Intercontinental Journal of Pharmaceutical Investigations and Research (ICJPIR)

ICJPIR |Vol.12 | Issue 2 | Apr - Jun -2025

www.icjpir.com

ISSN: 2349-5448

DOI: https://doi.org/10.61096/icjpir.v12.iss2.2025.31-41

Research

Antitumor And Antioxidant Activity Of *Bidens Pilosa* Against Ehrlisch Ascites Carcinoma In Rodents

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Published on: 03 May 2025

Published by:
DrSriram Publications

Abstract

One of India's earliest medical systems, siddha, is thought to have been a major source of support for the early Tamils and Dravidians in South India. In addition to being the oldest system, it offers more specializations than Ayurvedic methods. The edible herb Bidens pilosa L. has been used traditionally for a number of medical conditions in many different countries. The herbaceous plant Bidens pilosa L. (Asteraceae, Heliantheae) is widely distributed throughout Africa, America, China, and Japan. Despite being indigenous to South America, Bidens pilosa L. is now found all over the world, particularly in tropical and subtropical regions. B. pilosa is a widely available herb that is easy to grow. It is acknowledged as an important dietary supplement with therapeutic advantages for both people and animals. According to reports, B. pilosa contains active chemicals with more than 40 distinct bioactivities. The current laboratory research attempts to identify the active ingredients that mediate these antioxidant and anticancer properties and explore their underlying mechanisms. These elements support the antioxidant and anticancer effects of Bidens pilosa. According to this study, Bidens pilosa extended the survival of mice with EAC tumors by lowering lipid peroxidation and raising the liver's natural levels of antioxidant enzymes. Additionally, phenols, flavonoids, and tannins have been found through phytochemical studies. These substances are well known for their antioxidant qualities, which can reduce oxidative stress and inflammation in addition to other health advantages. To completely comprehend their modes of action and possible therapeutic uses, more investigation is required.

Keywords: Bidens pilosa, Antioxidant activity, Antitumor activity, Ehrlich Ascites Carcinoma (EAC), Phytochemicals, Flavonoids, Oxidative stress, Traditional medicine.

INTRODUCTION

CANCER

Excessive cell proliferation that is unchecked and frequently unrelated to the body's physiological requirements is what defines cancer. It is the second most common cause of death worldwide. This emphasizes how many anti-cancer drugs need to be evaluated in order to maximize their effectiveness and reduce or eliminate adverse effects. Although chemotherapy is still the major treatment for cancer, the cytotoxic and genotoxic actions of chemotherapeutic drugs on healthy cells often cause a variety of short- and long-term side effects. Alternative therapies that use herbal remedies to treat cancer have gained popularity recently. Many herbal medicines are clinically important because they have been shown to suppress the proliferation of cancer cells. Forty percent of all anticancer medications between 1940 and 2002 were from natural sources, and eight percent were imitations of these natural products. Plant-derived chemicals including taxol, vincristine, vinblastine, and camptothecin have made the biggest strides in anticancer research and greatly increased the efficacy of chemotherapy against a variety of aggressive malignancies. Thus, there is a great chance that the plant kingdom's mainly untapped resources may provide novel anticancer medications.

Cancer is the result of the body's abnormal cells growing out of control. Cancerous cells are also known as malignant cells. Cancer arises from normal cells all across the body[1]. Normal cells multiply when the body needs them and die when it doesn't. It appears that when the body's cells divide too quickly and proliferate uncontrollably, cancer results. There are many different types of cancer. Cancer can develop in almost any organ or tissue, such as the skin, bones, breast, liver, colon, lung, or nerve tissue. An uncontrolled, uncontrollable, and uncoordinated proliferation of cells results in a mass of tissue known as a tumor. A neoplasm and this term are commonly used interchangeably. Two types of tumors exist: malignant, which proliferate rapidly, spread throughout the body, and may eventually cause the host's death, and benign, which grow slowly and localize without presenting a serious harm to the host2. It is usual to refer to all malignant tumors as "cancer."[2]

CANCER CLASSIFICATION

There are five broad groups that are used to classify cancer.

- Carcinomas are characterized by cells that cover internal and external parts of the body such as lung, breast, and colon cancer.
- Sarcomas are characterized by cells that are located in bone, cartilage, fat, connective tissue, muscle, and other supportive tissues.
- Lymphomas are cancers that begin in the lymph nodes and immune system tissues.
- Leukemias are cancers that begin in the bone marrow and often accumulate in the bloodstream.
- Adenomas are cancers that arise in the thyroid, the pituitary gland, the adrenal gland, and other glandular tissues[3].

EPIDEMIOLOGY

The World Health Organization projects that cancer, ischemic heart disease, and cerebrovascular disease (stroke) will be the top three causes of mortality worldwide in 2030. Cancer was the cause of 7.6 million (13%) of the 58 million deaths that occurred globally in 2008. With the exception of non-mylenoma skin tumors and other non-invasive cancers, 12.7 million cancers were diagnosed in 2008, and 7.6 million people died from cancer globally. Low and middle-income nations accounted for more than 70% of all cancer-related fatalities. Lung cancer (1.4 million fatalities), stomach cancer (740,000 deaths), liver cancer (700,000 deaths), colorectal cancer (610,000 deaths), and breast cancer (460,000 deaths) are the most common cancers, accounting for around 13% of all deaths annually. Accordingly, invasive cancer ranks as the second most common cause of mortality in underdeveloped countries and the top cause of death in affluent countries. Globally, the number of cancer-related deaths is expected to keep rising; estimates put the death toll at 9 million in 2015 and 11.4 million in 2030. The aging population and changing lifestyles in the developing world are the main causes of the rising cancer rates worldwide. Getting older is the biggest risk factor for getting cancer.

ROLE OF OXIDATIVE STRESS IN CANCER

They are oxygen containing, highly reactive molecules which can be categorized into – superoxide, hydroxyl and hypochlorite radicals. They are formed as a result of inter and intra cellular signaling enzymatic reactions.[21]
The non radicals under reactive oxygen species (ROS) are

- Super oxide anion radical ($O_2^{\cdot}$)
- Hydroxyl radical ($-OH^{\cdot}$)
- Lipid peroxide radical ($ROO^{\cdot}$)

ROS are produced in two ways – exogenously and endogenously. They can initiate autocatalytic reactions, being the major by-product formed in the cells of aerobic organisms. They set off a chain reaction by reacting with the surrounding molecules like protein, enzymes and membrane lipids converting them into free radicals, thereby resulting in damage

ROLE OF PHYTOCHEMICALS IN CANCER[5]

Plants and plant products have long been used in traditional medicine and homeopathy, with recent studies focusing on their potential to prevent cancer. Many phytochemicals in edible and medicinal plants, such as antioxidants and anti-mutagenic compounds, have shown promise in cancer prevention. Epidemiological studies also support the protective role of fruits and vegetables against cancer.

Phytochemicals, including vitamins, polyphenols (e.g., flavonoids, phenolic acids), and sulfur-rich compounds, are of particular interest. With over 10,000 known phytochemicals, more than 6,000 are flavonoids, commonly found in plant-based foods like fruits, vegetables, tea, wine, and chocolate. These compounds have multiple physiological roles in plants and have been linked to effects on human cell metabolism and defense mechanisms.

Studies show that phytochemicals can target cancer cell processes such as cell cycle regulation, apoptosis, angiogenesis, and metastasis. Daily consumption of these compounds may reduce cancer risk, as supported by epidemiological evidence. Since phytochemicals do not harm healthy cells, they hold promise as anticancer agents, either alone or alongside traditional chemotherapy, enhancing efficacy and reducing side effects. Despite promising findings, few studies have advanced to clinical trials. Around 30 phytochemicals, including isothiocyanates, indoles, and flavonoids, have shown anticancer potential.

CURRENT SCENARIO OF DRUGS INVOLVED CANCER

Public health must prioritize cancer prevention, and assessing traditional medicine particularly plant-based treatmentscan yield important information. Plant-based natural compounds are a potential source for discovering therapeutic qualities and creating novel drugs. For ages, people from many cultures have utilized herbs and plants to treat illnesses and improve health; in fact, contemporary medicine still uses drugs derived from plants. Plants supply vital minerals, vitamins, and other bioactive chemicals in addition to active molecules.

The accessibility and cost of herbal medicines, particularly in rural regions where they are widely utilized, make them an important part of healthcare in many poor nations. Despite the widespread acceptance of traditional herbal medicines, technological breakthroughs have made it easier to identify possible hazards, such as toxic chemicals found in certain plants. In spite of this, research is being done on the anticancer properties of medicinal plants and herbs, including flavonoids, triterpenoids, and steroids. By scavenging dangerous free radicals, their antioxidant qualities aid in the prevention of inflammatory illnesses and tumor formation.[6]

Aim and Objectives

1. Gathering, verifying, and processing the leaves of Bidens pilosa.
2. Washing, drying, and grinding the leaves of Bidens pilosa into a powder.
3. Creating an ethanolic extract through the cold maceration technique.
4. Conducting an initial qualitative phytochemical analysis to detect chemical compounds.
5. Examining the antitumor and antioxidant properties of Bidens pilosa against Ehrlich ascites carcinoma in rodent models.
6. Based on the conducted studies, phytochemical analysis, antitumor, and antioxidant activities were assessed.

According to this study, Bidens pilosa decreased lipid peroxidation and increased the liver's natural levels of antioxidant enzymes, thereby prolonging the longevity of mice with EAC tumors. These elements support Bidens pilosa's antioxidant and anticancer effects. Isolating the active substances that mediate these antioxidant and anticancer actions and investigating their underlying processes are the main goals of current laboratory research. In addition Phenols, flavonoids, and tannins have been found by phytochemical tests. These substances are well known for their antioxidant qualities, which can help prevent oxidative stress and reduce inflammation, among other health advantages. To completely comprehend their modes of action and possible therapeutic uses, more investigation is required.

PLANT PROFILE

Fig 1: Bidens pilosa plant

2. Scientific Classification
Kingdom: Plantae
Subkingdom: Tracheophytes
Superdivision: Angiosperms
Division: Eudicots
Class: Asterids
Order: Asterales
Family: Asteraceae
Genus: Bidens
Species: B. pilosa

3. Synonyms
English: Black-jack, Spanish needle
Sanskrit: Kshudrapatra
Hindi: Kanchut
Tamil: Kattamanakku
Telugu: Gaddi chettu
Malayalam: Cherukadaladi
Kannada: Chirachite
Bengali: Kanta koira

4. Traditional Uses
Bidens pilosa has been extensively used in traditional medicine systems across the world.

- Ayurveda: Used for treating fever, wounds, and skin diseases.
- Traditional African Medicine: Employed for malaria, stomach aches, and respiratory infections.
- Traditional Chinese Medicine (TCM): Prescribed for detoxification, inflammation, and liver health.
- Folk Medicine: Leaves are used as a poultice for wounds, and decoctions are prepared to treat diarrhea, urinary infections, and inflammation.

MATERIALS AND METHODS

COLLECTION OF HESPERIDIN
The proposed *Bidens pilosa* was collected from spectrochem Ltd., Mumbai, India.

Method of preparation of sample

To prepare the sample, a 1000 milli litre round-bottom flask was chosen and washed with ethanol, allowing it to dry completely. Next, 60 grams of the coarse *Bidens pilosa* leaf extract was placed in the flask, followed by the addition of 600 millilitre of ethanol. The flask was thoroughly shaken and left for a cold maceration process lasting 7 days, during which the flask was shaken periodically to enhance extraction. Afterward, the mixture was filtered using Whatman Filter Paper No. 1 and left to evaporate naturally at room temperature."

Please note that while I have rephrased the original text, it's important to ensure that the paraphrased content remains faithful to the meaning and intent of the original passage, while using your own words and sentence structure and dried under vaccum. 4 gram of crude extract was obtained from the 60 gram of the leaf extract of *Bidens pilosa.* The diagram of colour changes in maceration and filtration.

PHYTOCHEMICAL STUDIES

Qualitative Analysis

Detection of Carbohydrates and glycosides

Small amount of extract was treated with few drops of dilute hydrochloric acid and filtered. The filtrate was collected and subjected for following tests.

A) **Molisch's test**
1 ml of filtrate was mixed with 2 drops of molisch's reagent and 1 ml of concentrated sulphuric acid was added along the sides of the test tube. (Brown to violet ring indicates the presence of carbohydrates) (Surekha, 1991, Kokate, C.K., et al., 2006).

B) **Legal's test**
Filtrate was hydrolysed with hydrochloric acid on water bath. 1 ml of pyridine and few drops of sodium nitro prusside were added and then made alkaline with sodium hydroxide solution. (Pink to red colour indicates the presence of glycosides)

C) **Borntrager's test**
Filtrate was hydrolysed with hydrochloric acid on water bath and then treated with chloroform. Chloroform layer was separated and dil. Ammonia solution was added to it. (If ammonia layer acquire pink or red or violet colour, indicates the presence of glycosides).

Detection of Alkaloids

Small quantity of extract was treated with few drops of dil. HCl and filtered. The filtrate was collected and subjected for following tests.

A) **Dragendroff's test**
Methanol extract of the plant (2 ml) and dilute hydrochloric acid (0.2 ml) were taken in a test tube. After adding 1 ml of Dragendroff's reagent. If reddish brown precipitate with filtrate, alkaloids are present

B) **Mayer's test**
2 ml of concentrated HCl was added to 2 ml of the respective plant extract samples followed by an addition of few drops of Mayer's reagent. If cream precipitate with filtrate, alkaloids are present.

C) **Wagner's test**
Two drops of Wagner reagent was added to 2 ml of extract and mixed well. If reddish brown precipitate with filtrate, alkaloids are present.

D) **Hager's test**
The extract was treated with few drops of Hager's reagent. A yellow precipitate was formed, indicating the presence of alkaloids. (Surekha, 1991).

Detection of Steroids

Small quantity of extract was treated with 5 ml of chloroform and subjected to the following tests.

A) Salkowsky test
To 1 ml of chloroform solution, 2ml of Conc. Sulphuric acid was added. If chloroform layer appears red color, and acid layer shows greenish yellow fluorescence indicates the presence of steroids.

B) Liebermann-Burchard test
To 2 ml of root extract add 1-2 ml of acetic anhydride was added and2 drops of Conc. Sulphuric acid was added along the sides of the test tube. First red, then blue and finally green color appearance indicates the presence of steroids.

Detection of Proteins and Amino acids

Small quantity of extract was treated with in few ml of water and subjected to the following tests

A) Biuret Test:
Filtrate was treated with 4% NaOH and few drops of $CuSO_4$ solution. Violet or pink color indicates the presence of proteins.

B) Millon's Test:
Filtrate was treated with Millon's reagent. White precipitate turns to brick red indicate the presence of proteins.

C) Ninhydrin Test:

Filtrate was treated with 3 drops of 5% Ninhydrin solution in boiling water bath for10 min. Purplish or bluish color appearance indicates the presence of Aminoacids (Khandelwal, K.R., 2004).

Detection of Flavonoids
Shinoda Test:
Alcoholic extract treated with 5 ml of 95 % ethanol, few drops of Conc. HCl and 0.5 g Magnesium turnings. Pink color indicates the presence of flavonoids. (Khandelwal, K. R., 2004). To small quantity of extract, lead acetate solution was added. Formation of yellow colored precipitate indicates the presence of flavonoids. Extract was treated with sodium hydroxide; yellow coloration indicates the presence of flavonoids. (Kumar et al., 2008).

Detection of Tannins
To 5 ml of extract, 1ml of 10 % aq. Potassium dichromate solution was added. Red precipitate indicates the presence of tannins. To 5 ml of extract, 1 ml of 5 % ferric chloride solution was added, deep blue-black color, tannins. Small quantity of extract was treated with Dil. HNO3, reddish to yellow color (tannins). Extract treated with Dil. Iodine solutions, transient red color (tannins). Extract treated with Dil. KMnO4, decoloration (tannins) (Khandelwal, K.R., 2004).

Detection of Saponins
Foam Test:
Dried root extract of plant was shaked with 20 ml distilled water vigorously. Persistent foam indicates the presence of Saponins.
Detection of Terpenoids

A) **Knoller's Test**
The extract was diluted with 2 ml of 0.1 % stannic chloride in pure thionylchloride. Pink to red to green to purple color indicates the presence of terpenoids.
Detection of Phenol:

A) Test by Ferric chloride:
Three drops of newly produced, 1% ferric chloride and potassium ferro cyanide were added to the aqueous extract solution to spike it. A bluish- green tinge was thought to be appealing. The extract of methanol was diluted with water. A few crystals of ferric sulphate were added to the mixture as well. The presence of phenolic compound is attested to by their deep violet hue.

RESULTS AND DISCUSSION

CYTOLOGICAL STUDIES OF EAC BEARING MICE

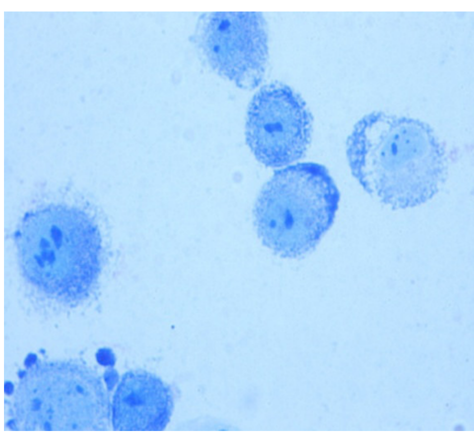
Fig 2.1

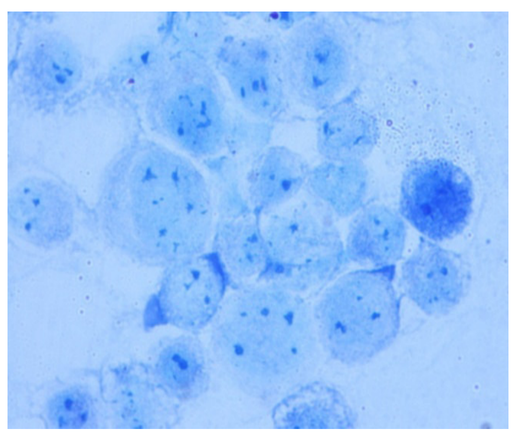
Fig 2.2

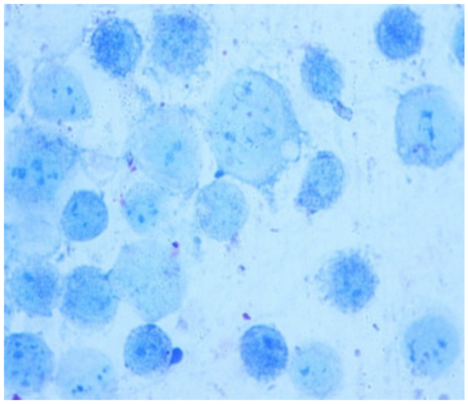

Fig 2.3

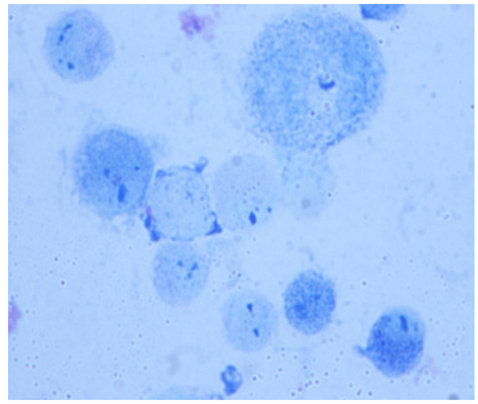

Fig 2.4

Figure: 2.1, Tumor Control
EAC control cells with cell wall, hypertrophied cells with pale cytoplasm and multiple nuclei, with actively dividing of cells.
Figure: 22, Bidens pilosa **(100mg/kg)**
Cell membrane damage, degeneration, cytoplasmic vaculation few membrane blebs.
Figure: 2.3, Bidens pilosa **(200mg/kg)**
Irregular cell wall with few places damaged, vaculation in the cytoplasm, hyper chromatic pykynotic nuclei and presence of few membrane blebs.
Figure: 2.4, Cyclophosphamide (20 mg/kg)
Cells showed huge number of vacuoles in cytoplasm in membrane blebs formation

IN-VITRO CYTOTOXICITY

In this study Bidens pilosa leaf extract has shown cytotoxic effect on EAC cell lines. The percentage of cell death observed is changed with respect to increased in concentration, that was 50µg/ml, 100µg/ml, 200µg/ml, 400µg/ml, 800µg/ml, and 1000µg/ml concentrations of Bidens pilosa showed 47%, 49%, 56%, 69%, 72% and 88% of cell death respectively (fig 1). IC_{50} was found to be 41.5ug/ml.

Table1: Determination of IC_{50} of Bidens pilosa **on EAC Cell lines**

Concentration(ug/ml)	%cell death
50	47
100	49
200	56
400	69
800	72
1000	88

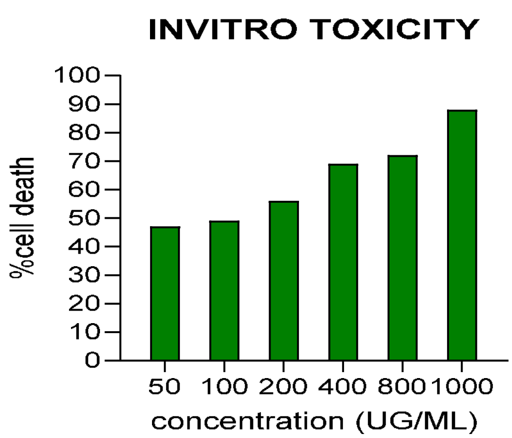

Fig 3: Determination of IC_{50} of Bidens pilosa **on EAC Cell lines**

Table 2: Effect of Bidens pilosa **on tumor growth parameters**

S.No.	Parameters	Group II (EAC control)	Group III (EAC+100mg/kg) Bidens pilosa	Group IV (EAC+200mg/kg) Bidens pilosa	Group V (EAC+CPA 20mg/kg)
1	Mean survival time(days)	15.67±0.600	26.00±0.577^{a}	31.50±0.763^{c}	36.00±0.577^{c}
2	Increased life span (%)	--	14.78	57.63	75.15
3	Tumor volume (ml)	12.44±0.160	8.65±0.197^{c}	7.58±0.174^{c}	6.43±0.182^{c}
4	Tumor weight (g)	10.74±0.155	10.09±0.180^{c}	8.33±0.167^{c}	5.33±0.174^{c}
5	Viable cell count ($x10^7$cells/ml)	18.34±0.305	16.00±0.294^{c}	15.69±0.266^{c}	14.68±0.186^{c}
6	Nonviable cell count($x10^7$cells/ml)	0.146±0.004	0.356±0.019	0.789±0.025^{c}	0.467±0.035^{c}

All values represent Mean ± SEM; n=6 for treatment groups. Statistical analysis was carried out using one-way ANOVA followed by Bonferroni test. EAC control vs Bidens pilosa -treated groups; values significant with ($p<0.05$) ^{a}, ($p<0.01$) ^{b} and ($p<0.001$) ^{c}.

Table 4: Effect of Bidens pilosa **on haematological parameters**

Parameters	Group I (Normal control)	Group II (EAC control)	Group III (EAC+100mg/kg) Bidens pilosa	Group IV (EAC+200mg/kg) Bidens pilosa	Group V (EAC+CPA 20 mg/kg)
Haemoglobin(%)	12.6±0.618	6.30±0.181^{c}	4.66±0.153	6.44±0.289^{d}	8.76±0.253^{f}
RBC($x10^6$cell/mm^3	14.5±0.527	8.02±0.127^{c}	7.48±0.178	8.67±0.182^{e}	8.35±0.184^{f}
WBC($x10^4$cells/mn	0.74±0.036	4.86±0.082^{c}	4.31±0.184^{d}	2.56±0.166^{f}	1.44±0.033^{f}
Lymphocytes(%)	63.4±0.750	47.9±0.397^{c}	36.1±0.479	54.03±0.831	49.73±1.274^{f}
Monocytes(%)	1.76±0.145	0.34±0.037^{c}	0.74±0.022	0.87±0.011	0.66±0.020^{d}
Neutrophils(%)	36.5±0.579	56.8±0.661^{c}	45.6±0.813^{f}	37.5±0.918^{f}	41.4±0.716^{f}

All values represent Mean ± SEM; n=6 for treatment groups. Statistical analysis was carried out using one-way ANOVA followed by Bonferroni test. Normal control vs. EAC control; values significant with ($p<0.05$) ^{a}, ($p<0.01$) ^{b} and ($p<0.001$) ^{c}, EAC control vs. Bidens pilosa -treated groups; values significant with ($p<0.05$) ^{d}, ($p<0.01$) ^{e} and ($p<0.001$) ^{f}

Table 5: Effect of Bidens pilosa **on biochemical parameters**

Parameters	GroupI (Normal control)	Group II (EAC control)	Group III (EAC+100mg/kg) Bidens pilosa	Group IV (EAC+200mg/kg) Bidens pilosa	Group V (EAC+CPA 20 mg/kg)
SGPT(U/L)	15.33±0.614	45.50±1.607^{c}	42.66±1.763^{d}	20.66±0.881^{f}	23.33±1.054^{f}
SGOT(U/L)	8.50±0.428	5.833±0.307^{c}	5.00±0.365	6.66±0.494^{e}	8.66±0.557^{f}
Albumin (gm%)	3.81±0.170	24.68±0.537^{c}	8.08±0.324^{f}	7.26±0.276^{f}	2.23±0.260^{f}
TotalProtein (gms%)	9.06±0.234	5.350±0.207^{c}	4.00±0.171	3.38±0.200	7.26±0.446^{f}

All values represent Mean ± SEM; n=6 for treatment groups. Statistical analysis was carried out using one-way ANOVA followed by Bonferroni test. Normal control vs. EAC control; values significant with ($p<0.05$) ^{a}, ($p<0.01$) ^{b} and ($p<0.001$) ^{c}, EAC control vs. Bidens pilosa -treated groups; values significant with ($p<0.05$) ^{d}, ($p<0.01$) ^{e} and ($p<0.001$) ^{f}

Table 6: Effect of Bidens pilosa **on antioxidant activity**

Parameters	**Group I (Normal control)**	**Group II (EAC control)**	**Group III (EAC+100mg/kg)** Bidens pilosa	**Group IV (EAC+200mg/kg)** Bidens pilosa	**Group V (EAC+CPA 20 mg/kg)**
LPO(ng of MDA/mg(protein)	0.176±0.007	1.04±0.034[c]	0.465±0.075[f]	0.786±0.026[e]	0.970±0.019
SOD(U/mg protein)	0.146±0.014	0.0654±0.008[c]	0.043±0.008	0.064±0.007	0.155±0.015[f]
CAT(U/mg protein)	0.876±0.019	0.165±0.020[c]	0.455±0.019[d]	0.575±0.018[f]	0.747±0.031[f]
GSH (mg/g wet tissue)	0.654±0.024	0.435±0.007[c]	0.434±0.031	0.486±0.033[d]	0.436±0.017[e]
GP_X(U/mg protein)	0.123±0.0013	0.056±0.0016[c]	0.165±0.016[f]	0.147±0.010[f]	0.075±0.011
GST(U/mg protein)	1.43±0.069	1.54±0.057[c]	0.776±0.026[f]	0.634±0.058[f]	0.984±0.047[f]

All values represent Mean ± SEM; n=6 for treatment groups. Statistical analysis was carried out using one-way ANOVA followed by Bonferroni test. Normal control vs. EAC control; values significant with ($p<0.05$) [a], ($p<0.01$) [b] and ($p<0.001$) [c], EAC control vs. Bidens pilosa treated groups; values significant with ($p<0.05$) [d], ($p<0.01$) [e] and ($p<0.001$) [f].

Table 7: *Bidens pilosa* leaf extract Phytochemical test

S.No	**PhytochemicalTest**	**Ethyl acetate extract**
1.	Alkaloids	_
2.	Flavonoids	+
3.	Tannins	+
4.	Phenols	+
5	Carbohydrates	_
6.	Proteins	_
7.	Steroids	_
8.	Saponins	_

[- absence; + presence]

The rising interest in plant-based medications can be attributed to the common perception that herbal remedies are safer alternatives to expensive synthetic drugs, which often come with side effects. This highlights the necessity for screening medicinal plants to identify those with promising biological activities. Additionally, the ongoing emergence of resistant strains underscores the urgent need for the discovery and development of new treatments for various diseases. In recent years, plant-derived natural products such as flavonoids, terpenoids, and steroids have garnered significant attention due to their wide-ranging pharmacological effects, including antioxidant and anticancer properties. There is an increasing focus on the study of specific flavonoids, triterpenoids, and steroids because of their potential health benefits. A key feature of these compounds is their antioxidant activity, which helps mitigate the progression of tumors and inflammatory diseases. Antioxidants are crucial in neutralizing free radicals, thereby offering protection against infections and degenerative conditions.[7] Plants have long been a vital source of effective traditional medications for various types of cancer. Although the specific compounds extracted from these plants may not always be used directly as drugs, they often serve as a foundation for the creation of new therapeutic agents.

Additionally, flavonoids are thought to play a role in cancer prevention by influencing signal transduction related to cell growth and the formation of new blood vessels. It is well-established that dietary choices and nutrition significantly impact cancer prevention. Numerous epidemiological studies indicate that a diet rich in fruits and vegetables is linked to a lower risk of developing most types of cancer.[8]

This study aimed to assess the impact of Bidens pilosa on EAC-bearing mice. The findings revealed that Bidens pilosa exhibited significant antitumor effects against the transplantable tumors in these mice. The key indicators for evaluating the efficacy of any anticancer drug include the extension of the animals' lifespan. A decrease in the number of ascitic tumor cells may suggest that Bidens pilosa influences peritoneal macrophages or other immune system components, thereby enhancing their ability to eliminate tumor cells, or it may directly affect tumor cell proliferation. Bidens pilosa significantly reduced tumor volume, viable cell count, and improved the survival time of EAC-bearing mice, demonstrating its role as an anti-neoplastic agent. Additionally, Hesperidin contributed to an increased percentage of lifespan extension and a reduction in viable cell count.

Myelosuppression is a common and significant side effect of cancer chemotherapy. Treatment with Bidens pilosa and the subsequent reduction of tumors led to notable enhancements in hemoglobin levels, as well as red and white blood cell counts. These findings are particularly important, as anemia frequently occurs in cancer patients and is exacerbated during chemotherapy due to the suppressive effects of many antineoplastic agents on erythropoiesis, which limits the administration of these drugs.[9]

Numerous epidemiological studies have indicated that the presence of tumors, whether in humans or experimental animals, can disrupt various functions of the liver and kidneys. Tumors are associated with elevated levels of SGPT, SGOT, and LPO, along with decreased levels of CAT, GSH, GPX, and GST in the liver tissues of the tumor control group. Liver damage results in increased SGPT and SGOT levels, which are released into the bloodstream. On day 14, the biochemical parameters of tumor-bearing mice showed significant deviations from those of the normal group, with increased SGPT, SGOT, and albumin levels, alongside a decrease in total protein. However, following treatment with Bidens pilosa, the serum biochemical parameters returned to levels comparable to the saline control, indicating a protective effect.[10]

Lipid peroxidation, driven by free radicals, is recognized as a key factor in the destruction of cell membranes and subsequent cellular damage. The oxidation of unsaturated fatty acids within biological membranes results in decreased membrane fluidity and compromises both the structure and function of the membrane. Malondialdehyde (MDA), a byproduct of lipid peroxidation, has been found to be elevated in cancerous tissues compared to healthy organs. An increase in thiobarbituric acid reactive substances (TBARS) signifies heightened lipid peroxidation, which contributes to tissue damage and indicates a failure of antioxidant defenses to mitigate the production of excess free radicals. The protective role of glutathione (GSH) against lipid peroxidation is well established, as it helps to maintain GSH levels. GSH can detoxify reactive oxygen species like hydrogen peroxide (H2O2) or directly reduce lipid peroxides. This study demonstrates that Bidens pilosa significantly lowers lipid peroxidation levels while enhancing glutathione content, suggesting its potential as an antitumor agent.[11,12]

Conversely, superoxide dismutase (SOD) is a widely recognized chain-breaking antioxidant present in all aerobic organisms. This metalloprotein is found throughout various cells and plays a crucial role in protecting against oxidative damage caused by reactive oxygen species (ROS). Catalase, another key component of the free radical scavenging system, is located in all major organs of both animals and humans, with particularly high concentrations in the liver and red blood cells. Both enzymes are vital for the removal of ROS generated during the redox processes of xenobiotics in liver tissues. Research indicates that catalase and SOD can be easily inactivated by lipid peroxides or ROS.[13] Additionally, studies have shown that mice with Ehrlich ascites carcinoma (EAC) exhibited reduced SOD activity, potentially due to a loss of manganese-dependent SOD activity in the liver. There have also been reports of inhibited catalase activity in tumor cell lines. In this study, the administration of Bidens pilosa significantly increased the levels of catalase and SOD, indicating its potential to restore these enzyme levels. In the tumor control group, the levels of glutathione peroxidase (GPX) and glutathione S-transferase (GST) were significantly lower compared to the normal control group. However, treatment with hesperidin notably restored GPX and GST levels to nearly normal.[14]

CONCLUSION

The present study demonstrated that Bidens pilosa increased the life span of EAC tumor bearing mice and decreased the lipid peroxidation and thereby augmented the endogenous antioxidant enzymes in the liver. The above parameters are responsible for the antitumor and antioxidant activities of Bidens pilosa. Ongoing laboratory investigations aim to identify the active compounds responsible for the antitumor and antioxidant effects, as well as to explore their mechanisms. Additionally, phytochemical analyses have indicated the presence of phenols, flavonoids, and tannins.

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