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Phytochemical analysis and anticancer profiling of *Bombax ceiba* fractions against Ehrlich ascites tumor model

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Abstract

Bombax ceiba L. (Malvaceae) is a folk medicinal plant widely utilized for the cure of various conditions including inflammation, arthritis, diabetes, dysmenorrhea, and sexual disorders. Despite its extensive ethnomedicinal applications, its antineoplastic potential remains underexplored. The objective of this study was to investigate the anticancer potential of the solvent fractions derived from the *B. ceiba* bark extract against Ehrlich ascites carcinoma (EAC) in mice Phytochemical screening demonstrated the existence of bioactive constituents such as phenols, tannins, glycosides, flavonoids, terpenoids, and saponins across different fractions. Acute toxicity assessment indicated that all fractions were well-tolerated up to a dose of 1000 mg/kg body weight, with no noted symptoms of toxicity or mortality. The trypan blue exclusion assay demonstrated that chloroform (CHF) and ethyl acetate (EAF) fractions exhibited potent antiproliferative activity, resulting in 83.46% and 82.28% inhibition of EAC cells growth, respectively, at a dose of 10 mg/kg. Furthermore, these fractions significantly ($p < 0.01$) reduced tumor weight and conferred protective effects on hematological parameters in EAC-carrying mice. Collectively, the results suggest that the chloroform and ethyl acetate fractions of *B. ceiba* bark possess promising anticancer properties, supporting its potential as a source of natural chemotherapeutic agents.

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Introduction

The global load of cancer keeps rising at an alarming rate, driven by factors such as unhealthy lifestyles, environmental exposures, and increasing life expectancy (Wu *et al.*, 2024). According to recent cancer statistics, approximately 10 million people died from cancer worldwide, with around 20 million new cases diagnosed in 2022 (Bray *et al.*, 2024). Despite significant advancements in oncology, current therapeutic strategies such as surgery, radiotherapy, and chemotherapy are often associated with limited efficacy, serious adverse effects, and suboptimal survival outcomes (Liu *et al.*, 2024). Consequently, there is an immediate demand to identify alternative, safer, and more effective anticancer agents.

Medicinal plants have long served as rich reservoirs of bioactive compounds, many of which have inspired or directly led to the development of successful anticancer drugs, including topotecan, irinotecan, paclitaxel, vinorelbine, and vinblastine (Asma ST *et al.*, 2022). The vast and largely untapped pharmacological potential of traditional medicinal plants provides promising opportunities for the discovery of novel antineoplastic agents.

Bombax ceiba L. (family: Malvaceae) is an ethnomedicinal plant widely distributed across Bangladesh, India, Sri Lanka, Pakistan, Australia, and parts of Africa. Different parts of the plant are traditionally employed for both therapeutic and commercial requirements. The flowers are valued for their ornamental and nutritional uses, particularly in African communities, while in Bangladesh, the young roots are used as a tonic and for the treatment of male impotence. Its fruits are traditionally employed for kidney stone prevention, and the bark and leaves are used to manage inflammation and diabetes (Das *et al.*, 2021).

Phytochemical investigations have revealed that *B. ceiba* is a rich source of bioactive constituents, including terpenoids, flavonoids, glycosides, esters, phenolic acids, coumarins, and lignans. The leaves have demonstrated anti-inflammatory and anticancer properties, attributed to compounds such as β-sitosterol, β-amyrin, and isoscopoletin (Taher *et al.*, 2024). The flowers exhibit antioxidant and antidiabetic activity and contain compounds such as gallic acid, quercetin, mangiferin, and protocatechuic acid. Notably, the bark contains lupeol, a triterpenoid with documented antiangiogenic and antimetastatic properties (Uzzal *et al.*, 2024).

Although *B. ceiba* has been noted for its hepatoprotective, anti-inflammatory, and antidiabetic potential, its antineoplastic potential remains largely unexplored. In light of its phytochemical richness and longstanding traditional use, the present study aims to assess the anticancer potential of various solvent fractions obtained from the crude methanol extract of *B. ceiba* bark using an Ehrlich ascites carcinoma (EAC) mouse model.

Materials and methods

Chemicals

Trypan blue was procured from HiMedia (India). Ferric chloride ($FeCl_3$), hydrochloric acid (HCl), potassium iodide (KI), potassium hexacyanoferrate [$K_2Fe(CN_6)$], sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), mercuric chloride ($HgCl_2$), and glacial acetic acid were obtained from Merck (Germany). Methanol, n-hexane, chloroform, and ethyl acetate were purchased from Duksan Pure Chemicals Ltd. (South Korea).

Plant Collection

Mature-fresh bark of *Bombax ceiba* L. was collected from the campus of Rajshahi University. The plant material was identified with an expert taxonomist Professor Md. Mahbubur Rahman, and sample specimen (PH-97) was stored in the Herbarium of the Department of Botany, Rajshahi University.

The collected bark was cut into small pieces, shade-dried with occasional sun exposure, and ground using a grinder (Walton, Bangladesh) in order to create coarse powder. The powdered material was kept at room temperature for subsequent utilization.

Extraction and fractionation

The plant sample was cold extracted with methanol as it produces high yield. Approximately 4.5 kg of bark powder was immerged in 20 L of methanol in amber-colored glass bottles for 12 days with frequent shaking. The extract was filtered twice utilizing Whatman Grade 1 filter paper and subsequently concentrated using a rotary evaporator (Bibby Sterlin, UK) to yield 103 g of crude methanol extract (CME). The CME was fractionated using the Kupchan partitioning method, sequentially with n-hexane, chloroform, and ethyl acetate to obtain the following fractions: n-hexane fraction (HF, 5 g), chloroform fraction (CHF, 7.5 g), ethyl acetate fraction (EAF, 9.5 g), and residual aqueous fraction (AQF, 80 g) (Bin Emran *et al.*, 2015).

Phytochemical analysis

Preliminary phytochemical screening was conducted using standard chemical tests to identify secondary metabolites (Mujeeb *et al.*, 2014), and (Chen *et al.*, 2022).

Saponins (Frothing test)

10 mg of each fraction was shaken vigorously with deionized water (5 mL). Stable froth confirmed the existence of saponins.

Tannins ($FeCl_3$ test)

About 2 mL of fraction was mixed with three drops of 5% $FeCl_3$. A dark green coloration demonstrated the tannins are present.

Glycosides (Keller-Kiliani test)

Each fraction (2 mL) was combined with glacial acetic acid (1 mL) and one drop of $FeCl_3$ (2%).Subsequently 1 mL of concentrated H_2SO_4 was added to the mixture. The creation of a brown ring at the interface and a bluish-green upper layer confirmed the existence of glycosides.

Steroids and Terpenoids (Salkowski test)

Approximately 100 mg of fraction was dissolved in 2 mL of chloroform. Then concentrated H2SO4 (2 mL) was carefully included along the tube wall. A reddish-brown interface indicated terpenoids.

Alkaloids

Myer's test

Addition of Meyer's reagent (potassium iodide: 5 g and mercuric chloride: 1.36 g in 100 mL water) produced a cream precipitate in the existence of alkaloids.

Hager's test

The fraction mixed with Hager's reagent (saturated picric acid solution) resulted in a yellow precipitate, indicating alkaloids.

Flavonoids

100 mg of fraction was dissolved in methanol, treated with 2% NaOH (1 mL), subsequently added dilute HCl. A color change from dark yellow to colorless indicated flavonoids.

Phenols

The fraction (1 mL) was treated with few drops of $FeCl_3$ and 1 mL of $K_2Fe(CN)_6$. A greenish-blue color confirmed the presence of phenols.

Ethics statement

The study was implemented in compliance with institutional guidelines and ethical standards. Approval for the animal experiments was acquired from the Ethics Committee of the Institute of Biological Sciences, University of Rajshahi (Issue No. 249 (35)/320/IAMEBBC/IBSc).

Animals

Male Swiss albino mice (weighing 25 ± 2 g) were procured from the Department of Zoology, University of Rajshahi. The mice were kept in iron cages that had proper ventilation, a controlled environment, and unrestricted usage to food and water.

Acute toxicity study

Acute toxicity was assessed in mice following an established protocol (Meguellati *et al.*, 2019). Mice were randomly allocated into six groups (n = 6). Group I given distilled water (control), while Groups

II–VI were administered CME, HF, CHF, EAF, and AQF at doses of 10, 100, and 1000 mg/kg body weight. Animals were noted for 48 hours for symptoms of toxicity, behavioral changes, and mortality to determine safe dosage limits.

Cell line maintenance

Ehrlich ascites carcinoma (EAC) cells were obtained from the Indian Institute of Chemical Biology (Kolkata, India) and maintained through bi-weekly intraperitoneal transplantation in Swiss albino mice.

Assessment of antiproliferative activity

Antiproliferative capacity of the fractions of *B. ceiba* bark was evaluated as per (Haque *et al*., 2016). Mice were segregated into 12 groups (n=6). On day 0, each mouse was intraperitoneally injected with 1.8×10^6 EAC cells. Treatment began 24 hours post-inoculation. Group I received 0.5% DMSO (vehicle control), while Group XII received bleomycin (0.3 mg/kg) as the standard. Groups II–XI were treated with CME, HF, CHF, EAF, and AQF at doses of 5 mg/kg and 10 mg/kg body weight. On day 6, mice were euthanized, and peritoneal fluid was collected using 0.9% NaCl. EAC cells were treated with trypan blue (0.4% w/v) and quantified using a hemocytometer beneath a light microscope (Leica, Germany). Cell growth inhibition (%) was determined utilizing the formula:

$$\text{Percentage Inhibition} = (1 - Es/Ec) \times 100$$

Where Es and Ec denote the number of viable EAC cells in treated and control groups, respectively.

Hematological analysis

The effects of *B. ceiba* bark fractions on hematological parameters, including hemoglobin concentration, red blood cell (RBC), and white blood cell (WBC) counts, were assessed as described previously (Rahman *et al*., 2021). The treatment of experimental mice with respective fractions was continued for 12 days. Blood samples were obtained via tail vein puncture. Hemoglobin levels were measured using a hemoglobinometer, and RBC and WBC counts were determined using a hemocytometer.

Measurement of tumor weight

The impact of *B. ceiba* fractions on tumor weight was assessed as per previous report [14] (Alam *et al*, 2016). The same treatment procedure was adopted for determining the tumor weight as done for cell growth restriction. The treatment of the EAC cells bearing mice with the fractions was started after 24 hours of tumor cells implanted and proceeded for 20 days. The weight of the control and treated mice was recorded and from this the tumor weight reduction capacity of the respective fractions was calculated.

Statistical analysis

Results are noted as mean ± standard error. Statistical comparisons were made utilizing one-way analysis of variance (ANOVA) subsequently Dunnett's post hoc test. Microsoft Excel 2010 and SPSS version 19 (IBM, USA) were employed to analyze the data.

Results

Acute toxicity study

The acute toxicity of the n-hexane (HF), chloroform (CHF), ethyl acetate (EAF), and aqueous (AQF) fractions derived from the crude methanol extract (CME) of *Bombax ceiba* bark was assessed in Swiss albino mice. Each fraction was administered intraperitoneally at doses of 10, 100, and 1000 mg/kg body weight. No signs of toxicity or mortality were noted in any of the treated groups up to the maximum dose of 1000 mg/kg, indicating that the extractive and its derived fractions are non-toxic at these concentrations.

Phytochemical analysis

Phytochemical screening of the various fractions of *Bombax ceiba* bark was performed using standard qualitative methods. The results demonstrated the existence of several classes of biomolecules including glycosides, phenols, tannins, flavonoids, terpenoids, steroids, and saponins, whereas alkaloids were absent in all fractions. Among the tested fractions, ethyl acetate fraction (EAF) demonstrated the richest phytochemical profile, followed by aqueous (AQF) and chloroform (CHF) fractions, which exhibited moderate levels of these compounds.

Table 1. Phytochemical screening of fractions derived from the bark extract of *Bombax ceiba*.

Phytochemicals	CME	HF	CHF	EAF	AQF
Alkaloids	-	-	-	-	-
Flavonoids	+ + +	+	+ +	+ + +	+ + +
Phenols	+ + +	+	+ +	+ + +	+ + +
Tannins	+ ++	+	+ +	+ + +	+ + +
Saponins	+ +	+	+ +	+ + +	+ + +
Terpenoids	+ + +	+	+ + +	+ + +	+ +
Steroids	+ +	+	+ + +	+ + +	+ +
Glycosides	+ + +	+	+ +	+ + +	+ + +

Note: - = absence, + = low, ++ = medium, +++ = high.

The n-hexane fraction (HF) contained the lowest abundance of phytoconstituents. These findings are shown in Table 1.

Antiproliferative activity

The antiproliferative activity of *Bombax ceiba* bark-derived fractions was evaluated in vivo against Ehrlich ascites carcinoma (EAC) cells in mice, utilizing the trypan blue exclusion assay. Mice were administered the n-hexane (HF), chloroform (CHF), ethyl acetate (EAF), and aqueous (AQF) fractions intraperitoneally at doses of 5 and 10 mg/kg body weight. As shown in Table 2, all fractions exhibited a dose-dependent inhibition of EAC cell proliferation. At the 10 mg/kg dose, the CHF fraction demonstrated the most pronounced antiproliferative effect, achieving 83.46% inhibition, closely followed by EAF (82.28%), AQF (66.93%), and HF (40.94%). Notably, significant activity was also observed at the 5 mg/kg dose, with CHF and EAF exhibiting 52.36% and 43.31% inhibition, respectively ($p < 0.01$ and $p < 0.05$). The crude methanol extract (CME) showed robust efficacy, producing 79.13% inhibition at 10 mg/kg, while the reference chemotherapeutic agent bleomycin (0.3 mg/kg) led to a 91.73% reduction in tumor cell burden. These findings underscore the potent and dose-dependent antiproliferative potential of the chloroform and ethyl acetate fractions, supporting their promise for further investigation as antitumor agents.

Table 2. Effect of fractions derived from the *Bombax ceiba* bark extract on EAC cells proliferation restriction in mice (n = 6).

Samples	Dose (mg/kg body weight)	EAC cell count in a mouse on day six	Cell proliferation restriction (%)
Control (EAC + Vehicle)	-	$(2.54 \pm 0.45) \times 10^8$	
CME	5	$(1.53 \pm 0.31) \times 10^8$	39.76^{*}
	10	$(0.53 \pm 0.22) \times 10^8$	79.13^{***}
HF	5	$(1.8 \pm 0.43) \times 10^8$	29.13^{*}
	10	$(1.5 \pm 0.19) \times 10^8$	40.94^{*}
CHF	5	$(1.21 \pm 0.16) \times 10^8$	52.36^{**}
	10	$(0.42 \pm 0.1) \times 10^8$	83.46^{***}
EAF	5	$(1.44 \pm 0.34) \times 10^8$	43.31^{*}
	10	$(0.45 \pm 0.11) \times 10^8$	82.28^{***}
AQF	5	$(1.74 \pm 0.24) \times 10^8$	31.50^{*}
	10	$(0.84 \pm 0.13) \times 10^8$	66.93^{**}
Bleomycin	0.3	$(0.21 \pm 0.08) \times 10^8$	91.73^{***}

Data are provided as mean ± standard error at significant values of $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

Effect on tumor weight

Administration of the *Bombax ceiba* bark extract fractions resulted in a significant reduction in tumor weight in EAC-bearing mice, as illustrated in Figure 1. Among all the tested fractions, the chloroform fraction (CHF) produced the most pronounced effect, reducing the average tumor weight to 7.45 g compared to 22.72 g in the untreated control group ($p < 0.01$). The ethyl acetate fraction (EAF) and the crude methanol extract (CME) also exhibited notable antitumor activity, lowering tumor weights to 9.12 g and 11.15 g, respectively. In contrast, the n-hexane (HF) and aqueous (AQF) fractions were less effective in reducing tumor burden. These findings further support the potent antitumor properties of the CHF and EAF fractions of *Bombax ceiba* bark.

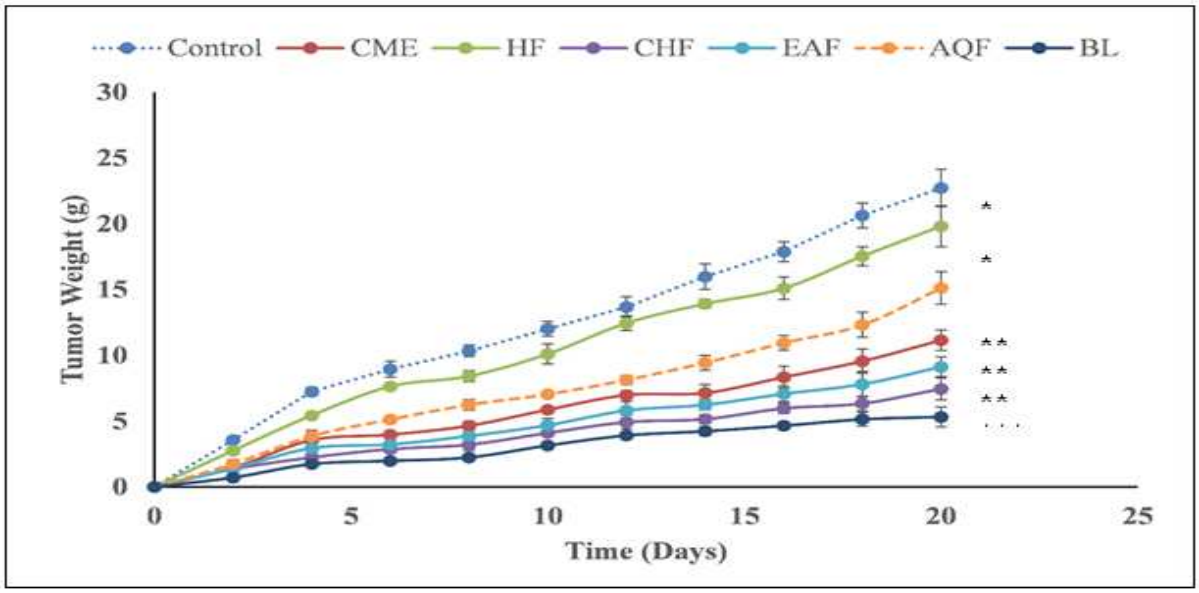

Fig. 1. Effect of *Bombax ceiba* bark extract fractions on tumor burden in mice bearing Ehrlich ascites carcinoma (EAC). Experimental groups received treatments with the solvent fractions n-hexane (HF), chloroform (CHF), ethyl acetate (EAF), and aqueous (AQF) obtained from the methanol extract (CME) of *B. ceiba*. Tumor weights were measured at the conclusion of the treatment period. Results are reported as mean ± standard error, (n = 6). Statistical significance relative to the untreated control group is denoted as $^{*}p < 0.05$, $^{**}p < 0.01$, *and* $^{***}p < 0.001$.

Impact on hematological parameters

EAC progression in mice resulted in marked hematological disruptions, including a significant elevation in white blood cell (WBC) count and reductions in red blood cell (RBC) count and hemoglobin (Hb) content, relative to normal controls. Treatment with fractions of *Bombax ceiba* bark extract notably mitigated these cancer-induced alterations, improving hematological indices toward physiological norms (Figure 2). Among the tested groups, the chloroform (CHF) and ethyl acetate (EAF) fractions demonstrated the most pronounced corrective effects. CHF significantly restored RBC levels and hemoglobin content while concurrently reducing WBC count ($p<0.01$). EAF also exhibited considerable hematoprotective activity, especially in normalizing RBC and WBC levels. The crude methanol extract (CME) and aqueous fraction (AQF) exerted moderate restorative effects, while n-hexane fraction (HF) the showed the least pronounced activity. These results highlight the hematological protective potential of *Bombax ceiba*, particularly the CHF and EAF fractions, in tumor-bearing conditions.

Discussion

Cancer persists as a primary cause of death worldwide, with its global burden continuing to rise (Piña-Sánchez *et al.*, 2021.) Natural sources, especially plant-derived molecules, have attracted significant attention from researchers in pursuit of safer and more efficient antineoplastic drugs compared to existing therapies. Several well-

established anticancer agents including paclitaxel, camptothecin, vincristine, curcumin, berberine, resveratrol, and topotecan originate from medicinal plants, underscoring the value of exploring traditional botanical resources (Chunarkar-Patil *et al.*, 2024) Despite considerable progress, vast opportunities remain to investigate underexplored traditional medicinal plants for their potential anticancer properties.

Bangladesh, a South Asian country situated on the Bay of Bengal, possesses a rich biodiversity with over 500 documented medicinal plant species (Khondker *et al.*, 2023). A significant portion of its population relies on traditional herbal remedies, with local healers (kabiraj) playing an important role in primary healthcare delivery (Rahman *et al.*, 2013) In light of this context, we focused our investigation on *Bombax ceiba,* a traditionally used plant growing in Rajshahi, Bangladesh, to assess its anticancer potential.

Plants generate a wide array of secondary metabolites, such as terpenoids, flavonoids, tannins, glycosides, phenols, coumarins, lignans, carotenoids, alkaloids, and steroids. These bioactive compounds are known for their diverse pharmacological actions, including neuroprotective, anti-inflammatory, antimicrobial, antidiabetic, and anticancer activities Makangara *et al.*, 2024. Our phytochemical investigation of *B. ceiba* bark fractions confirmed the presence of saponins, phenols, glycosides, terpenoids, steroids, flavonoids, and tannins compounds widely recognized for their therapeutic efficacy. For instance, flavonoids, commonly found in vegetables and herbs, possess broad-spectrum biological activities including antitumor, antioxidant, and anti-inflammatory effects (Roy *et al.*, 2022) Similarly, polyphenols such as curcumin, a compound derived from *Curcuma longa*, have demonstrated effectiveness against breast, liver, and lung cancers (Islam *et al.*, 2024). Glycosides like digoxin and digitoxin from *Digitalis purpurea* show both cardioprotective and anticancer potential (Kumavath *et al.*, 2021). Terpenoid-based drugs, such as paclitaxel, are derived from natural sources like *Taxus brevifolia* (Chaachouay *et al.*, 2024).

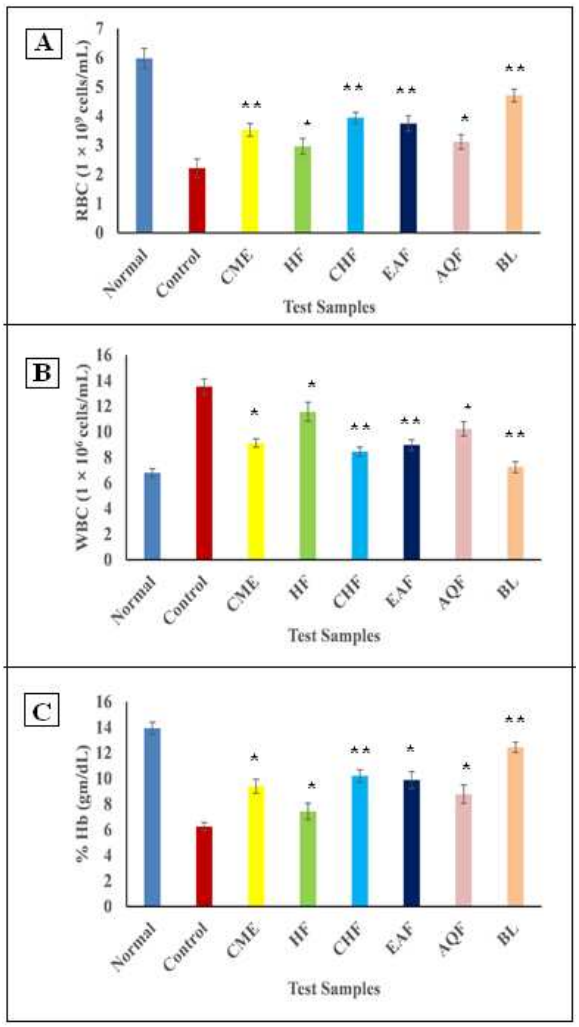

Fig. 2. Hematological effects of *Bombax ceiba* bark extract fractions in EAC-bearing mice. (A) RBC count, (B) WBC count, and (C) hemoglobin (Hb) levels following treatment with crude methanol extract (CME), n-hexane (HF), chloroform (CHF), ethyl acetate (EAF), aqueous (AQF) fractions, and the standard drug bleomycin (BL). Data are shown as mean ± standard error (n = 6), $^{*}p < 0.05$, $^{**}p < 0.01$.

To evaluate the anticancer potential of *B. ceiba*, we employed the Ehrlich ascites carcinoma (EAC) model, a widely accepted and robust in vivo system for screening antitumor agents due to its reproducibility and simplicity (Islam *et al.*, 2018). Our study demonstrated that the chloroform fraction (CHF) exhibited the strongest antiproliferative activity, inhibiting EAC cell growth by 83.46%, followed closely by the ethyl acetate fraction (EAF) at 82.28%. These findings align with previous studies, such as Einafshar *et al.*, who reported chloroform fractions as

the most effective against glioblastoma cells (Einafshar *et al.* 2024) and (Mostafa *et al.*, 2024) who found ethyl acetate fractions most active against EAC cells.

In addition to inhibiting cell proliferation, reduction in tumor mass is a critical indicator of anticancer efficacy. Our findings revealed that CHF and EAF significantly decreased tumor weight in EAC-bearing mice, demonstrating their potent antitumor effects. This observation is strengthened by prior research by Rahman *et al.*, who reported substantial tumor weight reduction following treatment with *Tabebuia pallida* leaves extract in a similar model (Rahman *et al.*, 2021.

Hematological abnormalities, including iron-deficiency anemia and myelosuppression, are commonly associated with cancer and its treatments (Busti *et al.*, 2018). Therefore, the ability of a potential anticancer agent to normalize hematological parameters is of particular importance. In our study, treatment with CHF and EAF not only mitigated tumor progression but also significantly restored hematological indices, including RBC count, WBC count, and hemoglobin levels. These findings suggest a dual benefit: direct antitumor activity and systemic protective effects features that further highlight the therapeutic potential of these fractions. Similar hematological improvements have been documented in earlier studies evaluating plant-based anticancer therapies (Rahman *et al.*, 2021. and Alam *et al.*, 2016).

Conclusion

This study provides compelling evidence that the chloroform (CHF) and ethyl acetate (EAF) fractions of *Bombax ceiba* bark extract possess potent anticancer properties. Both fractions significantly inhibited tumor cell proliferation, reduced tumor burden, and restored blood parameters in Ehrlich ascites carcinoma (EAC)-bearing mice, underscoring their therapeutic potential. Phytochemical screening revealed that CHF and EAF are enriched with flavonoids, polyphenols, glycosides, tannins, terpenoids, and steroids classes of bioactive compounds known for their diverse anticancer mechanisms. The observed effects are likely due to the synergistic activity of these phytoconstituents. These findings not only validate the traditional medicinal use of *B. ceiba* but also highlight its potential as a source of novel, plant-derived anticancer agents. Future investigations should focus on the isolation, structural elucidation, and mechanistic studies of the active constituents to further advance the development of targeted phytotherapeutics.

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