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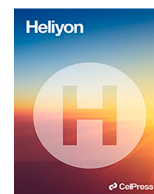

Research article

Unveiling the apoptotic potential of antioxidant-rich Bangladeshi medicinal plant extractives and computational modeling to identify antitumor compounds

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ABSTRACT

Nowadays, there has been a significant surge in the exploration of anticancer compounds derived from medicinal plants due to their perceived safety and efficacy. Therefore, our objective was to investigate the antioxidant and antiproliferative properties, along with the phytoconstituents, of methanol extracts from various parts of 15 selected Bangladeshi medicinal plants. Standard spectrophotometric methods and confocal microscopy were utilized to assess the antioxidant and antiproliferative potential of these extracts. Additionally, phytochemical profiling was executed through gas chromatography-mass spectrometry (GC-MS) analysis. Among the extractives, *Bombax ceiba* bark exhibited the highest scavenging capacity against DPPH (IC_{50}: 10.3 ± 0.7 μg/mL) and hydroxyl (IC_{50}: 3.9 ± 0.1 μg/mL) free radicals. Furthermore, the total antioxidants, reducing power, and polyphenols of *B. ceiba* bark were higher than those of other extracts. *B. ceiba* bark also showed significant antiproliferative capacity against MCF-7 cells (86.67 %) in the MTT assay, followed by *Cocos nucifera* roots (83.92 %), *Bixa orellana* leaves (44.09 %), and *Leea macrophylla* roots (25 %). Moreover, *B. ceiba* bark, *L. macrophylla* roots, *C. nucifera* roots, and *B. orellana* leaves-treated Ehrlich ascites carcinoma (EAC) cells demonstrated growth inhibition rates of 87.27 %, 80.45 %, 42.9 %, and 37.27 %, respectively. Fluorescence microscopic analysis of EAC cells treated with these extracts revealed apoptotic features such as condensed chromatin, cell shrinkage, nucleus fragmentation, and membrane blebbing compared to untreated EAC cells. The GC-MS analysis of *B. ceiba* bark identified 18 compounds, including various alcohols, alkenes, and esters. Additionally, a molecular docking study revealed oxalic acid, cyclohexyl dodecyl ester as the most potent compound (−6.5) active against breast cancer. In summary, our results demonstrate that *B. ceiba* bark possesses robust antioxidant and antiproliferative properties, along with potent antitumor compounds, which could be utilized in the treatment of carcinoma.

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1. Introduction

Cancer, the second major cause of global mortality, presents a significant threat of public health, despite significant progress in medical interventions [1]. As per the report of International Agency for Research on Cancer about 19.3 million new cases of cancer emerged globally in 2020, leading to 10 million deaths from this deadly disease [2]. Chemotherapy remains a cornerstone in managing advanced cancer stages and preventing potential metastasis. A range of chemotherapeutic agents derived from natural reservoirs, including paclitaxel, vincristine, vinblastine, bleomycin, and camptothecin, are currently employed in cancer therapy. Nevertheless, the constraints of chemotherapy, including non-specific toxicity to healthy tissues, low efficacy rates, and severe side effects, highlight the urgent need for novel, safe, and effective cancer-fighting medications. Consequently, special attention has been focused on natural sources like plants, microorganisms, and animals to uncover potent anticancer compounds [3].

In biological systems, imbalance between the generation and neutralization of reactive oxygen species (ROS) induces oxidative stress (OS) and plays a critical role in the generation of numerous pathophysiological conditions, including diabetes, carcinoma, Alzheimer's disease, coronary artery disease, aging, and renal disorders [4]. Numerous biological macromolecules, including DNA, are susceptible to OS damage, which can result in cell death [5]. Due to compromised cellular antioxidant defense mechanisms, this oxidative-driven cellular damage may spread more widely. Normally, an endogenous antioxidant system can protect against ROS-induced injury and assist in repairing injured cells. However, this endogenous systems may not be sufficient to protect our body from various ailments under increased OS; hence, dietary antioxidants are crucial [6].

An antioxidant is a stable compound capable of providing electrons to unstable free radicals, thereby scavenging and delaying cellular damage. Additionally, antioxidants may contribute to the elimination of toxic metabolic wastes from the body by neutralizing free radicals. Moreover, the consumption of antioxidant-rich foods through dietary supplements is linked to a reduced risk of developing diabetes mellitus, atherosclerosis, malignancies, arthritis, and aging [7]. Plant biomolecules, including alkaloids, glycosides, terpenoids, carotenoids, polyphenols, and vitamins demonstrate a wide range of biological actions and health benefits [8]. Although polyphenols are prevalent in both consumable and non-consumable plants, [9,10], there is limited scientific information available regarding the free-radicals neutralizing and anti-proliferative capacities of traditional plants. Furthermore, folk medicinal plants are still widely used for general well-being globally. To find new, novel sources of unique anticancer compounds for pharmaceuticals, it is therefore imperative and fascinating to carry out more research on these activities [11].

Medicinal herbs serve as a rich source of biomolecules and offer promising lead compounds for preventing and treating various illnesses, including inflammation, neurological disease, diabetes, carcinoma, microbial infections, and immunological disorders [12]. Globally, more than three thousand plant species have been identified with antitumor potential [13], and plants are responsible for approximately 25 % of modern drugs [14]. Moreover, traditional remedies, known for being readily available, cost-effective, and culturally fitting, serve as primary healthcare for approximately 80 % of Asians [15]. In Bangladesh, a Southeast Asian country with around 500 varieties of medicinal plants, the majority of rural residents are largely dependent on medicinal plant-based products for their treatment of various diseases [16]. Notably, the nation hosts 400 public and private farms dedicated to cultivating and promoting therapeutic plants and plant-derived goods [17]. Given their significant medicinal value, there exists immense potential to explore novel natural anticancer molecules from these plants. However, despite their widespread use, very few studies have documented the phytochemical analysis, as well as the antioxidant and antineoplastic potentials of these plants. Consequently, this study focuses on assessing the antioxidant and antitumor potentials of phytoconstituents extracted from different parts of fifteen Bangladeshi medicinal plants, including *Limonia acidissima* L., *Cocos nucifera* L., *Annona reticulata* L., *Hyptis suaveolens* L. Poit, *Leea macrophylla* Roxb., *Vernonia amygdalina* Del., *Abroma augusta* L., *Colocasia esculenta* L. Schott, *Argemone mexicana* L., *Bixa orellana* L., *Bombax ceiba* L., *Ocimum gratissimum* L., *Musa paradisiaca* L., *Ficus benghalensis* L., *and Cassia grandis* Lf.

Limonia acidissima L. (Rutaceae), known locally as Kodbel in Bangladesh, has been traditionally utilized to cure different disorders such as jaundice, diabetes, and diarrhea. Research has shown that its leaves and fruit pulp exhibit significant memory-enhancing capacity in Wistar rats and contain alkaloids, flavonoids, triterpenoids, steroids, saponins, phenolic acids, and volatile oils [18,19]. The roots of *Cocos nucifera* L. (locally known as Narikel, Family: Arecaceae) are widely employed in managing diarrhea and dysentery in folk medicine. Chemical analysis reveals the presence of phenolic compounds, flavonoids, lignans, and tannins [20]. *Annona reticulata* L. (known locally as Ata, Family: Annonaceae) bark is used for treating ulcers and toothaches. It also possesses notable hepatoprotective and anti-inflammatory properties due to the presence of kaur-16-en-19-oic acid as a key component [21]. *Hyptis suaveolens* L. Poit (known locally as Tokma, Family: Lamiaceae) seeds are traditionally used as a tonic and have significant anti-hyperuricemic activity due to the derivatives of caffeoylquinic acid [22]. *Leea macrophylla* Roxb. (Leeaceae), commonly referred to as Hathikana, is found mainly in the Rajshahi and Chittagong divisions of Bangladesh. Its leaves, stems, and roots are used for treating arthritis and cancer by tribal people. It also exhibits potent antioxidant and anti-diabetic properties, containing butylated hydroxytoluene, n-hexadecanoic acid, stigmasterol, γ-sitosterol, and γ-sitostenone [23]. *Vernonia amygdalina* Del. (known locally as Bitter leaf, Family: Asteraceae) leaves are largely utilized in the management of diabetes. They contain vernodalol, vernolide, vernoamyoside, 1-heneicosenol O-β-D-glucopyranoside, and luteolin [24]. *Abroma augusta* L. (known locally as Ulat kambal, Family: Malvaceae) leaves are used as a traditional remedy for jaundice and inflammation. Its chemical constituents include taraxerol, phenolic acids, flavonoids, and steroids [25]. *Colocasia esculenta* L. Schott, locally known as Kochu and belonging to the Araceae family, has stalks that are used to prevent anemia and are reputed for their antioxidant properties due to the presence of cyanidin 3-rutinoside as a major compound [26]. *Argemone mexicana* L. (known locally as Sial kata, Family: Papaveraceae) roots are traditionally used in treating infections and tumors, exhibiting potent anti-microbial activity. The main chemical constituents are berberine, protopine, and flavonoids [27,28]. *Bixa orellana* L. (known locally as Doigota, Family: Bixaceae) leaves are used in treating renal calculi and dysentery. Chemical analysis shows the presence of bixin, norbixin, flavonoids, tannins, and saponins [29]. *Bombax ceiba* L.

(locally called Shimul, Family: Malvaceae) is found across Bangladesh. Its bark and roots are traditionally used as tonics and for managing sexual dysfunction. Additionally, significant antioxidant capacities have been reported, with the plant containing lupeol, luteolin, rutin, quercetin, gallic acid, mangiferin, and epicatechin-7-*O*-β-xylopyranoside [30,31]. *Ocimum gratissimum* L. (known locally as Ram tulsi, Family: Lamiaceae) leaves are traditionally used for managing colds and coughs, and they possess potent hepatoprotective activity. The leaves contain essential oils (linalool, eugenol, thymol) and phytochemicals (rosmarinic acid, gallic acid, epicatechin, chlorogenic acid, luteolin, apigenin, quercetin, and kaempferol) [32]. *Musa paradisiaca* L. (known locally as Kala, Family: Musaceae) is commonly used in dysentery as a folk remedy and has significant anti-diabetic activity due to the presence of caffeic acid, flavonol, and anthocyanins [33]. The bark and fruits of *Ficus benghalensis* L. (locally called Bot, Family: Moraceae) have a long-standing tradition of use as a tonic and in the treatment of wounds, fractures, and gonorrhea. Chemical constituents include tannins, flavonoids, steroids, glycosides, and saponins [34]. Similarly, *Cassia grandis* Lf, known locally as Pink cassia and part of the Fabaceae family, has been extensively utilized to combat inflammation, diabetes, skin infections, and anemia as a complementary medicine. It contains linalool, polyphenols, saponins, tannins, steroids, coumarins, alkaloids, and glycosides [35]. Considering their traditional uses and pharmacological values, we have selected the aforementioned plants for phytochemical analysis and evaluation of their anti-oxidative and antiproliferative capacities.

2. Materials and methods

2.1. Utilized materials

In this study, each chemical and reagent used met the standards for analytical quality. The following substances were used: aluminum chloride ($AlCl_3$), ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}\cdot4H_2O$], ascorbic acid (AA), butylated hydroxytoluene

Table 1
Comparative polyphenols content, and anti-oxidative capacity of Bangladeshi medicinal plant extracts.

Plant Species (Accession No.)	Plant Parts	TPC (mg of GAE/g)	TFC (mg of CAE/g)	TAC (mg of AAE/g)	FRC (OD)	DPPH (IC_{50}: μg/mL)	Hydroxyl Radical (IC_{50}: μg/mL)
L. acidissima (PH-60)	Leaves	179 ± 5*	39 ± 1*	170 ± 3*	0.1 ± 0.02*	122.7 ± 4.7*	90.4 ± 1.2*
	Bark	141 ± 1*	42 ± 0*	150 ± 3*	0.1 ± 0.03	80.8 ± 4.3*	82.7 ± 5.2*
	Pulp	54 ± 1*	19 ± 3	100 ± 1*	0.05 ± 0.01	201.5 ± 2.5*	204.5 ± 2.4*
	Rind	45 ± 2*	19 ± 1*	96 ± 1*	0.03 ± 0.01	238.3 ± 5.5	242.4 ± 6.6
C. nucifera (PH-02)	Roots	214 ± 5**	179 ± 5**	214 ± 3**	0.41 ± 0.03*	23.5 ± 0.5**	9.4 ± 0.3**
A. reticulata (PH-175)	Bark	112 ± 8*	47 ± 3*	178 ± 4*	0.08 ± 0.01	91.3 ± 2.5*	92.3 ± 3.5*
H. suaveolens (PH-52)	Seeds	62 ± 1*	37 ± 1*	187 ± 7*	0.1 ± 0.01*	135.3 ± 7.6*	134.3 ± 5.6*
L. macrophylla (PH-103)	Leaves	100 ± 1*	94 ± 3*	134 ± 2*	0.4 ± 0.07*	31.3 ± 0.7**	27.2 ± 0.3**
	Stems	83 ± 2*	42 ± 4*	125 ± 4*	0.1 ± 0*	119.2 ± 6.9	121.2 ± 6.3*
	Roots	460 ± 6*	373 ± 2**	349 ± 3**	0.67 ± 0.05*	11.4 ± 1.8**	4.1 ± 0.1**
	Fruits	198 ± 7*	160 ± 2*	182 ± 3*	0.4 ± 0.02*	25.3 ± 1.7*	21.2 ± 1.9*
V. amygdalina (PH-37)	Leaves	79 ± 2*	51 ± 3*	124 ± 1*	0.07 ± 0.01	84.3 ± 5.6*	83.3 ± 4.6*
A. augusta (PH-190)	Leaves	78 ± 2*	28 ± 1*	126 ± 2*	0.12 ± 0.01*	282.5 ± 5.2	272.5 ± 4.2
C. esculenta (PH-70)	Stalks	35 ± 1*	15 ± 1	103 ± 3*	0.06 ± 0.02	380.2 ± 5.6	340.2 ± 6.1
A. Mexicana (PH-120)	Roots	37 ± 1*	16 ± 1	103 ± 2*	0.06 ± 0.01	438.7 ± 5	313 ± 4
B. orellana (PH-10)	Leaves	315 ± 6*	166 ± 4*	249 ± 4**	0.45 ± 0.02**	14.4 ± 1.3**	5.4 ± 0.6**
B. ceiba (PH-97)	Bark	558 ± 5**	508 ± 3**	357 ± 4**	0.86 ± 0.06**	10.3 ± 0.7**	3.9 ± 0.1**
	Roots	117 ± 1*	67 ± 2*	133 ± 4*	0.09 ± 0.01*	30 ± 1*	28 ± 1.2*
O. gratissimum (PH-22)	Leaves	88 ± 1*	82 ± 4*	139 ± 4*	0.08 ± 0.02	99.3 ± 1.1*	29.4 ± 1.1*
M. paradisiaca (PH-30)	Leaves	39 ± 2*	30 ± 2*	89 ± 1*	0.05 ± 0.01	601 ± 5	391 ± 3
F. benghalensis (PH-29)	Leaves	120 ± 1*	85 ± 1*	130 ± 2*	0.09 ± 0.02*	50.7 ± 1.9*	78.1 ± 2.9*
	Fruits	47 ± 1*	32 ± 2*	128 ± 5*	0.11 ± 0.02*	235.2 ± 5.5	123.7 ± 4.1*
C. grandis (PH-17)	Leaves	203 ± 4*	142 ± 6*	201 ± 4**	0.11 ± 0.01*	30 ± 1.8*	43.9 ± 3.9*
	Bark	334 ± 8*	258 ± 3*	209 ± 4**	0.5 ± 0.04**	19.3 ± 0.9**	16.4 ± 2**
Catechin (standard)	–	–	–	–	1.29 ± 0.03	–	–
Ascorbic Acid (standard)	–	–	–	–	–	5.1 ± 0.1	–
BHT (standard)	–	–	–	–	–	7 ± 0.2	4.67 ± 0.1

Here, TPC = Total Phenolic Compounds, TFC = Total Flavonoid Compounds, TAC = Total Antioxidant Capability, FRC = Ferric Reducing Capacity, GAE = Gallic Acid Equivalent, CAE = Catechin Equivalent, AAE = Ascorbic Acid Equivalent, IC_{50} = Half-inhibition concentration. Results are presented as mean ± SEM. The student *t*-test was performed with *$p < 0.05$, and **$p < 0.01$ significant values.

(BHT), gallic acid (GA), sodium dihydrogen phosphate dihydrate ($NaH_2PO_4{\cdot}2H_2O$), potassium ferricyanide [$K_3Fe(CN)_6$], tricholo acetic acid (TCA), ferric chloride ($FeCl_3$), thiobarbituric acid (TBA), EDTA, DAPI (4′,6-diamidino-2-phenylindole), MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide), and sulfuric acid (H_2SO_4) were sourced from Merck in Germany. DPPH (2,2-diphenyl-1-picrylhydrazyl) was commercially obtained from Tokyo Chemical Industry Co. Ltd. in Japan. Catechin (CA) was acquired from Active Fine Chemicals Limited, Bangladesh. Folin & Ciocalteus's reagent and hydrogen peroxide (H_2O_2) were procured from Alfa Aesar in the United States. Bleomycin sulfate was supplied by Beacon Pharmaceuticals Limited, Bangladesh.

2.2. Plant sample collection and authentication

The various parts of 15 medicinal plants were collected from Chapainawabganj (24.73°N 88.20°E) and Rajshahi (24°22′N 88°36′E) districts of Bangladesh in October–December 2020. The authenticity of the collected plants was confirmed by Professor AHM Mahbubur Rahman, a skilled taxonomist, and corresponding accession numbers (refer to Table 1) were recorded in the Herbarium of Rajshahi University under the Botany Department. To eliminate unwanted materials from these collected plant parts, distilled water (DW) was employed, followed by air drying at room temperature (RT) and intermittent sun drying over a period of twelve days. The subsequently dried plant materials were finely pulverized using an electric blender (Walton, Bangladesh) and stored at RT for future utilization.

2.3. Extraction

Before choosing the suitable solvent, the crushed plant samples were extracted using various solvents including, acetone, chloroform, n-hexane, methanol, and ethyl acetate. Methanol was ultimately chosen for extraction based on the extraction yield. The extraction of plant components was carried out using the ultrasound-assisted extraction (UAE) technique in a digital ultrasonic bath UBT-1080 (UNILAB, USA). In a wide-mouthed glass bottle sealed with a cap, 100 g of crushed plant materials were submerged in 700 mL of methanol. The mixture was then sonicated at a frequency of 40 kHz for 30 min at 30 °C. The resulting extracts were initially filtered through sterile cotton (Lab Asia, Bangladesh) twice and subsequently through filter papers (Whatman Grade 1, UK). The mass of the collected extracts was then reduced using a rota-evaporator (Bibby Sterlin Ltd. in the UK) at a regulated temperature of 40 °C with lowered pressure to obtain the final plant extractives (0.2–8 g) and conserved at 4 °C in a refrigerator for future utilization.

2.4. Evaluation of polyphenols content

2.4.1. Estimation of total phenols

The total phenols of the extractives were estimated by using Folin-Ciocalteu reagent (FCR) following the previously published report [36]. Gallic acid (GA) served as a reference standard in this assay. Briefly, 0.5 mL sample/standard combined with 2.5 mL FCR (diluted 10 times with DW) and 2.5 mL Na_2CO_3 (7.5 %). The mixture was vortexed and kept at 25 °C for 30 min. Following the incubation, the resulting solution's absorbance was recorded at 760 nm wavelength utilizing a spectrophotometer (UVmini-1240, Japan). The concentration of the extractives utilized as 100 μgmL^{-1}. The phenolic content of the extractives was assessed by using the formula, $C = c \times v/m$, Where, C = phenolic compounds of extractives in mg gallic acid equivalent (GAE) per gram of dry sample, c = GAE concentration of the extractives obtained from GA calibration curve ($y = 0.0084x - 0.079$, $R^2 = 0.9995$), v = volume of the solution (mL) and m = mass of the extractives (g).

2.4.2. Assessment of total flavonoids

The total flavonoids present in the extractives were evaluated following the $AlCl_3$ colorimetric technique as per the early published report [37]. Catechin (CA) employed as a reference standard in this test. In brief, 0.5 mL sample/standard added with 0.15 mL $NaNO_2$ (5 %) and 2.5 mL DW. After 5 min, the resulting solution was included with 0.3 mL $AlCl_3$ (10 %), 1.0 mL NaOH (0.001 M), and 0.55 mL DW. Then, the solution was incubated at RT (20 min) and absorbance was recorded using a spectrophotometer (UVmini-1240, Japan) at 420 nm. The concentration of the extracts utilized as 100 μgmL^{-1}. The total flavonoid content in the extractives was assessed by utilizing the formula, $C = c \times v/m$, Where, C = flavonoids content of extractives in mg catechin equivalent (CAE) per gram of dry sample, c = CAE concentration of the extracts taken from CA calibration curve ($y = 0.0026x - 0.0056$, $R^2 = 0.9998$), v = volume of the solution (mL) and m = mass of the extracts (g).

2.5. Evaluation of anti-oxidant capacity

2.5.1. Total anti-oxidants determination

The total anti-oxidants of the extractives was estimated using the phosphomolybdenum assay as per the early published report with ascorbic acid (AA) as a reference standard [38]. To create the standard calibration curve, 0.5 mL AA was combined with 3.0 mL of phosphomolybdenum reagent (0.6 M sulfuric acid, 0.028 M sodium dihydrogen phosphate, and 0.004 M ammonium molybdate). For completing the reaction, the resulting mixture was incubated for 10 min at 95 °C. After that, the solution was kept at RT for cooling, and the absorbance was recorded at 695 nm by a spectrophotometer (UVmini-1240, Japan). The concentration of the extracts was maintained at 100 μgmL^{-1}. The total anti-oxidant capability of the extractives was quantified in terms of ascorbic acid equivalents (AAE) using the equation, $y = 0.0034x - 0.0575$, $R^2 = 0.994$.

2.5.2. Assessment of ferric-reducing capacity

The ferric-reducing ability of the extractives was assessed utilizing the process reported earlier by M. M. Rahman et al., 2019 with slight modifications [39]. The antioxidants in the sample reduce Fe^{3+} to Fe^{2+} ions and form a complex exhibiting Perl's Prussian blue color. The absorbance of this complex linearly indicates the reducing power of the extractives. Shortly, 0.25 mL of extracts/standard added with 0.625 mL phosphate buffer (0.2 M, pH 6.6), and 0.625 mL potassium ferricyanide (1 %). The solution was incubated for 20 min at 50 °C and mixed to 0.625 mL trichloro acetic acid (10 %). The resulting mixture was centrifuged at 3000 rpm for 10 min and the supernatant was collected. The supernatant was added with 0.36 mL ferric chloride (0.1 %), and 1.8 mL DW. The resulting solution's absorbance was recorded by a spectrophotometer (UVmini-1240, Japan) at a wavelength of 700 nm. The concentration of both the extractives and the standard was set at 100 μg/mL through a trial-and-error approach.

2.5.3. DPPH free-radical neutralizing capacity

The extractives capability to neutralize the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical was assessed in accordance with a previously documented procedure [40]. Shortly, 3.0 mL DPPH (0.0001 M) solution was added to the 2.0 mL methanol extracts at different concentrations (6.25–200 μgmL^{-1}). After thoroughly vortexing, the solution was incubated at 25 °C about 30 min and the absorbance was recorded with a spectrophotometer (UVmini-1240, Japan) at a wavelength of 517 nm using methanol solution as a blank. Butylated hydroxytoluene (BHT) and AA were utilized as standard antioxidants. The inhibition capacity in percentage on DPPH of extractives/standards was analyzed by equation (1).

$$\% \text{ Inhibition} = (1\text{-Absorbance}_{sample}/\text{Absorbance}_{control}) \times 100 \tag{1}$$

The percentage inhibition values were then plotted against the extract concentrations, and the half-inhibition concentration (IC_{50}) was determined by extrapolation.

2.5.4. Hydroxyl free-radical neutralizing capacity

The hydroxyl free-radical neutralizing power of the extractives was analyzed as per the previously published report with minor modifications, utilizing BHT as a reference standard [41]. In summary, varying concentrations (6.25–200 μgmL^{-1}) of 0.1 mL extractives/standard added with 0.2 mL premixed $FeCl_3$: EDTA solution (1:1, 0.1 M), 0.1 mL H_2O_2 (0.001 M), and 0.5 mL of 0.028 M 2-deoxy-D-ribose in KH_2PO_4- K_2HPO_4 buffer (0.05 M). Subsequently, 0.1 mL of AA (0.003 M) was introduced, and the resulting solution was kept at 37 °C for 1 h. After that, 2.0 mL of 2.8 % TCA and 2.0 mL of 1 % TBA in KH_2PO_4-K_2HPO_4 buffer (0.05 M) were combined, and the solution was kept again at 90 °C for 20 min until a pink chromogenic color appeared. The solution was then allowed to cool at RT, and absorbance was recorded at 532 nm using a spectrophotometer (UVmini-1240, Japan). The neutralization capacity of extracts on hydroxyl radical was calculated by equation (2).

$$\% \text{ Inhibition} = (1\text{-Absorbance}_{sample}/\text{Absorbance}_{control}) \times 100 \tag{2}$$

The percentage inhibition values were plotted against extractive concentrations, and the half-inhibition concentration (IC_{50}) was determined through extrapolation.

2.6. In-vitro proliferation inhibition assay

2.6.1. Culture of cells

MCF-7 (breast cancer) cells were taken from Professor Zhao Xudong, a former faculty member at Kunming Institute of Zoology (China) through gifting. The collected cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) containing 1 % penicillin, 1 % streptomycin, and 10 % fetal bovine serum, and incubated in a 5 % CO_2 environment at 37 °C. The cell culture was maintained as per standard procedure [42].

2.6.2. Cell proliferation assessment

The antiproliferative potency of the antioxidant-rich extractives (*L. macrophylla* roots, *B. ceiba* bark, *C. nucifera* roots, *C. grandis* bark, and *B. orellana* leaves) on MCF-7 cells was performed following the early published report utilizing 3-(4,5-dimethylthiazol-2-yl) 2,5 diphenyl tetrazolium bromide (MTT) dye [43]. Briefly, MCF-7 cells (1 x 10^4) were seeded in each well of a 96-well plate with 0.15 mL DMEM media and kept in a CO_2 incubator for 24 h at 37 °C. Varying concentrations (125–500 μgmL^{-1}) of extractives and standard anticancer drug bleomycin sulfate were added in every plate well of plate and incubated again for 48 h. Subsequently, 0.02 mL of MTT (5 $mgmL^{-1}$) in phosphate buffer saline (PBS) was mixed in each well and incubated the plates at 37 °C for 8 h. After that, 0.2 mL of isopropanol (acidic) was incorporated to every plate well to solubilize the formazan crystals and kept 30 min at 37 °C. The resulting solution's absorbance was recorded by an Optica Microplate Reader (UK) at 570 nm. Dimethyl sulfoxide (DMSO, 0.1 %) was utilized as a negative control. The antiproliferative capacity of the extractives/standard was estimated from equation (3).

$$\text{Percentage (\%) of proliferation inhibition} = (1\text{-Absorbance}_{sample}/\text{Absorbance}_{control}) \times 100 \tag{3}$$

2.7. In-vivo evaluation of antiproliferative capacity

2.7.1. Test animals and ethics statement

For this test, male Swiss albino mice weighing 26 ± 2 g were segregated into 7 groups, each comprising 6 mice. These mice were procured from the animal center of the Genetic Engineering and Biotechnology department at Rajshahi University. The animal experiment was conducted as per the institutional approval and guidelines of Rajshahi University, Bangladesh (license No: 249 (35)/320/IAMEBBC/IBSc), maintaining the international law and ethics.

2.7.2. Cell collection and maintenance

The Ehrlich Ascites carcinoma (EAC) cells were acquired from the Indian Institute of Chemical Biology (West Bengal, India) and properly maintained in the laboratory by intraperitoneal inoculation of cells (1×10^5) biweekly in mice.

2.7.3. Cell proliferation determination

The in-vivo antiproliferative efficacy *of the* extractives was determined against EAC cells in mice following the earlier reported method [44]. EAC cells (1×10^6) were inoculated via intraperitoneal injections in every mouse of 7 groups on day "0". Treatment of mice bearing EAC cells with the samples commenced 24 h after cancer cell inoculation and persisted for five days. The extractives were initially dissolved in DMSO (0.5 % v/v) and then diluted in normal saline (0.98 %). Group one served as the control, receiving the vehicle identical to the sample extracts, and group two was treated with bleomycin sulfate (0.3 mg/kg/day) as a standard anticancer drug. Group three to seven were treated with the antioxidant-rich extractives, *L. macrophylla* roots, *B. ceiba* bark, *C. nucifera* roots, *C. grandis* bark, and *B. orellana* leaves*,* respectively, at 10 mg/kg/day (i. p). On day six, the intraperitoneal EAC cells were collected with normal saline (0.98 %) from the sacrificed mice and counted using a hemocytometer with trypan blue dye (0.4 %). The cell growth inhibition in percentage was determined by comparing the viable number of EAC cells between treatment and control groups using equation (4).

$$\text{EAC cell growth inhibition (\%)} = (1\text{–}T/C) \times 100 \tag{4}$$

Where, T = mean of collected EAC cells from treated mice and C = mean of collected EAC cells from controlled mice.

2.8. Studies of hematological parameters

The impact of the test samples on hematological profiles including red blood cell (RBC), white blood cell (WBC), and hemoglobin content of control and treatment mice was investigated using established procedures as outlined in previous literature [45]. The administration of antioxidant-rich extracts from *L. macrophylla* roots, *B. ceiba* bark, *C. nucifera* roots, *C. grandis* bark, and *B. orellana* leaves at a dosage of 10 mg/kg/day (i.p.) to tumor-bearing mice commenced one day after cancer cell inoculation and continued for a consecutive 12 days. The blood samples of mice were collected from tail, and hematological parameters (RBC, WBC, and hemoglobin content) were assessed using a hemocytometer and hematometer, respectively.

2.9. Determination of tumor weight

The tumor weight of mice was determined following the method reported earlier by Kabir et al., 2022 [46]. The same treatment procedure and mice were used to determine the tumor weight as those utilized to analyze the hematological parameters. Mice weight was recorded on a daily basis for up to 20 days following the treatment with antioxidant-rich extractives and standard anticancer drug bleomycin sulfate to determine the tumor weight.

2.10. Analysis of cellular morphology and nuclear damage

Apoptosis was assessed following a previously documented protocol [42]. The Olympus-IX71 fluorescence microscope (Seoul in Korea) was employed to analyze the morphological alterations between the treated-EAC cells and controlled-EAC cells. In brief, mice were treated with antioxidant-rich extractives of *L. macrophylla* roots, *B. ceiba* bark, *C. nucifera* roots, *C. grandis* bark, and *B. orellana* leaves at a dose of 10 mg/kg/day (i.p.) and continued for five days. At day six, the EAC cells were collected from both the treated and controlled mice. The collected EAC cells were taken in a glass slide and washed properly with PBS thrice. Subsequently, 0.1 μgmL^{-1} DAPI (4′, 6-diamidino-2-phenylindole) was added to the slide for staining of cells and kept in the dark place for 20 min at 37 °C. Lastly, again PBS was added to the stained EAC cells to eliminate unbound dye and observed under a microscope.

2.11. GC-MS analysis of Bombax ceiba bark

The chemical profiling of *Bombax ceiba* bark through gas chromatography-mass spectrometry (GC-MS) was conducted following a slightly modified method from Hossain et al., 2020 [47]. In this study, we used a Shimadzu GC-2010 Plus gas chromatograph connected to a Shimadzu GCMS-QP2020 mass spectrometer (both from Japan). A capillary column (SH-Rxi-5Sil MS; 30 m × 0.25 mm × 0.25 μm) was utilized. A continuous flow of pure helium (99.99 %) was maintained at a rate of 1 mL/min. The dried extract of *B. ceiba* bark was dissolved in GC-grade methanol (Thermo Fisher Scientific, India) and passed through a membrane filter at 0.45 μm in size.

Subsequently, a 2 μL aliquot was introduced into the system using the splitless mode, while maintaining the injector temperature at 220 °C. The temperature of the oven was 50 °C (2 min) and then maintained at 150 °C (3 min), 250 °C (5 min), and 300 °C (10 min). The final hold time was about 20 min. The detection of MS was conducted with electron ionization mode at an energy level of 70 electron volts (eV). The ion source was heated at 200 °C, while the temperature of the interface was 250 °C. The range of masses analyzed ranged from 50 to 550 *m/z* (mass-to-charge ratio). The phytoconstituents were identified through the comparison of the detected compounds in the spectrum with the GC-MS reference library NIST-14.

2.12. *In silico molecular docking study*

2.12.1. *Protein preparation*

For molecular docking analysis, we obtained the three-dimensional (3D) configuration of the HER2 protein from the RCSB PDB (PDB ID: 7PCD) [48]. Using The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC software, the protein structure was prepared by removing all water molecules, heteroatoms, and ligands present in the 3D structure.

2.12.2. *Ligand preparation*

To prepare the ligands, we acquired the SDF file of the well-known anticancer drug carboplatin, frequently utilized in breast cancer treatment [49]. Additionally, we obtained 18 compounds from the PubChem server, identified from *B. ceiba* bark through GC-MS profiling. All the ligands were energy minimized using the Pyrx software upon importing the compounds for molecular docking [50].

2.12.3. *Molecular docking and interaction analysis*

For molecular docking of the selected compounds and standard drug with the HER2 protein (PDB ID: 7PCD) [48], we employed the Pyrx Autodock Vina Wizard [50]. During grid box generation, we specified the major binding site residues of the protein (SER783, MET801, PRO802, and CYS805) [48] to conduct site-specific docking, aiming to enhance precision. The dimensions of the grid box were set to X: 23.9960, Y: 23.8281, and Z: 22.5053 (Angstrom). Following molecular docking, compound files were generated using Pymol software, and the interaction of the compounds with the protein was analyzed using BIOVIA, Dassault Systèmes, Discovery Studio, San Diego: Dassault Systèmes, 2021.

2.13. *Statistical analysis*

The experiments were conducted three times, and the results are presented as the average value plus or minus the standard error of the mean (SEM). Statistical and graphical analyses were performed utilizing Microsoft Excel 2007 (Roselle, IL, USA) and SPSS 20 software (IBM, USA). To compare the means between different groups, we used one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test at significance levels of 5 %, 1 %, and 0.1 %. Additionally, we employed a *t*-test to compare the means of two specific groups.

3. Results

3.1. *Quantification of polyphenols*

3.1.1. *Total phenolic compounds*

The quantity of phenolic compounds in the extract was measured using FCR and is presented in Table 1 as milligrams of GAE per gram of the sample [51]. The chosen Bangladeshi medicinal plant extracts displayed a wide range of phenolic content, ranging from 35 ± 1 to 558 ± 5 mg of GAE per gram of the sample. *B. ceiba* bark showed the highest concentration of phenolic compounds at 558 ± 5 mg of GAE per gram. Following closely were *L. macrophylla* roots (460 ± 6 mg of GAE/g), *C. grandis* bark (334 ± 8 mg of GAE/g), *B. orellana* leaves (315 ± 6 mg of GAE/g), *C. nucifera* roots (214 ± 5 mg of GAE/g), and *C. grandis* leaves (203 ± 4 mg of GAE/g), all significant sources of phenolic compounds. On the lower end of the spectrum were *L. macrophylla* fruits, leaves, and stems; *L. acidissima* leaves, bark, pulp, and rind; *F. benghalensis* leaves and fruits; *B. ceiba* roots; *A. reticulata* bark; *O. gratissimum* leaves; *V. amygdalina* leaves; *A. augusta* leaves; *H. suaveolens* seeds; *M. paradisiaca* leaves; *A. Mexicana* roots; and *C. esculenta* stalks.

3.1.2. *Total flavonoids content*

Aluminum chloride colorimetric assay was used to measure the overall flavonoid content of the extracts and are given in Table 1 as milligrams of catechin equivalents (CAE) per gram of extract. Among the extracts, *B. ceiba* bark was identified as the most abundant source of flavonoids, containing 508 ± 3 mg of CAE per gram. Following closely were *L. macrophylla* roots (373 ± 2 mg of CAE/g), *C. grandis* bark (258 ± 3 mg of CAE/g), *C. nucifera* roots (179 ± 5 mg of CAE/g), *B. orellana* leaves (166 ± 4 mg of CAE/g), *L. macrophylla* fruits (160 ± 2 mg of CAE/g), and *C. grandis* leaves (142 ± 6 mg of CAE/g) which also exhibited significant levels of flavonoids. On the other hand, *L. macrophylla* leaves and stems; *F. benghalensis* leaves and fruits; *O. gratissimum* leaves; *B. ceiba* roots; *V. amygdalina* leaves; *A. reticulata* bark; *L. acidissima* bark, leaves, rind, and pulp; *H. suaveolens* seeds; *M. paradisiaca* leaves; *A. augusta* leaves; *A. Mexicana* roots; and *C. esculenta* stalks showed lower levels of flavonoids.

3.2. Antioxidant activity

3.2.1. Total antioxidant capacity

The total antioxidants of the extractives were assessed using the phosphomolybdate method, which measures the development of the phosphomolybdate complex spectrophotometrically. The results are depicted in Table 1 as milligrams of ascorbic acid equivalents (AAE) per gram of sample. Among the extracts, *B. ceiba* bark exhibited the highest total antioxidant capacity at 357 ± 4 mg of AAE/g. Additionally, *L. macrophylla* roots (349 ± 3 mg of AAE/g), *B. orellana* leaves (249 ± 4 mg of AAE/g), *C. nucifera* roots (214 ± 3 mg of AAE/g), *C. grandis* bark (209 ± 4 mg of AAE/g), and *C. grandis* leaves (201 ± 4 mg of AAE/g) were significant sources of total antioxidants. *H. suaveolens* seeds; *L. macrophylla* fruits, leaves, and stems; *A. reticulata* bark; *L. acidissima* leaves, bark, pulp, and rind; *F. benghalensis* leaves, and fruits; *A. augusta* leaves; *A. Mexicana* roots; *C. esculenta* stalks, and *M. paradisiaca* leaves exhibited lower levels of total antioxidants.

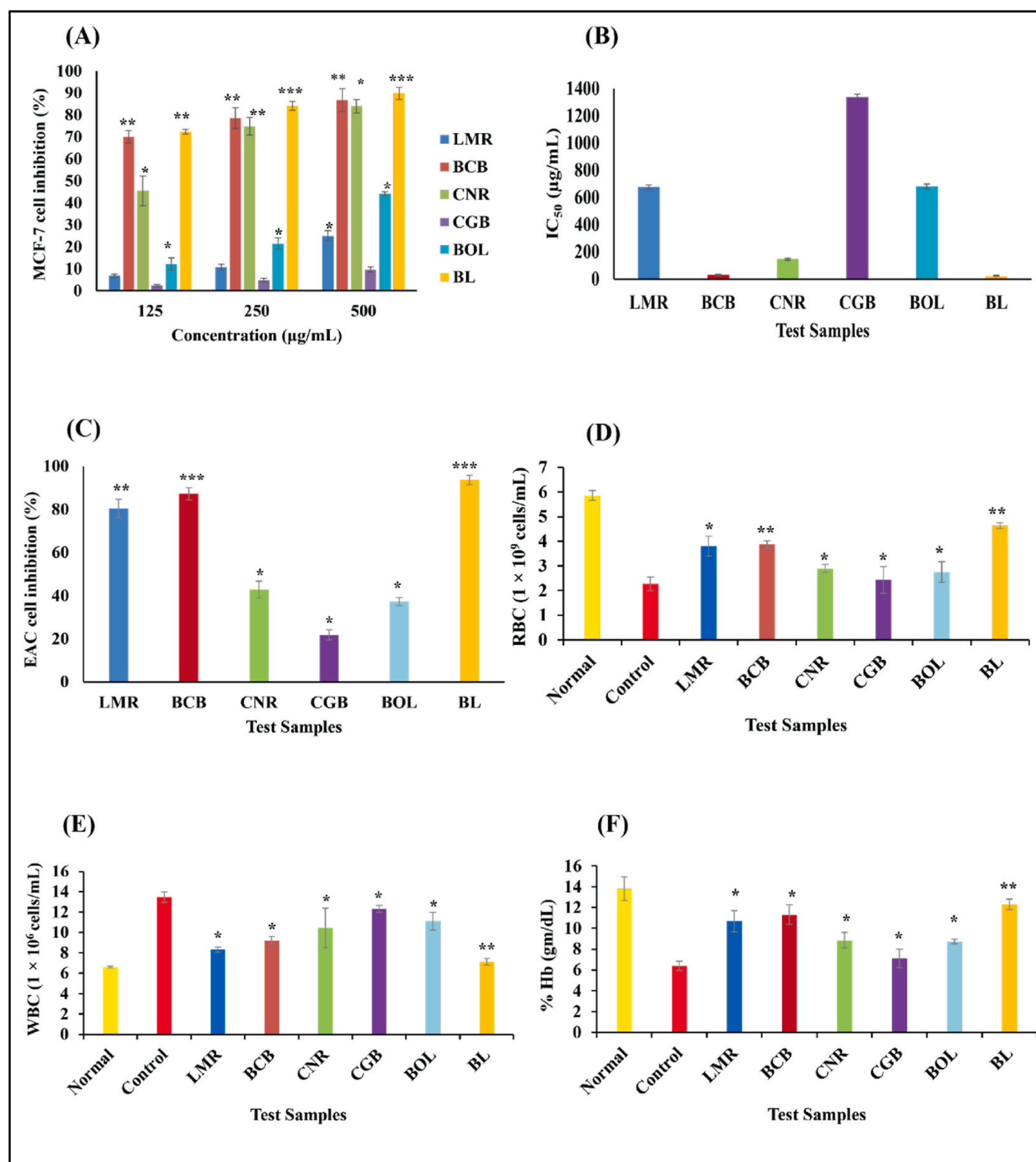

Fig. 1. Antiproliferative capacity of antioxidant-rich Bangladeshi medicinal plant extractives (A) Growth inhibition of MCF-7 cells after treatment with plant extractives using MTT assay, (B) IC_{50} of plant extractives, (C) Antiproliferative capacity of extractives against EAC cell in mouse model, (D) RBC level of control and extractives-treated EAC cell (E) WBC level of control and extractives-treated EAC cell, (F) Hemoglobin content of control and extractives-treated EAC cell. Data are showed as mean ± SEM, n = 6. Where significant values are *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$. LMR: *Leea macrophylla* roots, BCB: *Bombax ceiba* bark, CNR: *Cocos nucifera* roots, CGB: *Cassia grandis* bark, BOL: *Bixa orellana* leaves, BL: Bleomycin sulfate.

3.2.2. Ferric-reducing capacity

The ferric-reducing antioxidant test is a common technique for determining the antioxidant capacity of plant extracts. The presence of polyphenols in plant extracts enhances their anti-oxidative potential through the donation of hydrogen atoms or electrons to free radical chains. Table 1 summarizes the extracts' reducing capabilities. Among the samples tested, *B. ceiba* bark demonstrated the highest absorbance (0.86 ± 0.06) compared to the standard antioxidant catechin (1.29 ± 0.03). A higher absorption indicates a greater reducing power. *L. macrophylla* roots (absorbance 0.67 ± 0.05), *C. grandis* bark (absorbance 0.5 ± 0.04), *B. orellana* leaves (absorbance 0.45 ± 0.02), and *C. nucifera* roots (absorbance 0.41 ± 0.03) exhibited excellent reducing power. On the other hand, *L. macrophylla* fruits, leaves, stems; *A. augusta* leaves; *C. grandis* leaves; *F. benghalensis* fruits and leaves; *H. suaveolens* seeds; *L. acidissima* leaves, bark, pulp, and rind; *B. ceiba* roots; *A. reticulata* bark; *O. gratissimum* leaves; *V. amygdalina* leaves; *A. Mexicana* roots; and *C. esculenta* stalks displayed a lower reducing power.

3.2.3. DPPH free-radical scavenging activity

Table 1 compares the DPPH free-radical scavenging capacity of several extractives and conventional antioxidants, including AA and BHT. All of the extractives demonstrated free radical scavenging power, with a dose-dependent property, meaning that increasing the concentration of the extracts boosted their ability to scavenge DPPH. Among the extracts, *B. ceiba* bark showed highest free-radical scavenging potential (IC_{50}: 10.3 ± 0.7 μg/mL), which was quite comparable to the standard antioxidants AA (IC_{50}: 5.1 ± 0.1 μg/mL) and BHT (IC_{50}: 7 ± 0.2 μg/mL). Other extractives with excellent DPPH radical scavenging capacity included *L. macrophylla* roots (IC_{50}: 11.4 ± 1.8 μg/mL), *B. orellana* leaves (IC_{50}: 14.4 ± 1.3 μg/mL), *C. grandis* leaves (IC_{50}: 19.3 ± 0.9 μg/mL), and *C. nucifera* roots (IC_{50}: 23.5 ± 0.5 μg/mL). On the other hand, extractives such as *L. macrophylla* fruits, leaves, and stems; *B. ceiba* roots; *C. grandis* leaves; *F. benghalensis* leaves and fruits; *L. acidissima* bark, leaves, pulp, and rind; *V. amygdalina* leaves; *A. reticulata* bark; *O. gratissimum* leaves; *H. suaveolens* seeds; *A. augusta* leaves; *C. esculenta* stalks; *A. mexicana* roots; and *M. paradisiaca* leaves displayed comparatively low DPPH radical scavenging capability.

3.2.4. Hydroxyl radical scavenging capacity

Fenton's reaction was used to create hydroxyl radicals, and the extractives were monitored to neutralize these in a dose-dependent manner [52]. Table 1 shows the extracts' scavenging ability for hydroxyl radicals. Lower IC_{50} values are indicative of stronger antioxidant activity in plant extracts. *B. ceiba* bark represented the most potent hydroxyl radical's scavenger (IC_{50}: 3.9 ± 0.1 μg/mL) than the standard antioxidant BHT (IC_{50}: 4.67 ± 0.1 μg/mL). Additionally, *L. macrophylla* roots (IC_{50}: 4.1 ± 0.1 μg/mL), *B. orellana* leaves (IC_{50}: 5.4 ± 0.6 μg/mL), *C. nucifera* roots (IC_{50}: 9.4 ± 0.3 μg/mL), *C. grandis* bark (IC_{50}: 16.4 ± 2 μg/mL), *and L. macrophylla* fruits (IC_{50}: 21.2 ± 1.9 μg/mL) also exhibited significant scavenging activity against hydroxyl radicals. However, *L. macrophylla* leaves and stems; *B. ceiba* roots; *O. gratissimum* leaves; *C. grandis* leaves; *F. benghalensis* leaves and fruits; *V. amygdalina* leaves; *A. reticulata* bark; *L. acidissima* bark, leaves, pulp, and rind; *H. suaveolens* seeds; *A. augusta* leaves; *A. Mexicana* roots; *C. esculenta* stalks; and *M. paradisiaca* leaves exhibited poor neutralization of hydroxyl radicals.

3.3. In-vitro anti-proliferative capacity on MCF-7 cells

The cytotoxic potential of antioxidant-rich Bangladeshi medicinal plant extracts and standard bleomycin sulfate against MCF-7 (breast cancer) cells was assessed using an MTT colorimetric technique to evaluate their in-vitro anti-proliferative capacity [53, 54]. The plant extracts demonstrated significant inhibition of MCF-7 cell proliferation in a dose-dependent manner (Fig. 1A). Among these extractives, *B. ceiba* bark exhibited the highest inhibition of MCF-7 cell growth (86.67 %), while *C. grandis* bark showed the lowest suppression (9.68 %) of MCF-7 cell growth. At a concentration of 125 μg/mL, *L. macrophylla* roots, *B. ceiba* bark, *C. nucifera* roots, *C. grandis* bark, and *B. orellana* leaves inhibited MCF-7 cell proliferation by 6.79 %, 70 %, 45.45 %, 2.42 %, and 12.15 %, respectively. At a concentration of 500 μg/mL, *L. macrophylla* roots, *B. ceiba* bark, *C. nucifera* roots, *C. grandis* bark, and *B. orellana* leaves inhibited MCF-7 cell proliferation by 25 %, 86.67 %, 83.92 %, 9.68 %, and 44.09 %, respectively. The half-maximum inhibition concentration (IC_{50}) of *L. macrophylla* roots, *B. ceiba* bark, *C. nucifera* roots, *C. grandis* bark, and *B. orellana* leaves were found to be 677 μg/mL, 30 μg/mL, 145 μg/mL, 1335 μg/mL, and 680 μg/mL, respectively (Fig. 1B). The standard anticancer drug bleomycin sulfate demonstrated 89.76 % inhibition with an IC_{50} value of 23 μg/mL. The antiproliferative activity of the extractives followed the order: *B. ceiba* bark > *C. nucifera* roots > *B. orellana* leaves > *L. macrophylla* roots > *C. grandis* bark.

3.4. In-vivo anti-proliferative capacity on EAC cells

The anti-proliferative capacity of antioxidant-rich Bangladeshi plant extractives against EAC cells in the mice model is represented in Fig. 1C, compared to the standard anticancer drug bleomycin sulfate.

EAC cells were collected from mice in both control and treated groups, and the number of viable cells was quantified in comparison to the control group samples. Among the extractives, *B. ceiba* bark exhibited the highest antiproliferative effect (87.27 %) against EAC cells at a dose of 10 mg/kg body weight/day (i.p.), followed by *L. macrophylla* roots (80.45 %), *C. nucifera* roots (42.9 %), *B. orellana* leaves (37.27 %), and *C. grandis* bark (21.82 %), respectively. The standard anticancer drug bleomycin sulfate inhibited EAC cell growth by 93.63 % at a dose of 0.3 mg/kg body weight/day (i.p.).

3.5. Studies of hematological parameters

The effects of antioxidant-rich Bangladeshi plant extractives on the hematological parameters (hemoglobin content, RBC and WBC) of tumor-bearing mice are depicted in Fig. 1D–F. The hematological parameters of EAC-bearing mice varied significantly compared to normal mice. In tumor-bearing mice, the WBC count increased, whereas RBC count and hemoglobin content decreased. Treatment with *B. ceiba* bark, *L. macrophylla* roots, *C. nucifera* roots, and *B. orellana* leaves significantly reversed these parameters back towards normal levels.

3.6. Determination of tumor weight

The effects of antioxidant-rich extractives on tumor weight were investigated in EAC-bearing mice. The average tumor weight was recorded for up to 20 days. Among the extractives, *B. ceiba* bark exhibited a significant reduction ($p < 0.001$) in tumor weight (7.31 ± 0.25 g) compared to control mice (21.62 ± 0.98 g). *L. macrophylla* roots (9.53 ± 0.59 g), *C. nucifera* roots (11.58 ± 0.36 g), and *B. orellana* leaves (13.49 ± 0.82 g) also reduced the average tumor weight in mice. Additionally, the capacity of *B. ceiba* bark to reduce tumor weight was comparable to the standard anticancer drug bleomycin (5.19 ± 0.16 g). However, *C. grandis* bark showed no significant reduction in tumor weight (Fig. 2).

3.7. Morphological examination of EAC cells

When examined under a fluorescence microscope, the nucleus of EAC cells was found to be round, regular, and of normal shape in the control group (Fig. 3A). However, EAC cells treated with *B. ceiba* bark, *L. macrophylla* roots, *C. nucifera* roots, and *B. orellana* leaves extractives (Fig. 3C–F) exhibited morphological abnormalities, including fragmented DNA, nuclear condensation, irregular shapes, plasma membrane blebbing, and the presence of apoptotic body formation. The standard anticancer drug bleomycin sulfate also induced apoptosis in EAC cells (Fig. 3B). Conversely, *C. grandis* bark showed no apoptotic properties on EAC cells (Fig. 3G).

3.8. GC-MS analysis of Bombax ceiba bark extractive

Several medicinally useful compounds were detected during the examination of the phytochemical content of *B. ceiba* bark by GC-MS analysis, as shown in Fig. 4. The results revealed a total of 18 compounds were discovered by comparing their mass spectra with the known constituents documented in the NIST-14 library. All the identified compounds belong to alcohol, ether, alkene, alkane and ester classes. The predominant compounds were 2-methyl-2-dodecanol (46.461 %); 3-ethyl-2-methyl-2-heptanol (8.432 %); 2-methoxydecane (7.658 %); 2,6-dimethyl-2-octanol (7.651 %); 1,5-diethyl-2,3-dimethylcyclohexane (6.428 %); 2-methyl-1-[(6-methylheptyl) oxy]-2-propanol (2.446 %); oxalic acid, cyclohexyl dodecyl ester (2.149 %); 3,7-dimethyl-1,7-octanediol (2.131 %); undecylcyclohexane (1.765 %); (3-Methylpentyl) cyclohexane (1.683 %); (Ethoxymethoxy) cyclohexane (1.338 %); diethylene glycol tert-butyl ether methyl ether (1.311 %); 2-methyl-2-nonanol (1.306 %). (2E)-4,4,5-trimethyl-2-hexene (0.915 %); 4,5-dimethyl-2-hepten-3-ol (0.731 %); 2,3,7-trimethyl-2-octene (0.702 %); 3,3-dimethyloctane (0.348 %); and (Z) 1-allyl-2-methylcyclohexanol (0.115 %) were the minor compounds (Table 2**)**.

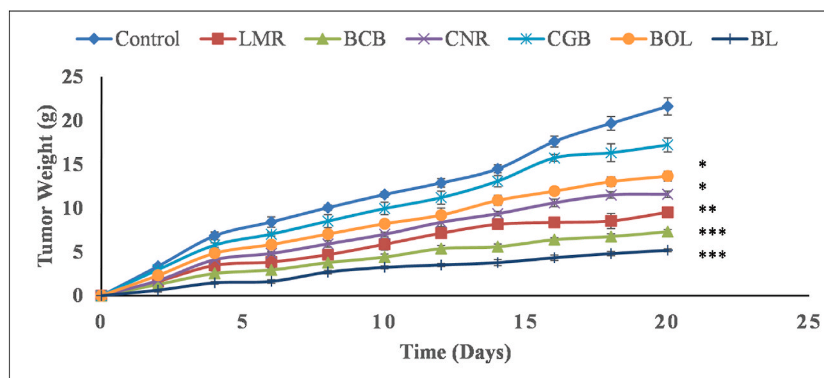

Fig. 2. Tumor growth inhibition capacity of antioxidant-rich Bangladeshi medicinal plant extractives and standard anticancer drug bleomycin sulfate. EAC-cell-bearing mice were treated with plant extracts (10 mg/kg body weight) and bleomycin sulfate (0.3 mg/kg body weight). Tumor weight was recorded for up to 20 days. Data are presented as mean ± SEM, n = 6. Statistical significance is indicated as follows: $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. LMR - *Leea macrophylla* roots, BCB - *Bombax ceiba* bark, CNR - *Cocos nucifera* roots, CGB - *Cassia grandis* bark, BOL - *Bixa orellana* leaves, BL - Bleomycin sulfate.

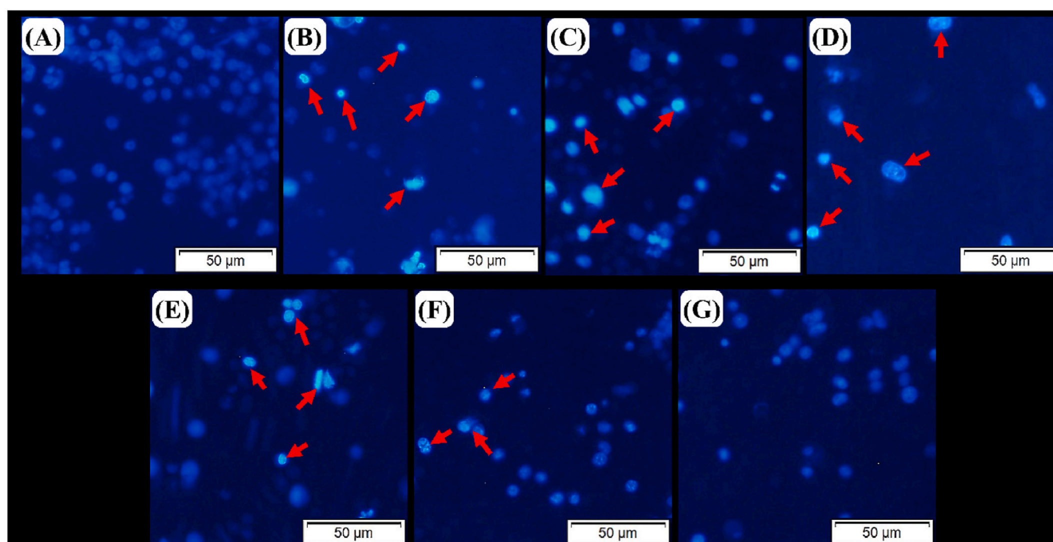

Fig. 3. Morphological observation of control and extractives-treated EAC cell by fluorescence microscopy (Olympus IX71). (A, B) indicate control and bleomycin-treated cells; (C–G) represent *L. macrophylla* roots, *B. ceiba* bark, *C. nucifera* roots, *B. orellana* leaves, and *C. grandis* bark-treated cells, respectively. Arrows indicate cells undergoing an apoptosis.

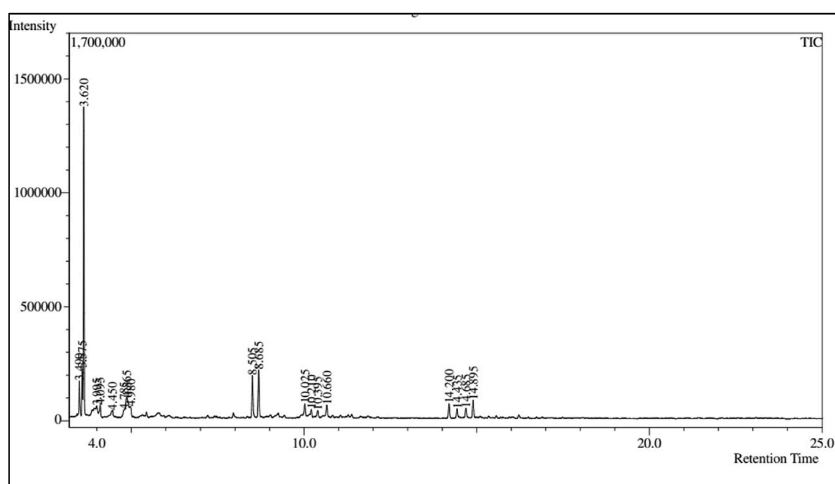

Fig. 4. GC-MS spectrum of *Bombax ceiba* bark extractive. An equipment gas chromatograph (GC-2010 Plus Shimadzu, Japan) combined with a mass spectrometer (GCMS-QP2020 Shimadzu, Japan) was used.

3.9. *In silico molecular docking study*

We determined the binding affinities of various compounds with the HER2 protein (PDB ID: 7PCD) by conducting the molecular docking study. Notably, oxalic acid, cyclohexyl dodecyl ester exhibited the highest binding affinity (−6.5) among the compounds, even surpassing that of the standard drug carboplatin (−5.1) (Table 3).

Upon visualization of the compound files in Discovery Studio, we observed that carboplatin and the top three compounds (oxalic acid, cyclohexyl dodecyl ester; undecylcyclohexane; and 1,5-diethyl-2,3-dimethylcyclohexane), based on their binding affinity, effectively bound within the binding pocket of the HER2 protein and interact with the binding site residues (Fig. 5A–D). Analysis of the interactions between the compounds and the protein revealed that both the standard drug carboplatin (Fig. 5A) and our top candidate, oxalic acid, cyclohexyl dodecyl ester (Fig. 5B), interacted with the SER783 residue, a critical binding site residue of the HER2 protein [48]. Interestingly, our top candidate exhibited closer interaction with the SER783 residue compared to the standard drug. Oxalic acid, cyclohexyl dodecyl ester demonstrated a total of 13 interactions with 11 different residues of the protein, whereas the standard drug carboplatin only displayed 7 interactions with 5 different residues of the protein (Supplementary Table 1).

Table 2
Identification of components of *Bombax ceiba* bark methanol extractives by GC-MS.

Sl. No.	Name of compounds	RT (Min)	Conc. (%)	Chemical Nature	Molecular Formula	Molecular Weight (g/mol)
1	(Z) 1-Allyl-2-methylcyclohexanol	3.456	0.115	Alcohol	$C_{10}H_{18}O$	154.25
2	2-Methoxydecane	3.575	7.658	Ether	$C_{11}H_{24}O$	172.31
3	2-Methyl-2-dodecanol	3.621	46.461	Alcohol	$C_{13}H_{28}O$	200.37
4	3,3-Dimethyloctane	4.000	0.348	Alkane	$C_{10}H_{22}$	142.28
5	Diethylene glycol tert-butyl ether methyl ether	4.097	1.311	Ether	$C_9H_{20}O_3$	176.25
6	4,5-Dimethyl-2-Hepten-3-ol	4.453	0.731	Alcohol	$C_9H_{18}O$	142.24
7	1,5-Diethyl-2,3-dimethylcyclohexane	4.884	6.428	Cycloalkane	$C_{12}H_{24}$	168.32
8	(3-Methylpentyl) cyclohexane	4.953	1.683	Cycloalkane	$C_{12}H_{24}$	168.32
9	2,6-Dimethyl-2-octanol	8.506	7.651	Alcohol	$C_{10}H_{22}O$	158.28
10	3-Ethyl-2-methyl-2-heptanol	8.686	8.432	Alcohol	$C_{10}H_{22}O$	158.28
11	Oxalic acid, cyclohexyl dodecyl ester	10.024	2.149	Ester	$C_{20}H_{36}O_4$	340.5
12	(2E)-4,4,5-Trimethyl-2-hexene	10.212	0.915	Alkene	C_9H_{18}	126.24
13	2,3,7-Trimethyl-2-octene	10.398	0.702	Alkene	$C_{11}H_{22}$	154.29
14	Undecylcyclohexane	10.661	1.765	Cycloalkane	$C_{17}H_{34}$	238.45
15	3,7-Dimethyl-1,7-octanediol	14.198	2.131	Alcohol	$C_{10}H_{22}O_2$	174.28
16	2-Methyl-2-nonanol	14.434	1.306	Alcohol	$C_{10}H_{22}O$	158.28
17	(Ethoxymethoxy) cyclohexane	14.687	1.338	Cycloalkane	$C_9H_{18}O_2$	158.24
18	2-Methyl-1-[(6-methylheptyl)oxy]-2-propanol	14.898	2.446	Alcohol	$C_{12}H_{26}O_2$	202.33

Table 3
Binding affinity of *Bombax ceiba* bark derived compounds and carboplatin (standard) with HER2 protein (PDB ID: 7PCD).

Name of Compounds	Binding Affinity
Oxalic acid, cyclohexyl dodecyl ester	−6.5
Undecylcyclohexane	−6.2
1,5-Diethyl-2,3-dimethylcyclohexane	−6.2
(3-Methylpentyl) cyclohexane	−5.8
2,3,7-Trimethyl-2-octene	−5.4
(Z) 1-Allyl-2-methylcyclohexanol	−5.2
Carboplatin (Standard)	−5.1
2-Methyl-2-dodecanol	−5
2-Methyl-1-[(6-methylheptyl) oxy]-2-propanol	−5
2,6-Dimethyl-2-octanol	−5
2-Methyl-2-nonanol	−4.9
3-Ethyl-2-methyl-2-heptanol	−4.9
(Ethoxymethoxy) cyclohexane	−4.9
3,7-Dimethyl-1,7-octanediol	−4.8
4,5-Dimethyl-2-hepten-3-ol	−4.8
(2E)-4,4,5-trimethyl-2-hexene	−4.7
2-Methoxydecane	−4.6
Diethylene glycol tert-butyl ether methyl ether	−4.5
3,3-Dimethyloctane	−4.5

4. Discussion

Recently, there has been an increasing fascination with employing traditional medicines for treating cancer, owing to their diverse biological activities and their safe, non-toxic nature [55]. Various valuable compounds sourced from medicinal plants, including curcumin, paclitaxel, camptothecin, vinblastine, vincristine, vinorelbine, and quercetin, among others, exhibit significant therapeutic potential in preventing and treating various types of cancers [56]. This research explores the antioxidant and anticancer properties of ethno-medicinal plants from Bangladesh. Consequently, the research delves into phytochemical profiling and assessing the antioxidant and antitumor characteristics of chosen traditional Bangladeshi plants.

For the assessment of the antioxidant capacities of the plant extractives, different methods are used as the assay conditions and principles are different in different techniques [37]. Hence, this study evaluated the antioxidant capacities of 15 traditional Bangladeshi plant extractives using different tests, including total antioxidant capacity, ferric reducing capability, DPPH, and hydroxyl radicals scavenging tests. The total antioxidants of these extractives were evaluated by their capability to reduce Mo (VI) to Mo (V), resulting in the production of a green-colored complex measured spectrophotometrically at 695 nm. The total antioxidants of the plant extracts ranged from 89 ± 1 to 357 ± 4 mg of AAE/g of extracts. All the medicinal plant extracts from Bangladesh demonstrated varying degrees of total antioxidant capacity, showing concentration-dependent behavior. Among the extracts, *B. ceiba* bark, *L. macrophylla* roots, *B. orellana* leaves, *C. nucifera* roots, and *C. grandis* bark represented an excellent total antioxidant capacity (Table 1). These results align with prior investigations and are attributed mainly to the presence of polyphenols in the samples [57].

The ferric-reducing assay, a widely utilized method for evaluating antioxidant capacity, relies on the conversion of Fe^{3+} to Fe^{2+} by

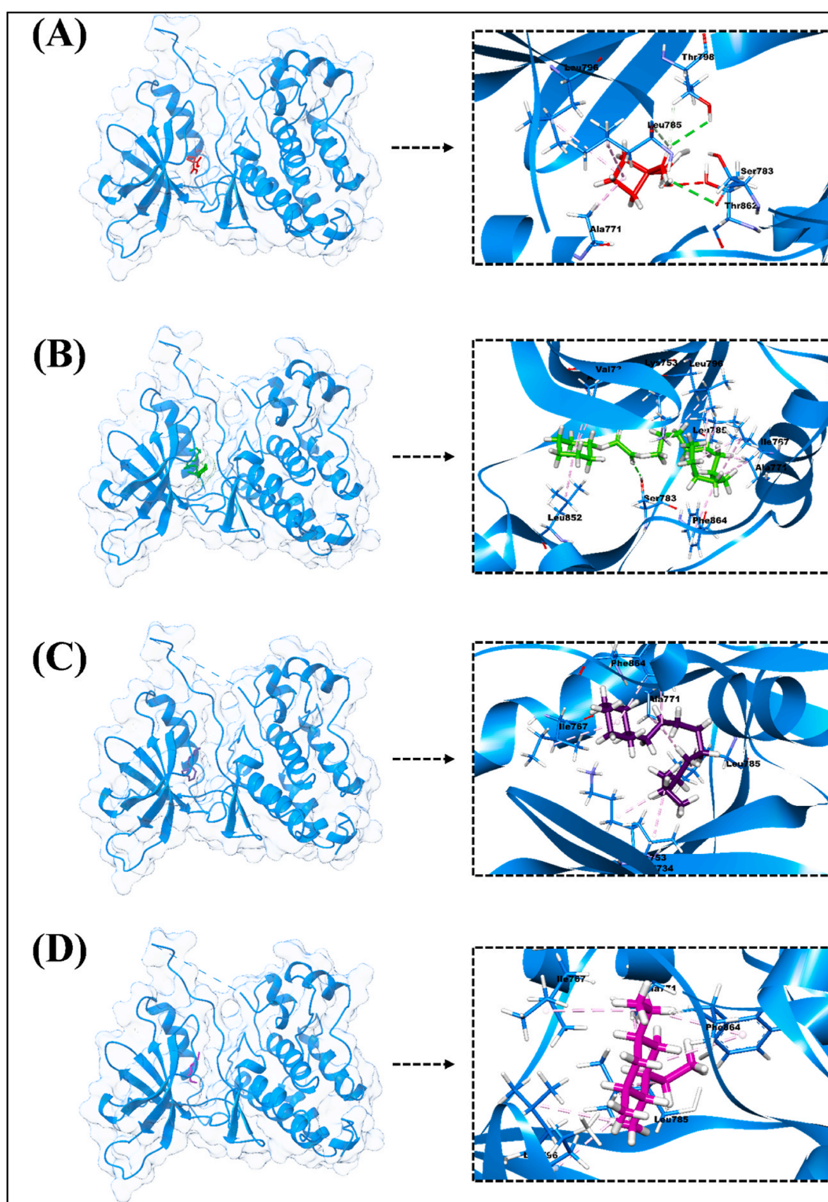

Fig. 5. Molecular docking and interaction close view of HER2 protein (PDB ID: 7PCD) with (A) Carboplatin (Standard Drug); (B) Oxalic acid, cyclohexyl dodecyl ester; (C) Undecylcyclohexane; and (D) 1,5-Diethyl-2,3-dimethylcyclohexane.

the antioxidant molecules present in plant extracts. This process forms Perl's Prussian blue-colored complex, measured spectrophotometrically at 700 nm. In this investigation, extracts from Bangladeshi medicinal plants exhibited varying degrees of reducing capability, ranging from moderate to excellent when compared with the synthetic standard antioxidant, CA. Specifically, *B. ceiba* bark, *L. macrophylla* roots, *C. grandis* bark, *B. orellana* leaves, and *C. nucifera* roots demonstrated significant reducing activity (refer to Table 1). The observed reduced activity of these extracts is likely attributed to their high polyphenol content, enabling them to donate electrons or hydrogen atoms to the free radical chain. Similar conclusions have been drawn in previous research by Aryal et al., in 2019 [58].

Free radicals are implicated in various harmful biological processes such as cancer, diabetes, neurological, and cardiovascular disorders. Antioxidants neutralize these free radicals, thereby protecting the body from a range of diseases and enhancing its natural defense mechanisms [59]. The DPPH free radical removal assay is a well-established method for assessing the antioxidant properties of plant extractives or pure compounds. In this assay, the antioxidant molecules reduce the deep purple-colored DPPH radicals to yield yellow-colored corresponding hydrazine. It is cost-effective, sensitive, reproducible, and requires a relatively short time to measure the antioxidant activity of a sample. Our investigation demonstrated that extracts from Bangladeshi medicinal plants displayed moderate

to high capacity in removing DPPH radicals compared to standard antioxidants such as AA and BHT. Notably, *B. ceiba* bark, *L. macrophylla* roots, *B. orellana* leaves, *C. grandis* bark, and *C. nucifera* roots exhibited significant DPPH radical-neutralizing ability (see Table 1). This outcome is likely attributed to the presence of polyphenols in the plant extractives, which have the capability to donate electrons or hydrogen atoms, thereby scavenging DPPH free radicals [60].

Hydroxyl radicals interact with the fatty acids within cell membranes, leading to DNA double-strand breaks and ultimately contributing to mutations and the development of cancer [61]. Consequently, the hydroxyl radical removal assay is commonly employed to assess the ability of plant extractives or pure compounds to scavenge free radicals. In this study, hydroxyl radicals were generated through Fenton's reaction, and the tested samples, containing antioxidant compounds, scavenged these radicals by either donating an electron or a hydrogen atom. Our results indicate that extracts from Bangladeshi medicinal plants possess moderate to high potential for removing hydroxyl radicals. Notably, extracts from *B. ceiba* bark, *L. macrophylla* roots, *C. grandis* bark, *B. orellana* leaves, and *C. nucifera* roots showed significant ($p < 0.01$) hydroxyl free-radical neutralizing capability compared to the standard antioxidant BHT (Table 1). Polyphenols in the extracts are responsible for this scavenging activity, which have the capability to donate electrons or hydrogen atoms to neutralize hydroxyl radicals. Consequently, these polyphenol-rich extracts effectively inhibit the interaction between hydroxyl radicals and the body's large molecules, suggesting potential applications in cancer treatment.

Phenolic compounds are natural molecules derived from plants, characterized by aromatic rings with attached hydroxyl groups, and have diverse biological actions [62]. Due to their capability to neutralize free radicals, phenolic compounds play a crucial role in plants. Plant-derived polyphenols have gained attention in the pharmaceutical and food industries as they inhibit the oxidation of materials and upgrade the quality of food and medicines [63]. Flavonoids, a subgroup of polyphenols, possess potent antioxidant properties and exert significant effects on human health. Numerous studies have highlighted the capability of flavonoids to scavenge ROS and their potential benefits in conditions such as diabetes, microbial infections, proliferation disorders, and inflammation [64, 65]. The antioxidant properties observed in Bangladeshi medicinal plants can largely be attributed to their significant polyphenol content, consistent with previous findings [66,67]. Notably, extracts from *B. ceiba* bark, *L. macrophylla* roots, *C. grandis* bark, *B. orellana* leaves, and *C. nucifera* roots exhibit strong antioxidant properties, possibly due to their rich content of phenolic and flavonoid compounds [60].

The overproduction of ROS is mainly responsible for cellular damage that leads to cancer formation. The plant-derived phytochemicals, including alkaloids, polyphenols, terpenoids, and others, can quench excess free radicals and protect the body from cellular injury. Several techniques have been employed to screen the anticancer capacity of these plant compounds and extracts. Among these techniques, the MTT colorimetric assay is a highly sensitive method utilized to assess cell viability, relying on the spectrophotometric measurement of a purple formazan product. It serves as a widely accepted standard for evaluating the cytotoxic effects of plant-derived compounds and extracts on cancer cell lines. In this study, we investigated the anti-proliferative potential of antioxidant-rich extracts from *B. ceiba* bark, *L. macrophylla* roots, *C. grandis* bark, *B. orellana* leaves, and *C. nucifera* roots against MCF-7 cells using the MTT assay. Our results revealed that *B. ceiba* bark exhibited the highest cytotoxicity at 86.67 %, followed by *C. nucifera* roots (83.92 %), *B. orellana* leaves (44.09 %), and *L. macrophylla* roots (25 %). The cytotoxicity of *B. ceiba* bark against MCF-7 cells was closely comparable to the standard drug bleomycin sulfate (89.76 %). The anti-proliferative capacity against MCF-7 cells of these plant extractives is mainly due to polyphenols and other phytochemicals [43]. Consistent with our findings, a study by Sharma et al. (2020) demonstrated similar cytotoxic effects of *B. ceiba* L. leaves against the human leukemia cell line (HL-60) using the MTT colorimetric technique [68]. Additionally, Padumadasa et al. (2016) reported comparable results for the cytotoxicity of *C. nucifera* L. inflorescence against HeLa cells [69].

In the subsequent phase, we assessed the in vivo cytotoxic effects of antioxidant-rich extracts from Bangladeshi medicinal plants on EAC cells in mice. The significant presence of antioxidants and cytotoxic properties within these medicinal plant extracts led to a notable inhibition of EAC cell proliferation. EAC cells are commonly utilized as a tumor model in cancer research [46]. Among the tested extracts, *B. ceiba* bark exhibited the most pronounced inhibition of EAC cell growth at 87.27 %, followed by *L. macrophylla* roots (80.45 %), *C. nucifera* roots (42.9 %), and *B. orellana* leaves (37.27 %). The in-vivo antiproliferative potential of *B. ceiba* bark closely resembled the standard anticancer drug bleomycin sulfate (93.63 %). This study marks the first report of both in vitro and in vivo anticancer activities of *B. ceiba* bark, *L. macrophylla* roots, *C. nucifera* roots, and *B. orellana* leaves. According to previous reports, methanol extract from *Litsea glutinosa* bark showed 85.76 % inhibition of EAC cell growth, while *Amaranthus hybridus* seed displayed 45 % inhibition of EAC cell growth [70,71]. Moreover, leaf and seed extracts of *Basella alba* reduced EAC cell proliferation by 62.54 % and 53.96 %, respectively [72]. Recent studies have reported that medicinal plant extractives exert their antiproliferative action through various mechanisms, including apoptosis [43,60].

Here, we also investigated the morphological changes in EAC cells treated with and without our studied medicinal plant extracts. Our observations revealed fragmented DNA, nuclear condensation, irregular cell shapes, plasma membrane blebbing, and cellular shrinkage in EAC cells treated with *B. ceiba* bark, *L. macrophylla* roots, *C. nucifera* roots, and *B. orellana* leaves, contrasting with the normal and rounded morphology of control cells. These morphological alterations serve as efficient indicators for evaluating the potency of molecules as anticancer agents, signifying the induction of apoptosis in EAC cells by the tested extracts, which suppress tumor development. The lack of apoptosis results in irregular cell multiplication and expansion, ultimately resulting in the development of cancer [73]. In an earlier study, Mostafa et al. reported that an antioxidant-rich ethyl acetate fraction of *Leea aequata* leaves exhibited antiproliferative action against MCF-7 and HeLa cells through apoptosis [60]. In another study, Al-Mamun et al. demonstrated that increased program cell death drives the antitumor activity of *Amaranthus hybridus* [71].

Furthermore, we assessed the effectiveness of our experimental extracts against EAC cells by examining alterations in hematological parameters and tumor growth suppression. Among the extracts, *B. ceiba* bark showed the highest tumor weight reduction, followed by *L. macrophylla* roots, *C. nucifera* roots, and *B. orellana* leaves, respectively. According to previous research, *Tabebuia pallida*

leaf extract significantly reduced tumor weight in ascites-bearing mice, demonstrating its antiproliferative action [43]. Moreover, Islam et al. reported similar results, showing that *Eucalyptus camaldulensis* stem bark extract exhibited anticancer activity with remarkable suppression of tumor weight in mice [74].

In EAC-bearing mice, reductions in red blood cell (RBC) and hemoglobin (Hb) levels may occur, potentially due to iron deficiency in hemolytic or myelopathic conditions [75]. Treatment with *B. ceiba* bark, *L. macrophylla* roots, *C. nucifera* roots, and *B. orellana* leaves significantly restored Hb, RBC, and white blood cell (WBC) counts to normal levels (Fig. 1D–F). These parameters are crucial for confirming the efficacy of *B. ceiba* bark, *L. macrophylla* roots, *C. nucifera* roots, and *B. orellana* leaves in cancer chemotherapy. Similar findings were observed in ascites-bearing mice treated with *Litsea glutinosa* extract [70].

As the *Bombax ceiba* bark exhibited the most significant anti-proliferative effects on MCF-7 and EAC cells compared to extracts from other medicinal plants in Bangladesh, phytochemical profiling of *B. ceiba* bark was performed by GC-MS to identify the compounds in the plant part. In the GC-MS analysis, we identified 18 compounds from the bark of this plant. Among these, 2-methyl-2-dodecanol and (Z) 1-allyl-2-methylcyclohexanol were the dominant components, notable for their antioxidant and antimicrobial properties [76,77]. According to a previous study, Sichaem et al. demonstrated that *B. ceiba* bark contained luteolin, gallic acid, quercetin, magniferin, rutin, salicylic aldehyde, and lupeol as major constituents [31]. In another study, Diab et al. reported antioxidant and anticancer potential of *Bombax ceiba* flowers [78]. These reports support our experimental findings regarding the anticancer potential of *B. ceiba* bark. The anticancer activity of the identified compounds was validated by computational molecular docking study.

Molecular docking and interaction analysis were conducted for 18 compounds alongside carboplatin (the standard) targeting the Her2 protein (PDB ID: 7PCD). Among the compounds, three compounds named oxalic acid, cyclohexyl dodecyl ester; undecylcyclohexane; and 1,5-diethyl-2,3-dimethylcyclohexane showed promising results against the cancer protein even better than the standard one. A recent study reported similar docking results concerning the antioxidant and anticancer activities of the ethyl acetate fraction of *Leea aequata* leaves [60]. These findings align closely with our experimental observations indicating the promising anti-proliferative activity of *B. ceiba* bark extract.

5. Conclusion

This study identified *Bombax ceiba* bark as the most promising source of polyphenols with significant antioxidant and anti-proliferative properties among various Bangladeshi medicinal plants. Additionally, *L. macrophylla* roots, *Cocos nucifera* roots, and *Bixa orellana* leaves also demonstrated high polyphenol content and notable antioxidant and antiproliferative activities. Phytochemical analysis of *B. ceiba* bark using GC-MS revealed 18 compounds, including alcohols, ethers, alkenes, alkanes, and esters. Among these, computer-aided modeling highlighted oxalic acid, cyclohexyl dodecyl ester as a potent antitumor compound, particularly effective against breast cancer. These findings emphasize the potential of *B. ceiba* bark as a source of anticancer compounds, suggesting its usefulness in carcinoma management and treatment. However, further research is crucial to fully understand its efficacy against cancer. Investigating the molecular mechanisms underlying its action is essential to confirm its effectiveness in combating carcinoma.

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Data availability

Data will be made available on request.

CRediT authorship contribution statement

Md Uzzal Haque: Writing – original draft, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **AHM Khurshid Alam:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Md Tanjil Islam Shovon:** Writing – review & editing, Investigation. **Khaled Mahmud Sujon:** Writing – review & editing, Investigation. **Md Mahmudul Hasan Maruf:** Investigation. **Syed Rashel Kabir:** Resources, Investigation. **Kazi Md Faisal Hoque:** Investigation. **Md Abu Reza:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Md. Uzzal Haque reports equipment, drugs, or supplies was provided by Beacon Pharmaceuticals Limited, Bangladesh. The authors declare that there are no conflicts of interest. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38885.

References

[1] U. Anand, A. Dey, A.K.S. Chandel, R. Sanyal, A. Mishra, D.K. Pandey, V. De Falco, A. Upadhyay, R. Kandimalla, A. Chaudhary, J.K. Dhanjal, S. Dewanjee, J. Vallamkondu, J.M. Pérez de la Lastra, Cancer chemotherapy and beyond: current status, drug candidates, associated risks and progress in targeted therapeutics, Genes Dis 10 (2023) 1367–1401, https://doi.org/10.1016/j.gendis.2022.02.007.

[2] H. Sung, J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, F. Bray, Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA. Cancer J. Clin. 71 (2021) 209–249, https://doi.org/10.3322/caac.21660.

[3] K. Mazumder, A. Aktar, P. Roy, B. Biswas, M.E. Hossain, K.K. Sarkar, S.C. Bachar, F. Ahmed, A.S.M. Monjur-Al-hossain, K. Fukase, A review on mechanistic insight of plant derived anticancer bioactive phytocompounds and their structure activity relationship, Molecules 27 (2022), https://doi.org/10.3390/molecules27093036.

[4] H. Sies, D.P. Jones, Reactive oxygen species (ROS) as pleiotropic physiological signalling agents, Nat. Rev. Mol. Cell Biol. 21 (2020) 363–383, https://doi.org/10.1038/s41580-020-0230-3.

[5] P.H. Evans, Free radicals in brain metabolism and pathology, Br. Med. Bull. 49 (1993) 577–587, https://doi.org/10.1093/oxfordjournals.bmb.a072632.

[6] K. Rahman, Studies on free radicals, antioxidants, and co-factors, Clin. Interv. Aging 2 (2007) 219–236.

[7] M.T. Fatima, A.A. Bhat, S. Nisar, K.A. Fakhro, A.S. Al-Shabeeb Akil, The role of dietary antioxidants in type 2 diabetes and neurodegenerative disorders: an assessment of the benefit profile, Heliyon 9 (2023) e12698, https://doi.org/10.1016/j.heliyon.2022.e12698.

[8] V. Lobo, A. Patil, A. Phatak, N. Chandra, Free radicals, antioxidants and functional foods: impact on human health, Pharmacogn. Rev. 4 (2010) 118–126, https://doi.org/10.4103/0973-7847.70902.

[9] F.C. Önder, M. Ay, S.D. Sarker, Comparative study of antioxidant properties and total phenolic content of the extracts of Humulus lupulus L. and quantification of bioactive components by LC-MS/MS and GC-MS. https://doi.org/10.1021/jf4031508, 2013.

[10] M.N. Uddin, R. Afrin, M.J. Uddin, M.J. Uddin, A.H.M.K. Alam, A.A. Rahman, G. Sadik, Vanda roxburghii chloroform extract as a potential source of polyphenols with antioxidant and cholinesterase inhibitory activities: Identification of a strong phenolic antioxidant, BMC Complement. Altern. Med. 15 (2015) 1–9, https://doi.org/10.1186/s12906-015-0728-y.

[11] G. Miliauskas, P.R. Venskutonis, T.A. Van Beek, Screening of radical scavenging activity of some medicinal and aromatic plant extracts, Food Chem. 85 (2004) 231–237, https://doi.org/10.1016/j.foodchem.2003.05.007.

[12] W. Naowaratwattana, W. De-Eknamkul, E.G. De Mejia, Phenolic-containing organic extracts of mulberry (Morus alba L.) leaves inhibit HepG2 hepatoma cells through G2/M phase arrest and inhibition of topoisomerase IIα activity, J. Med. Food 13 (2010) 1045–1056, https://doi.org/10.1089/jmf.2010.1021.

[13] J.G. Graham, M.L. Quinn, D.S. Fabricant, N.R. Farnsworth, Plants used against cancer - an extension of the work of Jonathan Hartwell, J. Ethnopharmacol. 73 (2000) 347–377, https://doi.org/10.1016/S0378-8741(00)00341-X.

[14] S.M.K. Rates, Plants as source of drugs, Toxicon 39 (2001) 603–613, https://doi.org/10.1016/S0041-0101(00)00154-9.

[15] O. Oyebode, N.B. Kandala, P.J. Chilton, R.J. Lilford, Use of traditional medicine in middle-income countries: a WHO-SAGE study, Health Policy Plan 31 (2016) 984–991, https://doi.org/10.1093/heapol/czw022.

[16] M.H. Rahman, B. Roy, G.M. Chowdhury, A. Hasan, M.S.R. Saimun, Medicinal Plant Sources and Traditional Healthcare Practices of Forest-dependent Communities in and Around Chunati Wildlife Sanctuary in Southeastern Bangladesh, Springer Nature Singapore, 2022, https://doi.org/10.1007/s42398-022-00230-z.

[17] N. Rashid, A. Paul, S. Islam, S. Sajib, K. Nasirujjaman, K. Hoque, M. Reza, Studies on antioxidant potential, phytochemical properties and toxicity of four popular medicinal plants of Bangladesh, J. Bio-Science 25 (2018) 27–37, https://doi.org/10.3329/jbs.v25i0.37495.

[18] K.K. Mali, G.V. Sutar, R.J. Dias, O.A. Devade, Evaluation of nootropic activity of limonia acidissima against scopolamine-induced amnesia in rats, Turkish J. Pharm. Sci. 18 (2021) 3–9, https://doi.org/10.4274/tjps.galenos.2019.30316.

[19] N. Sharma, Radha, M. Kumar, N. Kumari, N. Rais, A. Pundir, T. Anitha, V. Balamurugan, M. Senapathy, S. Dhumal, S. Natta, V.P. Deshmukh, S. Kumar, R. Pandiselvam, J.M. Lorenzo, M. Mekhemar, Beneath the rind: a review on the remarkable health benefits and applications of the wood apple fruit, Heliyon 10 (2024) e29202, https://doi.org/10.1016/j.heliyon.2024.e29202.

[20] E.B.C. Lima, C.N.S. Sousa, L.N. Meneses, N.C. Ximenes, M.A. Santos Júnior, G.S. Vasconcelos, N.B.C. Lima, M.C.A. Patrocínio, D. Macedo, S.M.M. Vasconcelos, Cocos nucifera (L.) (arecaceae): a phytochemical and pharmacological review, Brazilian J. Med. Biol. Res. 48 (2015) 953–964, https://doi.org/10.1590/1414-431X20154773.

[21] R. Kandimalla, S. Dash, S. Kalita, B. Choudhury, S. Malampati, K. Kalita, J. Kotoky, Bioactive guided fractions of Annona reticulata L. bark: protection against liver toxicity and inflammation through inhibiting oxidative stress and proinflammatory cytokines, Front. Pharmacol. 7 (2016) 1–12, https://doi.org/10.3389/fphar.2016.00168.

[22] F.C. Hsu, S.F. Tsai, S.S. Lee, Chemical investigation of Hyptis suaveolens seed, a potential antihyperuricemic nutraceutical, with assistance of HPLC-SPE-NMR, J. Food Drug Anal. 27 (2019) 897–905, https://doi.org/10.1016/j.jfda.2019.05.006.

[23] J. Mawa, M.A. Rahman, M.A. Hashem, M. Juwel Hosen, Leea macrophylla root extract upregulates the mRNA expression for antioxidative enzymes and repairs the necrosis of pancreatic β-cell and kidney tissues in fructose-fed Type 2 diabetic rats, Biomed. Pharmacother. 110 (2019) 74–84, https://doi.org/10.1016/j.biopha.2018.11.033.

[24] S. Degu, A. Meresa, Z. Animaw, M. Jegnie, A. Asfaw, G. Tegegn, Vernonia amygdalina: a comprehensive review of the nutritional makeup, traditional medicinal use, and pharmacology of isolated phytochemicals and compounds, Front. Nat. Prod. 3 (2024) 1–18, https://doi.org/10.3389/fntpr.2024.1347855.

[25] R. Khanra, S. Dewanjee, T. K Dua, R. Sahu, M. Gangopadhyay, V. DeFeo, M. Zia-Ul-Haq, Abroma augusta L. (Malvaceae) leaf extract attenuates diabetes induced nephropathy and cardiomyopathy via inhibition of oxidative stress and inflammatory response, J. Transl. Med. 13 (2015) 1–14, https://doi.org/10.1186/s12967-014-0364-1.

[26] N. Terasawa, A. Saotome, Y. Tachimura, A. Mochizuki, H. Ono, M. Takenaka, M. Murata, Identification and some properties of anthocyanin isolated from zuiki, stalk of Colocasia esculenta, J. Agric. Food Chem. 55 (2007) 4154–4159, https://doi.org/10.1021/jf063204t.

[27] N. More, A. Kharat, Antifungal and anticancer potential of Argemone mexicana L, Medicines 3 (2016) 28, https://doi.org/10.3390/medicines3040028.

[28] D.A. Orozco-Nunnelly, J. Pruet, C.P. Rios-Ibarra, E.L.B. Gamarra, T. Lefeber, T. Najdeska, Characterizing the cytotoxic effects and several antimicrobial phytocompounds of Argemone mexicana, PLoS One 16 (2021) 1–19, https://doi.org/10.1371/journal.pone.0249704.

[29] D.D.A. Vilar, M.S.D.A. Vilar, T.F.A.D.L.E. Moura, F.N. Raffin, M.R. De Oliveira, C.F.D.O. Franco, P.F. De Athayde-Filho, M.D.F.F.M. Diniz, J.M. Barbosa-Filho, Traditional Uses, chemical constituents, and biological activities of Bixa Orellana L.: a review, Sci. World J. 2014 (2014), https://doi.org/10.1155/2014/857292.

[30] Masood-Ur-Rehman, N. Akhtar, R. Mustafa, Antibacterial and antioxidant potential of stem bark extract of Bombax ceiba collected locally from south Punjab area of Pakistan, African J. Tradit. Complement. Altern. Med. AJTCAM 14 (2017) 9–15, https://doi.org/10.21010/ajtcam.v14i2.2.

[31] J. Sichaem, K. Inthanon, N. Funnimid, K. Phontree, H.V.T. Phan, T.M.D. Tran, N. Niamnont, K. Srikittiwanna, S. Sedlak, T.H. Duong, Chemical constituents of the stem bark of Bombax ceiba, Chem. Nat. Compd. 56 (2020) 909–911, https://doi.org/10.1007/s10600-020-03183-z.

[32] O.C. Ugbogu, O. Emmanuel, G.O. Agi, C. Ibe, C.N. Ekweogu, V.C. Ude, M.E. Uche, R.O. Nnanna, E.A. Ugbogu, A review on the traditional uses, phytochemistry, and pharmacological activities of clove basil (Ocimum gratissimum L.), Heliyon 7 (2021) e08404, https://doi.org/10.1016/j.heliyon.2021.e08404.

[33] R.O. Vilhena, I.D. Figueiredo, A.M. Baviera, D.B. Silva, B.M. Marson, J.A. Oliveira, R.G. Peccinini, I.K. Borges, R. Pontarolo, Antidiabetic activity of Musa x paradisiaca extracts in streptozotocin-induced diabetic rats and chemical characterization by HPLC-DAD-MS, J. Ethnopharmacol. 254 (2020) 112666, https://doi.org/10.1016/j.jep.2020.112666.

[34] R. Logesh, S. Vivekanandarajah Sathasivampillai, S. Varatharasan, S. Rajan, N. Das, J. Pandey, H. Prasad Devkota, Ficus benghalensis L. (Moraceae): a review on ethnomedicinal uses, phytochemistry and pharmacological activities, Curr. Res. Biotechnol. 6 (2023) 100134, https://doi.org/10.1016/j.crbiot.2023.100134.

[35] A.L. Prada, J.R.R. Amado, H. Keita, E.P. Zapata, H. Carvalho, E.S. Lima, T.P. de Sousa, J.C.T. Carvalho, Cassia grandis fruit extract reduces the blood glucose level in alloxan-induced diabetic rats, Biomed. Pharmacother. 103 (2018) 421–428, https://doi.org/10.1016/j.biopha.2018.04.059.

[36] N. Siddiqui, A. Rauf, A. Latif, Z. Mahmood, Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal Unani drug Gul-e-Zoofa (Nepeta bracteata Benth), J. Taibah Univ. Med. Sci. 12 (2017) 360–363, https://doi.org/10.1016/j.jtumed.2016.11.006.

[37] A.S.M. Ali Reza, M.S. Hossain, S. Akhter, M.R. Rahman, M.S. Nasrin, M.J. Uddin, G. Sadik, A.H.M. Khurshid Alam, In vitro antioxidant and cholinesterase inhibitory activities of Elatostema papillosum leaves and correlation with their phytochemical profiles: a study relevant to the treatment of Alzheimer's disease, BMC Complement. Altern. Med. 18 (2018) 1–8, https://doi.org/10.1186/s12906-018-2182-0.

[38] P. Prieto, M. Pineda, M. Aguilar, Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, Anal. Biochem. 269 (1999) 337–341, https://doi.org/10.1037/a0037168.

[39] M.M. Rahman, A.S.M.S. Hossain, M.G. Mostofa, M.A. Khan, R. Ali, A. Mosaddik, M.G. Sadik, A.H.M.K. Alam, Evaluation of anti-ROS and anticancer properties of Tabebuia pallida L. Leaves, Clin. Phytoscience 5 (2019), https://doi.org/10.1186/s40816-019-0111-5.

[40] S. Islam, S. Nasrin, M.A. Khan, A.S.M.S. Hossain, F. Islam, P. Khandokhar, M.N.H. Mollah, M. Rashid, G. Sadik, M.A.A. Rahman, A.H.M.K. Alam, Evaluation of antioxidant and anticancer properties of the seed extracts of Syzygium fruticosum Roxb. growing in Rajshahi, Bangladesh, BMC Complement. Altern. Med. 13 (2013) 1, https://doi.org/10.1186/1472-6882-13-142.

[41] M.A. Khan, A.A. Rahman, S. Islam, P. Khandokhar, S. Parvin, M.B. Islam, M. Hossain, M. Rashid, G. Sadik, S. Nasrin, M.N.H. Mollah, A.H.M.K. Alam, A comparative study on the antioxidant activity of methanolic extracts from different parts of Morus alba L. (Moraceae), BMC Res. Notes 6 (2013) 24, https://doi.org/10.1186/1756-0500-6-24.

[42] A.H.M.K. Alam, A.S.M.S. Hossain, M.A. Khan, S.R. Kabir, M.A. Reza, M.M. Rahman, M.S. Islam, M.A.A. Rahman, M. Rashid, M.G. Sadik, The antioxidative fraction of white mulberry induces apoptosis through regulation of p53 and NFκB in EAC cells, PLoS One 11 (2016) 1–18, https://doi.org/10.1371/journal.pone.0167536.

[43] M.M. Rahman, A.S.M.A. Reza, M.A. Khan, K.M. Sujon, R. Sharmin, M. Rashid, M.G. Sadik, M.A. Reza, T. Tsukahara, R. Capasso, A. Mosaddik, G.C. Gobe, A. K. Alam, Unfolding the apoptotic mechanism of antioxidant enriched-leaves of Tabebuia pallida (lindl.) miers in EAC cells and mouse model, J. Ethnopharmacol. 278 (2021) 114297, https://doi.org/10.1016/j.jep.2021.114297.

[44] A.S. Shimu, M. Miah, M. Billah, S. Karmakar, S.C. Mohanto, R. Khatun, M.A. Reza, K.M.F. Hoque, A comparative study of biological potentiality and EAC cell growth inhibition activity of Phyllanthus acidus (L.) fruit pulp and seed in Bangladesh, Saudi J. Biol. Sci. 28 (2021) 2014–2022, https://doi.org/10.1016/j.sjbs.2021.01.004.

[45] K. Dorji, S. Chhodenr, K. Wangchuk, S. Zangpo, S. Tenzin, C. Dawa, P.D. Samal, Nima, J. Tshering, C. Wangmo, S. Zangpo, K. Dorji, S. Tshewang, Routine clinical chemistry and haematological test reference intervals for healthy adults in the Bhutanese population, PLoS One 17 (2022) 1–21, https://doi.org/10.1371/journal.pone.0273778.

[46] S.R. Kabir, F. Islam, A.K.M. Asaduzzaman, Biogenic silver/silver chloride nanoparticles inhibit human cancer cells proliferation in vitro and Ehrlich ascites carcinoma cells growth in vivo, Sci. Rep. 12 (2022) 1–14, https://doi.org/10.1038/s41598-022-12974-z.

[47] K.H. Hossain, M.A. Rahman, M. Taher, J. Tangpong, D. Hajjar, W. Alelwani, A.A. Makki, A.S.M. Ali Reza, Hot methanol extract of Leea macrophylla (Roxb.) manages chemical-induced inflammation in rodent model, J. King Saud Univ. Sci. 32 (2020) 2892–2899, https://doi.org/10.1016/j.jksus.2020.07.014.

[48] B. Wilding, D. Scharn, D. Böse, A. Baum, V. Santoro, P. Chetta, R. Schnitzer, D.A. Botesteanu, C. Reiser, S. Kornigg, P. Knesl, A. Hörmann, A. Köferle, M. Corcokovic, S. Lieb, G. Scholz, J. Bruchhaus, M. Spina, J. Balla, B. Peric-Simov, J. Zimmer, S. Mitzner, T.N. Fett, A. Beran, L. Lamarre, T. Gerstberger, D. Gerlach, M. Bauer, A. Bergner, A. Schlattl, G. Bader, M. Treu, H. Engelhardt, S. Zahn, J.E. Fuchs, J. Zuber, P. Ettmayer, M. Pearson, M. Petronczki, N. Kraut, D. B. McConnell, F. Solca, R.A. Neumüller, Discovery of potent and selective HER2 inhibitors with efficacy against HER2 exon 20 insertion-driven tumors, which preserve wild-type EGFR signaling, Nat. Cancer 3 (2022) 821–836, https://doi.org/10.1038/s43018-022-00412-y.

[49] N. Pathak, A. Sharma, A. Elavarasi, J. Sankar, S.V.S. Deo, D.N. Sharma, S. Mathur, S. Kumar, C.P. Prasad, A. Kumar, A. Batra, Moment of truth-adding carboplatin to neoadjuvant/adjuvant chemotherapy in triple negative breast cancer improves overall survival: an individual participant data and trial-level Meta-analysis, Breast 64 (2022) 7–18, https://doi.org/10.1016/j.breast.2022.04.006.

[50] S. Dallakyan, A.J. Olson, Small-molecule library screening by docking with PyRx BT - chemical Biology, in: J.E. Hempel, C.H. Williams, C.C. Hong (Eds.), Methods and Protocols, Springer New York, New York, NY, 2015, pp. 243–250, https://doi.org/10.1007/978-1-4939-2269-7_19.

[51] A. Hameed, Z. Liu, H. Wu, B. Zhong, M. Ciborowski, H.A.R. Suleria, A comparative and comprehensive characterization of polyphenols of selected fruits from the Rosaceae family, Metabolites 12 (2022) 271.

[52] N. Rajapakse, E. Mendis, W.-K. Jung, J.-Y. Je, S.-K. Kim, Purification of a radical scavenging peptide from fermented mussel sauce and its antioxidant properties, Food Res. Int. 38 (2005) 175–182.

[53] C.A. Hoogstraten, J.A.M. Smeitink, F.G.M. Russel, T.J.J. Schirris, Dissecting drug-induced cytotoxicity and metabolic dysfunction in conditionally immortalized human proximal tubule cells, Front. Toxicol. 4 (2022) 842396.

[54] P. Senthilraja, K. Kathiresan, In vitro cytotoxicity MTT assay in Vero, HepG2 and MCF-7 cell lines study of Marine Yeast, J. Appl. Pharm. Sci. 5 (2015) 80–84.

[55] S. Kumar, R. Singh, D. Dutta, S. Chandel, A. Bhattacharya, V. Ravichandiran, S. Sukla, In vitro anticancer activity of methanolic extract of Justicia adhatoda leaves with special emphasis on human breast cancer cell line, Molecules 27 (2022), https://doi.org/10.3390/molecules27238222.

[56] M. Greenwell, P.K.S.M. Rahman, Medicinal plants: their use in anticancer treatment, Int. J. Pharm. Sci. Res. 6 (2015) 4103–4112, https://doi.org/10.13040/IJPSR.0975-8232.6(10).4103-12.

[57] M. Oktay, I. Gülçin, Ö.I. Küfrevioğlu, Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts, Lwt 36 (2003) 263–271, https://doi.org/10.1016/S0023-6438(02)00226-8.

[58] S. Aryal, M.K. Baniya, K. Danekhu, P. Kunwar, R. Gurung, N. Koirala, Total Phenolic content, Flavonoid content and antioxidant potential of wild vegetables from western Nepal, Plants 8 (2019), https://doi.org/10.3390/plants8040096.

[59] D. Huang, O.U. Boxin, R.L. Prior, The chemistry behind antioxidant capacity assays, J. Agric. Food Chem. 53 (2005) 1841–1856, https://doi.org/10.1021/jf030723c.

[60] M.G. Mostofa, A.S.M.A. Reza, Z. Khan, M.S. Munira, M.M. Khatoon, S.R. Kabir, M.G. Sadik, D. Ağagündüz, R. Capasso, M. Kazi, A.K. Alam, Apoptosis-inducing anti-proliferative and quantitative phytochemical profiling with in silico study of antioxidant-rich Leea aequata L. leaves, Heliyon 10 (2024) e23400, https://doi.org/10.1016/j.heliyon.2023.e23400.

[61] Y. Zhang, H. Fang, Q. Xie, J. Sun, R. Liu, Z. Hong, R. Yi, H. Wu, Comparative evaluation of the radical-scavenging activities of fucoxanthin and its stereoisomers, Molecules 19 (2014) 2100–2113, https://doi.org/10.3390/molecules19022100.
[62] M.P. Kähkönen, A.I. Hopia, H.J. Vuorela, J.P. Rauha, K. Pihlaja, T.S. Kujala, M. Heinonen, Antioxidant activity of plant extracts containing phenolic compounds, J. Agric. Food Chem. 47 (1999) 3954–3962, https://doi.org/10.1021/jf990146l.
[63] D. Lin, M. Xiao, J. Zhao, Z. Li, B. Xing, X. Li, M. Kong, L. Li, Q. Zhang, Y. Liu, H. Chen, W. Qin, H. Wu, S. Chen, An overview of plant phenolic compounds and their importance in human nutrition and management of type 2 diabetes, Molecules 21 (2016), https://doi.org/10.3390/molecules21101374.
[64] P. Montoro, A. Braca, C. Pizza, N. De Tommasi, Structure-antioxidant activity relationships of flavonoids isolated from different plant species, Food Chem. 92 (2005) 349–355, https://doi.org/10.1016/j.foodchem.2004.07.028.
[65] S. Sahreen, M.R. Khan, R.A. Khan, Comprehensive assessment of phenolics and antiradical potential of Rumex hastatus D. Don. roots, BMC Complement. Altern. Med. 14 (2014) 1–11, https://doi.org/10.1186/1472-6882-14-47.
[66] N. Saeed, M.R. Khan, M. Shabbir, Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L, BMC Complement. Altern. Med. 12 (2012), https://doi.org/10.1186/1472-6882-12-221.
[67] F. Sharififar, G. Dehghn-Nudeh, M. Mirtajaldini, Major flavonoids with antioxidant activity from Teucrium polium L, Food Chem. 112 (2009) 885–888, https://doi.org/10.1016/j.foodchem.2008.06.064.
[68] N. Sharma, S. Kispotta, P. Mazumder, Immunomodulatory and anticancer activity of Bombax ceiba Linn leaf extract, Asian Pac. J. Trop. Biomed. 10 (2020) 426–432, https://doi.org/10.4103/2221-1691.290134.
[69] C. Padumadasa, D. Dharmadana, A. Abeysekera, M. Thammitiyagodage, In vitro antioxidant, anti-inflammatory and anticancer activities of ethyl acetate soluble proanthocyanidins of the inflorescence of Cocos nucifera L, BMC Complement. Altern. Med. 16 (2016) 4–9, https://doi.org/10.1186/s12906-016-1335-2.
[70] S. Shafiq, R. Zahan, S. Yesmin, A. Khan, M.S. Mahmud, M.A. Reza, S.M. Albogami, M. Alorabi, M. De Waard, H.M. Saad, J.M. Sabatier, T. Naz, G.E.S. Batiha, Phytochemical analysis and understanding the antioxidant and anticancer properties of methanol extract from Litsea glutinosa: in vitro and in vivo studies, Molecules 27 (2022), https://doi.org/10.3390/molecules27206964.
[71] M.A. Al-Mamun, J. Husna, M. Khatun, R. Hasan, M. Kamruzzaman, K.M.F. Hoque, M.A. Reza, Z. Ferdousi, Assessment of antioxidant, anticancer and antimicrobial activity of two vegetable species of Amaranthus in Bangladesh, BMC Complement. Altern. Med. 16 (2016) 1–11, https://doi.org/10.1186/s12906-016-1130-0.
[72] M.S. Islam, M.S. Rahi, C.A. Jahangir, M.H. Rahman, I. Jerin, R. Amin, K.M.F. Hoque, M.A. Reza, In vivo anticancer activity of Basella alba leaf and seed extracts against ehrlich's ascites carcinoma (EAC) cell line, evidence-based complement, Altern. Med. 2018 (2018), https://doi.org/10.1155/2018/1537896.
[73] M.S. Islam, C.A. Jahangir, M.S. Rahi, M.M. Hasan, S.A. Sajib, K.M.F. Hoque, M.A. Reza, In-vivo antiproliferative activity of Morus latifolia leaf and bark extracts against Ehrlich's ascites carcinoma, Toxicol. Res. 36 (2020) 79–88, https://doi.org/10.1007/s43188-019-00011-7.
[74] F. Islam, H. Khatun, M. Khatun, S.M.M. Ali, J.A. Khanam, Growth inhibition and apoptosis of Ehrlich ascites carcinoma cells by the methanol extract of Eucalyptus camaldulensis, Pharm. Biol. 52 (2014) 281–290, https://doi.org/10.3109/13880209.2013.834365.
[75] R.S.Y. Wong, Apoptosis in cancer: from pathogenesis to treatment, J. Exp. Clin. Cancer Res. 30 (2011) 87, https://doi.org/10.1186/1756-9966-30-87.
[76] D.K. Arserİm, İ.H. Gecİbesler, M. Sudagidan, İ. Dursun, Determination of biological activity , lipophilic and volatile organic compounds of bingol propolis isolates bingol propolis izolatlarinin biyolojik aktivitesinin, lipofilik ve ucucu organik bilesenlerinin belierlenmesi, tr, J. Nat. Sci. 9 (2020) 92–102.
[77] I.A. Freires, C. Denny, B. Benso, S.M. De Alencar, P.L. Rosalen, Antibacterial activity of essential oils and their isolated constituents against cariogenic bacteria: a systematic review, Molecules 20 (2015) 7329–7358, https://doi.org/10.3390/molecules20047329.
[78] K.A. Diab, R. El-Shenawy, N.M. Helmy, S.A. El-Toumy, Polyphenol content, antioxidant, cytotoxic, and genotoxic activities of Bombax ceiba flowers in liver cancer cells Huh7, asian pacific, J. Cancer Prev. 23 (2022) 1345–1350, https://doi.org/10.31557/APJCP.2022.23.4.1345.