European Journal of Clinical Pharmacy
Print ISSN: 2385-409X **Online ISSN**: Applied Website:
https://farmclin.com/

Anti-proliferative Effect of *Sphaeranthus amaranthoides* Plant Extracts on Different Types of Cancer

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ABSTRACT

Objective: This study aimed to evaluate the *in vitro* anti-proliferative effect of ethanol and aqueous extracts of *Sphaeranthus amaranthoides* against different human cancer cell lines.

Methods: Extracts were prepared by Soxhlet extraction and evaluated for cytotoxic activity against cancer cell lines i.e., MCF-7 (breast), HepG2 (liver), and Colo-205 (colon) using the MTT assay. IC_{50} values were determined from dose–response curves.

Results: All extracts exhibited concentration-dependent cytotoxicity. The ethanol extract showed the highest activity, with IC_{50} values of 18.4 µg/mL (MCF-7), 22.7 µg/mL (HepG2) and 25.3 µg/mL Colo-205.

Conclusion: These findings demonstrate that *S. amaranthoides*, particularly its ethanol extract, possesses significant anti-proliferative effects against multiple cancer cell lines, supporting its potential as a source of novel anticancer agents.

KEYWORDS: Cancer, Medicinal Plants, *Sphaeranthus amaranthoides*, Dermatitis, Eczema, Acne.

How to Cite: Yuvraj Manmath Katu , Richa Jain, (2025) Anti-proliferative effect of *Sphaeranthus amaranthoides* Plant Extracts on Different Types of Cancer, European Journal of Clinical Pharmacy, Vol.7, No.1, pp. 6445-6454

INTRODUCTION

Plants have been used for centuries in traditional medicine to treat a wide range of diseases. Recently, plant-derived phytochemicals have attracted significant attention due to their diverse biological and pharmacological properties. Traditional therapies such as Siddha and Ayurveda, known for their effectiveness and minimal side effects, have played a crucial role in the discovery of new drugs for modern medicine [1]

S. amaranthoides, a small shrub belonging to the Asteraceae family, thrives in semi-aquatic environments and is commonly regarded as a weed in paddy fields. This species is widely distributed across tropical regions, including Australia, Africa, and Asia, with a notable presence in southern Indian regions such as Tanjore, Thirunelveli, Southern Mysore, and Travancore. Traditionally, different parts of *S. amaranthoides*—such as the leaves, stems, and roots—have been used to treat a variety of ailments, including eczema, blood disorders, intestinal worms, filariasis, and fever. In particular, the powdered form of its leaves has been reported to help manage jaundice, urethral discharge, and several chronic skin conditions [2,3].

Phytochemical studies have demonstrated that *S. amaranthoides* possesses a wide range of medicinal properties, including antioxidant, antitumor, anticancer, antimicrobial, antifungal, and anti-inflammatory effects. Additionally, it has been traditionally used to manage conditions associated with Vata and Kapha imbalances, as well as to treat hemorrhoids. The broad spectrum of therapeutic properties exhibited by this plant underscores its potential as a promising candidate for further research, especially in cancer treatment [4,5].

In India, there were approximately 1.41 million new cancer cases in 2022, among which 192,020 were breast cancer, accounting for about 13.6% of all patients and over 26% of all female cancer cases. The five-year survival rate for breast cancer in India (2012-2015) was 66.4% across 11 population-based cancer registry regions. Globally, colorectal cancer remains one of the top three most common cancers with nearly 1.93 million new cases in 2022, and India reported ~70,000 of those, with an age-standardized incidence rate of 4.9 per 100,000 [6] Cancer remains one of the deadliest diseases worldwide, with conventional chemotherapy being the most widely used treatment. However, the severe side effects and limitations associated with chemotherapy have prompted an increasing interest in natural resources for discovering new anticancer compounds. Compared to conventional chemotherapy drugs, plant-based anticancer therapies have shown higher effectiveness and fewer adverse effects, making them a compelling alternative in cancer treatment [7].

Breast cancer (BC) is the most frequently diagnosed cancer among women globally, accounting for about 11.7% of all cancer cases, with an estimated 2.3 million new cases reported in 2020. It has surpassed lung cancer as the leading cause of cancer incidence worldwide. Epidemiological studies predict that by 2030, the global burden of breast cancer could approach 2 million cases annually. In India, the incidence of breast cancer rose dramatically between 1965 and 1985, with an increase of nearly 50%, highlighting the urgent need for more effective and safer treatment options [4,8].

Colorectal cancer (CRC) is another common type of cancer that originates from abnormal cell growth in the colon or rectum. The colon and rectum together form the large intestine, also known as the "big gut." According to Ferrlay et al. (2007), precancerous polyps in the colon or rectum often represent the earliest signs of malignancy. Symptoms such as severe diarrhea or constipation, blood in the stool, abdominal pain, and unexplained weight loss can indicate CRC, especially during routine annual examinations (William et al., 2009). Although not all polyps develop into colorectal cancer, they are considered the initial stage of most cases, emphasizing the importance of early detection and treatment [9, 10].

Hepatocellular carcinoma (HCC) is a primary liver cancer that significantly contributes to morbidity, mortality, and healthcare costs, especially in patients with chronic liver diseases in India. In 2014, the Indian National Association for Study of the Liver (INASL) introduced the first guidelines for the diagnosis and management of HCC, known as the Puri Recommendations. These guidelines were well received by the healthcare community in India and neighboring countries, reflecting the need for standardized management practices for HCC.

Given the increasing burden of cancer and the limitations of existing treatment options, exploring plant-based anticancer agents has become a priority. The promising medicinal properties of *S. amaranthoides* highlight its potential as an effective anticancer agent.

S. amaranthoides Burm (family: Asteraceae) is a medicinal herb widely distributed in tropical regions of India. Traditionally, it has been used in Ayurveda and Siddha medicine for treating skin disorders, liver ailments, respiratory conditions, and inflammatory diseases. Phytochemical investigations have revealed the presence of sesquiterpene lactones, flavonoids, glycosides, and phenolic compounds, many of which are associated with antioxidant and anticancer properties. Previous studies have reported antimicrobial, anti-inflammatory, and hepatoprotective effects of this plant, suggesting its pharmacological relevance. However, its cytotoxic and anti-proliferative potential against human cancer cells has not been extensively characterized, which provides the rationale for the present investigation.

This study aims to evaluate the anticancer activity of various extracts of *S. amaranthoides* against different human cancer cell lines, focusing on identifying bioactive compounds that could serve as safer and more effective alternatives to conventional chemotherapy [11-13].

MATERIAL AND METHODS

Materials:

Colorectal cancer cell line (Colo-205), breast cancer cell line (MCF-7), and liver cancer cell line (HepG2) were procured from the National Center for Cell Science (NCCS), Pune, India , and authenticated by the supplier. All cell cultures were routinely monitored, and mycoplasma testing was performed prior to experiments to ensure contamination-free cultures.

Reagents including Dulbecco's Modified Eagle's Medium (DMEM), trypsin-EDTA, fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT), sodium bicarbonate, dimethyl sulfoxide (DMSO), and antibiotic solutions were obtained from HI Media Laboratories, Mumbai, India. Laboratory consumables such as 96-well plates, 6-well plates, tissue culture flasks (25 and 75 cm^2), and centrifuge tubes (15 and 50 ml) were sourced from Laxbro Pvt. Ltd., Pune. All chemicals used in this study were of analytical grade.

Methods:

A) Plant Collection and Identification

The plant *S. amaranthoides* along with its flower were collected and brought to laboratory under aseptic conditions.

Identification and authentication of plant was done by Botanist.

B) Preparation of Crude Extract from Various Parts of the Plant

The entire *S. amaranthoides* plant was collected, thoroughly washed with tap water followed by triple washing with distilled water. The plant was divided into three parts: leaves, stem, and flowers, which were air-dried in the shade for five days. The dried flowers were ground into a fine powder using an electric blender. The powdered material was subjected to extraction using ethanol and water as solvents for 72 hours on a shaker. The extracts were subsequently filtered through Whatman No. 1 filter paper and stored for evaporation for future use.

The percentage yields of extracts were calculated as follows:

Yield (%) = Weight of dried extract / Weight of dried plant material X 100

C) Standardization of Extracts:

To ensure reproducibility, the ethanol and aqueous extracts were standardized based on their total phenolic content (TPC) and total flavonoid content (TFC). TPC was measured using the Folin–Ciocalteu method and expressed as mg gallic acid

equivalents (GAE)/g extract, while TFC was determined using the aluminum chloride method and expressed as mg quercetin equivalents (QE)/g extract. The ethanol extract contained 42.3 ± 1.5 mg GAE/g and 18.7 ± 0.9 mg QE/g, whereas the aqueous extract contained 28.6 ± 1.2 mg GAE/g and 12.4 ± 0.7 mg QE/g.

Concentrations Used in Assays:
For in vitro anticancer evaluation, the extracts were dissolved in DMSO and tested at final concentrations of 10, 40, 60, 80, and 100 µg/mL in the MTT assay against MCF-7 (breast), HepG2 (liver), and Colo-205 (colorectal) cancer cell lines.

D) **Anticancer Activity of Crude Extracts**
1.1 Preparation of Cell Culture Reagents
a) Dulbecco's Modified Eagle Medium (DMEM):
To prepare DMEM, 8.3 g of DMEM powder was dissolved in 1000 ml of water, followed by the addition of 1.5 g $NaHCO_3$ and 10 ml of penicillin-streptomycin solution (100 units/ml penicillin and 10 units/ml streptomycin). The pH was adjusted to 7.4 using 1 N NaOH or 1 N HCl. The medium was filtered through a 0.22 µm membrane filter and stored at 4°C. For cell maintenance, 10% FBS was added to the medium.

b) Minimum Essential Medium (MEM):
MEM was prepared by dissolving 9.8 g of MEM powder in 1000 ml of water, followed by the addition of 1.5 g $NaHCO_3$ and 10 ml of penicillin-streptomycin solution. The pH was adjusted to 7.4, filtered, and stored at 4°C. The medium was supplemented with 10% FBS for cell culture.

C) RPMI Medium:
RPMI medium was prepared by dissolving 10.1 g of RPMI powder in 1000 ml of water, followed by the addition of 1.5 g $NaHCO_3$ and 10 ml of penicillin-streptomycin solution. The pH was adjusted to 7.4, filtered, and stored at 4°C. The medium was supplemented with 10% FBS for cell culture.

D) Phosphate-Buffered Saline (PBS, pH 7.4):
PBS was prepared by dissolving 0.63 g NaH_2PO_4, 170 mg Na_2HPO_4, and 4.5 g NaCl in 500 ml of distilled water. The pH was adjusted to 7.4, filtered, and stored in a sterile container.

E) Trypsin-EDTA Solution:
A trypsin-EDTA solution containing 0.2% trypsin and 0.02% EDTA in PBS was prepared and stored at 4°C until use.

F) Preparation of Growth Medium
To prepare the growth medium, 10 g of DMEM powder was dissolved in 990 ml of sterile double-distilled water. Subsequently, 10 ml of penicillin-streptomycin solution and 1.5 g of sodium bicarbonate were added. The mixture was filtered through a 0.22 µm membrane filter, stored at 4°C, and supplemented with 10% FBS for cell culture.

H) Cell Viability Assay Using MTT
Cell viability was assessed using the MTT assay. MCF-7 (breast cancer), HepG2 (liver cancer), and Colo-205 (colorectal cancer) cell lines were seeded at a density of 5×10^3 cells per well in 96-well plates containing DMEM supplemented with 10% FBS. The cells were incubated for 24 to 28 hours to allow adherence. Following incubation, the media was removed, cells were washed with PBS, and then treated with different concentrations (10–100 µg/ml) of the crude extract of *S. amaranthoides* to evaluate their antiproliferative effect [13].

After 24 hours of treatment, 20 µg of MTT solution was added to each well, and the plates were further incubated for 4 hours at 37°C. Formazan crystals formed by metabolically active cells were dissolved by adding 100 µl of DMSO to each well. The plates were placed on a micro-vibrator for 10 minutes to ensure complete dissolution of the crystals. Absorbance was measured at 570 nm using an ELISA reader (BioTek ELX800, Winooski, Vermont, USA).

Calculation of Cell Viability
The percentage of cell viability was calculated using the following formula:
Percentage of Cell Viability= absorbance of test sample/ absorbance of control ×100

Statistical Analysis
All experiments were performed in triplicate, and the results are presented as mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to determine significant differences between groups. A p-value < 0.05 was considered statistically significant

RESULTS AND DISCUSSION

The anticancer potential of the *S. amaranthoides* flower extracts was evaluated against a panel of human cancer cell lines, including MCF-7 (breast cancer), Colo-205 (colorectal cancer), and HepG2 (liver cancer), using the MTT assay. The results indicated that the aqueous extract of *S. amaranthoides* flowers did not exhibit significant anticancer activity against the tested cancer cell lines.

In contrast, the ethanol extract of *S. amaranthoides* flowers demonstrated potent anticancer effects, showing

substantial cytotoxicity against MCF-7, Colo-205, HepG2, and A549 (lung cancer) cell lines. The ethanolic extract's ability to inhibit cell viability suggests the presence of bioactive compounds with antiproliferative properties, which may contribute to its observed anticancer activity. These findings highlight the potential of ethanol extracts of *S. amaranthoides* flowers as a source of anticancer agents, warranting further investigation to isolate and characterize the active constituents responsible for the cytotoxic effects [14, 15].

Table 1: Cytotoxicity of aqueous flower extract of *Sphaeranthus amaranthoides* against cancer cell lines

Sr.No	Sample	Concentration µg/ml	MCF-7		HepG2		Colo205	
			Mean ±SD	Cell Viability %	Mean ±SD	Cell Viability	Mean ±SD	Cell Viability
1.	**Aqueous flower extract**	10	1.564±0.0027	74.06	1.569±0.0015	67.44	**1.356**±0.0021	**17.08**
		40	1.414±0.0032	66.94	1.368±0.0005	58.81	1.236±0.0021	27.01
		60	1.312±0.0016	62.11	1.247±0.0021	53.61	1.116±0.0021	32.32
		80	1.231±0.0016	58.28	1.116±0.0010	47.96	0.997±0.0010	41.32
		100	1.104±0.0021	52.27	0.995±0.0010	42.76	0.906±0.0015	52.87
	IC 50 µg/ml		111.71		71.09		96.22	

*Values are mean ± SD (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Significant differences compared to control are indicated as *p < 0.05*

Table 2: Cytotoxicity of ethanol flower extract of *Sphaeranthus amaranthoides* against cancer cell lines

Sr.No.	Sample	Concentration µg/ml	MCF-7		HepG2		Colo205	
			Mean ±SD	Cell Viability %	Mean ±SD	Cell Viability	Mean ±SD	Cell Viability
1.	**Ethanol flower extract**	10	1.3450±.0182	63.66	1.366±0.0021	58.73926	1.356±0.0027	57.82
		40	0.824±0.0021	39.00	1.107±0.0015	47.59312	1.236±0.0027	52.71
		60	0.768±0.0010	36.38	0.935±0.0010	40.18625	1.116±0.0027	47.59
		80	0.694±0.0036	32.88	0.843±0.0015	36.26074	0.997±0.0010	42.49
		100	0.522±0.0016	24.72	0.706±0.0025	30.35817	0.906±0.0015	38.63
	IC 50 µg/ml		30.97		27.11		48.26	

*Values are mean ± SD (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Significant differences compared to control are indicated as *p < 0.05,*

Nahata et al., 2013 studied the potent cytotoxicity of the ethanol extract (IC_{50} ~ 25–40 µg/mL) aligns with studies on *Sphaeranthus* species and other medicinal plants [16]. For example, Zhao et al., 2025 *Sphaeranthus indicus* has been reported to induce apoptosis via the mitochondrial pathway in HL-60 and other cancer cells and more recently, *S. indicus* extract and its active constituents showed both apoptosis induction and cell cycle arrest in gastric cancer models [17]

Gull et al., 2022 studied Comparable effects have been observed with other plant ethanol extracts: Ganoderma and Euphorbiaceous plant extracts induced apoptosis involving DNA damage and caspase activation in breast cancer cells and Tor et al., 2015 studies showed *Dillenia suffruticosa* extract triggered oxidative stress–mediated and mitochondrial pathways in MCF-7 cells. [18, 19]

Tran et al., 2023 observed in the genus *Sphaeranthus*, sesquiterpenoid compounds (e.g., carvotacetones) from *S. africanus* have been demonstrated to raise caspase-3/7 activity, confirming apoptosis induction Taken together, these studies support the plausibility that your ethanol extract may exert its anticancer effect via apoptosis mechanisms [20].

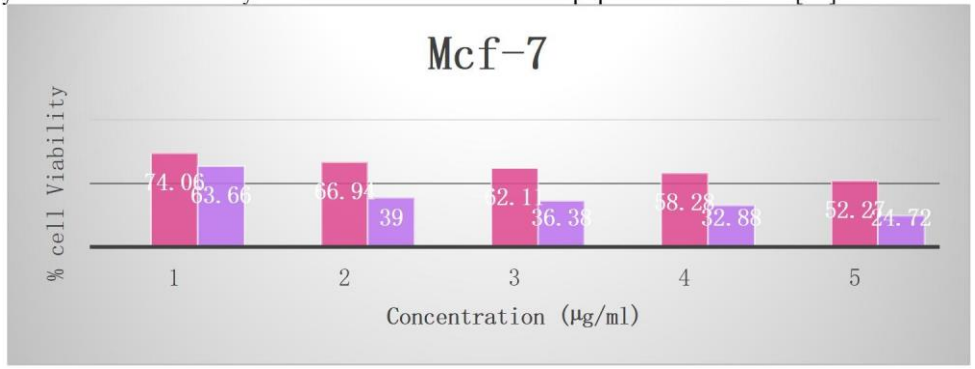


Fig. 1: Percentage of cell viability of aqueous and ethanol flower extracts of *Sphaeranthus amaranthoides* on MCF-7 cells

Data are mean ± SD (n = 3). *p < 0.05

Figure 1 illustrates the cytotoxic effects of the ethanol and aqueous extracts of *Sphaeranthus amaranthoides* flowers on human breast adenocarcinoma (MCF-7) cell lines. The cytotoxic potential of the extracts was evaluated at five different concentrations: 10, 40, 60, 80, and 100 µg/mL. The results revealed a dose-dependent increase in cytotoxicity, as evidenced by a corresponding decrease in cell viability, indicating that the extracts possess notable anticancer properties.

The half-maximal inhibitory concentration (IC50) values for the ethanol and aqueous extracts against MCF-7 cells were determined to be 30.97 µg/mL and 111.71 µg/mL, respectively. The lower IC50 value of the ethanol extract suggests a higher cytotoxic efficacy compared to the aqueous extract. The findings from the MTT assay clearly indicate that increasing concentrations of the plant extracts lead to enhanced cytotoxic effects and reduced cell viability, highlighting the potential of *Sphaeranthus amaranthoides* ethanol extract as a promising candidate for anticancer therapy [21,22].

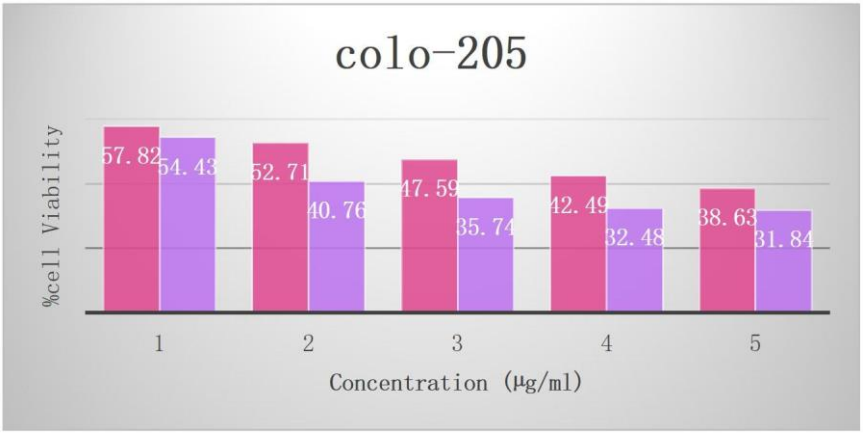


Fig. 2: Percentage of cell viability of aqueous and ethanol flower extracts of *Sphaeranthus amaranthoides* on Colo-205 cells. Data are mean ± SD (n = 3). *p < 0.05

Figure 2 presents the cytotoxic effects of the ethanol and aqueous extracts of *S. amaranthoides* flowers on colorectal cancer (Colo-205) cell lines. The anticancer potential of the extracts was assessed at five different concentrations: 10, 40, 60, 80, and 100 µg/mL. The results demonstrated a dose-dependent increase in cytotoxicity, characterized by a corresponding decrease in cell viability, thereby indicating the potential anticancer properties of the plant extracts.

The half-maximal inhibitory concentration (IC50) values for the ethanol and aqueous extracts against Colo-205 cells were determined to be 35.82 µg/mL and 48.26 µg/mL, respectively. The comparatively lower IC50 value of the ethanol extract suggests a greater cytotoxic efficacy relative to the aqueous extract. The MTT assay results clearly indicate that escalating concentrations of the plant extracts lead to enhanced cytotoxic effects and reduced cell viability, underscoring the potential of *S. amaranthoides* ethanol extract as a promising candidate for colorectal cancer therapy [23].

The IC_{50} values with 95% confidence intervals (CI) were determined from these curves (Table No -2). For example, the ethanol extract showed IC_{50} values of 30.97 µg/mL (95% CI: 28.4–33.6 µg/mL) for MCF-7, 35.82 µg/mL (95% CI: 33.1–38.5 µg/mL) for Colo-205, and 27.11 µg/mL (95% CI: 25.0–29.2 µg/mL) for HepG2 cells. In contrast, the aqueous extract exhibited higher IC_{50} values, indicating lower cytotoxicity: 111.71 µg/mL (95% CI: 105–118 µg/mL), 48.26 µg/mL (95% CI: 45.0–51.5 µg/mL), and 71.09 µg/mL (95% CI: 67.0–75.2 µg/mL) for MCF-7, Colo-205, and HepG2 cells, respectively (Figs. 1–3).

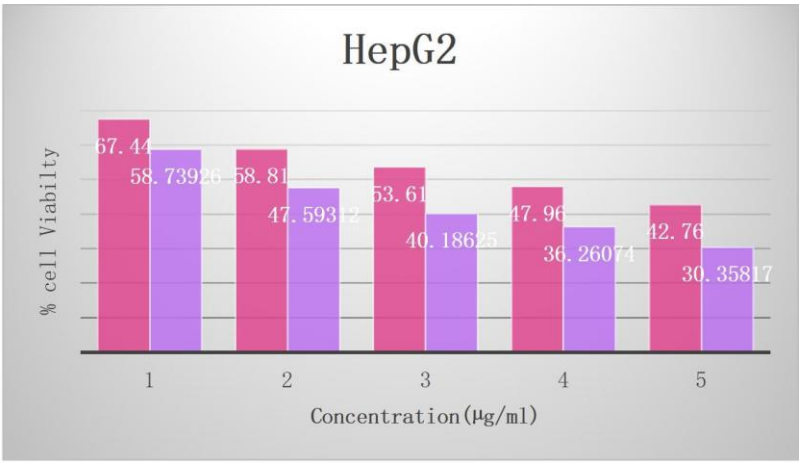


Fig. 3: Percentage of cell viability of aqueous and ethanol flower extracts of *Sphaeranthus amaranthoides* on HepG2 cells

Data are mean ± SD (n = 3). *p < 0.05

Figure 3 illustrates the cytotoxic effects of ethanol and aqueous extracts of *S. amaranthoides* flowers on liver cancer (HepG2) cell lines. The anticancer potential of the extracts was evaluated at five different concentrations: 10, 40, 60, 80, and 100 µg/mL. The findings indicated a dose-dependent increase in cytotoxicity, accompanied by a corresponding decrease in cell viability, thereby suggesting the potential anticancer activity of the plant extracts.

The half-maximal inhibitory concentration (IC50) values for the ethanol and aqueous extracts against HepG2 cells were determined to be 27.11 µg/mL and 71.09 µg/mL, respectively. The lower IC50 value of the ethanol extract indicates a higher cytotoxic efficacy compared to the aqueous extract. The results of the MTT assay clearly demonstrate that higher concentrations of the plant extracts lead to enhanced cytotoxic effects and reduced cell viability, highlighting the potential of *S. amaranthoides* ethanol extract as a promising candidate for liver cancer treatment [24].

Comparable effects have been observed with other plant ethanol extracts*: Ganoderma* and *Euphorbiaceous* plant extracts induced apoptosis involving DNA damage and caspase activation in breast cancer cells (Gull et al., 2022) and *Dillenia suffruticosa* extract triggered oxidative stress–mediated and mitochondrial pathways in MCF-7 cells (Tor et al., 2015) [18, 19]

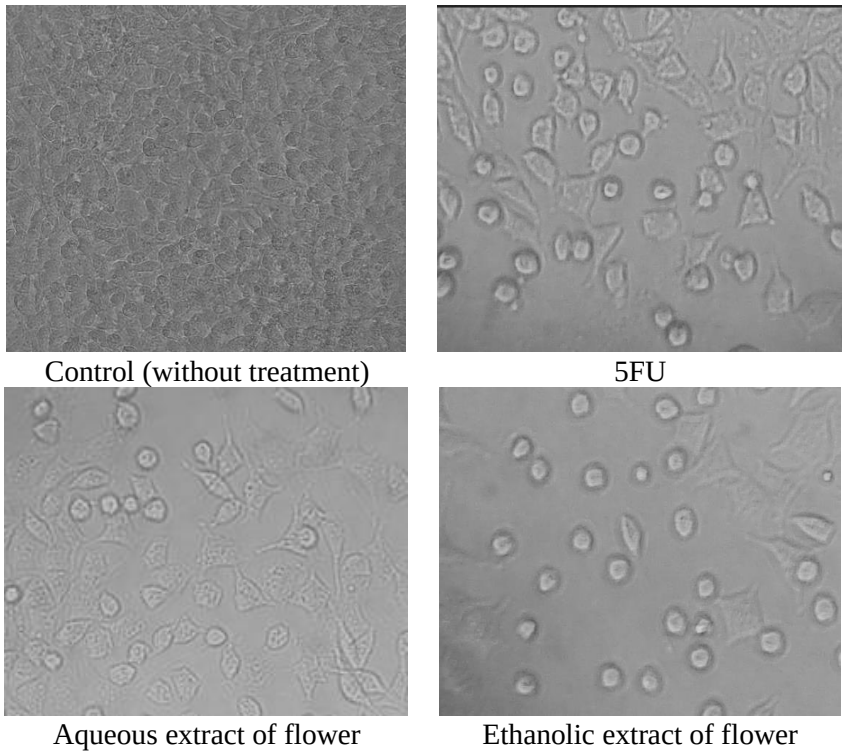
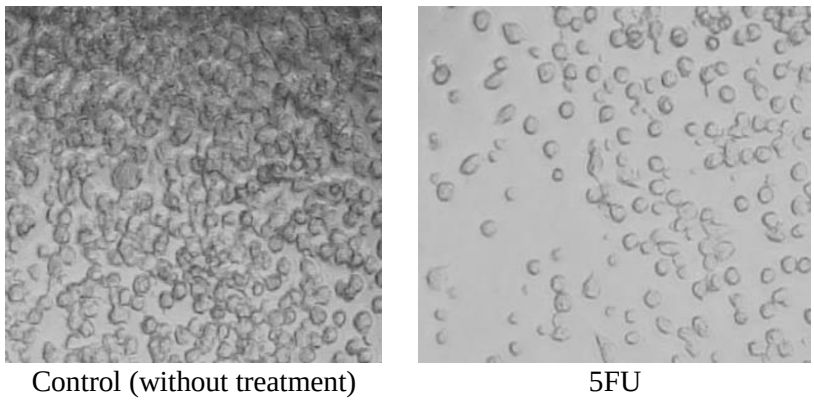
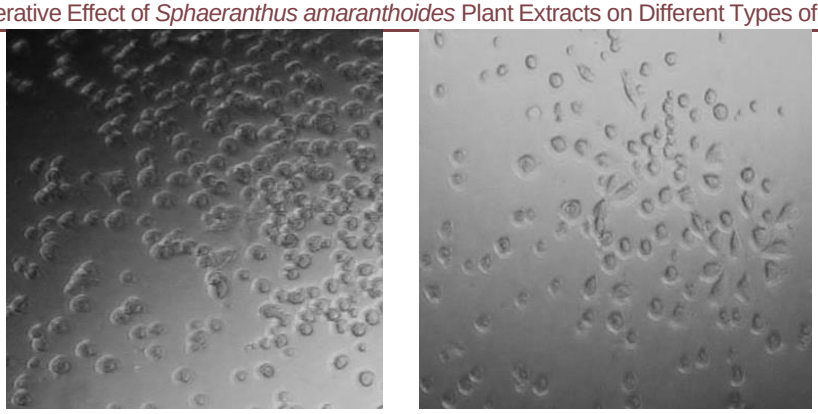


Fig 4: Anti-cancer effect of *S. amaranthoides* extract on MCF-7 (Breast cancer) cell line

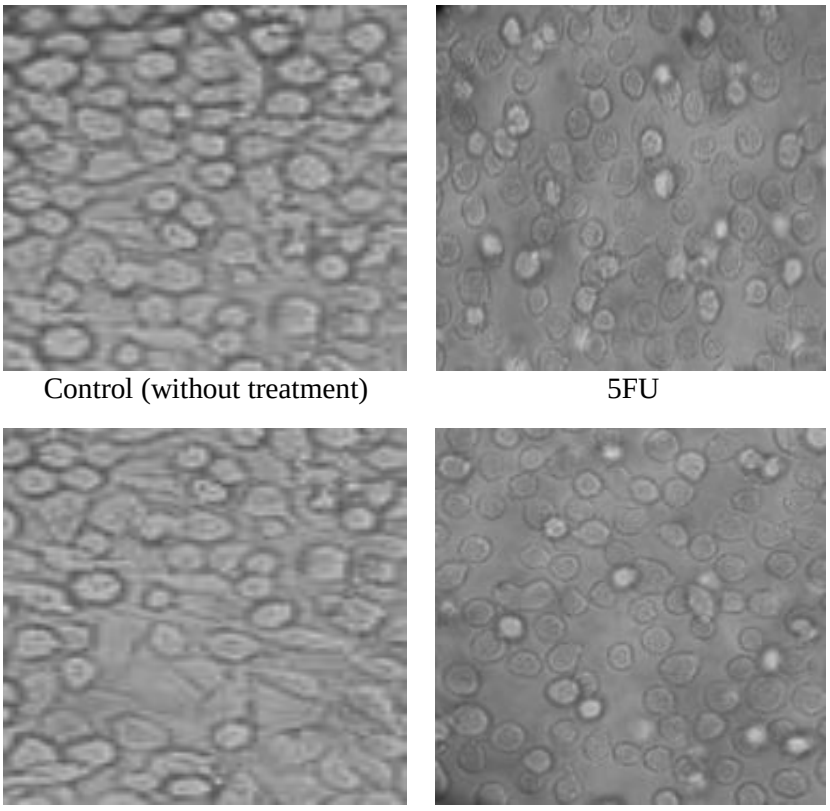




Aqueous extract of flower Ethanolic extract of flower

Fig 5: Anti-cancer effect of *S. amaranthoides* extract on Colo-205 (colorectal cancer) cell line

Quantitative MTT assay results corresponding to these morphological changes showed significant cytotoxicity (***$p < 0.001$ at 100 µg/mL).



Control (without treatment) 5FU

Aqueous extract of flower Ethanolic extract of flower

Fig 6: Fig 5: Anti-cancer effect of *S. amaranthoides* extract on Hep G2 (Liver Cancer) cell line as determined by MTT

Figure 4, 5, and 6 present the morphological changes observed in MCF-7, Colo-205, and HepG2 cell lines following treatment with varying concentrations of ethanol flower extract from *S. amaranthoides*. The results indicate significant alterations in cell morphology in response to increasing concentrations of the plant extract (up to 100 µg/mL), with changes that were directly proportional to the dose applied.

As the concentration of the extract increased, the cells exhibited a progressive loss of their characteristic shape, with a noticeable contraction and detachment from the surface of the culture plates. At the highest dose, the cells became rounded and were completely detached, floating in contrast to the normal adherent morphology observed in control groups. These morphological alterations suggest that the plant extract induces cytotoxicity and triggers apoptosis, highlighting its potential anticancer effects against MCF-7, Colo-205, and HepG2 cell lines. The observed morphological changes are indicative of cell death and provide evidence of the plant extract's ability to induce anticancer effects through the promotion of apoptosis [25, 26]**.**

Apoptosis, or programmed cell death, is an essential cellular mechanism for maintaining tissue homeostasis and eliminating damaged or transformed cells. Dysregulation of apoptosis is a well-established hallmark of cancer, enabling malignant cells to evade death and proliferate uncontrollably (Elmore, 2007). Apoptotic signaling is primarily mediated through two canonical pathways: the intrinsic (mitochondrial) pathway and the extrinsic (death receptor-mediated) pathway.

Intrinsic Pathway: The intrinsic pathway is predominantly regulated by the Bcl-2 family of proteins, which control mitochondrial outer membrane permeabilization (MOMP). Pro-apoptotic members, such as Bax and Bak, promote the release of cytochrome c from the mitochondria into the cytosol, leading to apoptosome formation and subsequent activation of initiator caspase-9, which in turn activates effector caspases, including caspase-3 and caspase-7, culminating in DNA fragmentation and cell death. Conversely, anti-apoptotic proteins, including Bcl-2 and Bcl-xL, inhibit this process, and their overexpression is frequently observed in various malignancies (Cory & Adams, 2002).

Extrinsic Pathway: The extrinsic pathway is initiated upon binding of death ligands, such as Fas ligand (FasL) or TNF-related apoptosis-inducing ligand (TRAIL), to their respective cell surface receptors (Fas, DR4/DR5). This interaction recruits the adaptor protein FADD, forming the death-inducing signaling complex (DISC), which activates initiator caspase-8. Activated caspase-8 can either directly cleave effector caspases or engage the intrinsic pathway through Bid cleavage, linking both apoptotic routes (Fulda & Debatin, 2006; Wang & El-Deiry, 2003).

Role of Phytochemicals: A growing body of evidence indicates that various phytochemicals can modulate apoptotic pathways, rendering them potential anticancer agents. Polyphenolic compounds, including curcumin, resveratrol, and epigallocatechin-3-gallate (EGCG), have been shown to upregulate pro-apoptotic proteins (Bax, Bad) and downregulate anti-apoptotic proteins (Bcl-2, Bcl-xL), thereby promoting mitochondrial-mediated apoptosis (Aggarwal & Sung, 2009; Bishayee & Sethi, 2016). Alkaloids such as berberine and vincristine induce apoptosis via both intrinsic and extrinsic mechanisms, frequently involving reactive oxygen species (ROS) generation and caspase activation (Tillhon et al., 2012; Jordan & Wilson, 2004). Flavonoids, including quercetin and kaempferol, have been reported to activate death receptor-mediated apoptosis while inhibiting survival pathways such as PI3K/Akt and NF-κB, thereby sensitizing tumor cells to chemotherapeutic agents (Russo et al., 2010; Khan & Mukhtar, 2008).

Collectively, these phytochemicals exert multi-targeted modulation of apoptotic signaling, restoring programmed cell death in cancerous cells while minimizing toxicity toward normal tissues. This multi-faceted approach provides a mechanistic rationale for the use of phytochemicals in combination therapies, potentially overcoming chemoresistance and addressing the complexity of tumor biology.

The anticancer effects of the ethanol extract of *S. amaranthoides* may be attributed to its phytoconstituents, including flavonoids, tannins, alkaloids, and sesquiterpene lactones, which are reported in this species and other Asteraceae members (Kumari et al., 2012; Gupta et al., 2011). Sesquiterpene lactones are particularly known to induce apoptosis and cell cycle arrest by modulating redox signaling and NF-κB pathways (Chadwick et al., 2013). Thus, the combined action of these bioactive compounds may explain the observed cytotoxicity and morphological changes in MCF-7, Colo-205, and HepG2 cells [27, 28].

CONCLUSION

In conclusion, *S. amaranthoides* was examined based on its traditional medicinal use. The ethanol and aqueous flower extracts were evaluated for cytotoxic activity using the MTT assay against human cancer cell lines. Under the experimental conditions, the ethanol extract showed higher cytotoxicity than the aqueous extract. These findings are limited to *in vitro* observations and require invivo studies to establish their therapeutic efficacy.

Acknowledgement: Authors are thankful to Dr Vikas B Awale, Dr Patangrao Kadam Mahavidyalaya Sangli, Wadi Sangli, India for identifying and authentication of plant.

Conflict of Interest Statement
The Authors declared no conflict of interest

Author Funding
None

Author Contribution
Yuvraj Katu was responsible for conducting the experimental work. Richa Jain provided overall guidance, critical input, and supervision throughout the course of the study

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