Evaluation of Invitro Antioxidant and Anticancer Activities of Bauhinia Purpurea Leaves

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Abstract:

Cancer is one of the most life threatening diseases, with more than 100 different types occurring due to some molecular changes within the cell. It is the third leading cause of death worldwide following cardiovascular and infectious diseases. In recent years, more interest has been paid to protect human beings against oxidative damage caused by free radicals which leads to ageing and human diseases like diabetes and cancer. The many medicinal plants are traditionally used for the treatment of many diseases.But there is no scientific report for proving this effect.Through,the literature Survey,we have selected the medicinal plant such as bauhinia purpurea (family: fabaceae). This plant is useful in treatment of various diseases like diarrhoea, dysentery, amoebic dysentery, inflammatory swelling,and hemorrhage - bleeding and skin disease.

Key Words: Cancer, Free radicals,Medicinal Plants,Swelling,hemorrhage

Introduction

Cancer is considered as the second reason for death after heart diseases, and breast cancer is the most common cancer in women between the ages of 40-44 years. Cancer is one of the most life-threatening diseases, with more than 100 different types occurring due to some molecular changes within the cell.[1] It is the third leading cause of death worldwide following cardiovascular and infectious diseases. It is estimated that 12.5% of the population dies due to cancer (WHO, 2004). The disease is widely prevalent, and in the West, almost a third of the population develops cancer at some point of time during their life.[2] Cancer is many diseases grouped into one umbrella term, all of whom share one common characteristic above all- the unnatural growth of cells. Cancer is an ancient disease and nowadays, the incidence of malignant cancer and cancer mortality is on the rise. According to the International Agency of Research for Cancer (IARC), the rise will be about 50% in the next 20 years. In low to middle income countries, a quarter of disease related deaths are caused due to cancer.[3] An estimate has been put forward that approximately 50% of cancer patients in the United States utilize medicinal agents that are derived from different plant parts or plant nutrients either exclusively or with conventional therapeutic procedures such as chemotherapy or radiation treatment. Since the 1940s a range of anticancer drugs have been introduced in the market and about 73% of them could be traced to be derived from natural products.[4]

Methodology

Selection of plant material

The many medicinal plants are traditionally used for the treatment of many diseases.But there is no scientific report for proving this effect.Through,the literature Survey,we have selected the medicinal plant such as bauhinia purpurea (family: fabaceae). This plant is useful in treatment of various diseases like diarrhoea, dysentery, amoebic dysentery, inflammatory swelling,and hemorrhage - bleeding and skin disease.

Collection of plant material

This plant was selected and collected from tirumala hills, Tirupati, Andhra Pradesh for the present study.

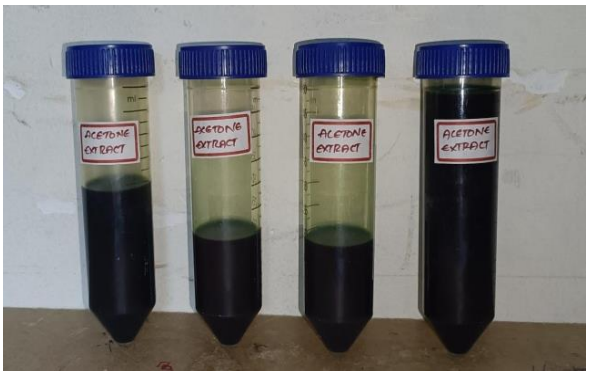
Authentication of plant material

The leaves of bauhinia purpurea were collected and authenticated by Dr.N.Savithramma professor of Botany, Co- Ordinator DDE Botany , Sree Venkateshwara University, Tirupati.

Extraction of plant material

The leaves were shade dried at room temperature for 10 days. Then these were milled intopowder by mechanical grinder. This powder was sequentially extracted to their increasingpolaritywithAcetone. And this extract was kept under observation for 24 hours for the isolation of phytoconstituents in the extract. After 24 hours the extract was filtered and the filterate was collected and then the filterate was subjected to the

phytochemical screening. The above extract was subjected to study the invitro antioxidant and anticancer activities[5].



SEM Analysis

The scanning electron microscope (SEM) uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that derive from electron-sampleinteractions reveal information about the sample including external morphology (texture), chemical composition, and crystalline structure and orientation of materials making up the sample.The surface characteristics of Acetone water extract of *Bauhinia purpurea* with was studied by SEM (Vegan 3 tescan).

Invitro Antioxidant Activity

Antioxidants are often used in oils and fatty foods to retard their autoxidation. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have restricted use in foods as they are suspected to be carcinogenic. Therefore, the importance of search for natural antioxidants has greatly increased in the recent years. Ethnomedical literature contains a large number of plants that can be used against diseases, in which reactive oxygen species and free radical play important role.In our present study, we evaluate the antioxidant activity by invitro antioxidant activity by three methods i.e., Hydroxyl radical scavenging activity, Nitric oxide radical scavenging activity and Total phenol content.

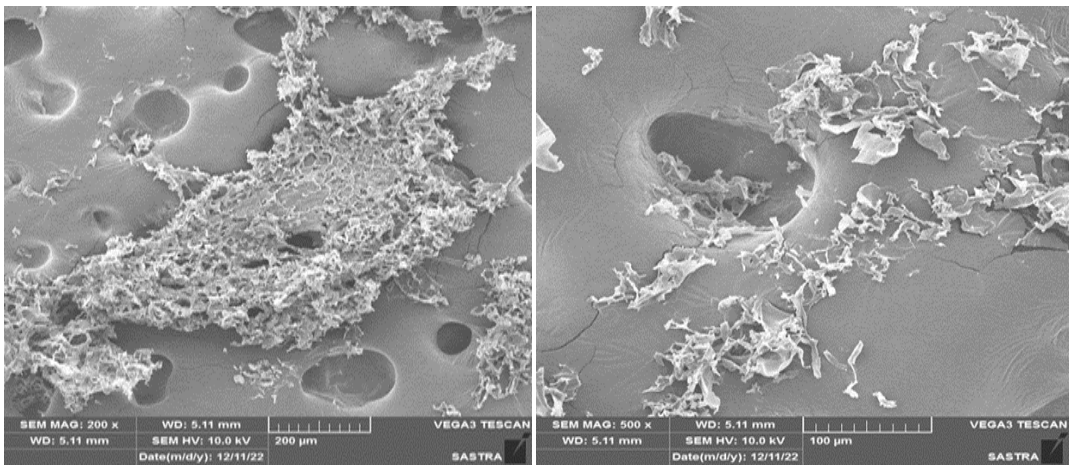
Invitro Anticancer Activity By HT-29 And AGS Cell Lines

Human colorectal adenocarcinoma cell line (HT-29) andadenocarcinoma gastric cell line (AGS)were exposed to acetone extract of *Bauhinia purpurea* for24h and cytotoxicity was determined with the MTT assay. The Percentage cancer cellinhibitionprofileswerefoundtobeconcentrationdependent.Themaximumconcentrationusedinthestudywas100 mg/ml.Ht-29 and AGS celllines,when subjectedtodifferentconcentrationsofplantextractsdisplayedweakinhibitionof19.75%. It was observed that a gradual increase in percentage inhibition was observed in all the cases

Results And Discussion

Scanning Electron Microscopy (SEM):

The specimens were scanned with an electron beam of acceleration potential of 10 kV and the images were collected as secondary electron mode.



Evaluation of Invitro Antioxidant Activity By Invitro Techniques

Hydroxyl radical scavenging activity

The percentage of hydroxyl radical scavenging activity of *Bauhinia purpurea* presented in Table 1. *Bauhinia purpurea* was exhibited a maximum hydroxyl radical scavenging activity of 48.01 % at µg/ml whereas for ascorbate (standard) was found to be 48.04 % at 100 µg/ml. The IC_{50} values of *Bauhinia purpurea* and ascorbate were found to be 600µg/ml and 100µg/ml respectively.

Table 1: Hydroxyl radical scavenging activity of *Bauhinia purpurea*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (*Bauhinia purpurea*)	Standard (Ascorbate)
1	25	22.32 ± 0.012	28.06 ± 0.017
2	50	26.12 ± 0.021	34.22 ± 0.076
3	75	30.12 ± 0.014	40.08 ± 0.014
4	100	35.28 ± 0.012	48.04 ± 0.033
5	200	37.07 ± 0.011	52.23 ± 0.048
6	400	43.22± 0.069	56.11 ± 0.031
7	600	48.01 ± 0.014	62.22 ± 0.014
		IC_{50} = 600 µg/ml	**IC_{50} = 100 µg/ml**

*All values are expressed as mean ± SEM for three determinations.

Nitric oxide radical scavenging activity

The reduction of nitric oxide radical by the *Bauhinia purpurea* and ascorbate were illustrated in Table 2. The maximum nitric oxide scavenging activity of *Bauhinia purpurea* and ascorbate at 600 µg/ml were found to be 76.19 % and 76.23% respectively. The IC_{50} value of *Bauhinia purpurea* and ascorbate were recorded as 600µg/ml and 100µg/ml respectively.

Table 2: Nitric oxide scavenging activity of *Bauhinia purpurea*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (*Bauhinia purpurea*)	Standard (Ascorbate)
1	25	25.22 ±0 .013	39.22 ± 0.026
2	50	32.13 ± 0.040	48.28 ± 0.069
3	75	40.18 ± 0.032	56.14 ± 0.021
4	100	46.17 ± 0.037	76.23 ± 0.022
5	200	58.21 ± 0.019	82.20 ± 0.027
6	400	64.11 ± 0.022	91.24 ± 0.044

7	600	76.19 ± 0.012	102.32 ± 0.041
		IC_{50} = 600 µg/ml	**IC_{50} = 100 µg/ml**

*All values are expressed as mean ± SEM for three determinations.

Total phenol content

Phenolic compounds are known as powerful chain breaking antioxidants. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. The phenolic compounds may contribute directly to antioxidant action. The total phenolic content of *Bauhinia purpurea* and ascorbate were illustrated in Table 3.

Table 3: The total Phenolic content of *Bauhinia purpurea*

S.No	Extracts	Total phenol content (mg/g Gallic acid) (±SEM)*
1	*Bauhinia purpurea*	1.38 ± 0.080

Based on the result the *Bauhinia purpurea* was found higher content of phenolic components.

InVitroAnticancerActivity
MTT assay of AGS cell lines
Sample ID
Cell lines : AGS
Incubation Time : 48hr

Concentration (ug/mL)	OD1	OD2	OD3	% Cell Death			Mean	SD	SEM	% Live Cells
100	0.096	0.102	0.102	71.34328	69.55224	69.55224	70.14925	0.344687	0.199005	29.85075
50	0.125	0.124	0.126	62.68657	62.98507	62.38806	62.68657	0.298507	0.172343	37.31343
25	0.135	0.145	0.132	59.70149	56.71642	60.59701	59.00498	1.950689	1.126231	40.99502
12.5	0.145	0.144	0.169	56.71642	57.01493	49.55224	54.42786	3.789377	2.187798	45.57214
6.25	0.221	0.224	0.235	34.02985	33.13433	29.85075	32.33831	1.712869	0.988925	67.66169
3.125	0.235	0.265	0.248	29.85075	20.89552	25.97015	25.57214	2.821967	1.629264	74.42786
1.7	0.269	0.278	0.299	19.70149	17.01493	10.74627	15.8209	3.328503	1.921712	84.1791
Control	0.325	0.325	0.356							100
Control Mean	0.335333									

Fig 1: Cell Viability Data

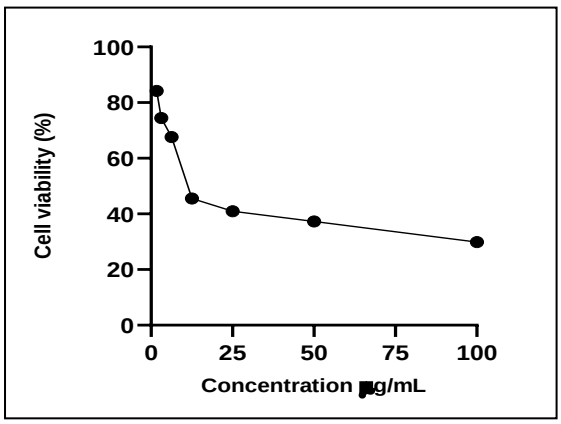
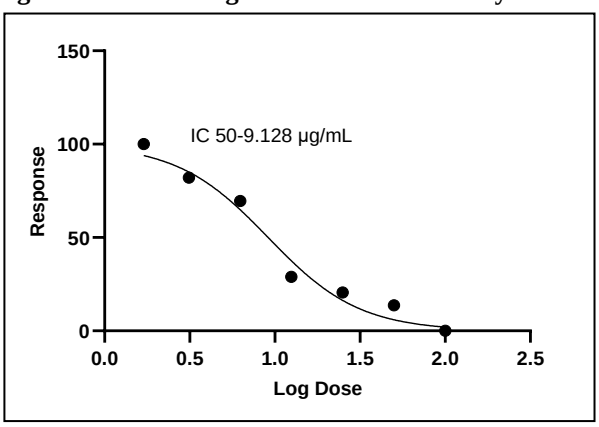
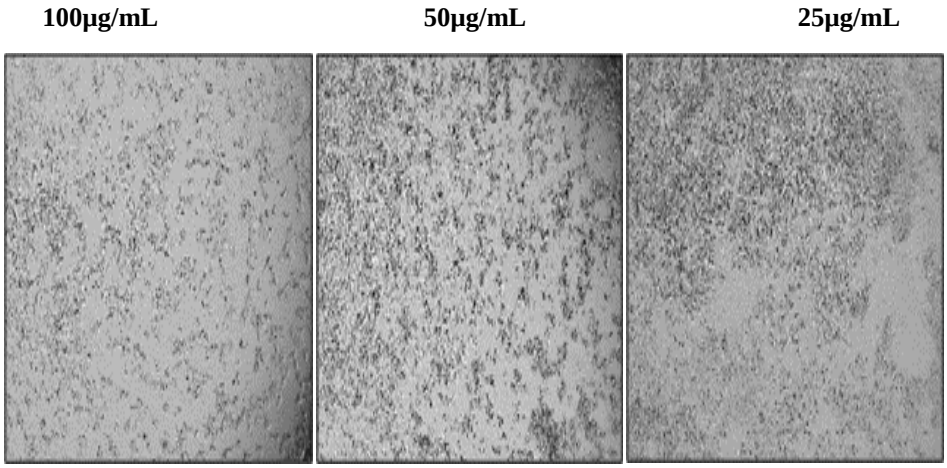
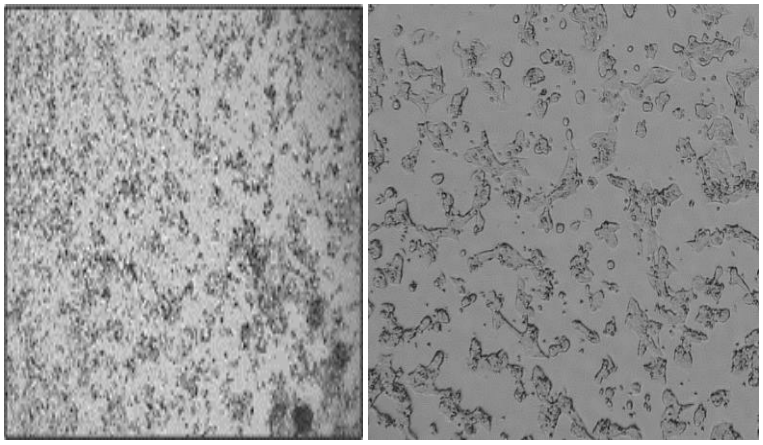


Fig 2 Non Linear Regression Curve Fit Analysis







MTT assayof HT-29 cell lines

Sample ID
Cell lines : HT-29
Incubation Time : 48hrs

Concentration (ug/mL)	OD1	OD2	OD3	% Cell Death			Mean	SD	SEM	% Live Cells
100	0.052	0.059	0.069	85.75342	83.83562	81.09589	83.56164	1.508923	0.871177	16.43836
50	0.085	0.096	0.098	76.71233	73.69863	73.15068	74.52055	0.689483	0.398073	25.47945
25	0.166	0.152	0.154	54.52055	58.35616	57.80822	56.89498	0.738165	0.42618	43.10502
12.5	0.198	0.201	0.211	45.75342	44.93151	42.19178	44.29224	1.433334	0.827536	55.70776
6.25	0.202	0.212	0.221	44.65753	41.91781	39.45205	42.00913	1.450685	0.837554	57.99087
3.125	0.265	0.245	0.265	27.39726	32.87671	27.39726	29.22374	2.79	1.610807	70.77626
1.7	0.289	0.296	0.258	20.82192	18.90411	29.31507	23.0137	5.243791	3.027504	76.9863
Control	0.366	0.389	0.365							100
Control Mean	0.373333									

Fig 1: Cell Viability Datas

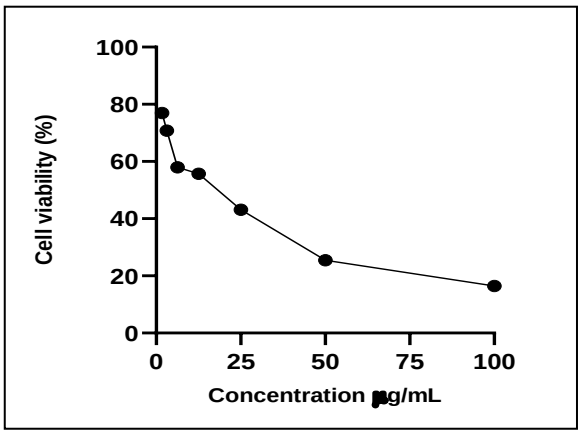
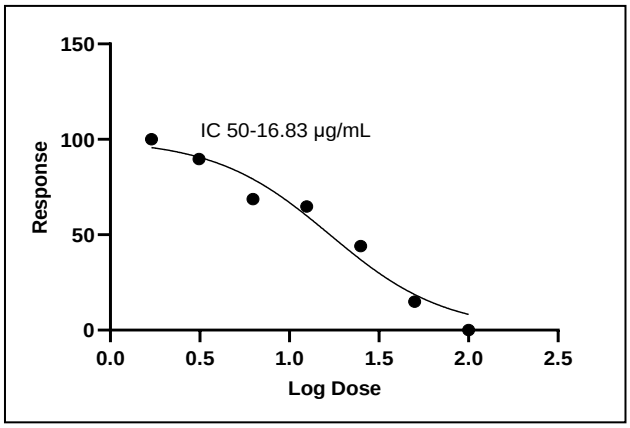
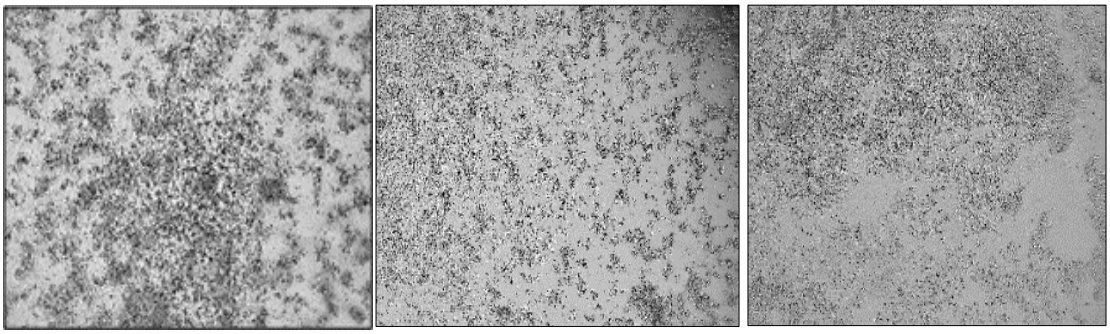


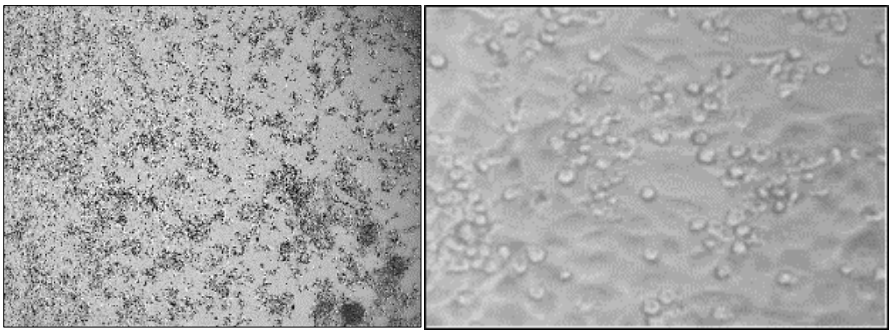
Fig 2 Non Linear Regression Curve Fit Analysis



100µg/mL **50µg/mL** **25µg/mL**



12.5µg/mL **HT-29 cell lines (untreated)**



Summary conclusion

Natural products discovered from medicinal plants have played an important rolein the treatment of cancer.The present study points to antioxidant and the potential anticancer activity of acetoneextractof *Bauhinia pupurea*. The leaves of bauhinia purpurea were collected and authenticatedby Dr. N.Savithramma professor of Botany, Co- Ordinator DDE Botany , Sree Venkateshwara University, Tirupati. At first we collected the leaves from our surrounding plants and we kept it for drying for 20 days. Then we make powder of the dried leaves, and acetone extract of *Bauhinia Purpurea* leaves was done. Then we performed the phytochemical screening tests and we get positive results for flavonoids, tannins, steroids/terpenoids, glycosides and saponins.

Conclusion

The present study was clearly indicated the *Bauhinia purpurea* showed strong antioxidant activity by inhibiting Hydroxyl radical scavenging, Nitric oxide radical scavenging activities when compared with standard Ascorbate. In addition, the *Bauhinia purpurea* was found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidants. Therefore, further investigations need to

be carried out to isolate and identify the antioxidant compounds present in the plant extract. And it also showed anticancer activity by increasing percentage death cell and decreasing the live cells percent. As the concentration of *Bauhinia Purpurea* leaves extract and optical density increases the percentage death cell also increases. So it showed anticancer activity.

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