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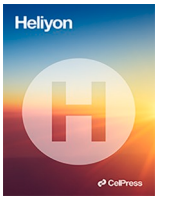

Research article

Phytochemical profile, antioxidant, cytotoxic and anti-inflammatory activities of stem bark extract and fractions of *Ailanthus excelsa* Roxb.: *In vitro, in vivo* and *in silico* approaches

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ABSTRACT

This study aimed to assess the phytochemical composition, *in vitro* antioxidant, cytotoxicity, and *in vivo* anti-inflammatory activities of the methanolic extract of Ailanthus excelsa (Simaroubaceae) stem bark and its fractions. Quantitative phytochemical analysis revealed that methanolic extract and all fractions contained a high level of flavonoids (20.40–22.91 mg/g QE), phenolics (1.72–7.41 mg/g GAE), saponins (33.28–51.87 mg/g DE), and alkaloids (0.21–0.33 mg/g AE). The antioxidant potential was evaluated *in vitro* using a range of assays, i.e., DPPH•, ABTS radical scavenging ability, and total antioxidant capacity. The chloroform and ethyl acetate fractions showed stronger antioxidant activity than the methanol extract. In vitro cytotoxic activity was investigated in three human tumor cell lines (A-549, MCF7 and HepG2) using the SRB assay. In addition, the *in vivo* anti-inflammatory effect was assessed by carrageenan-induced paw edema in rats. The chloroform fraction showed a more pronounced effect by effectively controlling the growth with the lowest GI50 and TGI concentrations. The human lung cancer cell line (A-549) was found to be more sensitive to the chloroform fraction. Furthermore, the chloroform fraction exhibited significant anti-inflammatory activity at a dose of 200 mg/kg in the latter phase of inflammation. Besides, methanol extract and ethyl acetate fraction revealed a significant cytotoxic and anti-inflammatory effects. The chloroform fraction of stem bark showed a strong anti-inflammatory effect in experimental animals and significant COX-2 inhibitory potential in the *in vitro* experiments. GC-MS analysis of chloroform fraction identified the phytochemicals like caftaric acid, 3,4-dihydroxy phenylacetic acid, arachidonic acid, cinnamic acid, 3-hydroxyphenylvaleric acid, caffeic acid, hexadeconoic acid, and oleanolic acid. The *in-silico* results suggest that identified compounds have better affinity towards the selected targets, viz. the BAX protein (PDB ID: 1F16), p53-binding protein Mdm-2 (PDB ID: 1YCR), and topoisomerase II (PDB ID: 1QZR). Amongst all, caftaric acid exhibited the best binding affinity for all three targets. Thus, it can be

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concluded that caftaric acid in combination with other phenolic compounds, might be responsible for the studied activity. Additional *in vivo* and *in vitro* studies are required to establish their exact molecular mechanisms and consider them as lead molecules in developing of valuable drugs for treating oxidative stress-induced disorders, cancers, and inflammations.

1. Introduction

Plant-derived drugs continue to be an important resource for fighting severe diseases, especially in developing countries. About 60–80% of the world's population still depends on traditional medicine to treat different diseases [1]. Cancer remains the most prominent health concern, making it the second-largest cause of death. In recent decades, extensive and systematic research has revealed much about its biology [2,3]. Despite the advancements in developing anticancer medications, mortality from cancer is increasing globally. Thus, discovering novel treatments is still crucial and necessary to establish multi-target cancer therapy, including oxidative stress and inflammation. Reactive oxygen and nitrogen species and various pro-inflammatory cytokines contribute significantly to the development of cancer by damaging DNA or other biomolecules. The core role of cyclooxygenase in inflammation initiation, progression, and overexpression is one of the culprits in forming a carcinogenic state in the body. Chronic inflammation increases the risk of resistance and tumor recurrence, such as brain and breast cancer and other types of cancer. Thus, controlling or eliminating inflammation may represent a valid strategy for cancer prevention and therapy [4]. Plants play a significant role in developing anticancer drugs, as more than 60% of anticancer agents are extracted from natural sources [5].

Ailanthus excelsa Roxb. is a deciduous tree belonging to the Simaroubaceae family, known as the "Tree of Heaven" and used medicinally in the Indian medicine system [6]. It has traditionally been used to treat wounds, skin rashes, fever, bronchitis, asthma, diarrhoea, and dysentery [7]. Moreover, the stem bark of this plant is considered as a substitute for *Holarrhena antidysenterica* in treating dysentery [8]. The Nilgiri tribes of India utilize its bark as antifertility medicine [9]. Furthermore, pharmacological studies revealed its potential as an antileukemic [10], antifungal, antibacterial [11,12], antifertility [13] and antimalarial [14] agent. Previous phytochemical studies have reported the presence of quassinoids [15,16], alkaloids, terpenoids, flavonoids, and steroids [17] in different parts of the plant. The characteristic compounds reported from *A. excelsa* stem bark are quassinoids like excelsin, 1,4-dihydroexcelsin [16,18], 2,4-dihydroexcelsin, 3,4-dihydroexcelsin [19], 13,18-dehydroexcelsin, glaucarubin [20], glaucarubol [21], ailanthinone, 1,12-deoxy-13-formyl ailanthiol, ailanex A, ailanex B, polyandrol, and glaucarubolone [12,22]. The compounds ailanthione, glaucarubinone, glaucarubol 15-isovalerate, and 13,18-dehydroglaucarubol 15-isovalerate isolated from the root bark of the plant reported to possess cytotoxic effects [23]. The phytosterol compounds such as β-sitosterol and stigmasterol [24]; indole alkaloid compounds such as canthin-6-one, 1-methoxy canthin-6-one, 5-methoxy canthin-6-one, and 8-hydroxy canthin-6-one [23]; and flavonoids such as kaempferol, luteolin, and apigenin [25,26] were reported from plant. Quassinoids, alkaloids, and flavonoid compounds have been predicted to be responsible for anti-inflammatory, antiamoebic, anticancer, amoebicidal, and cytotoxic properties [27].

The stem bark of *A. excelsa* alone or in conjunction with *Ficus benghalensis* and *Azadirachta indica* is used as a traditional therapy to treat cancer in Chhattisgarh and the Marathwada region of India [28,29]. Despite the significant traditional claims, studies on chemical investigation and cytotoxic activities of stem bark of the plant are still lacking. In the light of its traditional use and the existing literature, this study is intended to validate the therapeutic applications of *A. excelsa*. Hence, the aim of the study was designed a) to evaluate the antioxidant activity and cytotoxicity potential of *A. excelsa* extract and its fractions b) to undertake GC-MS analysis of bioactive fractions and identification of major phytochemicals, c) to carry out the molecular docking of identified phytochemicals from bioactive fraction on various cancer protein targets.

2. Materials and methods

The A. excelsa stem bark (AEB) was collected in 2019 from the Shirpur vicinity (21.3496° N, 74.8797° E), Maharashtra and authenticated by Dr. Sanjay Kshirsagar, Taxonomy Department, SSVPS College, Dhule, Maharashtra, India (Herbarium No. RCP-64). The plant material was washed, cleaned, shade dried, powdered, and passed through a 40-mesh sieve. The dried and powdered raw material was kept in a closed bottle until used for the extraction.

2.1. Preparation of the extract and fractions

The powdered plant material was extracted with methanol using a hot continuous percolation method for approximately thirty cycles to yield methanol extract (5.23 ± 0.13%). The methanol extract (AEBME) was suspended in a sufficient quantity of water (400 mL) and partitioned with chloroform followed by ethyl acetate to get a chloroform (AEB-*ChF*) and ethyl acetate (AEB-*EAF*) fractions, respectively. The extract and its fractions were concentrated under reduced pressure to dryness at 40 °C using a rotary evaporator (Rotavapor R-215; Buchi, Switzerland). The percentage yield of AEB-*ChF* and AEB-*EAF* were 2.03 ± 0.10% and 1.39 ± 0.09%, respectively. The dried extract and fractions were kept at 0–4 °C in closed container prior to use.

2.2. Phytochemical studies

2.2.1. Total phenolic content (TPC)

The TPC of AEBME and its fractions AEB-*ChF,* AEB-*EAF* were determined using the Folin-Ciocalteau reagent colourimetric assay [30]. Briefly, 0.1 mL of AEBME, AEB-*ChF,* and AEB-*EAF* were mixed separately with 0.2 mL of Folin-Ciocalteu reagent (1:5 with distilled water). After this, 2 mL of distilled water and 2 mL of 15% Na_2CO_3 were added to the mixture. After 10 min of incubation at 50 °C in a water bath, absorbance was measured at 660 nm using a UV-spectrophotometer (Agilent, Cary 60). The total phenolic content in the extracts was calculated using the gallic acid standard calibration curve (10, 20, 30, 40, and 50 μg/mL). The total phenolic content is expressed as milligrams per gram of gallic acid equivalents (GAE).

2.2.2. Total flavonoid content (TFC)

Briefly, 0.5 mL of AEBME, AEB-*ChF,* and AEB-*EAF* solutions were thoroughly mixed with 1.5 mL of ethanol, 0.1 mL of 10% $Al(NO_3)_3$, 0.1 mL of 0.1 M CH_3COONa, and 2.8 mL of water. The resultant mixture was incubated for 40 min at room temperature. The absorbance of the reaction mixture was measured at 415 nm using UV-spectrophotometer (Agilent, Cary 60) [30]. The TFC of the extract and fractions was calculated using the standard quercetin calibration curve (10, 20, 40, 60, and 80 μg/mL). Results were expressed as milligrams per gram quercetin equivalents (QE).

2.2.3. Total alkaloid content

The AEBME and its fractions (AEB-*ChF* and AEB-*EAF*) (0.1 mg/mL) were prepared by dissolving them in 2 N HCl and washing with chloroform. The acidic solution was made alkaline with 0.1 N NaOH, and the alkaline mixture was treated with 5 mL of phosphate buffer solution (pH 4.7) and 5 mL of bromocresol green solution. The resultant mixture was extracted with chloroform and made volume up to 10 mL. The blank was prepared by replacing the test or standard solution with methanol. The absorbance of the reaction mixture was measured at 470 nm using a UV-spectrophotometer (Agilent, Cary 60) against the blank.

Similarly, the standard alkaloid compound, atropine, was prepared in gradual concentrations (0.4, 0.6, 0.8, 1.0, and 1.2 μg/mL). Total alkaloid contents in the extract and fractions were calculated using the atropine standard calibration curve [31]. The total alkaloid content was calculated using a regression equation and expressed as atropine equivalents (AE) (mg/mL).

2.2.4. Total steroid content

Total steroid content was determined as described by Moyo et al., [32]. Briefly, 0.2 mL of each AEB extract and its fractions (AEB-*ChF*, AEB-*EAF*) were added to 0.80 mL of absolute methanol and 0.35 mL of vanillin (8% in ethanol) in a 15 mL glass test tube. Then, 1.25 mL of sulfuric acid (72%) was added, mixed for a few seconds, and transferred into an electric water heating bath at 60 °C for 10 min. After incubation, the reaction mixtures were cooled on an ice bath and absorbance was recorded at 544 nm using a UV-spectrophotometer (Agilent, Cary 60). The standard curve of diosgenin was obtained based on different concentrations (20, 40, 60, 80, and 100 μg/mL), and total steroid content (TSC) in the extract and fractions was presented as a milligram per gram of diosgenin equivalents (DE).

2.3. Antioxidant activity

The antioxidant potential of AEBME, AEB-*ChF*, and AEB-*EAF* was assessed using DPPH, the ABTS radical scavenging assay [33], and the total antioxidant capacity by phospholybdenum method [34]. The total antioxidant capacity was represented as milligrams per gram of ascorbic acid equivalents (AAE), whereas the antioxidant activity was measured as the IC_{50} concentration in DPPH, the ABTS radical scavenging assays. The detailed procedure is given as supplementary file.

2.4. Cytotoxic studies

2.4.1. Cell lines and their maintenance

The Human Breast Cancer Cell Line (MCF-7), Human Lung Cancer Cell Line (A-549), and Human Hepatic Cancer Cell Line (HepG2) were grown in RPMI 1640 medium containing 10% foetal bovine serum and 2 mM L-glutamine. In the present screening experiment, 100 μl cells were inoculated into 96-well microtiter plates, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C with 5% CO_2, 95% air, and 100% relative humidity for 24 h before adding AEB extract, fractions, and adriamycin (Doxorubicin, standard drug).

2.4.2. Cytotoxic activity using Sulforhodamine B (SRB) assay

An SRB colourimetric assay determined cell proliferation and viability [35]. Briefly, MCF-7, A-549, and HepG2 cells were plated onto a 96-well flat-bottom tissue culture plate at 10^4 cells/well density and incubated for 24 h. Subsequently, cells were treated with 10, 20, 40 and 80 μg/mL concentrations of the AEBME, AEB-*ChF,* AEB-*EAF* fractions, and the standard drug, adriamycin. The cells in the control group received only the medium containing 0.1% DMSO. After 48 h, the cells were fixed with ice-cold trichloroacetic acid (50 μL per well, 10% w/v) and incubated for 1 h at 4 °C. The supernatant was discarded; the plates were washed in distilled water and air-dried. 50 μL SRB (0.4% w/v in 1% aqueous acetic acid) solution was added to each well of the 96-well plates in each well and allowed staining to occur at room temperature for 20 min. After staining, the unbound SRB dye was recovered, and the residual dye was removed by washing 5 times with 1% acetic acid and then the plates were air-dried. Subsequently, the bound stain was eluted with

200 μL of 10 mM Tris Base (pH 10.5). It was then shaken for 5–10 min on a shaker platform. Lastly, the plates were read in a microplate reader with a working wavelength of 540 nm. TGI and GI_{50} were calculated and compared to adriamycin as the reference standard (Pharmacia Ltd, India).

2.5. Animals and acute toxicity study

Wistar albino rats weighing 150−200 g were used for the study. All animals were housed at an ambient temperature in polypropylene cages at ambient temperature of 25 ± 2 °C, relative humidity 55−65%; and placed under a 12 h light/dark cycle. Animals were fed a normal diet and water *ad-libitum* and deprived of food for 12 h before the experiment. All the experimental procedures in animals were approved by *Institutional Animal Ethical Committee* of R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur (Protocol approval No: *IAEC/RCPIPER/2018-19/30*).

2.5.1. Acute toxicity

Acute toxicity studies of AEBME extract, AEB-*ChF,* AEB-*EAF* were determined in mixed sex *Swiss* albino mice according to OECD guidelines No. 425 [36]. The animals were fasted overnight. One group was maintained as control and was given 0.5% Tween-80. The test extracts were administered orally at a maximum dose of 2000 mg/kg. The animals were observed continuously for 1 h for any gross behavioural changes and death, if any, and then intermittently for the next 6 h, and then again at 24 h after dosing with test extracts.

2.5.2. Anti-inflammatory activity

The anti-inflammatory activity was determined as per the previously described method [30]. In this experiment, *Wistar* rats of mixed sex were divided into six groups, each group consisting of six animals. The normal control group received vehicle (vehicle, 10 mL/kg, p.o.). The standard group received indomethacin (10 mg/kg, p.o.), while the test groups were treated with AEBME, AEB-*ChF* (100 and 200 mg/kg, p.o.) and after 1 h of drug administration, 0.1 mL of 1% suspension of carrageenan (edematogenic agent) was injected underneath the plantar tissue of the right hind paw of all rats to induce inflammation. The paw volume was measured using a plethysmometer (Ugo Basil-2740). The paw volume was measured at 0 h and 1, 2, 3, and 6 h. The percentage of inhibition was calculated according to formula (1):

$$\%\ \text{Inhibition} = \frac{\text{Paw volume of control animal} - \text{Paw volume of control treated}}{\text{Paw volume of control animal}} \times 100 \Bigg) \quad (1)$$

2.6. Cyclooxygenase (COX) inhibitor screening assay

A colorimetric COX (ovine) Inhibitor Screening Assay Kit (Cayman Chemical Co., Cat. No. 760111) was used to evaluate the inhibitory activity against COX-1 and COX-2 [37]. The enzymes were pre-incubated for 5 min at 25 °C with the AEBME, AEB-*ChF*, AEB-*EAF* and standard indomethacin (5, 10,50, 100, and 500 μg/mL) before the addition of arachidonic acid (final concentration 1.1 mM) and N,N,N′,N'-tetramethyl-*p*-phenylenediamine, (TMPD) followed by incubation for 5 min at 25 °C. The COX-1 or COX-2 inhibitory activities of the test compounds were measured by monitoring the production of TMPD oxidized at 590 nm. The IC_{50} concentration of test extracts and standard drug was calculated using the regression equation.

2.7. Gas chromatography-mass spectrometry of AEB-ChF

The gas chromatography-mass spectrometry (GC-MS) analysis of AEB-*ChF* was performed using an Agilent 7890 chromatograph equipped with a Flame Ionization Detector and HP-2 fused silica columns (25 m × 0.32 mm, 0.25 μm film thickness). The AEB-*ChF* of AEBME was diluted in an appropriate solvent (1/100, v/v) and filtered. The particle-free diluted test solution (1 μL) was injected using a syringe into the injector with a split of 50:80-1M-8-200-3M-8-250-3M-16-300-1M-HP1. The initial column temperature was 35 °C with a hold time of 3 min. The temperature was programmed to rise at 8 °C/min to a final temperature of 300 °C. Ultra-high purity helium (99.99%) was used as the carrier gas at a constant flow rate of 1.0 mL/min with 70 eV ionizing energy. The electron multiplier voltage was obtained from auto-tune. All data was obtained by collecting the full-scan mass spectra within the scan range of 40–550 amu. The compounds were identified by comparing the peaks with those standards in the NIST MS 2.0 spectral library attached to the GC-MS instrument.

2.8. Molecular docking

The molecular docking computational tool, GLIDE (Schordinger Inc.2018-4, LLC) was used for ligand docking investigation of phytochemicals identified from bioactive AEB-*ChF* at different anticancer targets like BAX protein (PDB ID: 1F16), p53-binding protein Mdm-2 (PDB ID: 1YCR), and Topoisomerase II (PDB ID: 1QZR). The 3D structures of these proteins were acquired from the protein data bank. The receptor grid can be set up and generated from the receptor grid generation panel. Grid files represent physical properties of a receptor volume (specifically the active site) that are searched when attempting to dock a ligand. The ligand has been excluded before calculating the grid for the co-crystallized ligand-enzyme. Once the ligand has been excluded, the volume for which grids will be calculated. An enclosing box was generated by considering the centroid of some active site residues, and the size of the enclosing box was kept to 20 A°. Define the Glide constraints for the grid to be generated. Glide constraints are receptor-ligand interactions important

to the binding mode based on structural or biochemical data. The protein preparation was done using the 'protein preparation wizard' in Maestro 8.0 for preparation and refinement. After ensuring chemical correctness, water molecules in the structures were erased, and hydrogen atoms were added where they had been introduced and where they were lacking. Utilizing the OPLS3 force field [38], the energy of the crystal structure was minimized [1]. The phytoconstituent structures were built using Maestro 8.0 and converted to 3D structures from 2D using the Ligprep 2.2 module. The resulting structures were saved in Maestro format. The molecular docking tool, GLIDE (Schordinger Inc., USA) (2008), was used for ligand docking studies. The low-energy conformation of the ligands was docked on the grid generated from protein structures utilizing the standard precision (SP) docking mode. The final evaluation is performed with a glide score (docking score) and the absolute best pose is produced as the output for the specific ligand was also done with Maestro.

2.9. Statistical analysis

The results were expressed as mean ± S.E.M./S.D. in phytochemical and pharmacological studies, respectively; statistical comparisons were made between drug-treated and control groups. GraphPad Prism 5.0 software (GraphPad 5, San Diego, USA) was used to statistically analyze the disease activity index data using one-way ANOVA followed by Dunnett's test. The values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Phytochemical studies

The total phenolic, flavonoid, steroid, and alkaloid content of the AEB extract and its fractions are shown in Table 1. AEB-*EAF* exhibited the highest total phenolic content (15.90 ± 0.53 mg/g GAE), followed by AEBME (7.41 ± 0.81 mg/g GAE) and AEB-*ChF* (1.72 ± 0.20 mg/g GAE). The total flavonoid content values showed similar trends to the total phenolic content values. The AEB-*EAF* displayed the highest total flavonoid content (22.91 ± 0.12 mg/g QE), and the lowest was that of the AEB-*ChF*. The total alkaloid content was significantly higher in AEB-*ChF* (0.33 ± 0.01 mg/g AE) than in other tested samples. The total steroid content in the AEB extract and fractions was exhibited in the range of 51.87 ± 0.74 to 33.28 ± 0.45 mg/g of diosgenin equivalents with the increasing order of AEB-*ChF* > AEBME > AEB-*EAF*.

3.2. Antioxidant activity

The extract and fractions under the study exhibited significant DPPH and ABTS radical scavenging activity (Table 2). The IC_{50} values of the test extract, fractions, and standard for DPPH assay was ranked as ascorbic acid > AEB-*ChF* > AEBME > AEB-*EAF*. AEB-*ChF* showed the lowest IC_{50} (79.50 ± 0.73 μg/mL) compared to AEBME and AEB-*EAF*. The AEB-*EAF* showed significantly stronger ABTS scavenging potency (IC_{50}: 51.29 ± 0.75 μg/mL) than the AEBME and AEB-*ChF*. The present results show that the ABTS radical scavenging ability of samples can be ranked as AEB-*EAF* > AEB-*ChF* > AEBME. The phosphomolybdenum assay is a quantitative method for evaluating the water-soluble antioxidant capacity. The total antioxidant capacity of AEB-*EAF* was found to be the highest (78.53 ± 0.16 mg/g AAE) and demonstrated the highest electron-donating capacity.

3.3. Cytotoxic activity

The cytotoxic effect of AEBME, AEB-*ChF,* AEB-*EAF* and adriamycin was evaluated using SRB assay on three different cell lines: human breast cancer cell line (MCF-7), human lung cancer cell line (A-549) and human hepatic cancer cell line (HepG2). The activity of test samples activity was compared with the standard anticancer drug adriamycin using percentage growth inhibition (% GI), TGI, and GI_{50} values. Fig. 1 depict the % GI of the AEB-*ChF* which was comparatively higher than all the test substances in the studied cancer cell lines. However, its sensitivity was more against human lung cancer (Fig. 1b) and human hepatic cancer cell lines (Fig. 1a), while AEBME was highly potent at all the concentration in human breast cancer (Fig. 1c). Adriamycin was highly effective in all cancer types at all doses. The results mentioned in Table 3 depict the TGI and GI_{50} values. A stronger inhibitory effect of AEB-*ChF* was observed by demonstrating the lowest TGI for A-549, MCF-7, and HepG2 cell lines than AEB-*EAF and* AEBME. The effect was comparable to that of the standard drug adriamycin. As per the observation, the results indicated the cytotoxic activity in descending order of AEB-*ChF* > AEB-*EAF* > AEBME. To address AEB-*ChF* mechanistic pathway, evaluation of anti-inflammatory activity and molecular docking was

Table 1
Total phenolic, flavonoid, steroids, and alkaloidal content of AEB.

Extracts/ fraction	Total Phenolics (mg/g gallic acid equivalent)	Total flavonoids (mg/g quercetin equivalent)	Total steroids (mg/g diosgenin equivalent)	Total alkaloids (mg/g atropine equivalent)
AEBME	7.41 ± 0.81	21.37 ± 0.18	41.46 ± 0.51	0.26 ± 0.01
AEB-*ChF*	1.72 ± 0.20	20.40 ± 0.02	51.87 ± 0.74	0.33 ± 0.01
AEB-*EAF*	15.90 ± 0.53	22.91 ± 0.12	33.28 ± 0.45	0.21 ± 0.01

Values are mean ± SEM, n = 3.
AEBME: *Ailanthus excelsa* methanol extract; AEB-*ChF*: *Ailanthus excelsa* Chloroform Fraction; AEB-*EAF*: *Ailanthus excelsa* Ethyl Acetate Fraction.

Table 2
Antioxidant effect (IC_{50}) on free DPPH radicals, ABTS radicals and total antioxidant capacity of *A. excelsa* bark extract and its fractions.

Extracts/fraction	IC_{50} (μg/ml)		Total antioxidant capacity (mg/g ascorbic acid equivalent)
	Scavenging ability on DPPH radicals	Scavenging ability on ABTS radicals	
AEBME	192.33 ± 0.27	67.77 ± 0.90	74.52 ± 0.67
AEB-*ChF*	79.50 ± 0.73	61.97 ± 1.88	25.97 ± 0.02
AEB-*EAF*	211.14 ± 1.36	51.29 ± 0.75	78.53 ± 0.16
Ascorbic acid	52.14 ± 2.41	9.94 ± 0.61	–

Values are mean ± SEM, n = 3.
AEBME: *Ailanthus excelsa* methanol extract; AEB-*ChF*: *Ailanthus excelsa* Chloroform Fraction; AEB-*EAF*: *Ailanthus excelsa* Ethyl Acetate Fraction; IC_{50}: Inhibitory concentration 50%.

done.

3.4. Anti-inflammatory activity

The AEB-*ChF* showed noticeably superior cytotoxic potential, therefore the *in vivo* anti-inflammatory evaluation of the AEBME and AEB-*ChF* was carried out. In acute toxicity studies, AEBME and AEB-*ChF* appeared to be non-toxic, as no lethality was observed at the highest dose, i.e., 2000 mg/kg p. o. in mice. Therefore, 100 and 200 mg/kg were selected as the test doses for the anti-inflammatory study. The *in vivo* anti-inflammatory activity of AEBME and AEB-*ChF* dependent on both the amount of testing extract treated (100 and 200 mg/kg) and the length of time from carrageenan-induced rat paw inflammation (1, 2, 3, and 6 h), as shown in Table 4. At a higher dose (200 mg/kg), AEBME and AEB-*ChF* had a statistically significant ($p < 0.01$) inhibitory effect on the progression of rat paw edema in the 3rd and 6th hours after carrageenan administration when compared to control. The percentage of inhibition of rat paw edema for the indomethacin-treated group (10 mg/kg) at the sixth hour was 58.13%. The AEB-*ChF* showed almost the same percentage of inhibition at a dose of 200 mg/kg, i.e., 56.91%. The result was almost comparable to the reference drug, indomethacin at the sixth hour. While the AEBME (200 mg/kg) showed 51.91% at sixth hour and exhibited a substantial percentage of inhibition in comparison to the indomethacin group. However, it should be noted that the results obtained cannot be directly compared with the positive control (indomethacin) because a much lower dose of indomethacin was used hence the dose-wise anti-inflammatory response of indomethacin is better. It should also be noted that indomethacin is a pure, laboratory-synthesized substance with proven anti-inflammatory action, whereas extracts are multicomponent systems.

3.5. Cyclooxygenase (COX) inhibitor screening assay

The percentage inhibition of prostaglandin synthesis by COX-1 and COX-2 of AEBME, AEB-*ChF* with indomethacin as the control are shown in Table 5. The AEBME had a moderate activity for both COX-1 (IC_{50}: 107.98 ± 3.71 μg/mL) and COX-2 (IC_{50}:97.21 ± 2.76 μg/mL) while, AEB-*ChF* had highest activity against COX-1 (IC_{50}: 60.62 ± 1.16 μg/mL) and COX-2 (IC_{50}: 56.45 ± 0.74 μg/mL).

3.6. GC-MS analysis

The GC-MS analysis of AEB-*ChF* identified a range of phytochemicals shown in Fig. 2 and Table 6. The elution time of compounds with their base mass-to-charge ratio (*m/z*) and their content by percentage was also determined. Based on the abundance, oleanolic acid (19.40%), arachidonic acid (19.37%), and caftaric acid (18.82%) were identified as major phytoconstituents, followed by cinnamic acid (13.67%), caffeic acid (9.01%), hexadecanoic acid (8.10%), 3,4-dihydroxy phenyl acetic acid (6.56%), and 3-hydroxyphenyl valeric acid (5.08%) (Fig. 3).

3.7. Molecular docking studies

The effects of the identified phytochemicals from bioactive fractions were investigated *in silico* molecular docking studies of the crystal structures of targeted proteins. The different target proteins were considered for molecular docking because of structural diversity of the identified compounds. The molecular docking simulations were used to examine the molecular interactions between identified compounds and the active binding sites of the Bcl2-associated X (BAX) protein, the p53-binding protein Mdm-2, and topoisomerase II. The most stable docking conformations of the GC-MS identified compound are presented in Fig. 4. The inhibitory activities of the AEB-*ChF* compounds towards selected targeted proteins were ranked based on their lowest binding energy and glide score involved in the complex formation at the active sites. The binding energies of the docked compounds to BAX, Mdm-2, and Topoisomerase II were found to be in the range between −6.852503 and −59.009532 kcal/mol (Table 7).

As can be seen from Table 7, the polyphenolic compounds significantly bind to selected target proteins. The docking analyses of identified molecules revealed several non-covalent interactions between investigated molecules and target proteins. The most prominent interactions are hydrogen bonds (Fig. 4).

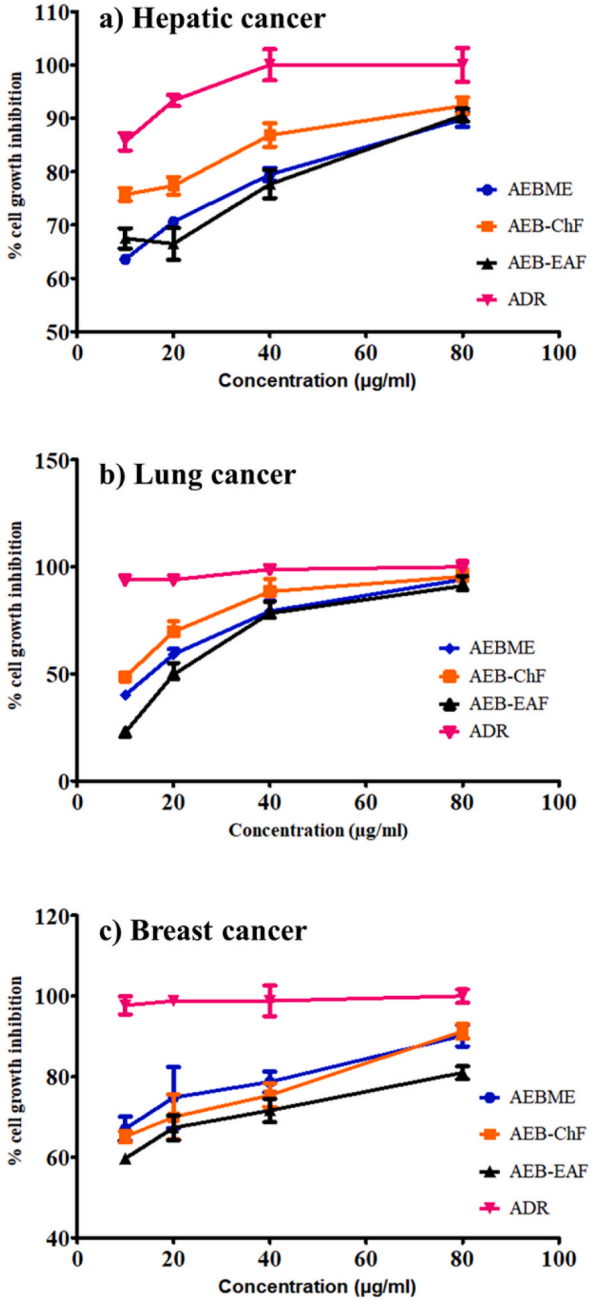

Fig. 1. Effect of A. excelsa stem bark extract and its fractions on the percent of the growth in (a) hepatic, (b) lung, and (c) breast cancerous cell lines. Different Cell lines were treated with vehicle, 10, 20, 40, and 80 µg/mL concentrations of the AEBME, AEB-*ChF*, AEB-*EAF* and the positive control (adramycin) for 48 h. Cell viability was analyzed using the SRB assay. Among the tested samples, AEB-*ChF* showed growth inhibition (91.30 ± 3.14%; 92.32 ± 2.77%) in breast and hepatic cancer cell lines (MCF-7; HepG2). The AEB-*ChF* has shown maximum cytotoxic effect (95.48 ± 3.31%)) in lung cancer cell line (A-549) at 80 µg/mL among the tested materials, which was comparable to standard adriamycin (100%).

3.7.1. Molecular docking for Bcl2-associated X (BAX) protein

The bioactive phytochemicals from AEB-*ChF* were docked in the protein BAX's pocket (PDB ID: 1f16) (Fig. 4a; Table 7). The phytochemical with the lowest expected Glide energy (−22.131972 kcal/mol), Glide Van der Waals binding energy (−14.57039 kcal/mol), and Glide model (−31.989926 kcal/mol) was 3,4-dihydroxyphenylacetic acid and it exhibits a hydrophobic H bond interaction with the active site residue GLU61 with a docking score of −5.033217 kcal/mol (GvdW: −14.57039 kcal/mol). The phenyl ring is also stabilized because of the π-cation interaction between LUS58 and the phenyl ring. Oleanolic acid lagged behind 3,4-dihydroxyphenyl acetic acid in terms of Glide energy (−22.67939 kcal/mol), Glide Van der Waals binding energy (−17.201836 kcal/mol), and Glide

Table 3
Effect of *A. excelsa* bark extract and its fractions on the parameter for the selected cancer cell line.

Sr. No.	Parameter calculated (μg/ml)					
	Treatment	Human Breast cancer cell line MCF-7		Human Lung cancer cell line A-549		Human Hepatic Cancer cell line (HepG2)	
		TGI	GI_{50}	TGI	GI_{50}	TGI	GI_{50}
1	AEBME	>80	<10	>80	12.2	>80	41.4
2	AEB-*ChF*	78.2	<10	58.3	<10	71.9	<10
3	AEB-*EAF*	>80	<10	>80	25.9	>80	<10
4	ADR	>58.3	<10	42.0	<10	62.3	<10

GI_{50}-growth inhibition by 50%, all the values are the average of three experiments; TGI- the drug concentration resulting in total growth inhibition; AEBME: *Ailanthus excelsa* methanol extract; AEB-*ChF*: *Ailanthus excelsa* Chloroform Fraction; AEB-*EAF*: *Ailanthus excelsa* Ethyl Acetate Fraction; ADR: Adriamycin.

Table 4
Effects of *A. excelsa* bioactive bark extract and chloroform fraction against carrageenan-induced paw edema in rats.

Treatment	% Rise in paw edema (%Inhibition)			
	1 h	2 h	3 h	6 h
Control (1% Carrageenan)	18.50 ± 0.91	33.93 ± 3.71	37.56 ± 1.25	52.22 ± 0.63
Standard (Indomethacin)	14.28 ± 0.42[b] (27.78)	15.85 ± 0.41[a] (56.84)	18.19 ± 0.36[a] (57.97)	31.2 ± 0.81[a] (58.13)
AEBME (100 mg/kg)	13.18 ± 0.27[c] (15.58)	23.71 ± 0.48[b] (18.25)	25.58 ± 0.45[a] (24.49)	44.89 ± 0.75[b] (44.24)
AEBME (200 mg/kg)	20.87 ± 0.36[b] (24.39)	18.66 ± 0.38[a] (34.69)	17.26 ± 0.27[a] (47.38)	26.20 ± 0.15[a] (51.91)
AEB-*ChF* (100 mg/kg)	15.82 ± 1.28[d] (17.55)	21.64 ± 1.38[b] (21.14)	23.81 ± 0.59[a] (28.19)	35.77 ± 2.02[b] (49.11)
AEB-*ChF* (200 mg/kg)	13.85 ± 0.22[b] (22.39)	16.73 ± 0.26[a] (32.46)	20.91 ± 0.61[a] (48.42)	24.30 ± 0.67[a] (56.91)

Values are mean ± SEM, n = 6.
[a]$P < 0.01$; [b]$P < 0.05$; [c]$P > 0.05$ when compared with control. Statistical differences were calculated using one-way ANOVA followed by Dunnett's test. AEBME: *Ailanthus excelsa* methanol extract; AEB-*ChF*: *Ailanthus excelsa* Chloroform Fraction.

Table 5
IC_{50} determination of *A. excelsa* bioactive extract and fraction as inhibitors of COX-1 and COX-2.

Extract/Fractions	IC_{50} (μg/mL)	
	COX-1	COX-2
AEBME	107.98 ± 3.71	97.21 ± 2.76
AEB-*ChF*	60.62 ± 1.16	56.45 ± 0.74
Indomethacin	5.34 ± 0.94	31.69 ± 0.65

Values are means of three determinations, mean ± SEM; IC_{50}: Inhibitory concentration 50%; COX-1: Cyclooxygenase-1; COX-2: Cyclooxygenase-2; AEBME: *Ailanthus excelsa* methanol extract; AEB-*ChF*: *Ailanthus excelsa* Chloroform Fraction.

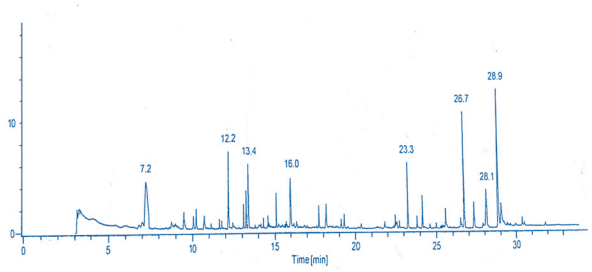

Fig. 2. GC-MS chromatogram of chloroform fraction of methanolic extract of A. excelsa stem bark.

emodel (−26.313992 kcal/mol). According to the glide score, the phytochemicals are ordered in decreasing order of kcal/mol as, caftaric acid (−6.110514), cinnamic acid (−5.064212), 3,4-dihydroxyphenylacetic acid (−5.033217), 3-hydroxyphenylvaleric acid (−3.971972 kcal/mol), oleanolic acid (−2.283242).

3.7.2. Molecular docking for p53-binding protein Mdm-2

Computational docking studies were carried out to identify the most favourable binding mode of the AEB-*ChF* identified

Table 6
The major phytochemicals identified in the chloroform fraction of methanolic extract of *A. excelsa* stem bark by GC-MS analysis.

Peak No	RT	% Area	Proposed compound	Molecular name	MW	Observed *m/z*	Compound class
1	7.2	19.37	Arachidonic acid	$C_{20}H_{32}O_2$	151	310.20	Fatty acid
2	12.2	8.10	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	256.08	Fatty acid
3	13.4	5.08	3-Hydroxyphenylvaleric acid	$C_{11}H_{14}O_3$	194.05	194.12	Medium-chain fatty acid
4	16.0	18.82	Caftaric acid	$C_{13}H_{12}O_9$	312.22	311.20	Phenolic acid
5	23.3	6.56	3,4-Dihydroxy phenylacetic acid	$C_8H_8O_4$	168.04	167.96	Phenolic acid
6	26.7	13.67	Cinnamic acid	$C_9H_8O_2$	148.15	149.11	Phenolic acid
7	28.1	9.01	Caffeic acid	$C_9H_8O_4$	180.16	180.10	Phenolic acid
8	28.9	19.40	Oleanolic acid	$C_{30}H_{48}O_3$	457	457.20	Triterpenoid

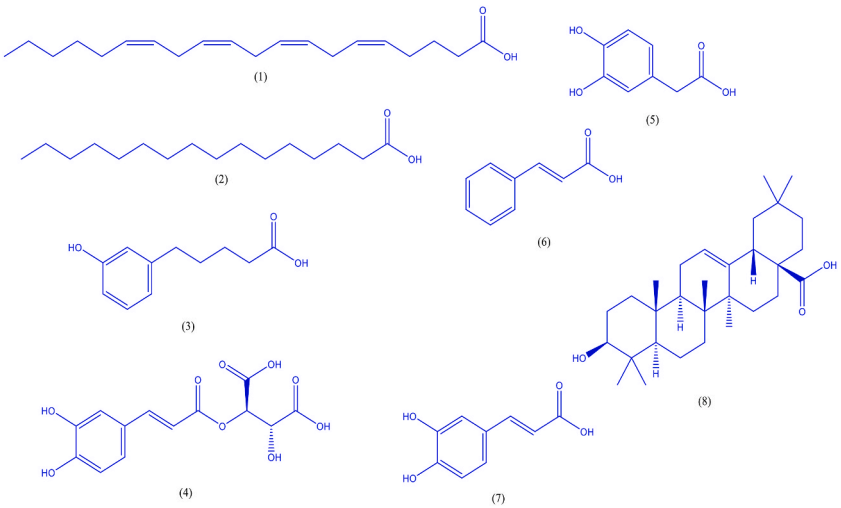

Fig. 3. Chemical structure of proposed phytochemicals: (1) Arachidonic acid, (2) Hexadecanoic acid, (3) 3-Hydroxyphenylvaleric acid, (4) Caftaric acid, (5) 3,4-Dihydroxy phenylacetic acid, (6) Cinnamic acid, (7) Caffeic acid, and (8) Oleanolic acid.

phytochemicals inside the binding pocket of the p53-binding protein Mdm-2 (PDB ID: 1ycr) (Fig. 4b, Table 7). Caftaric acid seems to have the lowest Glide energy (−38.708663 kcal/mol), Glide Van der Waals binding energy (−24.661393 kcal/mol), and Glide emodel (−59.009532 kcal/mol) among the compounds studied. Caftaric acid establishes five H bonds with GLN59, TRP23, and LYS24 at the active site of the p53-binding protein Mdm-2, with a glide score of −6.446272 kcal/mol. In terms of Glide energy (−35.850734 kcal/mol), GvdW (−34.867109 kcal/mol), and hydrophobic energy, oleanolic acid lagged behind caftaric acid. The phytochemicals can be arranged in descending order based on Glide score with the p53-binding protein Mdm as 3,4-dihydroxy phenylacetic acid (−6.8944 kcal/mol) > caffeic acid (−6.532139 kcal/mol) > caftaric acid (−6.446272 kcal/mol) > cinnamic acid (−6.290406 kcal/mol) > 3-hydroxyphenylvaleric acid (−6.218742 kcal/mol) > arachidonic acid (−4.848428 kcal/mol) > Oleanolic acid (−4.335555 kcal/mol) > hexadecanoic acid (−0.613185 kcal/mol).

3.7.3. Molecular docking for topoisomerase II

The identified phytochemicals from AEB-*ChF* were docked in the pocket of topoisomerase II (PDB ID: qzr) (Fig. 4c, Table 7). Caftaric acid had the lowest Glide energy (−32.650378 kcal/mol), Glide Van der Waals binding energy (−20.705144 kcal/mol), and Glide model (−41.693192 kcal/mol) among the identified phytochemicals. The finding anticipated hydrogen bond interactions with topoisomerase II active site residues Thr27 and Phe362. Arachidonic acid was the next to caftaric acid in glide energy at −31.897395 kcal/mol with ΔG_{vdW}: −29.451471 kcal/mol hydrophobic energies. Based on the glide score with topoisomerase-II, the studied phytochemicals can be ranked as caftaric acid (−5.144799 kcal/mol) > 3,4-Dihydroxy phenylacetic acid (−5.025057 kcal/mol) > arachidonic acid (−4.575415 kcal/mol) > cinnamic acid (−4.394239 kcal/mol) > 3-hydroxyphenylvaleric acid (−4.190921 kcal/mol) > caffeic acid (−4.069054 kcal/mol) > oleanolic acid (−3.438539 kcal/mol).

4. Discussion

A. excelsa is popular as a matchstick plant and is widely used for commercial purposes, especially in the manufacturing of matchsticks. Traditionally, the stem bark of the plant was also used as anticancer agent in combination with other herbs. The stem bark of *A. excelsa* was extracted with methanol, a universal solvent capable of extracting almost all types of secondary metabolites (polar to non-polar phytochemicals). The qualitative phytochemicals analysis of *A. excelsa* revealed alkaloids, phenolics, flavonoids, tannins and

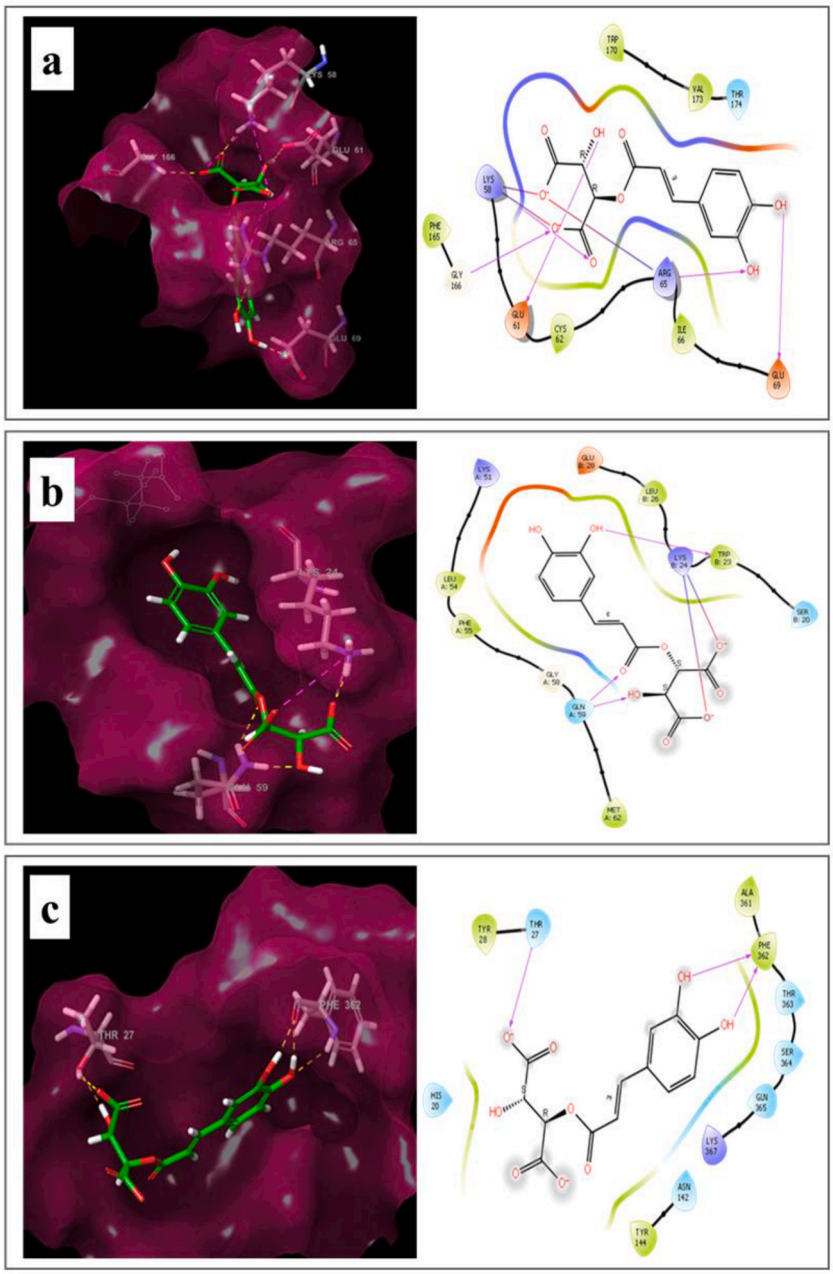

Fig. 4. The 3D structures of the target proteins, (a) protein BAX's pocket (PDB ID: 1f16); (b) p53-binding protein Mdm-2 (PDB ID: 1ycr); (c) topoisomerase II (PDB ID: qzr) with caftaric acid. Pink coloured lines indicates the interactions between the structure and amino acids. (i) 3,4-dihydroxyphenylacetic acid shows maximum interactions with ARG65, GLN69, ARG65, LYS58, GLY166, GLU61 (ii) Caftaric acid establishes five H bonds shown by dotted lines with GLN59, TRP23, and LYS24 at the active site. Dihydroxy phenyl group located deep in the active pocket (iii) Caftaric acid have hydrogen bond interactions with topoisomerase II active site residues Thr27 and Phe362. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

steroids in the extract and its fractions. Phenolics and flavonoids were predominantly observed in AEB-EAF and AEBME. It is a known fact that the ability of different polarity solvents to segregate various phytochemicals results in quantitative changes in TPC, TFC, TSC, and total alkaloid concentration in the extract and fractions. The study revealed that the highest number of phenolics and flavonoids accumulated in AEB-*EAF*, whereas AEB-*ChF* exhibited the highest steroid and alkaloid content along with a significant amount of phenolics when compared with crude AEBME. The natural antioxidants present in plants inhibit the harmful consequences of oxidative stress. The antioxidant potential of AEBME extract, AEB-*EAF*, AEB-*ChF* was measured using the change in absorbance of DPPH and ABTS free radicals. The antioxidant activity of the extract/fractions is strongly related to the solvent used in extraction. This is due to

Table 7
Binding energies (kcal/mol) of selected phytoconstituents with BAX protein, p53-binding protein Mdm-2 and Topoisomerase-II.

Sr No	Name of compounds	Glide score	Glide energy	ΔG_{vdW}	Glide emodel	Residue compounds interactions with Distance (Å)
BAX Protein						
1	Caftaric acid	−6.110514	−17.3262	−5.810756	−27.0157	ARG65 (2.50), GLN69 (1.54), ARG65 (4.98), LYS58 (4.99, 1.82) GLY166 (2.37), GLU61 (2.01)
2	Cinnamic acid	−5.064212	−9.675033	−2.257539	−14.22024	LYS58 (2.60), GLY166 (2.52)
3	3,4-Dihydroxy phenylacetic acid	−5.033217	−22.131972	−14.57039	−31.989926	GLU61, LUS58
4	3-Hydroxyphenylvaleric Acid	−3.971972	−9.795775	−5.699152	−11.826515	ARG65
5	Oleanolic acid	−2.283242	−22.67939	−17.201836	−26.313992	GLU69, LYS64
6	Hexadecanoic acid	−0.087506	−0.161309	−4.806025	−6.852503	LYS58, GLY166
7	Arachidonic acid	–	–	–	–	–
8	Caffeic acid	–	–	–	–	–
p53-binding protein Mdm-2						
1	3,4-Dihydroxy phenylacetic acid	−6.8944	−30.816044	−7.805232	−48.347489	GLU28 (1.41,1.83)
2	Caffeic acid	−6.532139	−32.381845	−7.88628	−50.239991	GLN59 (2.30), GLU28 (1.41)
3	Caftaric acid	−6.446272	−38.708663	−24.661393	−59.009532	TRP23 (1.90), LYS24 (2.63, 4.85)
4	Cinnamic acid	−6.290406	−25.103569	−18.701722	−36.946576	LYS (3.94), GLN (1.75)
5	3-Hydroxyphenylvaleric acid	−6.218742	−31.80615	−14.708821	−44.77022	GLN59 (1.79), GLU28 (1.50)
6	Arachidonic acid	−4.848428	−32.843575	−26.909223	−42.888886	LYS B24, GLN A59
7	Oleanolic acid	−4.335555	−35.850734	−34.867109	−43.58063	–
8	Hexadecanoic acid	−0.613185	−29.609475	−22.371072	−26.458239	GLN A59
Topoisomerase II						
1	Caftaric acid	−5.144799	−32.650378	−20.705144	−41.693192	PHE362(1.81, 1.90), THR27 (2.03)
2	3,4-Dihydroxy phenylacetic acid	−5.025057	−23.103731	−14.32758	−30.873393	THR (2.16)
3	Arachidonic acid	−4.575415	−31.897395	−29.451471	−40.717253	THR27 (1.84)
4	Cinnamic acid	−4.394239	−18.192259	−13.645976	−23.754793	THR27 (2.15). π–π stacking TYR144 (4.39)
5	3-Hydroxyphenylvaleric acid	−4.190921	−23.565729	−18.517351	−28.768431	THR27
6	Caffeic acid	−4.069054	−23.432642	−15.417704	−29.811307	GLN365, THR27
7	Oleanolic acid	−3.438539	−28.625332	−25.919349	−33.425927	THR371, TYR144
8	Hexadecanoic acid	–	–	–	–	–

the fact that antioxidant compounds dissolve differently in varied polarity solvents [39]. Polyphenols, terpenes and PUFA are major antioxidants in natural products, and their antioxidant potential has been demonstrated in previous studies [40–42]. Research has shown that plant polyphenolics, triterpenoids and long chain fatty acids possess anti-inflammatory activities associated to their antioxidant properties [43–45].

As the inflammatory process is linked to the risk of cancer progression, anti-inflammatory activity may be considered an additional favourable quality for extracts with anticancer potential [5]. AEBME and AEB-*ChF* exerted a significant anti-inflammatory action in the rat paw edema test at both dose levels. AEB-*ChF* demonstrated potent anti-inflammatory activity. The inhibition of edema observed was pronounced in the later phase of inflammation, indicating activity is possibly mediated through suppression of cyclooxygenase enzyme, tumor necrosis factor- α, interleukin-1, and interleukin-6 [46–48]. In recent years, COX-2 inhibitors become a new target and hotspot for cancer research and receives a lot of attention. Among the tested plant samples, AEB-*ChF* displayed significantly higher COX-2 inhibition. Because only the non-polar fractions were active, this indicate the phytochemicals present in this fraction expected to be responsible for COX inhibition. On the basis of findings from several researchers, it may be assumed that COX inhibitory effects on carcinogenesis in various rodent models represent one of the mechanisms of cytotoxicity [49]. Researchers believe that suppressing COX enzymes, particularly COX-2, is responsible for the chemopreventive efficacy [50–52]. In most preclinical investigations, selective COX-2 inhibitors suppress tumor growth rather than causing tumor regression. However, several studies proposed for multiple cellular mechanisms for cytotoxicity, i.e. COX-independent mechanisms [53], which include induction of apoptosis, suppression of angiogenesis, STAT3, upstream activation of JAK1/2 kinases, cSRC activation of caspase 3, cell cycle arrest in the S phase, and so on [54–57].

There have been no previous reports on the cytotoxic potential of *A. excelsa* stem bark and its phytochemical correlation. The results of this study are comparable to previous reports concerning the inhibitory effects of *A. excelsa* leaves extract on the growth of human cancer cell lines, including lung large cell carcinoma COR-L23 (ECACC No. 92031919), amelanotic melanoma C32 (ATCC No. CRL-1585), renal cell adenocarcinoma ACHN (ATCC No. CRL-1611), malignant melanoma A375 (ECACC No. 88113005), and lung carcinoma A-549 (ECACC No. 86012804) cells [6]. In the present study, the anticancer activity of AEB-*ChF* was observed in terms of cell viability in a concentration-dependent manner. AEB-*ChF* was found to be potent cytotoxic against all the studied cancerous cell lines compared with AEBME and AEB-*EAF*. The concentration of AEB-*ChF* and the reduction in cell viability were directly proportional, with an increased concentration of AEB-*ChF* resulting in the highest reduction in cell viability. The human lung cancer cell line demonstrated the substantial reductions in cell viability (A-549) (Fig. 1).

AEB-*ChF* shown strong antioxidant, anti-inflammatory, and cytotoxic effects when compared to AEBME and AEB-*EAF,* at the same concentration and dose. This suggests that bioactive substances would concentrate in AEB-*ChF*. The GC-MS analysis of AEB-*ChF* has identified many polyphenols, polyunsaturated fatty acids, and triterpenoid. It showed different types of phytochemicals that had not yet been reported in *A. excelsa* stem bark (Table 6). This might be because the chloroform fraction was obtained using a different method of fractionation, specifically solvent-solvent fractionation, from a methanol extract. These phytochemicals are capable of reducing the free radicals and reducing oxidative stress, chronic inflammation, inhibiting cell proliferation and inducing apoptosis through morphological changes like cell shrinkage, chromatin condensation, nuclear fragmentation, and a probable cell cycle modulation [58–60]. The literature reported the different phytochemicals from stem bark of *A. excelsa* contains majorly quassanoids, phytosteols, alkaloids, flavonoids. It was reported that, the phytochemicals isolated from stem bark of *A. excelsa* are majorly quassanoids, along with long-chain hydrocarbons, fatty acids, alkaloid, triterpenoids, and plant sterols. Quassinoids such as 3,4-dihydroxyexcelsin, excelsin, ailanex A, ailanex B, glaucarubine, glaucarubolone, glaucarubinone, and ailanthinone were also reported from the stem bark of *A. excelsa* [16,21,23,27,61,62]. The other phytochemicals reported from the stem bark are fatty acids, aliphatic alcohols, phytosterols and alkaloid, i.e., decasanoate, *n*-eintracontane, *n*-tetratriacontanoic acid, *n*-eintriacontan-8β-ol, stigmasterol, and canthin-6-one [63,64]. In a recent HPTLC study, lupeol and stigmasterol were identified in the stem bark extracts [65].

Molecular docking studies were conducted to clarify the possible mechanism of action of AEB. *In silico* structure-based molecular docking simulation focuses on the affinity and specificity of the identified phytocomponent towards targets. *In silico* study is a useful tool to explore the accommodation of ligands in the binding site, generating the calculated binding affinity in terms of scoring energy. Hence, this study was extended to molecular docking of identified compounds for different cancer target proteins, i.e., Bcl2-associated X protein (BAX), p53-binding protein mouse double minute 2 (Mdm-2), and Topoisomerase II.

Different stimuli, primarily oxidative stress, provoke apoptosis. It is induced intrinsically when the subclasses of the Bcl-2 protein family interact with the outer mitochondrial membrane activated by cytotoxic stress via stimulation of BAX that results in the liberation of cytochrome C (an apoptogenic compound). Cytochrome C is the main activator of caspase-9, causing a cascade of proteolytic activities that lead to apoptosis. Thus, it could be a potential target for cancer therapy [66].

The p53 protein is a tumor suppressor gene mutated in approximately more than 50% of human cancers. The activity of p53 can be regulated by multiple cellular proteins, particularly by Mdm2, which is not only a physiological antagonist of p53 but also a direct target for the transcriptional activation by p53. This establishes a negative autoregulatory feedback loop where p53 activates its own inhibitor [67]. Thus, the p53-binding protein Mdm-2 could be a key target in cancer.

DNA topoisomerases are the enzymes that unwind DNA double helix structures before replication, transcription, recombination, segregation, and other processes. When DNA topoisomerase II cuts the double-stranded DNA, it may remain covalently attached to the broken end of the DNA. This faulty recombination of DNA leads to abnormal DNA translocations, mutagenesis, and DNA abnormalities, leading to cancer. For this reason, DNA topoisomerase II is considered a potential target for anti-cancer drug development [68].

Based on the results presented in Table 7, it is clear that ligand efficiency is not a determining factor for the value of the binding energy. On the other hand, the main contribution to the binding energy comes from the sum of the dispersion, repulsion, and hydrogen bond energies. A higher number of OH groups leads to a higher probability of hydrogen bond formation, which directly leads to significantly lower values of binding energy. It should be noted that electrostatic interactions also significantly contribute to the stabilization of the complex with caftaric acid compared to other studied phytochemicals.

A molecular docking approach was utilized for the binding behaviour of arachidonic acid, cinnamic acid, hexadecanoic acid, 3,4-dihydroxy phenylacetic acid, caftaric acid, caffeic acid, oleanolic acid, hydroxyphenyl valeric acid against BAX protein, p53-binding protein Mdm-2, and Topoisomerase II docking. The finding was justified by the fact that all compounds exhibited good docking energy values. Among all the identified compounds of AEB-*ChF*, caftaric acid showed the best docking energy values and good interaction behaviour with all the selected targets compared to other compounds. Thus, caftaric acid can be considered as a major active phytochemical with the cytotoxic potential of *A. excelsa*. Additional *in vivo* studies with caftaric acid should be performed to see how it act in a living system and to confirm their action, as metabolic transformations must also be considered.

Furthermore, Out of the identified compounds in GC-MS analysis, cinnamic acid and caffeic acid are well established for its potential to induce apoptosis through different mechanisms [69,70]. Furthermore, arachidonic acid is reported for the significant rise in PPARγ, and inhibits human breast cancer cell proliferation [71]. Also, oleanolic acid acts via many mechanisms, such as reducing oxidative stress, inducing apoptosis, and inhibiting proliferation. Recent studies have reported that oleanolic acid also suppresses angiogenesis, STAT3, upstream activation of JAK1/2 kinases, cSRC activation of caspase 3, and induces cell cycle arrest in the S phase [56,57].

5. Conclusion

A. excelsa stem bark extract and fractions demonstrated significant antioxidant activity, cytotoxic potential against human cancer cell lines, A-549, MCF-7, and HepG2 cell lines. It was also shown to possess significant anti-inflammatory activity in rat model. Furthermore, molecular docking studies of identified phytochemicals showed caftaric acid had strong binding affinity to p53-binding protein Mdm-2, BAX protein, and topoisomerase II, which could be attributed to the cytotoxic activity demonstrated by AEB. These results suggest that caftaric acid could be a promising lead compound; for development of anticancer agents through interaction with Mdm-2, BAX, and topoisomerase II.

In view of the obtained results, we recommend that stem bark extract and fractions of *A. excelsa* could deliberate tolerance to oxidative stress and encompasses promising drug for cancer and related inflammatory diseases. The major limitation of the present study is that isolation, purification, and characterization of active principles from the plant was not carried out for its cytotoxicity and

anti-inflammatory activity. Along with the results of molecular docking studies for major compounds identified from the plant, isolation of bioactive compounds from active fractions could lead to the development of effective drugs in treating cancer and inflammatory conditions. So, it is recommended to isolate and purify bioactive compounds from the *A. excelsa* stem bark extract by adopting different extraction methodologies and to elucidate the possible mechanisms of action and pathways responsible for the anticancer and anti-inflammatory activities of compounds to serve as an effective drug candidate against the inflammatory conditions.

Author contribution statement

Sapkal Priyanka R., Firke Sandip D., Redasani Vivekkumar K.: Performed the experiments.
Tatiya Anilkumar U: Conceived and designed the experiments.
Gurav Shailendra S., Muniappan Ayyanar: Analyzed and interpreted the data; Wrote the paper.
Jamkhande Prasad G., Mutha Rakesh E.: Contributed reagents, materials, analysis tools or data.
Kalaskar Mohan G., Surana Sanjay J.: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of competing interest

The authors 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.2023.e15952.

Abbreviations

AEB-*ChF*	*Ailanthus excelsa* Chloroform Fraction
AEB-*EAF*	*Ailanthus excelsa* Ethyl Acetate Fraction
AEBME	*Ailanthus excelsa* Bark methanol extract
ABTS	2,2′-Azino-Bis (3-Ethylbenzothiazoline-6-Sulfonic acid)
ADR	Adriamycin
AE	Atropine Equivalents
AEB	*Ailanthus excelsa* stem Bark
BAX	Bcl2-associated X
COX	Cylcooxygenase
Mdm-2	Mouse double minute 2
DE	Diosgenin Equivalents
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
GAE	Gallic Acid Equivalents
GC-MS	Gas Chromatography Mass Spectroscopy
GI50	Growth Inhibition by 50%
IC50	Inhibitory Concentration by 50%
NO	Nitrous Oxide
QE	Quercetin Equivalents
SRB	Sulforhodamine B
TAC	Total Antioxidant Capacity
TFC	Total Flavonoid Content
TGI	Total Growth Inhibition
TPC	Total Phenolic Content
TSC	Total steroid Content

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