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Evaluation Of In Vitro Antioxidant and Anticancer Properties of *Bauhinia Purpurea* Leaves.

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Abstract

"Cancer is one of the most life-threatening diseases, with over 100 different types arising from molecular changes within cells. It is the third leading cause of death globally, following cardiovascular and infectious diseases. In recent years, there has been growing interest in protecting humans against oxidative damage caused by free radicals, which contribute to aging and diseases such as diabetes and cancer. Many medicinal plants have been traditionally used to treat various ailments, but scientific validation of these effects is often lacking. Based on a literature review, we have selected the medicinal plant *Bauhinia purpurea* (family: Fabaceae) for further study. This plant has been traditionally used to treat a range of conditions, including diarrhea, dysentery, amoebic dysentery, inflammatory swelling, hemorrhage, and skin diseases."

Keywords: Cancer, Free radicals, Medicinal Plants, Swelling, hemorrhage.

Introduction

Cancer is the second leading cause of death after heart disease, with breast cancer being the most common type among women aged 40-44. This life-threatening disease encompasses over 100 different types, all resulting from molecular changes within cells. Cancer ranks as the third leading cause of death worldwide, following cardiovascular and infectious diseases, and it is estimated that 12.5% of the

global population succumbs to cancer (WHO, 2004). The disease is prevalent, especially in Western countries, where nearly one-third of the population is expected to develop cancer at some point in their lives. Cancer is not a single disease but a group of diseases that share a common characteristic: the uncontrolled growth of cells. Though cancer is an ancient disease, the incidence of malignant cancers and cancer-related mortality is on the rise.

According to the International Agency for Research on Cancer (IARC), the incidence of cancer is expected to increase by about 50% over the next 20 years. In low- to middle-income countries, cancer accounts for a quarter of all disease-related deaths. It is estimated that around 50% of cancer patients in the United States use medicinal agents derived from various plant parts or nutrients, either alone or in conjunction with conventional treatments like chemotherapy or radiation. Since the 1940s, numerous anticancer drugs have been developed, with about 73% of them being traced back to natural products.

Methodology

Selection of plant material

Many medicinal plants have been traditionally used to treat a variety of diseases, though scientific evidence supporting these uses is often lacking. Based on a literature review, we have selected the medicinal plant *Bauhinia purpurea* (family: Fabaceae) for further investigation. This plant has traditionally been used to treat a range of conditions, including diarrhea, dysentery, amoebic dysentery, inflammatory swelling, hemorrhage, 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.Savithamma 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 and then milled into a powder using a mechanical grinder. This powder was sequentially extracted with acetone, following increasing polarity. The extract was then left to stand for 24 hours to allow for the isolation of phytoconstituents. After 24 hours, the extract was filtered, and the filtrate was collected and subjected to phytochemical screening. This extract was subsequently used to study its *in vitro* antioxidant and anticancer activities.

SEM Analysis

The scanning electron microscope (SEM) utilizes a focused beam of high-energy electrons to produce various signals at the surface of solid specimens. These signals, generated from electron-sample interactions, provide detailed information about the sample, including its external morphology (texture), chemical composition, and the crystalline structure and orientation of the materials comprising the sample. The surface characteristics of the acetone-water extract of *Bauhinia purpurea* were analyzed using SEM (Vega 3 TESCAN).

Invitro Antioxidant Activity

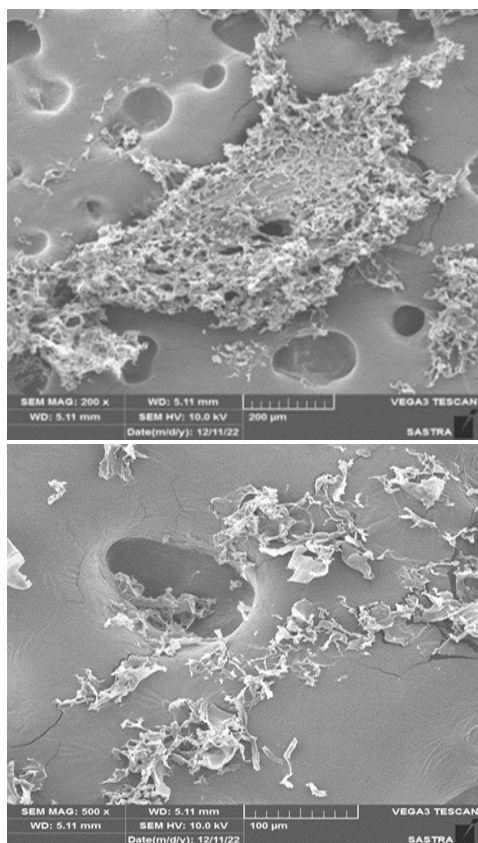
Antioxidants are commonly used in oils and fatty foods to slow down autoxidation. However, the use of synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), is limited in foods due to concerns over their potential carcinogenicity. As a result, there has been a growing

emphasis on finding natural antioxidants. Ethnomedical literature documents a vast number of plants that may be effective against diseases where reactive oxygen species and free radicals play a significant role. In our current study, we evaluate the antioxidant activity of *Bauhinia purpurea* through in vitro assays, specifically by assessing hydroxyl radical scavenging activity, nitric oxide radical scavenging activity, and total phenol content.

Results And Discussion

Scanning Electron Microscopy (SEM)

When a specimen is scanned with an



*Evaluation of Invitro Antioxidant Activity
By Invitro Techniques*

electron beam at an acceleration potential of 10 kV in a Scanning Electron Microscope (SEM), the resulting images are produced by detecting secondary electrons (SEs).

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of *Bauhinia purpurea*, as presented in Table 1, reached a maximum of 48.01% at a certain concentration ($\mu\text{g/ml}$), while the standard ascorbate showed a similar activity of 48.04% at 100 $\mu\text{g/ml}$. The IC₅₀ values for *Bauhinia purpurea* and ascorbate were determined to be 600 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$, respectively.

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

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

*All values are expressed as mean $\pm$ SEM for three determinations.

Nitric oxide radical scavenging activity

The reduction of nitric oxide radicals by *Bauhinia purpurea* and ascorbate is illustrated in Table 2. The maximum nitric oxide scavenging activity at 600 µg/ml was found to be 76.19% for *Bauhinia purpurea* and 76.23% for ascorbate. The IC₅₀ values for *Bauhinia purpurea* and ascorbate were recorded as 600 µg/ml and 100 µg/ml, respectively.

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

S.No	Concentration (µg/ml)	% of activity(±SEM) *	
		Sample (<i>Bauhinia purpurea</i>)	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.

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

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

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

In Vitro Anticancer Activity MTT assay of AGS cell lines

Sample ID

Cell lines: AGS

Incubation Time: 48hr

Fig 1: Cell Viability Data Analysis

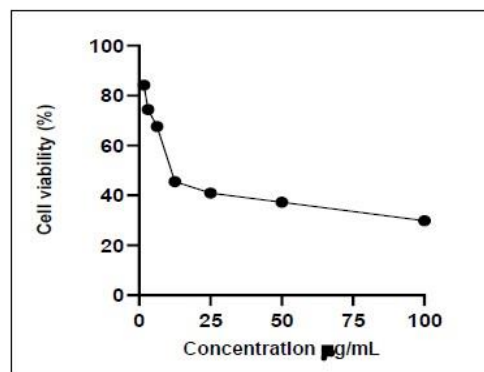
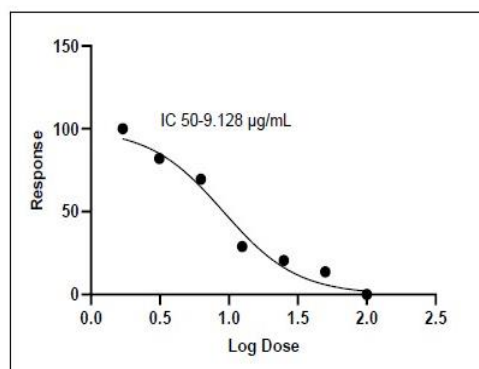
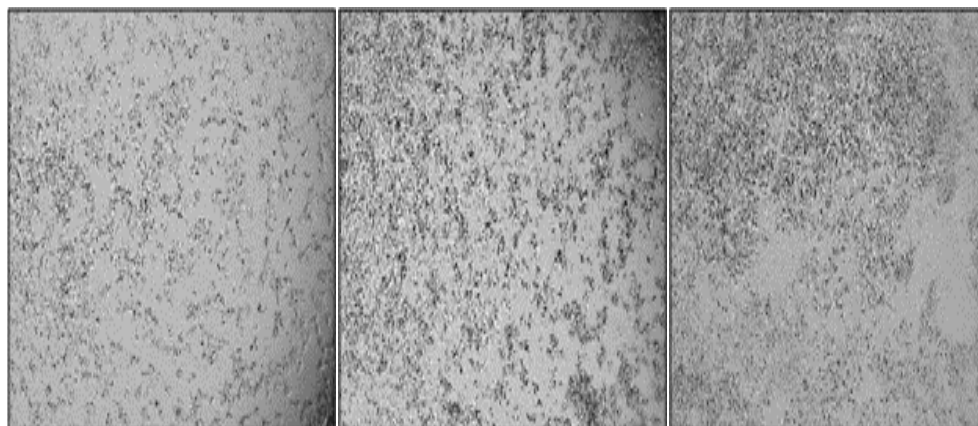
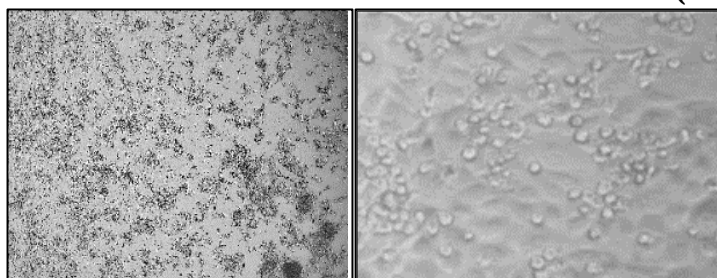


Fig 2 Non-Linear Regression Curve Fit



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									

100µg/mL**50µg/mL****25µg/mL****12.5µg/mL HT-29****cell lines (untreated)***MTT assay of 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	0.394073	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 Data's Analysis

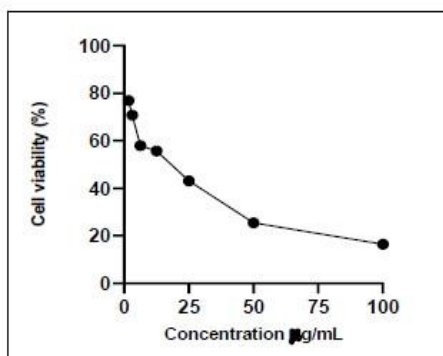
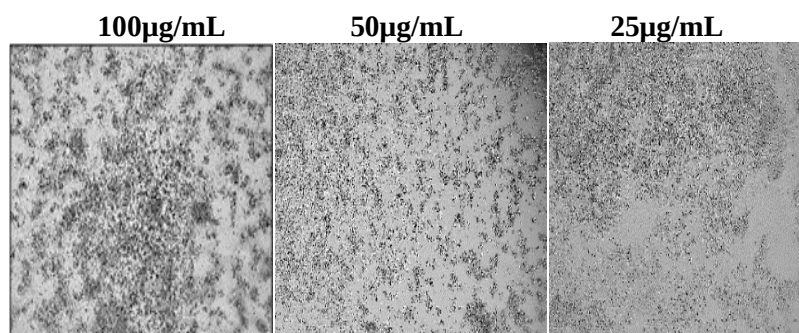
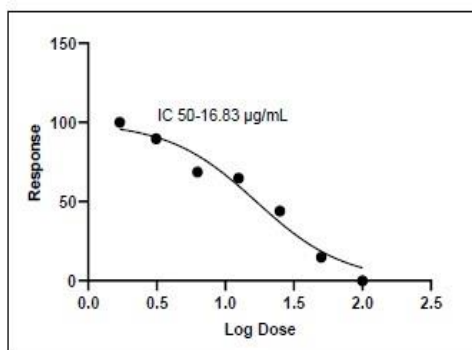
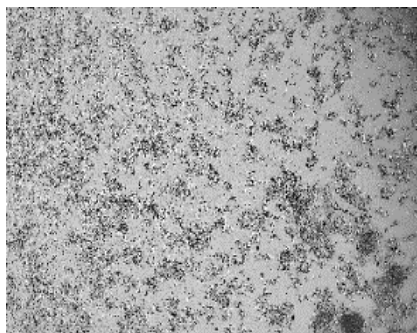


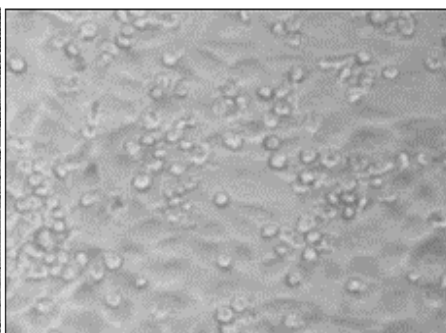
Fig 2 Non-Linear Regression Curve Fit



12.5µg/mL HT-29



Cell lines (untreated)



Summary conclusion

Natural products derived from medicinal plants have played a crucial role in cancer treatment. The present study highlights the antioxidant and potential anticancer activities of the acetone extract of *Bauhinia purpurea*. The leaves of *Bauhinia purpurea* were collected and authenticated by Dr. N. Savithramma, Professor of Botany and Coordinator of DDE Botany at Sree Venkateshwara University, Tirupati. First, we collected the leaves from surrounding plants and allowed them to dry for 20 days. After drying, the leaves were powdered, and an acetone extract of *Bauhinia purpurea* leaves was prepared. We then performed phytochemical screening tests, which yielded positive results for flavonoids, tannins, steroids/terpenoids, glycosides, and saponins.

Conclusion

The present study clearly indicates that *Bauhinia purpurea* exhibits strong antioxidant activity by inhibiting hydroxyl radical scavenging and nitric oxide radical scavenging activities, comparable to the standard ascorbate. Additionally, *Bauhinia purpurea* was found to contain a significant number of

total phenols, which play a major role in its antioxidant properties. Further investigations are necessary to isolate and identify the antioxidant compounds in the plant extract. The extract also demonstrated anticancer activity by increasing the percentage of cell death and decreasing the percentage of live cells. As the concentration of ***Bauhinia purpurea*** leaf extract and optical density increased, the percentage of cell death also rose, confirming its anticancer potential.

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