Antiviral, cytotoxic activities and chemical profile of two different species of *Abies nordmanniana* from Turkiye

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Abstract: *Abies* is an important genus of the Pinaceae family, with about 50 species found in the highlands of Asia, Europe, North Africa, and North and Central America. The principal aim of the present work was the investigate the chemical content and biological potential of the resin and cone from *Abies nordmanniana* subsp. *bornmulleriana* and *Abies nordmanniana* subsp. *equi-trojani*, respectively. The flavonoid and, phenolic contents by LC-HRMS (Liquid Chromatography-High Resolution Mass Spectrometry), the essential oil and fatty acid compositions by GC-MS (Gas Chromatography-Mass Spectrometry) and GC-FID (Gas Chromatography-Flame Ionization Detector) of resin and cones were evaluated. Cytotoxicity of the extracts and essential oils were screened against certain cancer cell lines, namely, human prostate adenocarcinoma cells (PC3), human lung adenocarcinoma cell line (A549), human pancreatic cancer cell line (PANC-1), human hepatocellular carcinoma cells (HepG2), human breast cancer cells (MDA-MB231) and normal human lung fibroblast cells (CCD-34-LU), by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] assay. According to the MTT results, hexane extracts of both cone (CH) and resin (RH), ethanol-water (CEW), dichloromethane (CD), and acetone (CA) extracts of the cone mostly inflict cytotoxicity in HepG2 cell line. Antiviral activities of *Abies nordmanniana* subsp. extracts at doses of 5 μg/g and 10 μg/g were also carried out as virucidal activity against avian coronavirus by *in ovo*. *Abies nordmanniana* subsp. extracts exhibited concentration-dependent antiviral activity on SPF-ECEs. Significantly, cone acetone extract (CA), cone ethanol extract (CE), and cone dichloromethane extract (CD) of *Abies nordmanniana* subsp. exhibited strong inhibition of the virus at a concentration of 10 μg/g. The most potent virucidal activity was observed with ethanol-water extract of conifer form (CEW). According to these results, it was proved that *Abies nordmanniana* species could be a potential, sustainable, and renewable drug source, especially considering the impressive antiviral and significant cytotoxic activity potentials.

Keywords: *Abies,* resin, cone, cytotoxicity, antiviral activity, avian coronavirus.

1. Introduction

Abies, an important genus of the Pinaceae family [1,2], are evergreen, tall coniferous trees, containing 51 species native to the northern hemisphere. They are distributed naturally in the temperate and boreal regions of the world and are mainly found in North and Central America, Europe, North Africa, and Asia (Himalayas, South China, and Taiwan) [3,4]. This genus establishes pure or mixed forests in high mountains or lower and even sea-level regions [5]. The history of the *Abies* genus dates back to the Hittites. At present, it is the most common form of traditional treatment, especially against bronchitis and shortness of breath [6]. In some regions, the resin obtained from the trunk of the tree is used as an antiseptic and wound healer, and its cones are used for many purposes as traditional medicine [7,8]. *Abies* genus has also beneficial applications in pharmacological studies owing to its content of bioactive constituents [9]. It has been reported that 277 compounds were found in 19 *Abies* genera in the isolation studies carried out for chemical content determination since 1938. They are mostly terpenoids, triterpenoids, flavonoids, lignans, and a few other constituents including phenols, steroids, fatty acids, and fatty alcohols [9,10]. Several biological activities have also been performed such as antimicrobial [11–13], antitumor [14–16], antibacterial [17–21], antifungal [22], anti-ulcerogenic [23,24], anti-inflammatory [25,26], antihypertensive [23], antitussive [25,27], and CNS (central nervous system) effects [28,29]. Taking into consideration the diversity of use among the folk medicine of the *Abies* genus, the fundamental aim of this study is to elucidate the content of pharmacologically active compounds in the resin of *Abies nordmanniana* subsp. *bornmulleriana* from Bolu and cones of *Abies nordmanniana* subsp. *equi-trojani* from Canakkale of Türkiye. Flavonoid, phenolic, essential oil, and fatty acid components of resin and cones were evaluated by

LC-HRMS, GC-MS, and GC-FID, respectively. Cytotoxicity of the extracts and essential oils were examined against diverse cancer cell lines, namely PC-3, A-549, PANC-1, HepG2, MDA-MB-231, and healthy cell line CCD-34Lu, by MTT assay. Antiviral activities were carried out as virucidal activity against avian coronavirus by *in ovo*.

2. Materials and Methods

2.1. Plant Material

Abies nordmanniana subsp. *bornmulleriana*'s resin samples were collected from their natural habitats along Seben of Bolu-Turkiye (40º24'41"N, 31º34'12"E) and cone forms of *Abies nordmanniana* subsp. *equi-trojani* were collected from their growth areas Bozcaada-Bayramic of Canakkale-Turkiye (39º48'34"N, 26º36'43"E) in June 2021.

2.2. Chemicals

100 mg/L dihydrocapsaicin (97%, Sigma-Aldrich) solution was prepared as a stock solution freshly. The solution was used as an internal standard (IS). In LC-HRMS analysis; following compounds were used as standards for method validation; (-)-epigallocatechin gallate (>97% TRC Canada), (-)-epigallocatechin (>97% TRC Canada), chlorogenic acid (≥95% Sigma-Aldrich), fumaric acid (≥99% Sigma-Aldrich), (-)-epicatechin (≥90 % Sigma-Aldrich), ascorbic acid (≥99% Sigma-Aldrich), (-)-epicatechin gallate (>97% TRC Canada), verbascoside (86.31% Hwi Analytik Gmbh), caffeic acid (≥98% Sigma-Aldrich), (+)-trans taxifolin (>97% TRC Canada), luteolin-7-glucoside (>97% TRC Canada), luteolin-7-rutinoside (>97% Carbosynth limited), rosmarinic acid (≥96% Sigma-Aldrich), vanilic acid (≥97% Sigma-Aldrich), apigenin-7-glucoside (>97% EDQM CS), dihydrokaempferol (>97% Phytolab), hyperoside (>97% TRC Canada), ellagic acid (>97% TRC Canada), quercetin (≥95% Sigma-Aldrich), quercitrin (>97% TRC Canada), myricetin (>95% Carl Roth GmbH + Co), scutellarein (>97% TRC

Canada), caffeic acid phenethyl ester (CAPE, ≥97% Sigma-Aldrich), salicylic acid (≥98% Sigma-Aldrich), naringenin (≥95% Sigma-Aldrich), luteolin (95% Sigma-Aldrich), nepetin (98% Supelco), (-)-sinensetin (>97% TRC Canada), apigenin (>97% TRC Canada), hispidulin (>97% TRC Canada), acacetin (>97% TRC Canada), pyrogallol (98% Biosynth), chrysin (≥96% Sigma-Aldrich).

2.3. Preparation of extracts and essential oils

Collected resin samples were studied with 3 different extraction methods. First resin samples (0.6324 g) were extracted with ethanol (35 mL) with a homogenizer (Silverson, L5M-A, USA) overnight at room temperature. The second samples (3.004 g) were water-distilled for 8h using a Clevenger-type apparatus to obtain essential oils and the third sample (2.996 g) was refluxed with *n*-hexane (500 mL) for 6h using a Soxhlet apparatus. All resin extracts were concentrated under vacuum till dryness at 40 ºC. The collected cone samples were dried under dark conditions, they were then ground and prepared for extraction. They were extracted three times with dichloromethane, acetone, and ethanol (10 mL each) with a homogenizer for 5 h at room temperature, separately. The extract rich in fatty acids was prepared with Soxhlet apparatus using *n*-hexane (500 mL) for 6 h. Also, the essential oil of samples was obtained by water-distillation with a Clevenger apparatus using 10 g crushed dried cones. The essential oils were dried over anhydrous granular sodium sulfate. All extracts and essential oils were stored at +4 °C away from light until chemical analysis and biological activity studies.

2.4. Preparation of samples for LC-HRMS analysis and optimization of the method

50-100 mg of the dried extracts were dissolved in methanol: water (60:40) in a 5 mL volumetric flask. Sonication is used to obtain a clear solution. Then, internal standard

[(100 μL of dihydrocapsaicin solution), (100 mg/L stock solution)] was added and the volume was diluted with the mobile phase. The flask was mixed gently and heated to get a clear solution. The clear solution was filtered via a 0.45 μm Millipore Millex-HV filter. 1 mL of the final solution was transferred into a capped auto-sampler vial. Injection of LC was settled to take 2 μL of the sample for each run. The temperature of the auto-sampler was kept at 15 ºC during the experiments [30–33]. LC-HRMS experiments were succeeded on a Thermo ORBITRAP Q-EXACTIVE mass spectrometry, equipped with a Troyasil C18 column (150 x 3 mm i.d., 5 μm particle size). For the separation of flavonoids and phenolics, the mobile phases A and B were formed of 1% formic acid-water and 1% formic acid-methanol, respectively. The gradient of the mobile phase was programmed as 50% A and 50% B during 1.00 min, 100% B during 5 min, and finally 50% A and 50% B during 9 min. The column temperature was fixed at 22 °C while the flow rate was 0.35 mL/min. [31,34]. Environmental conditions were set as relative humidity 50 ± 15 % RH and temperature 22.0 ± 5.0 °C. Acidified methanol and water gradient were found as the best mobile phase. This phase was also determined as a proper mobile phase for ionization abundance and separation of compounds. By the ESI source, the best ionization of small and relatively polar compounds was observed. The ions between *m/z* 100-900 were scanned in high resolution mode of the instrument. Compounds were identified by comparison of the retention time of standard compounds (in the range of purity 95%-99% See chemicals section). HRMS data of Bezmialem Vakif University, Drug Application and Research Center Library (ILMER). Dihydrocapsaicin (purity 95%) was used as an internal standard for LC-HRMS to reduce repeatability problems caused by external effects. The detailed mass parameters of each compound were given in Supporting Information in Table SI-1 [30,31,34,35].

2.5. GC-FID Analysis

Methyl esters of fatty acids were analyzed with Agilent GC-FID combined system using SUPELCO SP TM-2560 column (100 m × 0.25 mm ×0.20 μm). 5 mg of each extract was mixed with 2 mL 2 M potassium hydroxide in methanol and vortexed. 2 mL of isooctane was added on the mixture and vortexed again. Then, the samples were centrifuged at 3000 rpm for 4 min. Approximately 1 mL of the supernatant was vialed and 1 μL of the sample was injected into the GC-FID system. The oven temperature was programmed to start from 140 °C and increase by 4 °C every 5 min so that the final temperature reaches 240 °C and hold there for 5 min. Helium was used as carrier gas at a constant flow rate (1mL/min). Supelco Fame Mix 37 library data was used for the identification of compounds. The standard containing *n*-alkanes (Supelco 49452-U) was used for the calculation of relative retention indices.

2.6. GC-MS Analysis

The essential oil analysis was carried out using HP 6890 Series GC system, equipped with an INNOWAX column (Hewlett Packard No: 19091N-116). The dimension of the column was 60 m × 0.32 mm i.d., 0.25 μm film thickness. The temperature of the injection part was settled at 150 °C. The essential oils were diluted with *n*-hexane and 1μL of each sample was injected in the split mode with a split ratio of 50:1. The oven temperature was programmed to initiate 60 °C for 4 min, then 4 °C/min to 230 °C and to be held there for 5 min. Pure helium was used as carrier gas with a flow rate of 0.7 mL/min.

2.7. *In vitro* cytotoxicity assay

In vitro cytotoxicity assay screening of the extracts and essential oils based on metabolic cell viability was done using a modified MTT [3-(4,5-Dimethyl-2-thiazolyl)-2,5-

diphenyl-2H-tetrazolium bromide)] assay [36] that is based on the cleavage of the yellow tetrazolium salt, which forms water-insoluble, purple formazan crystals that affect the mitochondrial reductase activity of viable cells. For this purpose, 1×10^5 cells/well cells were seeded in 96-well plates in Dulbecco's modified Eagle's F12 medium (DMEM/F12) supplemented with 10% fetal bovine serum (FBS) and 0.1% penicillin/streptomycin at 100 µL volume and kept in the incubator overnight. Then, the extracts were dissolved in DMSO and diluted with PBS into the desired concentration (CH, CE, CD, CA, CEW, RH, RE, and REO (50, 5, and 0.5 µg/mL) and CEO (50, 5, 0.5, 1, 0.1, and 0.01 µg/mL) were added to the cells for 48 h at 37 °C. As a positive control, doxorubicin (20, 2, and 0.2 µg/mL) was used. After 48 h, the MTT assay was applied to determine cell viability. For MTT assays, 20 µL of MTT solution (from the stock concentration of 2.5 mg/mL) was added to all wells and incubated at 37 ºC for 3 h. Then, 150 µL DMSO was added to all wells. Then, the optical density (OD) was measured at 570 nm. The IC_{50} value was calculated using the GraphPad Prism 8 program based on the calculated percent viability and absorbance values. The percentage of viability was determined by the following formula:

$$\%Viable\ cells == \frac{[(absorbance\ of\ treated\ cells) - (absorbance\ of\ blank)]}{[(absorbance\ of\ control) - (absorbance\ of\ blank)]} \times 100$$

2.8. *In ovo* antiviral activity assay

2.8.1. Preparation of virus

Between nine and eleven days old specific pathogen-free embryonated chicken eggs (SPF-ECE) and 1% chicken RBC (red blood cell) were bought from Bornova Veterinary Control Institute in Izmir, Turkiye, for the antiviral activity assay. The infectious bronchitis virus (IBV) D274 strain which was first isolated from the Netherlands was

graciously given by Dr. Fethiye Çöven [37]. The embryo infectious dose (EID_{50}) of the IBV was determined to originate from Reed and Muench (1938) method [38] which is the gold standard method for calculating EID_{50}. The stock virus was diluted between 10^{-1} and 10^{-12} by using PBS and each dilution was inoculated into the chorioallantoic fluid (CAF) of four SPF-ECEs. After 48 h incubation, the CAF was collected from each egg, and a hemagglutination test was performed. According to the number of virus infected eggs, the EID_{50} titer of the stock virus was determined by using Reed and Muench formula. 100 EID_{50}/0.1 mL virus solution which was used in this experiment was diluted from stock virus by using phosphate-buffered saline (PBS) [39].

2.8.2. Inoculation of sample-virus mixture

Samples dissolved in DMSO were diluted with PBS to obtain the final concentrations of 5 μg/g and 10 μg/g. Enfluvir was dissolved in DMSO-pure water (1:9) for use as an antiviral agent and administered at a final concentration that contained less than 5% DMSO. The stock virus was mixed with the sample at a ratio of 1:1 and 0.1 mL of each mixture was injected into the chorioallantoic fluid of SPF-ECEs. After injection, SPF-ECEs were incubated for 48 h at 37 °C, with 55% humidity [40].

2.9. Hemagglutination (HA) assay

Following the WOAH procedure (2018), two-fold dilutions of CAF were used in the HA experiment to evaluate the viral titer. The HA assay was conducted using V-bottom 96-well microplates, and PBS was utilized for dilution [41]. To perform the test, 25 µL PBS was first added into all wells. Then, 25 µL CAF collected from each egg separately, was placed in the first column and diluted 2-fold to the last column. To have the same volume for all wells, a 25 µL sample was discarded to the last column. After 2-fold dilution, 25

μL 1% (v/v) chicken red blood cells (RBCs) were added into all wells, and plates were incubated for around 45 minutes at room temperature. HA activity was determined by the presence or absence of a tear shape. Lace-like formation represents HA positive, while tear-shaped (or button-shaped) formation is interpreted as HA negative. The Local Ethical Committee of Animal Experiments at Ege University authorized the HA assay methodology to determine antiviral activity (Date: 2020 No: 2020-051).

3. Results and discussion

In this work, resin samples from the trunk of *Abies nordmanniana* subsp. *bornmulleriana* and the cone from lateral branches of *Abies nordmanniana* subsp. *equi-trojani* from Türkiye were examined, separately. The samples were chosen due to their ethnobotanical features such as the folk and traditional usage of materials in certain regions. The most common form of traditional use of resin from *Abies nordmanniana* subsp. *bornmulleriana* is used as gum in Bolu, especially against bronchitis and shortness of breath. The most common public use of the cone from *Abies nordmanniana* subsp. *equi-trojani* is the use of water vapor or jam obtained from the cones against lung diseases such as bronchitis and COPD (chronic obstructive pulmonary disease) in Canakkale.

Abies species have been used as traditional medicine and food ingredients due to their specific odor and biological activities. Former studies, that investigated the chemical composition of *Abies* extracts, have mostly identified monoterpenes and monoterpenoids in resin and essential oil, triterpenoids in needles and bark [42]. Besides, many extracts have been identified as rich sources of flavonoids and phenolics [9]. Not only the extracts but also the essential oils of these species have been studied and are widely used especially in the treatment of colds, as well as indigestion, venereal diseases, and lung disorders [11]. Essential oils of *Abies* genus exhibited considerable diversity in both the

composition and percentages of compounds between species and from tree to tree [43]. The chemical content of essential oils of cone and resin were examined separately, in this study (Table 1). Seventeen compounds comprising 99.99% of the total oil were identified of which limonene (33.50%) and *α*-pinene (28.79%) were recognized as major constituents of cone. Limonene is popularly used for digestive problems, abdominal pain, and as a cough suppressant [44] and is considered a safe method of treatment with low toxicity since it is rapidly absorbed and metabolized without posing a mutagenic, carcinogenic or nephrotoxic risk in the gastrointestinal tract [45]. In addition, its use in respiratory tract diseases among the public was reported by Hirota et al. [46], and whether it can reduce the allergic airway; aimed to evaluate whether it could improve asthma symptoms and demonstrate that it has a potent therapeutic effect on allergic airway and asthma. Limonene also offers a range of biological activities described in the literature, such as antidepressant [47], antinociceptive [48], antidiabetic [49], antiulcerogenic activity [50], and anticancer activity both *in vitro* and *in vivo* [51]. The other major compound, *α*-pinene, in the essential oil of the conifer form, especially with its woody and pine scent, is used for respiratory problems traditionally. It has a variety of biological activities such as gastro-protective [52], insecticide, anti-inflammatory, antiviral [53], neuroprotective [54], and antifungal [55]. Cone essential oils composition also includes *β*-pinene (3.55%) which is the isomer of *α*-pinene. The percentage of *α*-pinene in cone essential oil is considerably higher than in *β*-pinene. Comparison of resin and cone essential oils exhibited quite different chemical contents. While the major compounds in the essential oil components of the conifer form were limonene (33.50 %) and *α*-pinene (28.79%), verbenone (18.16%) and 2,5-dimethyl furan (10.79%) were the main components in the resin essential oil (Table 1). Verbenone, a natural monoterpenoid

bicyclic ketone, is an essential component of essential oils [54]. Due to its pleasant aroma, it is used in perfumes, herbal teas, aromatherapy, spices, and herbal medicines. It is also used in natural control against a pest that damages pine trees and as an antimicrobial agent [56].

The hexane extract of both cone and resin was prepared with the Soxhlet apparatus, 5 different fatty acids were detected for gum, while this amount is 4 for cones. The amount of these fatty acids is in the form of oleic acid (41.00%), palmitic acid (20.81%), linoleic acid (15.66%), stearic acid (14.24%) and behenic acid (8.28%). These amounts change in coniferous form as oleic acid (55.74%), palmitic acid (20.83%), stearic acid (12.49%), and linoleic acid (10.94%) (Table 2). Oleic acid is the most abundant compound for both gum (41.00%) and cone (55.74%) extracts. Oleic acid is known for both its antiviral and cytotoxic activities [57]. The antitumor activity of oleic acid has been investigated by Venepally et al. (2017), and it is shown that oleic acid is the most promising fatty acid against A-549, PC-3, MDA-MB-231, HepG2 [58]. In addition, it is known that oleic acid has an anti-inflammatory effect by reducing adhesion molecules, reducing autoimmune disorders, lowering blood pressure, and reducing the risk of cancer [59]. The evident variation of content was also observed in the amount of linoleic acid. Besides, behenic acid was detected only in the hexane extract of resin. Differentiation of the components of these two extracts caused differences in their cytotoxic activities in some cell lines. In PANC-1 cell lines, the IC_{50} value could not be detected in resin hexane extract, while this value was 48.6± 5.28 in cones. The main difference was observed in the HepG2 cell line. Both extracts showed more activity compared to the control group doxorubicin, while the activity of the resin (17.725± 2.09) extract was better than the cones (36.67± 3.68). Samra et al., (2021) tested the activity of behenic acid against the HepG2 cancer line and proved

that it showed potential cytotoxic activity compared to doxorubicin [60]. The fact that the resin is more active against HepG2 cell lines may be related to its behenic acid content. In vitro cytotoxicity assays were performed based on metabolic cell viability using the MTT test which is still one of the most useful and well-liked viability assays. The MTT assay uses mitochondrial reductase to change the water-soluble yellow dye MTT into an insoluble purple formazan. The concentration of the formazan is then evaluated by optical density at 570 nm after it has been solubilized [28]. Doxorubicin was used as a positive control to evaluate the adequate cytotoxic dose of the samples on cancerous cell lines (See Sup. Info Figure. S1-S6).

All *Abies nordmanniana* subsps' IC_{50} values were calculated for cells in the measurable range (0.5-50 μg/mL). The percent vitality graph according to the MTT test result is given in Table 3. According to the results, none of the samples inflicted cytotoxic effects on CCD-34Lu, MDA-MB-231, PC-3, and A-549 cell lines even at the highest concentration of 50 μg/ml. However, most of them (CH, RH, CEW, CD, and CA) had a cytotoxic effect on HepG2 cells. The IC_{50} value of RH (17.725± 2.09 μg/mL) in HepG2 cells was found to be almost nearly twice more cytotoxic when compared to doxorubicin (36.85 ± 0.02 μg/mL) and also only CH in the concentration of 48.6 ± 5.28 μg/mL showed a cytotoxic effect on PANC-1 cells. As the healthy cell line model, we used CCD-34Lu cells to evaluate the samples' cytotoxic side effects on them. Based on the results, various kinds of samples may not have any cytotoxic influence on CCD-34Lu cells, which demonstrated *Abies nordmanniana* subsp. would be potential candidate molecules for future cancer studies.

The phenolic and flavonoid contents of resin ethanol extract, cone ethanol, ethanol-water (1:1), dichloromethane, and acetone extracts were determined by LC-HRMS (Table 4).

In the ethanol extract of resin, eleven different phenolics and flavonoids were determined. The major compound was fumaric acid (57050.00 mg/kg) which is not detected in any extracts of conifer form. Following fumaric acid, other common compounds in resin ethanol extracts, are salicylic acid (282.45 mg/kg) and rosmarinic acid (158.69 mg/kg), respectively.

Ethanol-water extract of the cone has the most diverse chemical contents, with eighteen phenolics and flavonoids. The most abundant of these were ascorbic acid (2508.50 mg/kg), followed by vanillic acid with 1691.89 mg/kg and quercitrin with 283.28 mg/kg. The seventeen different compounds were detected in the ethanol extract of the cone and the major compound was detected as vanillic acid (1152.93 mg/kg). In comparison of the ethanol and ethanol-water extracts were compared, no significant change was observed in the amount of vanillic acid, but the presence of water had a significant effect on the increase of the amount of ascorbic acid, a type of water-soluble vitamin. The most abundant phenolics in the dichloromethane extract of the cone are rosmarinic acid (130.13 mg/kg), salicylic acid (103.91 mg/kg), and caffeic acid (38.10 mg/kg). Cone acetone extract has the most diverse content of eighteen compounds, just like the ethanol-water extract, but they are quite different from each other in quantity and content. The quantity major compound, vanillic acid, was 1554.36 mg/kg, and salicylic acid and ascorbic acid were following it with 352.66 mg/kg and 56.54 mg/kg, respectively.

Antiviral activity against the avian coronavirus was used to evaluate the antiviral potential of cone and resin extracts. After 48 hours incubation of SPF-ECEs with virus-extract embryos were examined and CAF was collected to perform HA assay. Egg mortality, % mortality, and $\log_2$ HA titer are shown in Table 5 and Figure 1. The virucidal antiviral activity of several *Abies nordmanniana* subsp. samples at doses of 5 μg/g and 10 μg/g

were assessed. When comparing SPF-ECEs groups, extracts of *Abies nordmanniana* subsp. displayed concentration-dependent antiviral activity. In the virus control group, there was only one dead embryo among all SPF-ECEs, as shown by the daily viability check of the embryos (Table 5) (See Sup. Info. Fig. SI-7.). The reason for the death may be considered as some injection manipulation during virus inoculation when taking into account healthy embryos in the virus control group. The HA titer of virus control was calculated as 2048, which means the virus was replicating. Based on the results, enfluvir reduced the $\log_2$ HA titer compared to the control group dose-dependently by 1 $\log_2$ HA titer at 10 μg/g. The acetone extract of the cone (CA), exhibited virucidal activity by reducing the $\log_2$ HA titer at 10 μg/g, but when the embryos were investigated there was also one dead embryo in tested four SPF-ECE's. Remarkably, the cone dichloromethane extract (CD), and cone ethanol extract (CE) showed significant inhibition of the virus at a concentration of 10 μg/g, without having any toxicity effect on the SPE-ECE's. Both extracts reduced the HA titers 10-fold based on $\log_2$ HA titers at 10 μg/g. The most effective HA titer inhibition was observed in ethanol-water extract of conifer form (CEW), the sample decreased the virus activity in comparison with virus control at both concentrations of 5 μg/g and 10 μg/g. However, the concentration of 10 μg/g exhibited toxicity on embryos with three dead embryos in a group of four eggs which means a high concentration of CEW can be toxic while a low concentration is non-toxic for chicken embryos. The rest of the extracts had slight virucidal effects on the SPE-ECE's with an average reduction of 2-fold based on $\log_2$ HA titers at 5 μg/g and 10 μg/g. The most abundant component in the cone ethanol-water extract (CEW) was ascorbic acid. Ascorbic acid (Vitamin C) in high doses is proven to be virucidal. Vitamin C reduced the viral load of Ebstein-Barr virus (EBV)-infected cells, based on an experimental model

[61]. The protective and mitigating effects of it against the virus may have been beneficial in decreasing the virus activity [62]. In some *in vivo* studies, the contradictory ability of vitamin C to generate reactive oxygen species through the reduction of transition metals is highlighted. The significant toxicity of CEW in SPF-ECEs at 10 μg/g concentration can be attributed to vitamin C's potential for generating reactive oxygen species. Despite the data based on *in vitro* studies, there is a lack of clinical evidence to support the theory which suggests that vitamin C may be effective in reducing viral load. The use of vitamin C as an additional therapy for serious infections caused by the flu, RSV, herpes, and other prevalent viral disorders must be thoroughly evaluated in clinical trials [61]. Dichloromethane, acetone, and ethanol extracts have a considerable amount of salicylic acid. In 2020, Geiger et al. proved that salicylic acid interferes with viral replication of SARS-CoV-2 [63]. This suggests that salicylic acid, a metabolite of the acetylsalicylic acid which is the active compound of Aspirin, has a potential virucidal effect against IBV D274. The main chemical content difference between dichloromethane, ethanol, and acetone extracts is fumaric acid. While fumaric acid was observed as the main component with the highest percentage in the ethanol resin extract, it was not detected in the other extracts. Considering its synergistic effect with salicylic acid, fumaric acid may have suppressed the antiviral effect of salicylic acid. According to *in ovo* results in our laboratory, enfluvir did not exhibit the highest antiviral activity against IBV. This might be explained by the fact that the majority of antivirals only work against specific viruses. In conclusion, three *Abies nordmanniana subsp*. samples, CD, CA, and CE had significant virucidal effects on SPF-ECEs at the concentration of 10 μg/g. While CEW had the most potent antiviral effect against the virus, 10 μg/g concentration of the extract displayed a damaging effect on SPF-ECEs, according to dose-dependent fatalities. The *in*

ovo antiviral activity results suggested that *Abies nordmanniana* subsp. samples had strong virucidal effects against the avian coronavirus strain D274 of the infectious bronchitis virus (IBV). However, because most antivirals are exclusively effective against a certain virus, we specifically want to draw your attention to the findings. Further and extended studies, which include testing the extracts' virucidal effects against different viruses, will provide a broader perspective on the virus inhibition mechanism of *Abies nordmanniana* subsp. extracts. It is important to prioritize research on natural products since the need for fast produced natural-derived supplements is proven with the emergence of SARS-Cov-2. With further research, the understanding of natural sources will increase and the production of naturally derived products will be accelerated.

4. Conclusions

Abies nordmanniana spp. has been widely used as a traditional product among the public since ancient times. For the treatment of viral diseases and cancer, which are some of the biggest problems of recent years, plants and the products obtained from them have become an undeniable solution in the search for various sources and potential drugs [64,65]. In this study, it was proved that *Abies nordmanniana* spp. could be a potential drug source, especially considering the impressive antiviral activity of the green product ethanol-water extract and the significant cytotoxic activity of the fatty acid extract against HepG2 cell lines compared to the control group doxorubicin. The cones and resin of this tree are evergreen and perennial, making it also a great potential for sustainable products.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

N.B. Sarikahya, A. Nalbantsoy: Conceptualization and supervision; G.S. Okkali, G. Gucur: Discussion of chemical analysis; F.O. Coven, S. Zeinali, A.D. Caglar, E. Uyar: Biological activity investigation and discussion, LC-HRMS analysis: A.C. Goren, writing—original draft: N.B. Sarikahya. All authors have read and agreed to the published version of the manuscript.

Supporting Information

Supporting information for this article is available as Word file.

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TABLES

Table 1. The essential oil composition (%) of resin from *A. nordmanniana* subsp. *bornmulleriana* and cone from *Abies nordmanniana* subsp. *equi-trojani.*

RRI[a]	Compounds	Cone[b]	Resin[b]	Identification methods[c]
1023	*α*-pinene	28.79	-	GC-FID, GC-MS
1190	*β*-pinene	3.55	-	GC-FID, GC-MS
1197	limonene	33.50	-	GC-FID, GC-MS
1557	1-octanol	1.16	-	GC-FID, GC-MS
1647	*trans*-pinocarveol	2.86	2.24	GC-FID, GC-MS
1713	*cis*-verbenol	3.17	-	GC-FID, GC-MS
1692	*α*-terpineol	1.96	7.72	GC-FID, GC-MS
1699	verbenone	3.27	18.16	GC-FID, GC-MS
1728	carvone	3.03	-	GC-FID, GC-MS
1783	myrtenol	1.49	6.88	GC-FID, GC-MS
1829	*trans* (+)-carveol	3.15	8.11	GC-FID, GC-MS
1844	*p*-cymen-8-ol	1.31	7.04	GC-FID, GC-MS
1859	*cis*-carveol	1.74	-	GC-FID, GC-MS
1967	caryophyllene oxide	1.19	5.90	GC-FID, GC-MS
2265	limonene glycol	5.17	-	GC-FID, GC-MS
2312	*trans*-sobreol	2.98	2.17	GC-FID, GC-MS
1960	2,5-diethylfuran	-	10.79	GC-FID, GC-MS
2185	*Z*-3-pinen-2-ol	-	1.90	GC-FID, GC-MS
2118	*p*-mentha-1,5-dien-8-ol	-	5.7	GC-FID, GC-MS
2190	*p*-mentha-1,7-dien-8-ol	-	4.05	GC-FID, GC-MS
2250	(E,E)-7,11,15, trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene	-	9.71	GC-FID, GC-MS
2298	nerolidol	-	3.39	GC-FID, GC-MS

2294	Spiro[alpha,alpha-dimethylcyclohexane-1-methanol-1,2-oxirane] isomer	-	2.59	GC-FID, GC-MS
2386	15-crown-5	-	1.54	GC-FID, GC-MS
2410	21-crown-7	-	2.01	GC-FID, GC-MS
	undefined	1.67	0.1	GC-FID, GC-MS

[a]Relative retention indices were calculated against *n*-alkanes (Supelco 49452-U) on the HP-Innovax (19091N-116) capillary column. [b]% values were calculated from FID data.[c]GC and GC-MS identifications based on the basis of computer matching of the mass spectra of the peaks with the Nist-Wiley and Arge-Far essential oil libraries.

Table 2. Fatty acid composition (%[a]) of hexane extracts of resin from *A. nordmanniana* subsp. *bornmulleriana* and cone from *Abies nordmanniana* subsp. *equi-trojani*

Fatty acids[b]	**Resin**	**Cone**
16:0	20.81	20.83
18:0	14.24	12.49
18:1 n9c	41.00	55.74
18:2 n6c	15.66	10.94
22:0	8.28	-

[a]Percentage of total fatty acids.
[b]Carbons and double bond numbers of fatty acids

Table 3. IC_{50} values for cytotoxic activities of *Abies nordmanniana* subsp. on different cell lines in μg/mL.

	CCD-34Lu	**MDA-MB-231**	**PANC-1**	**PC-3**	**HepG2**	**A549**
CH	>50	>50	48.6 ± 5.28	>50	36.67±3.68	>50
CE	>50	>50	>50	>50	>50	>50
CD	>50	>50	>50	>50	29.08 ± 5.61	>50
CA	>50	>50	>50	>50	43.95 ± 0.3	>50

CEW	>50	>50	>50	>50	32.53 ± 2.25	>50
CEO	>50	>50	>50	>50	>50	>50
RH	>50	>50	>50	>50	17.725± 2.09	>50
RE	>50	>50	>50	>50	>50	>50
REO	>50	>50	>50	>50	>50	>50
Doxorubicin	8.012 ± 0.01	15.22 ± 0.02	5.207 ±0.01	4.23± 0.02	36.85 ± 0.02	1.21 ± 0.01

*CH: hexane extract of cone, CE: ethanol extract of cone, CD: dichloromethane extract of cone, CA: acetone extract of cone, CEW: ethanol-water (1:1) extract of cone, CEO: Essential oils of cone, RH: hexane extract of resin, RE: ethanol extract of resin, REO: essential oils of resin.

Table 4: Phenolic and flavonoid contents of (mg/kg extract) resin ethanol extract, cone ethanol, ethanol-water (1:1), dichloromethane, and acetone extracts of *Abies nordmanniana* subsp.

	RE	**CEW**	**CE**	**CD**	**CA***	**U % ****
Ascorbic acid	71.46	2508.50	36.27	18.98	56.54	3.94
Fumaric acid	57050.00	<LOD	<LOD	<LOD	<LOD	2.88
(-)-Epicatechin gallate	<LOD	3.33	<LOD	<LOD	<LOD	3.05
Verbascoside	3.22	<LOD	<LOD	6.60	1.13	2.93
Chicoric acid	<LOD	10.83	7.18	<LOD	11.86	2.28
Caffeic acid	40.12	8.00	10.14	38.10	19.48	3.74
(+)-*trans* taxifolin	<LOD	5.56	4.11	0.15	6.07	3.35
Vanilic acid	<LOD	1691.89	1152.93	<LOD	1554.355	3.49
Luteolin 7-glucoside	7.88	<LOD	<LOD	9.19	<LOD	4.14
Rutin	<LOD	47.33	1.53	1.20	1.69	3.07
Rosmarinic acid	158.69	32.78	24.05	130.13	35.44	3.77
Hyperoside	<LOD	263.44	51.78	<LOD	44.12	3.46
Dihydrokaempferol	<LOD	21.72	19.73	1.54	24.28	2.86
Apigenin 7-glucoside	1.61	5.00	0.49	1.84	0.92	3.59
Quercitrin	<LOD	283.28	59.73	<LOD	49.76	3.78
Myricetin	<LOD	18.22	4.33	<LOD	6.35	4.18
Quercetin	1.25	63.44	51.45	0.30	65.51	2.95
Salicylic acid	282.45	259.89	270.68	103.91	352.66	1.89
Naringenin	3.04	37.44	37.92	9.79	50.96	4.20
Apigenin	5.55	2.61	1.75	0.41	2.12	2.87

Hispidulin	<LOD	1.44	1.75	<LOD	1.69	3.41

*RE: Ethanol extract of resin (*A. nordmanniana* subsp. *bornmulleriana*) , CEW: Ethanol-water (1:1) extract of cone, CE: Ethanol extract of cone, CD: Dichloromethane extract of cone, CA: Acetone extract of cone (*A. nordmanniana* subsp. *equi-trojani*),
**U%: The percent relative uncertainties of the reported compounds

Table 5. The virucidal effects of *Abies nordmanniana* subsp. extracts on IBV after 48h incubation.

Sample*	Concentration μg/g	Egg mortality	Mortality%	HA titer (Mean)	HA titer (log_2) (Mean)
Positive control (Only virus)		1/4	25	2048	11
Negative control (Untreated ECE)		0/4	0	0	0
Vehicle control (5% DMSO)		0/4	0	2048	11
CH	5 μg/g	1/4	25	512	9
	10 μg/g	0/4	0	1024	10
CE	5 μg/g	1/4	25	512	9
	10 μg/g	1/4	0	2	1
CD	5 μg/g	0/4	0	512	9
	10 μg/g	0/4	0	2	1
CA	5 μg/g	0/4	0	256	8
	10 μg/g	1/4	25	2	1
CEW	5 μg/g	0/4	0	2	1
	10 μg/g	3/4	75	2	1
CEO	0.5% v/v	0/4	0	1024	10
	5% v/v	1/4	25	1024	10
RH	5 μg/g	1/4	25	512	9
	10 μg/g	0/4	0	512	9
RE	5 μg/g	2/4	50	256	8
	10 μg/g	1/4	25	512	9
REO	5 μg/g	1/4	25	1024	10

	10 μg/g	1/4	25	1024	10
Enfluvir	5 μg/g	1/4	25	4096	12
	10 μg/g	0/4	0	1024	10

*CH: hexane extract of cone, CE: ethanol extract of cone, CD: dichloromethane extract of cone, CA: acetone extract of cone, CEW: ethanol-water (1:1) extract of cone (*A. nordmanniana* subsp. *equi-trojani*), CEO: Essential oils of cone, RH: hexane extract of resin, RE: ethanol extract of resin, REO: essential oils of resin (*A. nordmanniana* subsp. *bornmulleriana*)

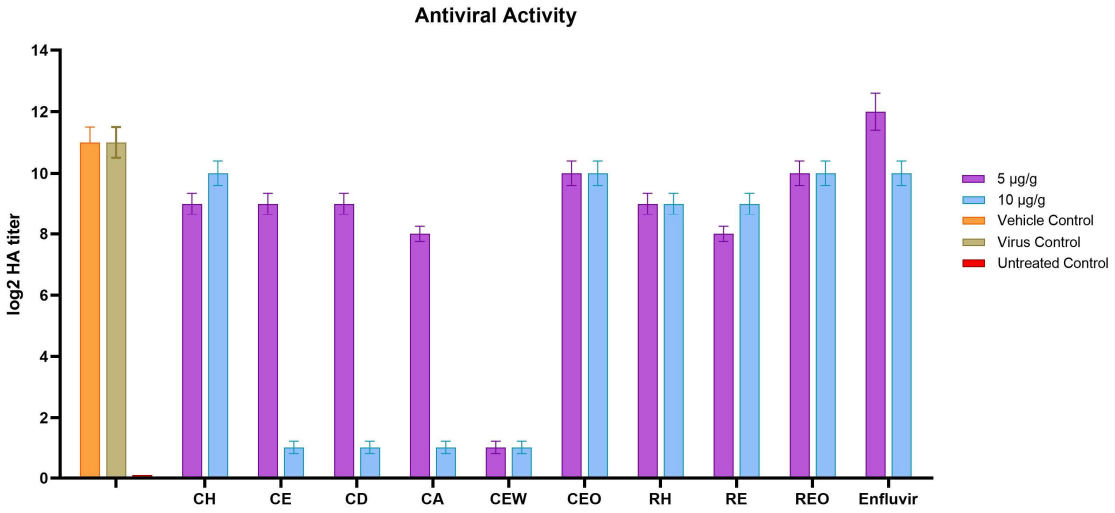

Figure 1. HA titers of SPF-ECE embryos after incubation with CH: hexane extract of cone, CE: ethanol extract of cone, CD: dichloromethane extract of cone, CA: acetone extract of cone, CEW: ethanol-water (1:1) extract of cone, CEO: essential oil of cone, RH: hexane extract of resin, RE: ethanol extract of resin, REO: essential oil of resin samples, enfluvir and control groups on IBV after 48h incubation.

Supporting Information

Table S1. Mass parameters and linear regression equation of compounds[a-b].

Compounds	Molecule Formula	m/z	Ionization Mode	Linear interval	Linear regression equation	LOD/ LOQ	R^2	Recovery
Ascorbic acid	$C_6H_8O_6$	175.0248	Negative	0.5-10	y=0.00347x-0.00137	0.39/1.29	0.9988	96.2
(-)-Epigallocatechin	$C_{15}H_{14}O_7$	307.0812	Positive	0.3-5	y=0.00317x+0.000443	0.17/0.57	0.9947	102.22
(-)-Epigallocatechin gallate	$C_{22}H_{18}O_{11}$	459.0922	Positive	0.3-7	y=0.00182x+0.000026	0.1/0.33	0.9989	94.76
Chlorogenic acid	$C_{16}H_{18}O_9$	353.0878	Negative	0.05-10	y=0.00817x+0.000163	0.02/0.06	0.9994	96.68
Fumaric acid	$C_4H_4O_4$	115.0037	Negative	0.1-10	y=0.00061x-0.0000329	0.05/0.17	0.9991	97.13
(-)-Epicatechin	$C_{15}H_{14}O_6$	289.0718	Negative	0.05-10	y=0.0172x+0.0002269	0.01/0.03	0.9993	95.66
(-)-Epicatechin gallate	$C_{22}H_{18}O_{10}$	441.0827	Negative	0.05-10	y=0.00788x-0.0001875	0.01/0.03	0.9995	96.54
Verbascoside	$C_{29}H_{36}O_{15}$	623.1981	Negative	0.1-10	y=0.00758x+0.000563	0.03/0.1	0.9995	96.19
Caffeic acid	$C_9H_8O_4$	179.0350	Negative	0.3-10	y=0.0304x+0.00366	0.08/0.27	0.9993	94.51
(+)-*trans* taxifolin	$C_{15}H_{12}O_7$	303.0510	Negative	0.3-10	y=0.0289x+0.00537	0.01/0.03	0.9978	91.66
Luteolin 7-glucoside	$C_{21}H_{20}O_{11}$	447.0933	Negative	0.1-7	y=0.0162x+0.00226	0.01/0.03	0.9961	96.31
Rutin	$C_{27}H_{30}O_{16}$	609.1461	Negative	0.05-10	y=0.00329x-0.00005576	0.01/0.03	0.999	96.97
Rosmarinic acid	$C_{18}H_{16}O_8$	359.0772	Negative	0.05-10	y=0.00717x-0.0003067	0.01/0.03	0.9992	99.85
Dihydrokaempferol	$C_{15}H_{12}O_6$	287.0561	Negative	0.3-7	y=0.0756x+0.0118	0.01/0.03	0.995	95.37
Apigenin 7-glucoside	$C_{21}H_{20}O_{10}$	431.0984	Negative	0.3-7	y=0.0246x+0.00306	0.01/0.03	0.9962	96.07
Ellagic acid	$C_{14}H_6O_8$	300.9990	Negative	0.05-10	y=0.0085x-0.000612	0.03/1	0.9994	101.49
Quercitrin	$C_{21}H_{20}O_{11}$	447.0933	Negative	0.05-10	y=0.0179+0.0003331	0.01/0.03	0.999	97
Myricetin	$C_{15}H_{10}O_8$	317.0303	Negative	0.1-10	y=0.0202x+0.00165	0.01/0.03	0.9993	100.1
Scutellarein	$C_{15}H_{10}O_6$	285.0405	Negative	1+7	y=0.028x+0.00845	0.01/0.03	0.9983	95.86
Quercetin	$C_{15}H_{10}O_7$	301.0354	Negative	0.1-10	y=0.0509x+0.00467	0.01/0.03	0.9978	96.41
Salicylic acid	$C_7H_6O_3$	137.0244	Negative	0.3-10	y=0.0361x+0.00245	0.01/0.03	0.9982	92.88
Naringenin	$C_{15}H_{12}O_5$	271.0612	Negative	0.1-10	y=0.0281x+0.00182	0.01/0.03	0.9995	86.65
Luteolin	$C_{15}H_{10}O_6$	285.0405	Negative	0.1-10	y=0.117x+0.00848	0.01/0.03	0.9981	96.98
Apigenin	$C_{15}H_{10}O_5$	269.0456	Negative	0.3-10	y=0.104x+0.0199	0.01/0.03	0.9998	81.55
Hispidulin	$C_{16}H_{12}O_6$	301.0707	Positive	0.05-10	y=0.02614x+0.0003114	0.01/0.03	0.9993	98.36

Chrysin	$C_{15}H_{10}O_4$	253.0506	Negative	0.05-7	y=0.0964x-0.0002622	0.01/0.03	0.999	87.92
Acacetin	$C_{16}H_{12}O_5$	283.0612	Negative	0.05-7	y=0.046x+0.0001875	0.01/0.03	0.9995	87.52
Pyrogallol	$C_6H_6O_3$	125.0244	Negative	0.5-10	y=0.5283x-0.06866	0.35/1.17	0.9954	98.38
Dihidro Kafeik Asit	$C_{18}H_{23}NO_7$	366.1547	Negative	0.5-10	y=0.06102x-0.00989	0.14/0.46	0.999	100.77
Chrysoeriol	$C_{16}H_{12}O_6$	299.0561	Negative	0.5-10	y=0.1023x-0.002224	0.15/0.5	0.9974	96.42
Sclareol	$C_{20}H_{36}O_2$	273.2575	Positive	0.5-10	y=0.3233x+0.0004172	0.12/0.39	0.9984	100.59

[a] Dihydrocapsaicin used as an internal standard
[b] LOD: limit of detection; LOQ: limit of quantification

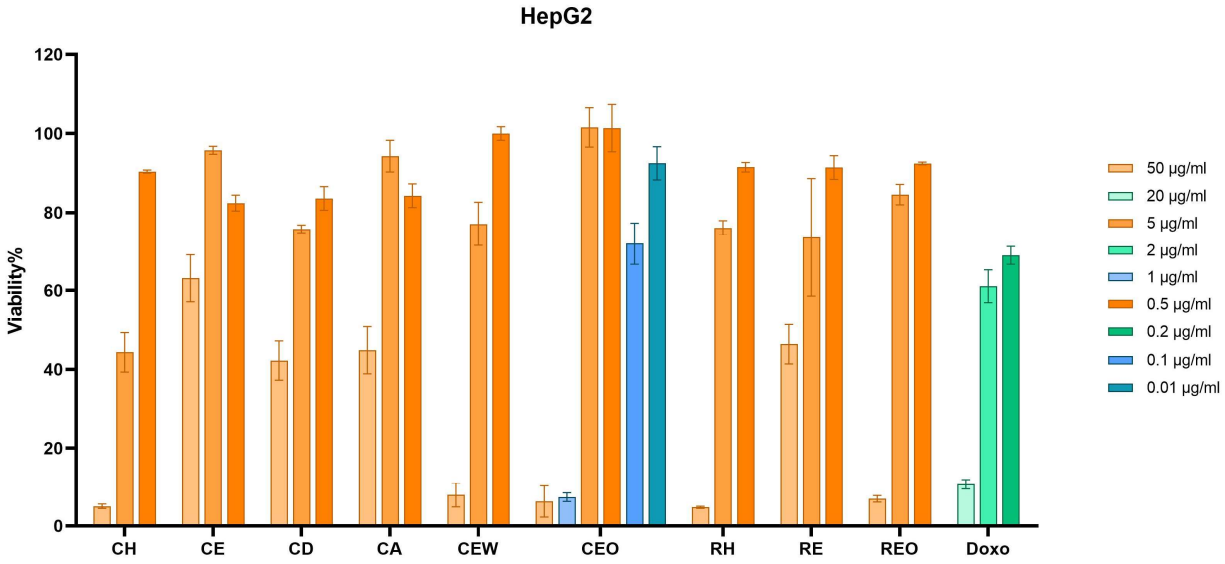

Figure S1. The cell viability of CH: hexane extract of cone, CE: ethanol extract of cone, CD: dichloromethane extract of cone, CA: acetone extract of cone, CEW: ethanol-water (1:1) extract of cone, CEO: essential oil of cone, RH: hexane extract of resin, RE: ethanol extract of resin, REO: essential oil of resin samples and doxorubicin in HepG2 cell line.

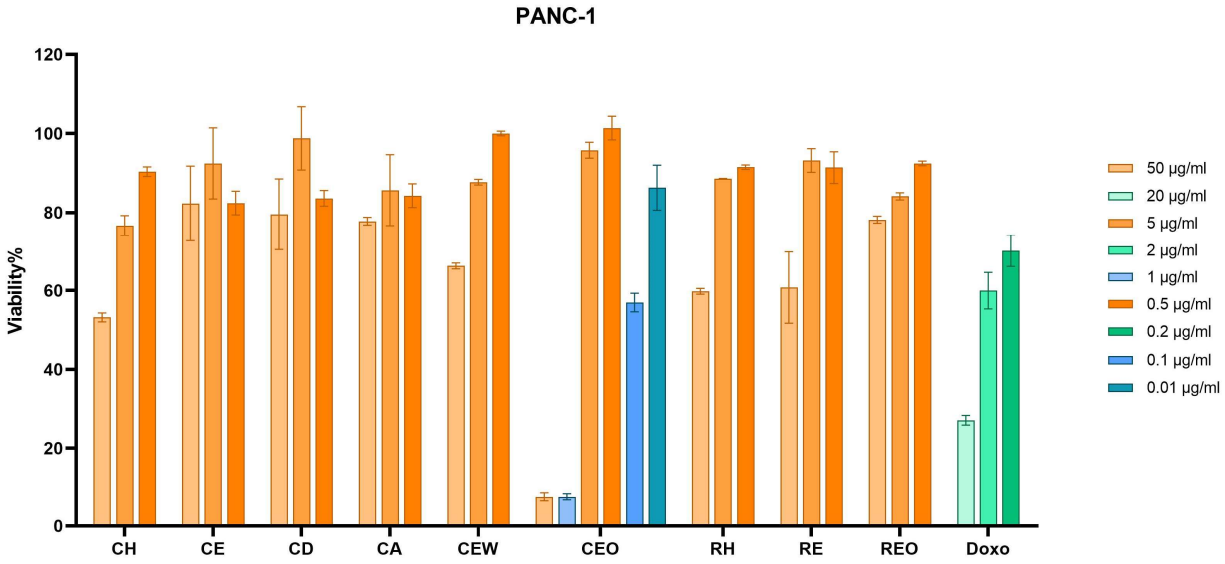

Figure S2. The cell viability of CH: hexane extract of cone, CE: ethanol extract of cone, CD: dichloromethane extract of cone, CA: acetone extract of cone, CEW: ethanol-water (1:1) extract of cone, CEO: essential oil of cone, RH: hexane extract of resin, RE: ethanol extract of resin, REO: essential oil of resin samples and doxorubicin in PANC-1 cell line.

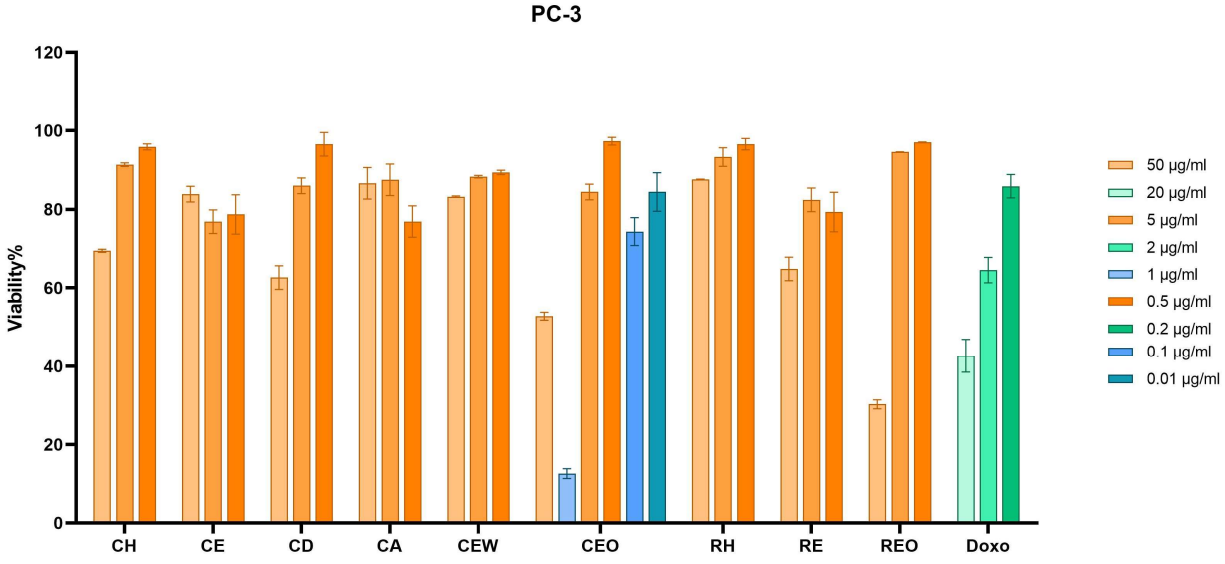

Figure S3. The cell viability of CH: hexane extract of cone, CE: ethanol extract of cone, CD: dichloromethane extract of cone, CA: acetone extract of cone, CEW: ethanol-water (1:1) extract of cone, CEO: essential oil of cone, RH: hexane extract of resin, RE: ethanol extract of resin, REO: essential oil of resin samples and doxorubicin in PC-3 cell line.

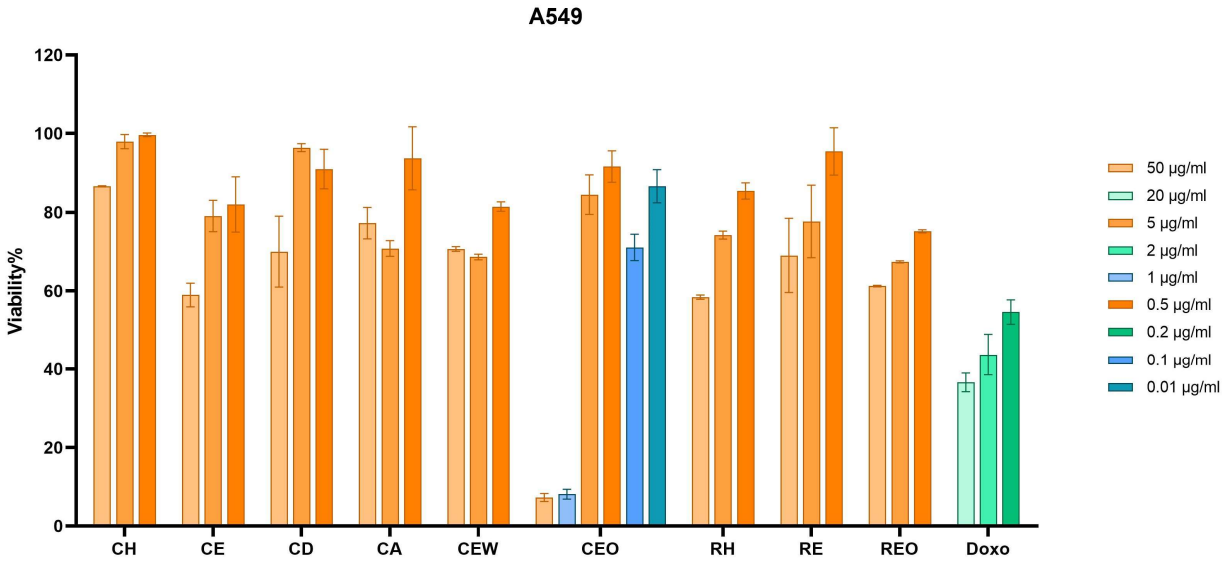

Figure S4. The cell viability of CH: hexane extract of cone, CE: ethanol extract of cone, CD: dichloromethane extract of cone, CA: acetone extract of cone, CEW: ethanol-water (1:1) extract of cone, CEO: essential oil of cone, RH: hexane extract of resin, RE: ethanol extract of resin, REO: essential oil of resin samples and doxorubicin in A549 cell line.

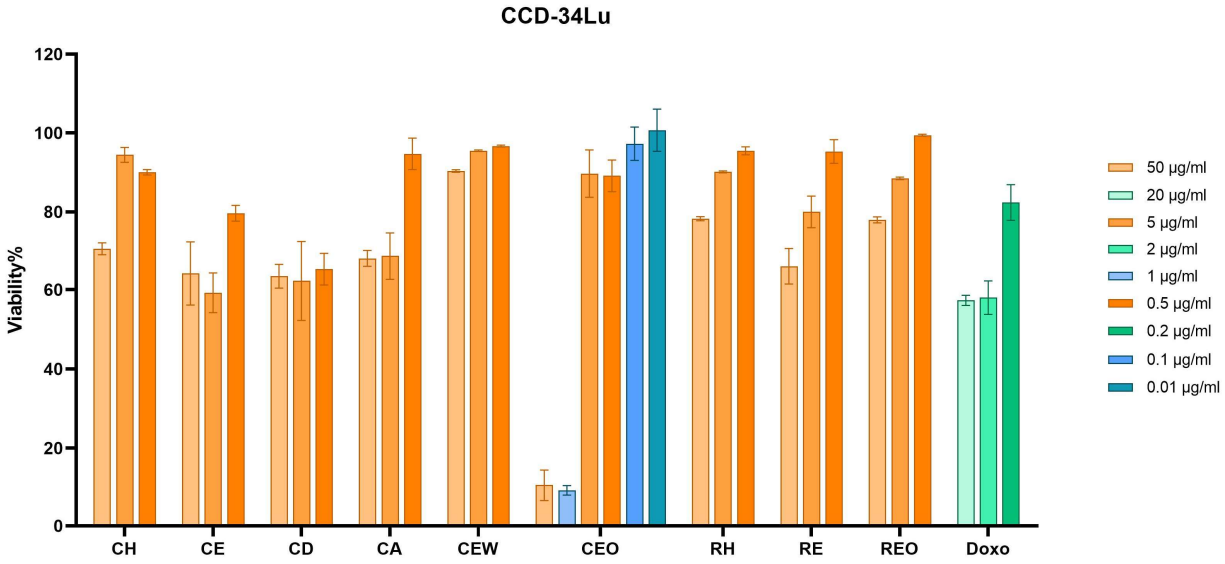

Figure S5. The cell viability of CH: hexane extract of cone, CE: ethanol extract of cone, CD: dichloromethane extract of cone, CA: acetone extract of cone, CEW: ethanol-water (1:1) extract of cone, CEO: essential oil of cone, RH: hexane extract of resin, RE: ethanol extract of resin, REO: essential oil of resin samples and doxorubicin in CCD-34Lu cell line.

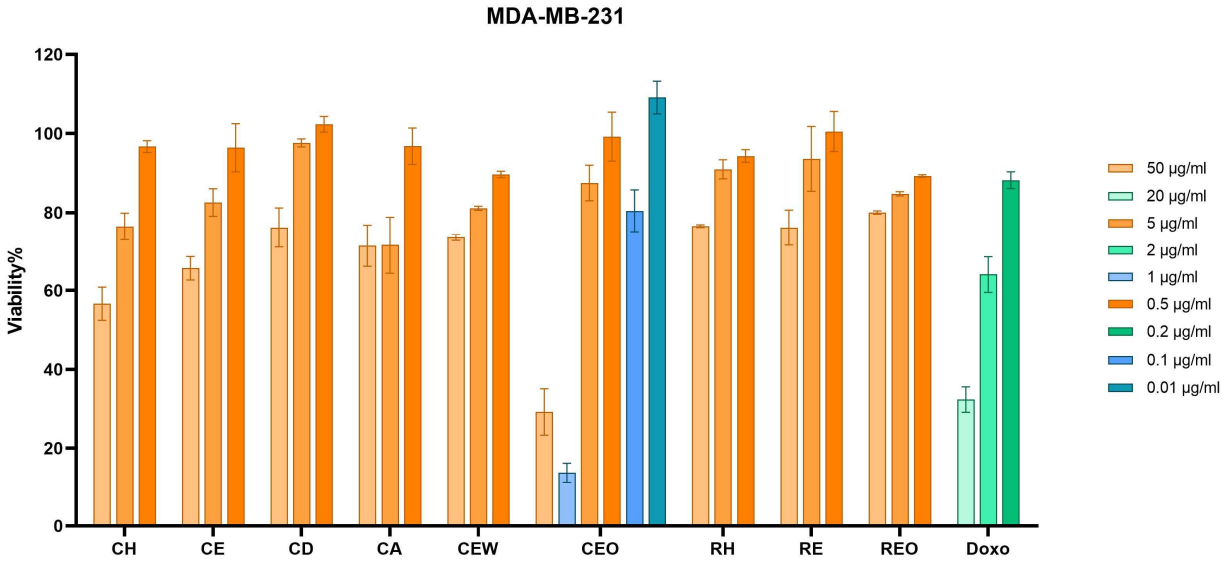

Figure S6. The cell viability of CH: hexane extract of cone, CE: ethanol extract of cone, CD: dichloromethane extract of cone, CA: acetone extract of cone, CEW: ethanol-water (1:1) extract of cone, CEO: essential oil of cone, RH: hexane extract of resin, RE: ethanol extract of resin, REO: essential oil of resin samples and doxorubicin in MDA-MB 231 cell line.

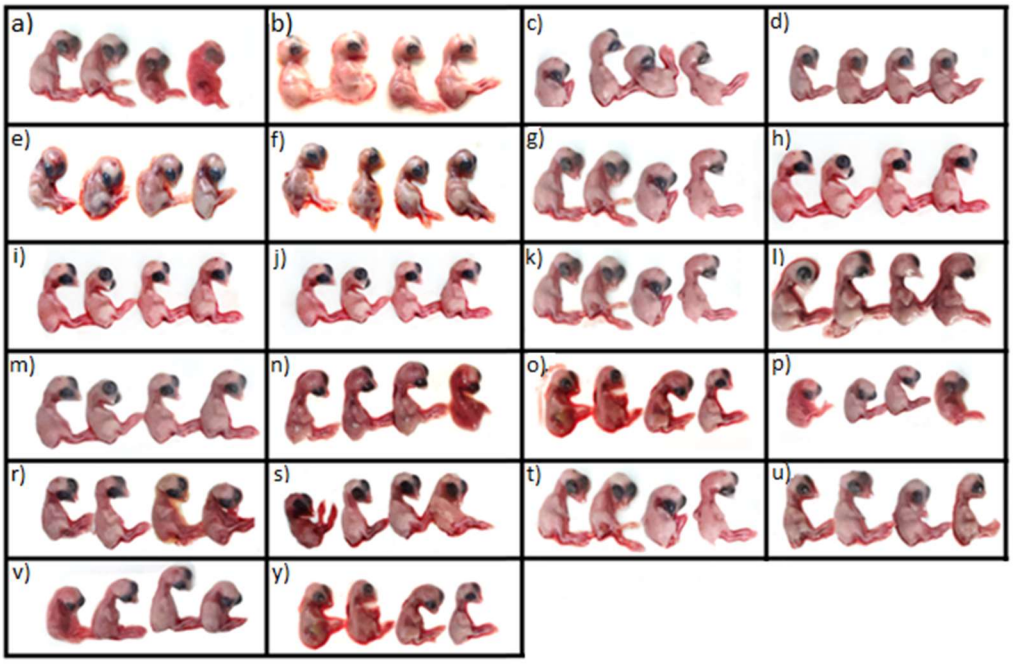

Figure S7. Embryos removed from treated SPF-ECE's

a) Positive control (Only virus), **b)** Negative control (Untreated ECE), c) Enfluvir (5 µg/g), **d)** Enfluvir (10 µg/g), **e)** CEO (0.5% v/v), **f)** CEO (5% v/v), **g)** CH (5 µg/g), **h)** CH (10 µg/g), **i)** CD (5 µg/g), **j)** CD (10 µg/g), **k)** CA (5 µg/g), **l)** CA (10 µg/g), **m)** CE (5 µg/g), **n)** CE (10

µg/g), **o)** CEW (5 µg/g), **p)** CEW (10 µg/g), **r)** RE (5 µg/g), **s)** RE (10 µg/g), **t)** RH (5 µg/g), **u)** RH (10 µg/g), **v)** REO (5 µg/g), **y)** REO (10 µg/g)

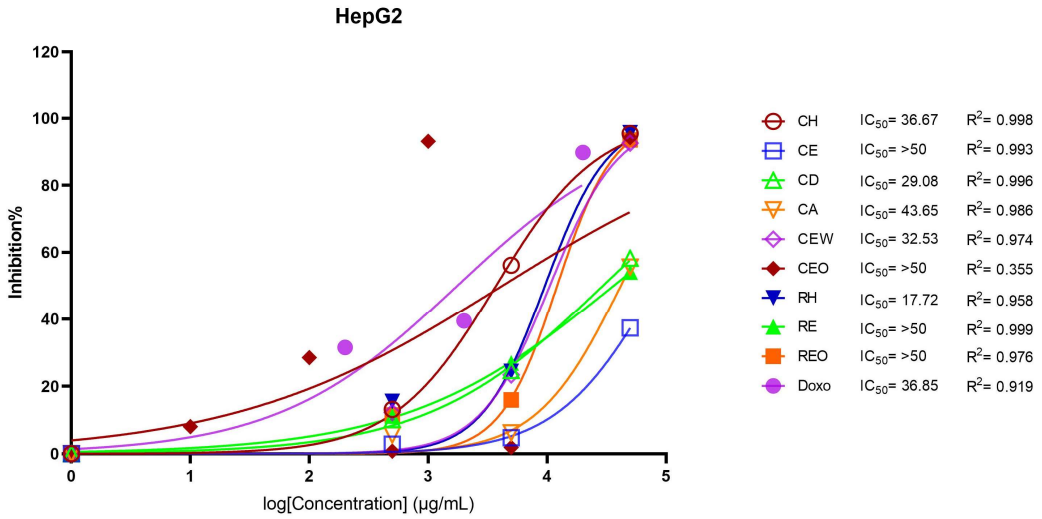

Figure S8. The IC_{50} results with R^2 values of CH: hexane extract of cone, CE: ethanol extract of cone, CD: dichloromethane extract of cone, CA: acetone extract of cone, CEW: ethanol-water (1:1) extract of cone, CEO: essential oil of cone, RH: hexane extract of resin, RE: ethanol extract of resin, REO: essential oil of resin samples and doxorubicin in HepG2 cell line.

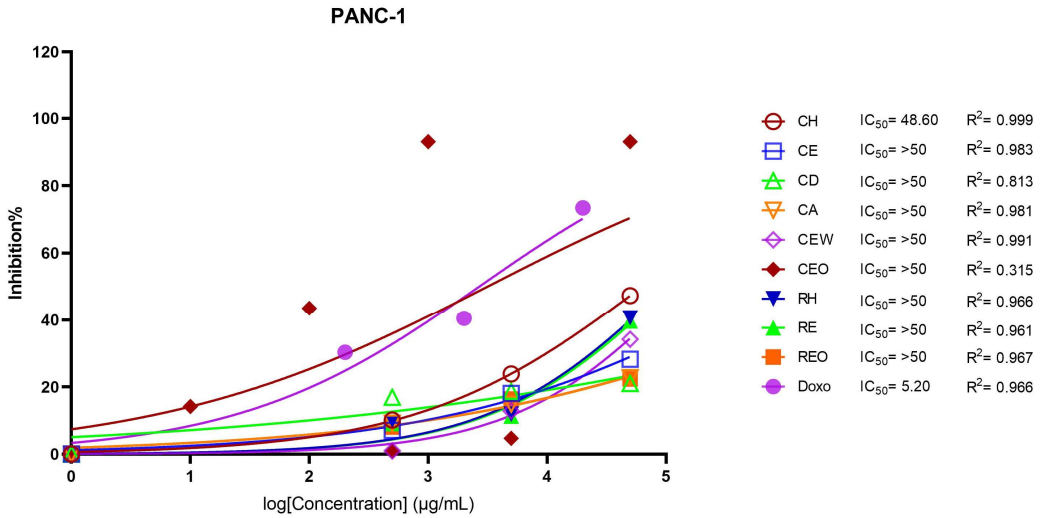

Figure S9. The IC_{50} results with R^2 values of CH: hexane extract of cone, CE: ethanol extract of cone, CD: dichloromethane extract of cone, CA: acetone extract of cone, CEW: ethanol-water (1:1) extract of cone, CEO: essential oil of cone, RH: hexane extract of resin, RE: ethanol extract of resin, REO: essential oil of resin samples and doxorubicin in PANC-1 cell line.

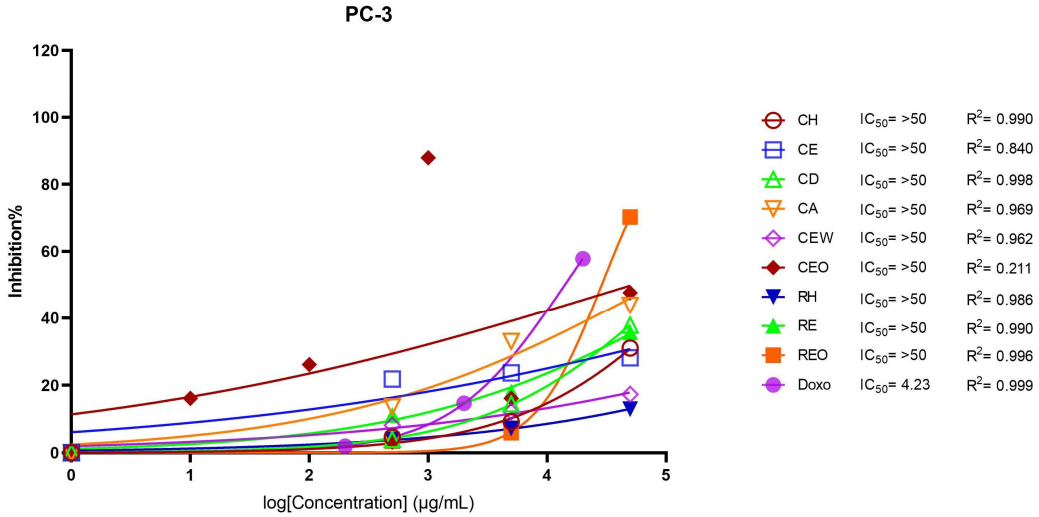

Figure S10. The IC_{50} results with R^2 values of CH: hexane extract of cone, CE: ethanol extract of cone, CD: dichloromethane extract of cone, CA: acetone extract of cone, CEW: ethanol-water (1:1) extract of cone, CEO: essential oil of cone, RH: hexane extract of resin, RE: ethanol extract of resin, REO: essential oil of resin samples and doxorubicin in PC-3 cell line.

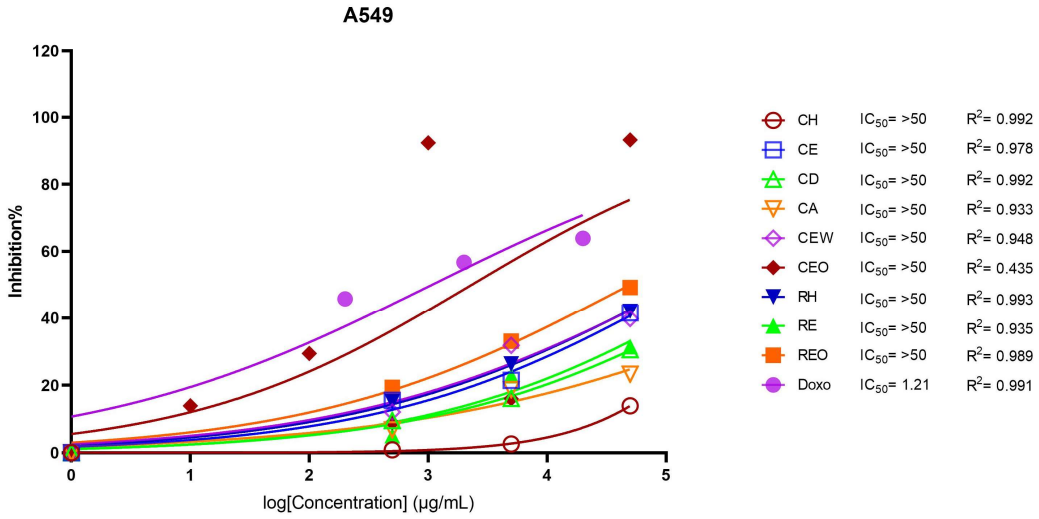

Figure S11. The IC_{50} results with R^2 values of CH: hexane extract of cone, CE: ethanol extract of cone, CD: dichloromethane extract of cone, CA: acetone extract of cone, CEW: ethanol-water (1:1) extract of cone, CEO: essential oil of cone, RH: hexane extract of resin, RE: ethanol extract of resin, REO: essential oil of resin samples and doxorubicin in A549 cell line.

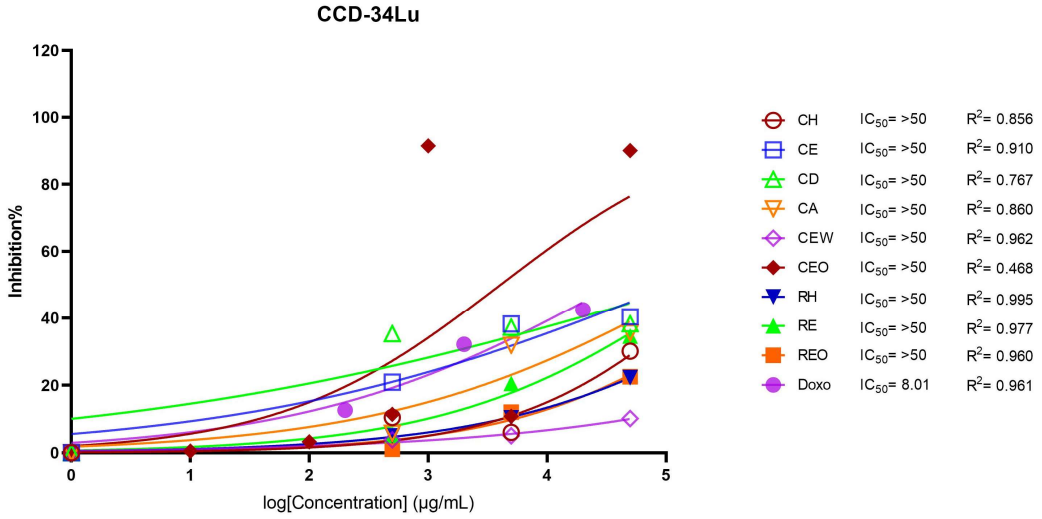

Figure S12. The IC_{50} results with R^2 values of CH: hexane extract of cone, CE: ethanol extract of cone, CD: dichloromethane extract of cone, CA: acetone extract of cone, CEW: ethanol-water (1:1) extract of cone, CEO: essential oil of cone, RH: hexane extract of resin, RE: ethanol extract of resin, REO: essential oil of resin samples and doxorubicin in CCD-34Lu cell line.

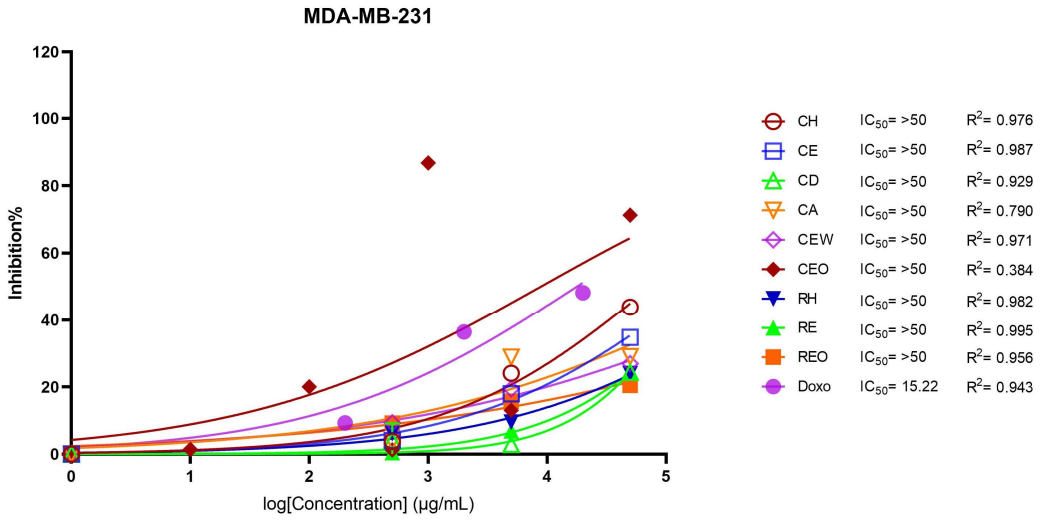

Figure S13. The IC_{50} results with R^2 values of CH: hexane extract of cone, CE: ethanol extract of cone, CD: dichloromethane extract of cone, CA: acetone extract of cone, CEW: ethanol-water (1:1) extract of cone, CEO: essential oil of cone, RH: hexane extract of resin, RE: ethanol extract of resin, REO: essential oil of resin samples and doxorubicin in MDA-MB 231 cell line.