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Evaluation of anticancer potential of leaves of *Ananas comosus*

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ABSTRACT

Leaves of Ananas comosus was evaluated earlier for number of activities except for its anticancer potential on HT29 cell line. The flavonoid enriched fraction [FEF] of hydro alcohol extract of leaves was evaluated for cytotoxicity by MTT assay method in HT 29 cell line. It was found to have remarkable cytotoxic effect which was proportionately increasing with increase in concentration.

Keywords: Ananas comosus, Cytotoxic, HT 29 cell line and MTT assay

INTRODUCTION

Though there are many methods like Chemotherapy, Radiation, Surgery and combination of above methods for cancer treatment, health care researchers throughout the world are continuing with their efforts to find out better remedy. As all the above methods and drugs have one or other problem, focus is shifted to find out a natural drug. Ananas comosus is one of the potential candidates for the same as promising constituent like Bromolain [1, 2, 3, 4] has been isolated from it and successfully marketed.

Ethno pharmacological information of it also shows its use ranging from purgative, anthelmintic, hiccups, diuretic, diaphoretic, abortificient, menorrhagia to uterine fibroids [5, 6, 7] More recent studies have proved its use in soft tissue inflammation and oedema. Bromolain isolated from it is used as an anti-inflammatory and wound healer. Hence crown leaves of Ananas comosus which are thrown as waste was taken up for present study.

MATERIALS AND METHODS

The crown leaves of Ananas comosus was collected from the fruits purchased from the local market and authenticated by the Taxonomist of D.B Jain College, Chennai. The leaves were cut into small pieces and thoroughly dried in shade without contamination by dust or insects. It was powdered in a mill without producing heat and passed through no.10 sieve to get uniform particles. They were then subjected to extraction using soxhlet apparatus [8] It was first extracted with Petroleum Ether [60^{o} -80^{o}c] to defat it and then after drying the marc extracted with Ethyl alcohol 60%

Total extract thus obtained was further extracted with Ethyl acetate to enrich the flavonoid content, though other constituents soluble in Ethyl acetate were also extracted with flavonoids [9] This extract was used for pharmacological evaluation.

PHARMACOLOGICAL EVALUATION

Cytotoxicity Assay by MTT Method

Traditionally, the in vitro determinations of toxic effects of unknown compounds have been performed by counting viable cells after staining with a vital dye. Alternative methods used are measurement of radioisotope incorporation as a measure of DNA synthesis, counting by automated counters and others which rely on dyes and cellular activity. The MTT system is a means of measuring the activity of living cells via mitochondrial dehydrogenases. The MTT method is simple, accurate and yields reproducible results. The key component (3-[4, 5- dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) or MTT, is a water soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by mitochondrial dehydrogenase enzymes of viable cells. This water insoluble formazan can be solubilized using DMSO, acidified isopropanol or other solvents (Pure propanol or ethanol). The resulting purple solution is spectrophotometrically measured. An increase or decrease in cell number results in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxcity caused by the test material.

Procedure for Determining Cell Cytotoxicity

Cell Seeding

For adherent cells: 100-200µl of desired cell suspension was seeded in a 96-well plate at required cell density (25,000-50,000 cells per well), without the test agent. The cells were allowed to adhere to the culture plate for about 24 hours. (Appropriate concentrations of the test agent were added).

The plate was incubated for at 37°C in a 5% CO2 atmosphere for 24 hrs. After 24 hours the growth medium was removed, freshly prepared plant extracts were serially diluted (300µg, 100µg, 10 µg, 1 µg, 0.1 µg) and 100µl of each dilution was seeded in 96-well plate in triplicate

After the incubation period, the plates were removed from incubator and MTT reagent was added to a final concentration of 10% of total volume. This volume was same as the volume used while determining optimum cell density.

The plate was wrapped with aluminum foil to avoid exposure to light. The plates were returned to the incubator and incubated for 2 to 4 hours.

(Note: Incubation time varies for different cell lines. Within one experiment, incubation time was kept constant while making comparisons.)

For adherent cells, the culture medium was aspirated without disturbing the monolayer. Then solubilization solution was added in an amount equal to the culture volume

Gentle stirring in a gyratory shaker was done to enhance dissolution. Occasionally, pipetting up and down was carried out to completely dissolve the MTT formazone crystals especially in dense cultures. The absorbance was noted on a Spectrostar Nano ELISA Plate reader at 570nm.

RESULTS AND DISCUSSION

The alcohol60% extract was used to prepare **Flavonoid enriched fraction [FEF]** for evaluation of cytotoxicity by **MTT assay method in HT-29 cell line.**

It was found that the Flavonoid enriched fraction of leaves of Ananas comosus has a moderate anticancer activity. There was a linear increase in the activity with increase in the concentration of the test material i.e., in higher doses there was an improvement in the activity. A decrease in the number of cells resulted in a concomitant change in the amount of formazan formed, indicating the degree of cytotoxicity caused by the test material. The IC_{50} value was found to be 57.67 which mean that the FEF of leaves of Ananas comosus has moderate and significant cytotoxic activity.

STATISTICAL ANALYSIS

IC50 Value

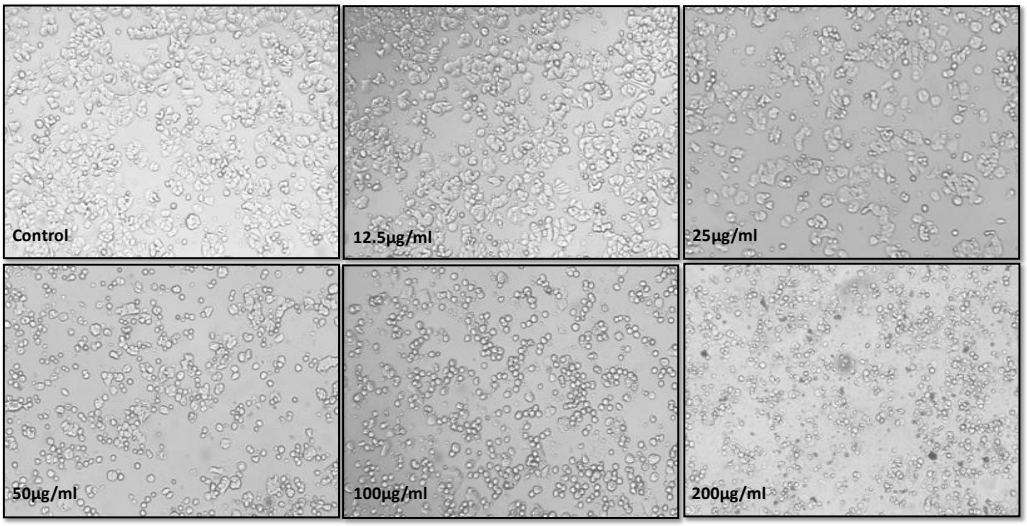
The half maximal inhibitory concentration (IC50) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half. The

IC50 of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist on reversing agonist activity. IC50 values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist.IC50 values for cytotoxicity tests were derived from a nonlinear regression analysis (curve fit) based on sigmoid dose response curve (variable) and computed using Graph Pad Prism 6 (Graph pad, SanDiego, CA, USA)

Nonlinear regression

In statistics, nonlinear regression is a form of regression analysis in which observational data are modeled by a function which is a nonlinear combination of the model parameters and depends on one or more independent variables. The data are fitted by a method of successive approximations

Cytotoxic effect of Sample - 1 on HT-29 cells



Cytotoxic effect of Sample - 1 on HT-29 cells

	1	2	3	4	5	6
A	0.228	0.205	0.213		0.171	650
B	0.208	0.221	0.235		0.229	650
C	0.215	0.208	0.237		0.192	650
D	0.189	0.178	0.193		0.206	650
E	0.197	0.205	0.183			650

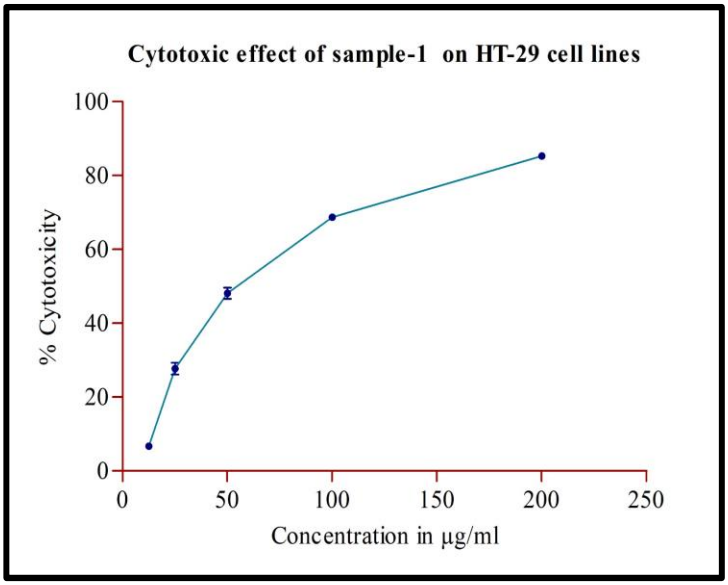
	1	2	3	4	5	6
A	1.826	1.813	1.843		1.983	570
B	1.471	1.487	1.452		1.893	570
C	1.083	1.125	1.141		1.906	570
D	0.748	0.713	0.723		1.927	570
E	0.434	0.465	0.448			570

Control	1.812	1.714	
	1.664	1.721	
		Mean	1.72775

		OD (570nm - 630nm)		
Concentration		R1	R2	R2
12.5		1.598	1.608	1.63
25		1.263	1.266	1.217
50		0.868	0.917	0.904
100		0.559	0.535	0.53
200		0.237	0.26	0.265

% Cytotoxicity				% Cytotoxicity		
R1	R2	R2		Mean	SD	SEM
7.509767	6.93098	5.657647		6.699465	0.947516	0.547049
26.89915	26.72551	29.56157		27.72874	1.589647	0.917783
49.76125	46.92519	47.67762		48.12135	1.469178	0.84823
67.64578	69.03487	69.32427		68.66831	0.897276	0.518043
86.28274	84.95153	84.66213		85.2988	0.864314	0.499012

	Nonlin fit	A
		% Cytotoxicity
		Y
1	log(inhibitor) vs. normalized response -- Va	
2	Best-fit values	
3	LogIC50	1.738
4	HillSlope	1.397
5	IC50	54.67
6	Std. Error	
7	LogIC50	0.01274
8	HillSlope	0.05744
9	95% Confidence Intervals	
10	LogIC50	1.710 to 1.765
11	HillSlope	1.273 to 1.521
12	IC50	51.32 to 58.25
13	Goodness of Fit	
14	Degrees of Freedom	13
15	R^2	0.9908
16	Absolute Sum of Squares	108.8
17	Sy.x	2.893
18	Number of points	
19	Analyzed	15



There is further scope in this project that the constituent or constituents responsible for this significant anti-cancer (cytotoxicity) potential of FEF and alcohol 60% extracts of leaves of Ananas comosus can be isolated in pure form and their identification including structure can be elucidated. After which they can be used for conforming above, as well as other Pharmacological activities.

CONCLUSION

The Flavonoid Enriched Fraction of the alcohol 60% extract of the leaves of Ananas comosus has remarkable cytotoxic activity at higher concentration. As mentioned above constituent(s) responsible for it can be isolated and further studied. Works towards that is in progress in our laboratory.

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