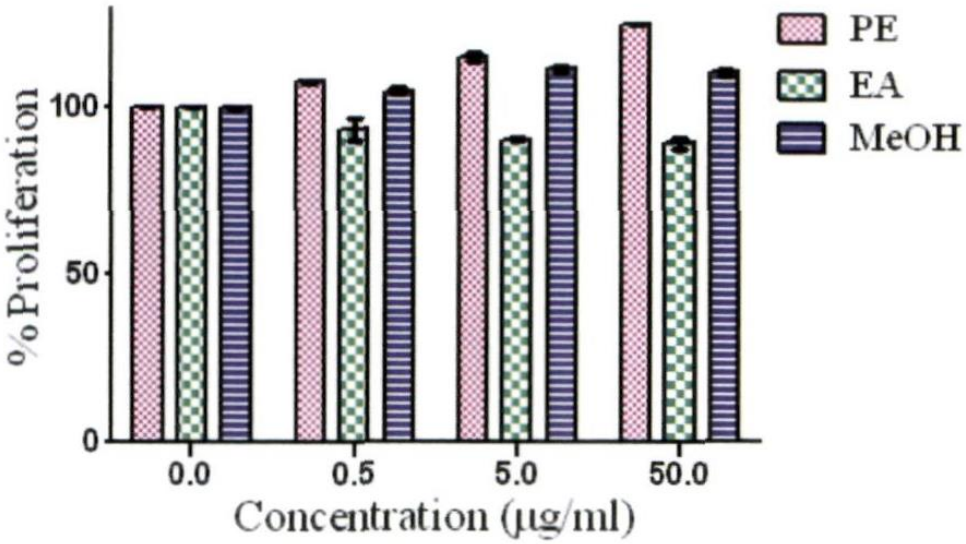

Figure 1c: Screening of various plant extracts of *Semen armeniacae* on Colo205 by the MTT assay

The values of percent proliferative response and percent cytotoxicity for the different extracts examined on Colo205 are summarised in Table 1.

Table 1-"Values of % proliferative response as well as % cytotoxicity of the various extracts studied on Colo205"

NAME OF THE PLANT	EXTRACT	% PROLIFERATION, 24h			% CELL GROWTH INHIBITION*		
		0.5 µg/ml	5 µg/ml	50µg/ml	0.5 µg/ml	5 µg/ml	50µg/ml
Vanda tessellata	PE	133.14±0.34	132.51±1.34	121.54±0.98	PR	PR	PR
	DCM	125.71±2.13	107.74±2.06	131.04±1.04	PR	PR	PR
	MeOH	128.68±1.2	129.04±0.11	121.46±1.02	PR	PR	PR
Ipomoea aquatica	PE	101.53±1	105.38±0.45	93.48±0.98	NR	NR	7
	DCM	84±1.0l-	41.04±0.67	2.78±1.08	16	59	97
	MeOH	94.44±0.45	63.59±0.07	18.19±0.07	6	36	82
Semen armeniacae	PE	107.78±0.5	115.05±1.12	124.95±0.11	NR	PR	PR
	EA	93.44±3.2	90.65±0.15	89.61±1.78	7	9	10
	MeOH	104.99±1.05	111.61±0.88	110.36±1.06	NR	PR	PR

"NR= No Response, PR= Proliferative",
"% **cell growth inhibition= 100 % proliferation"**

•"Indicates values rounded off to the next approximate number".

Regarding Vanda tessellata extracts, the proliferative capacity of the plants is one hundred percent. Despite its low cytotoxicity (down to 5 ug/ml), the PE extract of Ipomoea aquatica exhibited minimal cytotoxicity at high concentrations (50 ug/ml). In contrast, the DCM and MeOH extracts demonstrated significant cytotoxicity. The cell lines demonstrated this pronounced toxicity. The EP and MeOH extracts of Semen armeniacae exhibited proliferation in cell lines at all concentrations, whereas the EA extract exhibited weak cytotoxicity.

Singh et al. (2020) and Vaidya (2019) described this study's screening of plants for anticancer activity against colorectal adenocarcinomas. Anticancer efficacy in vitro was assessed using the MTT assay on extracts obtained from three plants. In vitro analysis revealed that dichloromethane and petroleum ether extracts of Vanda tessellata exhibited the most promising activity among the three plants examined.

4. Conclusion

Natural products provide an avenue through which new chemical constituents with particular reactivity can be identified. Through systematic exploration, novel chemical moieties derived from

plants can be isolated and potentially employed in the treatment of aforementioned disorders. This is particularly crucial in the context of cancer treatment, given the rapid development of drug resistance by cancer cells.

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