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Antioxidant and antimicrobial activities of Dandelion root extract (*Taraxacum officinale*) and its cytotoxic effect on MDA-MB-231 breast cancer cells

Ridwan Olanrewaju Shittu[1] · Ismaila Ceesay[1] · Pwadubashiyi Coston Pwavodi[2]

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Abstract
Breast cancer (BC) is one of the leading causes of death globally, resulting in 2.26 million cases worldwide. Treatments to downregulate these tumor cells include surgery, immunotherapy, and chemotherapy. However, habituation is expressed by the tumor cells after using the latter. Different plants have been used to treat diseases ranging from bacterial infection to cancer. Natural health products confirmed to be potent and still undergoing clinical trials include the dandelion plant, also known as *Taraxacum officinale*. This study evaluated Dandelion root extract's (DRE) antioxidant, antimicrobial, and cytotoxic effects. The Gas Chromatography-Mass Spectrometry (GC–MS) result revealed the presence of bioactive compounds such as P-hydroxybenzoic, chlorogenic acid, vanillic acid, caffeic acid, lupeol, leutolin, apigenin, coumestrol and much more that are present in the DRE extract. Antioxidant activity using a diphenylpicryl hydrazine (DPPH) scavenging assay was employed, and the result suggested a stable radical IC_{50} value of 2.860 ± 0.001 mg/mL. Trypan blue exclusion assay was used to determine the cytotoxic effect on MDA-MB-231 cells. Antimicrobial activity was assessed by disc diffusion assay, and the average diameter values obtained were 25 mm for gentamicin. No antimicrobial activity was observed for the extracts at different concentrations. DRE showed no inhibitory effect against two gram-negative and gram-positive bacteria pathogens. Our study indicates that DRE has proven novel in killing breast cancer cells, has a mildly vigorous antioxidant activity to scavenge free radicals, and has no inhibitory effect against some bacteria.

Article Highlights
This study deals with the aqueous extraction of the dandelion root and its use in analyzing its cytotoxic effect on MDA-MB-231 breast cancer cells. The study also explored the antioxidant and antimicrobial activities of the plant extract. Different characterizations were carried out on the plant extract to test the presence of bioactive compounds in the plant. The study revealed that the plant has a potential cytotoxic effect on the cancer cells and validates the reason it is taken locally as an herbal treatment for cancer patients. The plant had less effect on the bacteria gram-positive and negative.

Keywords Antimicrobial · Antioxidant · Breast cancer · Cytotoxicity · Dandelion root extract

✉ Pwadubashiyi Coston Pwavodi, pwavodicoston@gmail.com; Ridwan Olanrewaju Shittu, laurexy@gmail.com; Ismaila Ceesay, ismailceesay99@gmail.com | [1]Faculty of Engineering, Department of Bioengineering, Cyprus International University, Haspolat, 98258 Nicosia, Northern Cyprus, Turkey. [2]Faculty of Engineering, Department of Biomedical/Medical Engineering/Bioengineering, Cyprus International University, Haspolat, 99258 Nicosia, North Cyprus, Turkey.

1 Introduction

Worldwide, breast cancer affects 2.1 million women annually, making it the most prevalent type in females. In addition, breast cancer has the most remarkable fatality rate of all malignancies. An estimated 600,000 women globally died from this illness in 2018, making up about 15% of all fatalities from cancer among women. Statistics show that breast cancer is becoming increasingly prevalent practically everywhere despite being more common among females in more developed nations [1].

Natural products (NPs) and natural health products (NHPs) have been recognized as crucial in creating numerous medications. Additionally, most available chemotherapies, including paclitaxel, come from natural sources [2]. The latest evidence on using NHPs for particular conditions provides some associated scientific support for their use [3]. One of the most prevalent and well-known weeds, dandelions (Taraxacum species), are everywhere worldwide [4].

Due to this plant's medicinal properties, traditional Native Americans and traditional Chinese medicine use it extensively. Extracts from dandelions may be used to treat conditions ranging from digestive problems to different types of cancer. However, the anticancer properties of dandelion extracts have only been the subject of a few scientific investigations, and more needs to be understood about the mechanism of action [5]. DRE demonstrated potent anticancer action in human leukemia, pancreatic cancer, colorectal cancer, and drug-resistant human melanoma cells [6]. Dandelion root extracts have been shown to suppress colorectal cancer cell proliferation selectively but have no detrimental effects on normal colon epithelial cells. Phytochemical analysis of DRE has identified taraxasterol (TS), which may reduce colorectal cancer cell viability and inhibit colonic epithelial cell inflammation. The flowering perennial *Taraxacum officinale*, known as dandelion, has long been used to treat cancer, diabetes, and hepatic, renal, and stomach disorders. Research has indicated that dandelion root has antitumor properties in breast, liver, pancreatic, lung, and colorectal cancers. Multiple studies have suggested that flavonoids possess a wide spectrum of pharmacological functions, including scavenging free radicals, regulating the cardiovascular and cerebrovascular system, reducing blood glucose levels, exerting antioxidant effects, antitumor properties, anti-inflammatory and analgesic effects, and improving immunity. Research on the impact of Dandelion on esophageal squamous cell carcinoma revealed that the alcohol extract of Dandelion efficiently suppressed the proliferation of esophageal squamous cell carcinoma cells and hindered their metastasis by targeting EMT-related proteins [7–10].

Triterpenes and steroids can be found in the roots of dandelion plants [11]. Many nations have employed it as a traditional and contemporary herbal system. These components can be utilized as anti-inflammatory medications, diuretics, digestive stimulants, insulin stimulants, anticancer, antidiabetic, and antioxidant agents, and they are non-toxic to guard against liver and testicular harm [1]. Dandelion plants' chemical constituents are primarily sesquiterpene lactones, phenolic acids, triterpenoids, polysaccharides, phytosterols, volatile oils, and other compounds. They are also a rich source of vitamins, choline, minerals, and other trace elements, all confer various health benefits on the plants. The Taraxacum genus is abundant in sesquiterpenoid and its lactones, including α-pinene lactones, guaiacol lactones, and patchoulene lactones with their derivatives. The more abundant components are germacrene, eudesmane, and guaiane-type sesquiterpene lactones. A high concentration of secondary metabolites is synthesized in the dandelion root: lactescence taraxinic acid β-D-glucopyranosyl ester (TA-G). These components are responsible for its antioxidant effects. Dandelion plants are rich in polyphenolic bioactive compounds that exist in the flowers, leaves, and roots, including chlorogenic acid, quercetin, chicory acid, hydroxycinnamic acid, dicaffeoylquinic acid, and their derivatives. The polyphenolic content is notably higher in flowers and leaves compared to roots. The phenolic content is chicoric, caftaric, chlorogenic, and flavonoids such as apigenin, luteolin, quercetin, isorhamnetin, and kaempferol. These components are responsible for the antioxidant properties of the plant. Dandelion contains abundant triterpenoids, including compounds like taraxasterol, pseudotaraxasterol, lupeol, and their acetates. Pentacyclic triterpenoids are the predominant type in dandelion. This discovery offers a potential for bioengineering dandelion with high triterpenoid content and contributes to our understanding of the regulatory mechanisms of triterpene biosynthesis in plants. Flavonoids are another group of components of dandelion, including quercetin, rutin, and luteolin, as well as their glycoside derivatives, which play a crucial role in plant growth, development, and antimicrobial defense. The flavonoid constitutions were predominantly found in Taraxacum mongolicum, Taraxacum officinale, and Taraxacum officinale WEB. Ex WIGG, and Taraxacum formosanum. These compounds significantly contribute to the antioxidant activity of dandelion. The dandelion is a rich source of phytosterols, with sitosterol being the predominant component, followed by substantial amounts of stigmasterol and campesterol. Both dandelion pollen and root exhibit diverse sterol profiles, including β-sitosterol, stigmasterol, and pollen alkanesterol in pollen,

and β-sitosterol along with β-sitosterol-β-D-glucoside in the root. Phytosterols can inhibit cholesterol absorption, promote its degradation and metabolism, and inhibit cholesterol biosynthesis [9, 12].

Despite that, the potency of DRE on MDA-MB-231 breast cancer has not been fully elucidated or reported in the study of DRE on breast cancer cells. However, subsequent studies have found that it has cytotoxic effects at various doses on MCF cells. The concentrations were increased against a stable reactive oxygen species to validate the antioxidant activity of DRE. Lastly, the antimicrobial activity of the plant extract was determined against a gram-negative and gram-positive bacteria strain. This study examined the time-dose response of DRE in the aggressive breast cancer subtype.

In this study, we report the anti-cancer activity of the DRE obtained with an in-vitro (MDA-MB-231 breast cancer cell lines) study. We hypothesized that due to the compositional complexity mixture of bioactive compounds in DRE, the DRE might be able to activate different signaling events and more efficiently induce program cell death processes by targeting different metabolic vulnerabilities of cancer cells. Accordingly, we have shown that DRE treatment triggered cell death in all cell models examined. Additionally, the antimicrobial and antioxidant properties of DRE were investigated. After a careful literature search, we discovered no work on using the root extract on the MDA-MB-231 breast cancer cells in the past 10 years, which gives information as in our study (Table 1).

The structure of the paper Antioxidant and antimicrobial activities of Dandelion root extract (*Taraxacum officinale*) and its cytotoxic effect on MDA-MB-231 breast cancer cells is as follows:

Section one, which is the introduction, highlights the prevalence and severity of breast cancer worldwide, making it a leading cause of cancer death in women worldwide despite the high prevalence in developed countries. The section also emphasizes the importance of natural products in cancer therapy. Dandelion (Taraxacum species), which is consumed historically in traditional medicine, suggests anticancer potential. Some studies have found its effectiveness in the treatment of leukemia, pancreatic, colorectal, and breast cancers, although the mechanisms remain poorly explored.

The Materials and Methods section describes the methods and equipment used to study the effects of dandelion root extract (DRE). The section outlines in detail all the assay and Equipment used for the study, from the collection of the plant samples to how it was authenticated, prepared, and used for the study. The section also outlines how the Breast cancer cells (MDA-MB-231) were cultured and exposed to DRE for cytotoxicity assay by trypan blue exclusion assay. The DPPH assay evaluated antioxidant activity, and antimicrobial properties were tested against gram-positive and gram-negative bacteria using the disk diffusion method. Statistical analysis was performed with SPSS software, and graphs were plotted.

The results and discussion section explains the characteristics and effects of dandelion root extract (DRE). The GC–MS analysis suggested the presence of several bioactive compounds. The DRE exhibited a dose-dependent cytotoxic effect on MDA-MB-231 breast cancer cells. The Antioxidant activity was also measured by the DPPH assay to investigate the free-radical damaging effect. Lastly, antimicrobial assays were carried out to investigate the inhibition effect of the extract against Gram-positive or Gram-negative bacteria.

The last section of the study is the conclusion that summarizes the results from the effect of the dandelion root extract (DRE) from the different assays carried out.

2 Materials and method

2.1 Materials

GCMS-QP2010 Plus System (Shimadzu, Japan), 35 mm cell culture dishes (Thermo Scientific Nunc Cell Culture Dishes; Thermo Scientific; USA), and a UV–visible spectrum were evaluated using a UV–visible spectrophotometer (Shimadzu UV-2450), Dulbecco's Modified Eagle Medium (DMEM) (Gibco by Life Technologies™, USA), 1,1-diphenyl-2-picrylhyorazyl (DPPH), and an inverted microscope (Leica DFC295).

2.2 Source of plant

The leaves together and flowers of *Taraxacum officinale* were collected from the wild from Badagry local government area, Lagos state (Nigeria: 6° 25′ 53. 688″ N) and then identified by a Pharmacognosist at the faculty of Pharmacy, Cyprus International University. The herbarium specimen with the number CIU/PHAR/ASTE/001 was prepared and then deposited at the Faculty of Pharmacy, Cyprus International University's herbarium for future reference.

Table 1 Showing some other works about Dandelion plant parts and the type of cell lines used

Dandelion plant species	Part	Extraction solvent	Cancer/bacterial subtype	In vitro/in vivo	Effects	References
Taraxacum officinale	Leaves	Water	CAL 27 cells/E. coli K-12 MG1655	In vitro	No effect on E. coli, Little effect was shown on CAL 27 cells	[13]
Taraxacum officinale	Whole plant	Water	SGC-7901 cells	In vitro	Dandelion induces apoptosis, possibly via regulation of Bax, Bcl2, Survivin, Pten, and Erk Gene expression	[13]
Taraxacum mongolicum	Whole dried plant	Ethanol	MDA-MB-231, MDA-MB-231–468, MCF-10A cell lines	In vivo/in vitro	Downregulating the CHKA expression and inhibiting PI3K/AKT pathway	[1]
Taraxacum mongolicum	Whole plant	Water	MDA-MB-231, HCC1937, 4TI	In vivo and in vitro	CDK1, CCNB1, CCNB2, and CDK6 were cell cycle-related targets by the dandelion	[14]
Taraxacum officinale	Root	Water	PANC-1	In vitro	Caspase 3 activity causes apoptosis	[15]
Taraxacum Mongolicum	Whole plant	Ethanol	MDA-MB-231 and MDA-MB-231–468, human monocytic leukemia U937	In vitro	Inhibition of IL-10/STAT3/PD-L1 immuno-suppressive signaling pathway	[16]
Taraxacum officinale	Root	Water	HT-29, HCT116 and NCM460	In vivo and in vitro	Reduced the expression of FLICE-like inhibitory protein (cFLIP), an inhibitor of the extrinsic apoptosis pathway and activating caspase 8	[6]

2.3 Ethics and consent approval

Local ethical permissions were obtained from the local authorities before collecting the plant used for the study.

2.4 Plant preparation

Dandelion root water extracts were made utilizing a number of filtration processes: lyophilized, composed to a certain stock concentration, and sterilized. Roots that had been washed and cleaned were macerated in water (Fig. 1). After that, the mixed mass was run through a nylon mesh to remove very big fibers, and the filtrate underwent a 5000rmp centrifugation at ambient temperature for 6 min. Afterward, a Buchner funnel filters the supernatant before being lyophilized to create a fine powder. The extract yield obtained after lyophilization was (43% w/w yield); the blended composition was passed through a Whatman No 1 filter paper, separating little fiber materials from the filtrate. Afterward, the powder is reconditioned to a specific stock concentration using sterile 1× Phosphate Buffer Saline (PBS), and the DRE is further diluted when applied to breast cancer cells in a medium.

2.5 Cell culture

The breast cancer cell lines MDA-MB-231 were obtained from Imperial College London, United Kingdom, and their use was approved by the ethical committee of the Biotechnology Research Center (BRCEC2011-01). Cells were grown in DMEM supplemented with 10% L-glutamine, 2 mM glutamine, and penicillin/streptomycin (Gibco by Life Technology, Carlsbad, CA, USA). The cells were continuously kept in a humid chamber at 37 °C and 5% CO_2; analytical grades were utilized for all the substances used in the cell culture.

2.6 Gas chromatography-mass spectrometry (GC–MS) analysis

The phytochemical screening was evaluated on a GCMS—QP2010 plus, a product of Shimadzu, and a column type of Teknokroma TRB-5. The rate used was 5 °C/min at an initial temperature of 50 °C and a final temperature of 280 °C with a 2-min hold time. The total duration was 31 min, using helium as a carrier gas. The mobile phase flow rate was programmed at 1 mL/min, and the ionizing voltage was relative to tuning. The mass scan range was 35–1000 amu at 70 eV using an

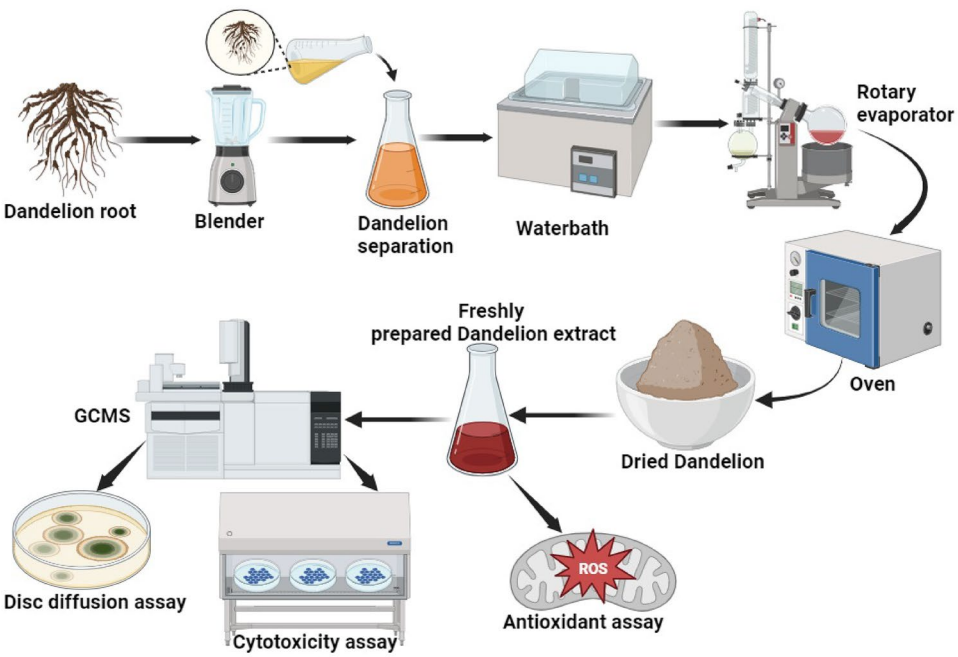


Fig. 1 Shows the procedure that was used for all the assays that were performed

injection volume of 1 µL. The spectra of the compounds in the extract were compared with the already analyzed and known components found in the GC–MS database [17].

2.7 Cytotoxicity assay

Using the breast cancer cell lines MDA-MB-231, the trypan blue dye exclusion assay was used to assess the DRE cytotoxic activity. The test was carried out to establish whether the cell lines were toxic by the DRE. The cells were plated in 35 mm dishes, incubated overnight, and then exposed to different concentrations of DRE (0.5, 1.0, 2.5 and 5 mg/mL) (Fig. 1). Trypan blue dye (4%) was applied to the 35 mm cell culture dishes following the treatment. The dishes were then incubated for 10 min. A Leica DFC295 inverted microscope was used to view 30 randomly selected fields of view with at least 20 cells in each field at a magnification of 100 to count the number of live and dead cells [18].

2.8 Antioxidant assay

The DPPH assay is a common and simple method to measure the antioxidant activity of a substance. The DPPH assay is based on the reduction of a purple-colored radical, DPPH, to a colorless product by receiving a hydrogen atom from an antioxidant. The color change can be monitored by measuring the absorbance at 517 nm. The DPPH assay does not require expertise because it is easy to perform, fast, and reliable. The assay only involves mixing the sample with the DPPH solution and measuring the absorbance after a specific time. The assay does not need sophisticated equipment or reagents; the results can be compared with standard antioxidants. The DPPH assay is widely used to screen for the antioxidant activity of natural products, drugs, extracts, and other biological sources [19]. The stability of the 1,1-diphenyl-2-picrylhyorazyl (DPPH) free radical was used to measure the antioxidant activity of the extracts, with a couple of slight modifications. Briefly stated, 50 µL of the sample DRE was dissolved in a water solution with different concentrations of 0.1, 0.5, 1.0, 1.5, and 2.5 mg/mL, then 200 mL of a 0.2 M solution of DPPH in 100% methanol was added. The reaction mixtures were made in triplicate and left at 37 °C in the dark for 15 min with the samples in a 96-well plate. Ascorbic acid was employed as the standard due to its high antioxidant activity and to compare its curve with DRE activity. Methanol was used as the blank, and DPPH in methanol without the extracts served as the positive control. After incubation, a UV–visible spectrophotometer measured the purple color's deterioration at 517 nm. An increase in the DPPH was shown by a reduction in the DPPH solution absorbance, which was calculated as a percentage of inhibition (I%) according to Eq. (1).

$$I\% = \frac{Ac - As}{Ac} \times 100, \tag{1}$$

Ac represents the absorbance of the control, and As is the absorbance of the sample. The scavenging activity of the samples was assessed using the percentage scavenging activities at five different concentrations. The effective concentration, which is the concentration (mg/mL) of the DRE needed to remove 50% of the radicals from the samples, was determined using the IC_{50} format, which is the value defined as the substrate concentration that causes the loss of 50% of the activity of DPPH, against the percentages of inhibition according to different dilutions of the extracts used for the analysis [20].

2.9 Determination of antimicrobial activity

Disc diffusion assay was used to determine the antimicrobial activity of DRE against four bacteria, which include two gram-positive, Bacillus subtilis (ATCC 25911), Staphylococcus aureus (29,213), and two gram-negative, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27259). 27 g/L of nutrient agar was dissolved in water to create the agar. The sterile nutritional agar was placed into sterile Petri dishes. Afterward, it was infected with microbial cells, including 200 µL of microbial cell suspension in 25 mL agar media. 8 mm diameter sterile filter paper discs were saturated with 20 µL of DRE. The paper discs were placed on the surface of the contaminated agar plates after being given time to evaporate. Plants were chilled for 2 h to allow the extract's pre-diffusion into the agar. The plates were then incubated at 37 °C for the following day. Positive controls included the antibiotic gentamicin; the negative controls were carried out using paper discs with 20 µL of distilled water. The inhibition zones were evaluated after the incubation period. [21]

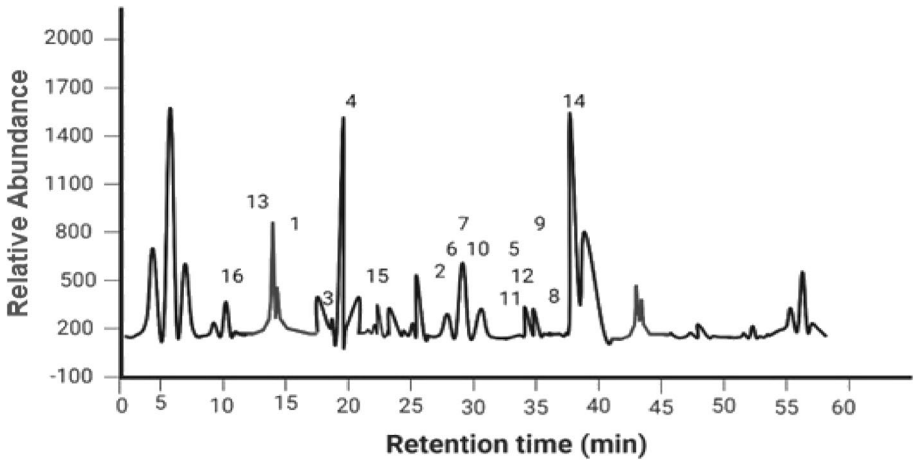


Fig. 2 Shows the chromatogram obtained from GC–MS analysis of DRE, which displays different peaks according to the concentration of the compounds. The Y-axis represents the relative abundance, and the X-axis represents the time each component elutes from the column. The rate used was 5 °C/min at an initial temperature of 50 °C and a final temperature of 280 °C with a 2-min hold time. The total duration was 31 min, using helium as a carrier gas.

Table 2 Shows the bioactive compounds present in DRE and the total ion chromatogram

Names	Active	Retention Time (min)	Number	Formular	Mass (g/mole)
P-Hydroxylbenzoic	+	15.01	1	$C_7H_6O_3$	138.12
Chlorogenic acid	+	27.06	2	$C_{16}H_{18}O_9$	354.31
Vanillic	+	19.08	3	$C_8H_8O_4$	168.15
Caffeic acid	+	19.16	4	$C_9H_8O_4$	180.16
Lupeol	+	34.19	5	$C_{30}H_{50}O$	426.72
Leutolin	+	29.13	6	$C_{15}H_{10}O_6$	286.24
Apigenin	+	29.02	7	$C_{15}H_{10}O_5$	270.24
α-Amyrin	+	35.75	8	$C_{30}H_{50}O$	426.72
Taraxasterol	+	35.01	9	$C_{30}H_{50}O$	426.72
Vitamin E	+	30.08	10	$C_{29}H_{50}O_2$	430.71
Germanicol	+	34.11	11	$C_{30}H_{50}O$	426.72
Olean-18-en-28-oic acid,3-oxo, methylester	+	34.02	12	$C_{30}H_{48}O_3$	456.70
2*H*-1-Benzopyran-2-one	+	14.76	13	$C_9H_6O_2$	146.15
urs-20-en-3-ol(3β,18α,19α),	+	38.60	14	$C_{30}H_{48}O_3$	456.70
Coumestrol	+	22.52	15	$C_{15}H_8O_5$	268.22
6S-2,3,8,8-Tetramethyltricyclo [5,2,2,0](1,6)] undec-2-ene	+	11.06	16	$C_{15}H_{24}$	204.35

2.10 Statistical analysis

All experiments were performed at least three times independently, and IL, USA, was used for statistical analysis. The study used the SPSS 17.0 software (SPSS Inc., Chicago). Multiple group comparisons were analyzed with one-way ANOVA and Student's t-test. The data analyzed were expressed as mean ± standard deviation. The differences were considered to be statistically significant when $p < 0.05$.

3 Results and discussion

3.1 GC–MS analysis

Hundreds of the bioactive compounds found in DRE were identified by GC–MS (Fig. 2). Twenty of these compounds were chosen, and the data library built into the GC–MS was used to analyze them. Polyphenols, flavonoids, and

triterpenes are a few of the substances discovered (Table 2). The substances found in DRE matched the findings by [20]. The dandelion plant is reported to contain an array of secondary metabolites, such as hydroxycinnamic acids (HCAs), flavonoids, sesquiterpene lactones (SLs), triterpenes, and coumarins. It is also a rich source of vitamins, phytosterols, polysaccharides (inulin), various amino acids, and minerals, particularly potassium. These various components have been suggested to show various In vitro and in vivo biological effects, such as antioxidant, anti-inflammatory, anti-carcinogenic, anti-hyperglycemic, anti-thrombotic, antimicrobial, and antiviral [22].

3.2 In vitro cytotoxic activity

Trypan blue exclusion assay was used to determine the viability of the aggressive breast cancer cells following MDA-MB-231 cell culture. DRE was applied to MDA-MB-231 cells in doses of 0.5, 1.0, 2.5 and 5 mg/mL compared to the MDA-MB-231 control, which had 100% viability. The results (Fig. 3A) revealed that DRE significantly reduced the viability of the cells in a dose-dependent form; treatment with 5, 2.5, 1.0, and 0.5 mg/mL resulted in 48%, 60%, 73%, and 93% viability, respectively, at 72 h' time point when compared with the MDA-MB-231 control of 100% viability ($P < 0.001$, $n \geq 3$).

Dandelion has been utilized by several cultures as a form of traditional medicine to treat conditions like cancer, diabetes, and digestive problems. The majority of FDA-approved chemotherapeutic medications are derived from plants. Recent synthetic medication production consumed by cancer patients has resulted in numerous adverse effects in patients, including gradual hair loss, body size reduction, and immune system issues. This study established that dandelion root can cause apoptosis in MDA-MB-231 cells, that DRE has antioxidant properties against reactive oxygen species, and that it has no antibacterial properties against a variety of microorganisms. The graph indicates that the MDA-MB-231 cells and phosphate buffer saline (PBS) used as controls are 100% viable. Treatment with 0.5 mg/mL DRE reduced the

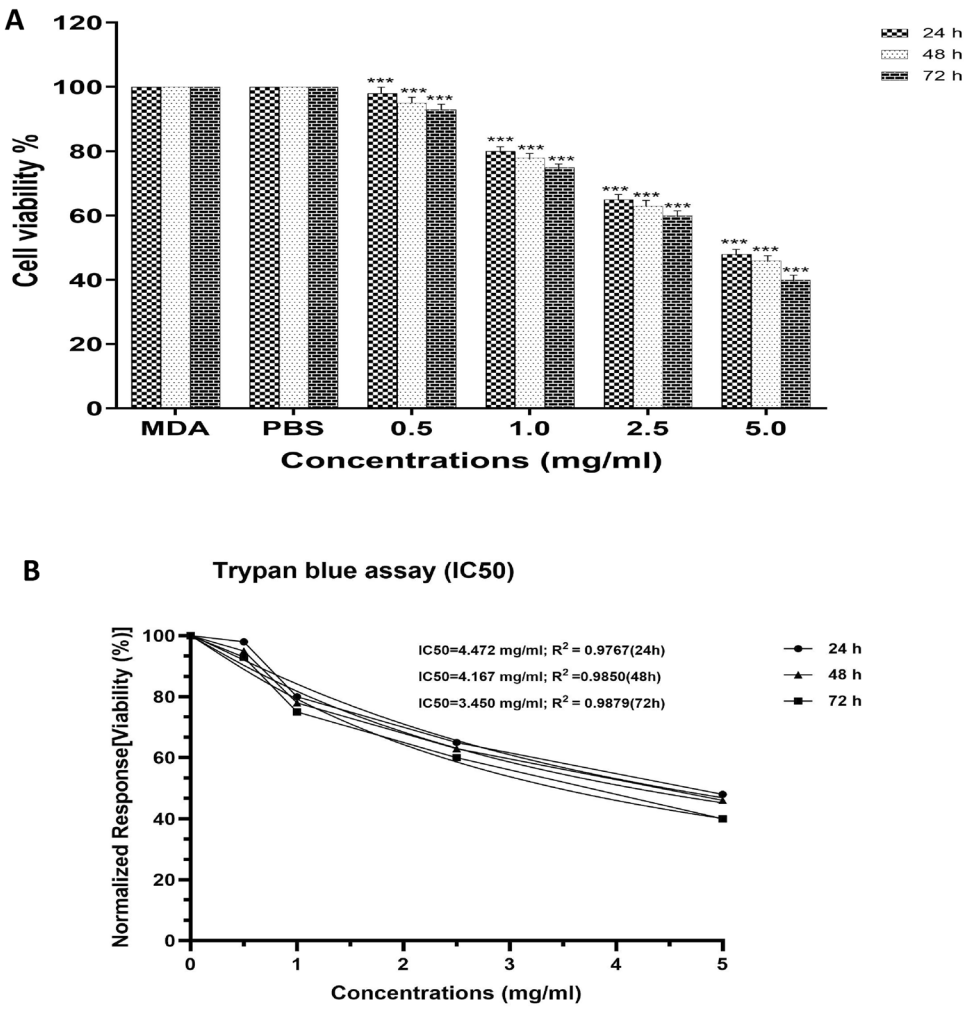


Fig. 3 **A** shows the cytotoxic effect of DRE on MDA-MB-231. The bar chart reveals the cell viability percentage on the Y-axis against the plant extract concentration on the X-axis at different time points. The experiment was repeated three times, as shown by the error bar. **B** IC_{50} was determined by nonlinear regression [curve fit (Inhibitor) vs. normalized response–Variable slope] using GraphPad prism software

cell population to (98%, 95%, and 93%) after 24, 48 and 72 h of incubation. A doubling dose of the first concentration, 1.0 mg/mL, was successfully administered to the cells, resulting in a notable difference (80%, 78%, and 75%) at (24, 48 and 72 h) respectively. MDA MB-231, the positive control, exhibits 100% viability after three consecutive days in the incubator. The concentration of 2.5 mg/mL killed the breast cancer cells to about (63, 65, and 60%) at (24, 48 and 72 h). The final concentration of 5 mg/mL killed the breast cancer cells to about (48, 52, and 56%) at (24, 48 and 72 h). The negative control PBS, used to dissolve DRE, produces 100% viability as well. There was no significant difference between the first concentration of 0.5 mg/mL and the control after incubating for 24 h. A little effect was visible after 48 h, killing 5% of the tumor. 72 h' time point killed 7% of the cells. However, the more time the tumor cells were exposed to the treatment, the lesser their population and changes in their physiological appearance. The double increments of the first concentration of the extract improve the mechanical action against the tumor cells by the apoptotic reaction. At 24 h, there was a decrease in the viable cell population after cell count. More so, 48 h shows a significant difference. Likewise, the 72 h show that the breast cancer cell growth was highly inhibited compared to the control. Lastly, the final concentration, 5 mg/mL, shows a high significant difference at both 24 and 48 h, while the 72 h shows the highest inhibitory effect of DRE on breast cancer aggressive subtype; it reveals that close to half of the total cells were degraded. DRE has not been fully elucidated on MDA-MB-231 breast cancer, also known as the aggressive subtype. The IC_{50} value shows the concentration of dandelion root extract needed to reduce 50% of cell viability (Fig. 3B). The values determined indicate the extract's efficacy in controlling or stopping the growth of MDA-MB-231 cancer cells. It was observed that after 24 h of exposure, a concentration of 4.472 mg/mL of dandelion root extract led to a 50% reduction in the viability of MDA-MD-231 cells. After 48 h, a lower concentration of 4.167 mg/mL was needed to achieve a 50% inhibition response compared to the dose used earlier; this indicates that the cells showed increased sensitivity to the extract with longer exposure time. By the end of 72 h, the required concentration for inhibition further dropped to 3.450 mg/mL, indicating the cytotoxic effect of the dandelion root extract as exposure duration extended. Additionally, the IC_{50} values demonstrate a consistent decrease over time from 24 h (at 4.472 mg/mL) to 3.450 mg/mL by the end of 72 h. The results suggest that the dandelion root extracts can kill or slow down the growth of MDA-MB-231 breast cancer cells progressively over time. A study by Sigsted et al. only explains the "Evaluation of aqueous extract of *Taraxacum officinale* on growth and invasion of breast and prostate cancer cells." The results published by this author show that the subtype of breast cancer used was Mcf, and the cells were ultimately killed by extracts of flower and root of DRE at a concentration of 50 µL/mL (Fig. 4). Also, a study by [11] enumerated that concentrations 1.0, 2.5, 5.0, and 10 mg/mL of DRE inhibited the growth of drug-resistant human melanoma cells effectively. Recent research has shown that DRE can protect colonic cells [23] and kill gastric cancer [5]. Some components were being extracted from DRE to treat breast tumors. Leutolin, an example of a flavonoid, downregulated various genes like AP2B1, APP, GPNMB, and DLST in breast cancer [24]. Our study has proven that DRE inhibited the growth of breast cancer cells in a dose-dependent manner. Cytotoxic activity using the trypan blue assay demonstrated that the tumor cells undergo intrinsic and extrinsic apoptotic pathways by activating AMPK [25]. After checking the cells under the microscope, blebs were observed on the tumor cells after treatment with DRE following 72 h of incubation (Fig. 4). Also, pieces of the degraded cells were seen under the scanning electron microscope. This may indicate another mechanistic way by which apoptosis is exhibited. The degradation of the breast cancer cells might be due to the presence of ROS groups and the action of some bioactive compounds like polyphenols and triterpenoids in the extract. Also, the MDA-MB-231 cells may have been induced by DRE through G2/M cell cycle arrest and alteration in the amino acids (Figs. 5, 6) that make up the endoplasmic reticulum [26].

4 Breast cancer typical apoptotic morphology

4.1 Evaluation of the antioxidant activity of DRE using 1, 1- diphenyl-2-picryl hydrazyl (DPPH).

In the antioxidant activity test (Fig. 7), the DDPH free radical scavenging activity assay was performed. The chemical composition of the DRE demonstrated antiradical activity. When the concentration was 0.1 mg/mL, the percentage inhibition rate of DRE increased to about 18%. An increase to 0.5 mg/mL raised the inhibition to 27% when the second concentration doubled to 1.0 mg/mL. The percentage inhibition was raised to 30%, showing that increased concentration produces more hydrogen to scavenge the DPPH radical. At the highest 2.0 mg/mL concentration, the inhibition value increased to 50%. The result from the characterization of DRE reveals the highest percentage of hydroxycinnamic acids, which include chlorogenic and caffeic acids (Fig. 8) and are known to play a significant role in various diseases like macromolecule dysfunctions, which were elucidated by [27]. More so, these compounds have been used in vivo and were

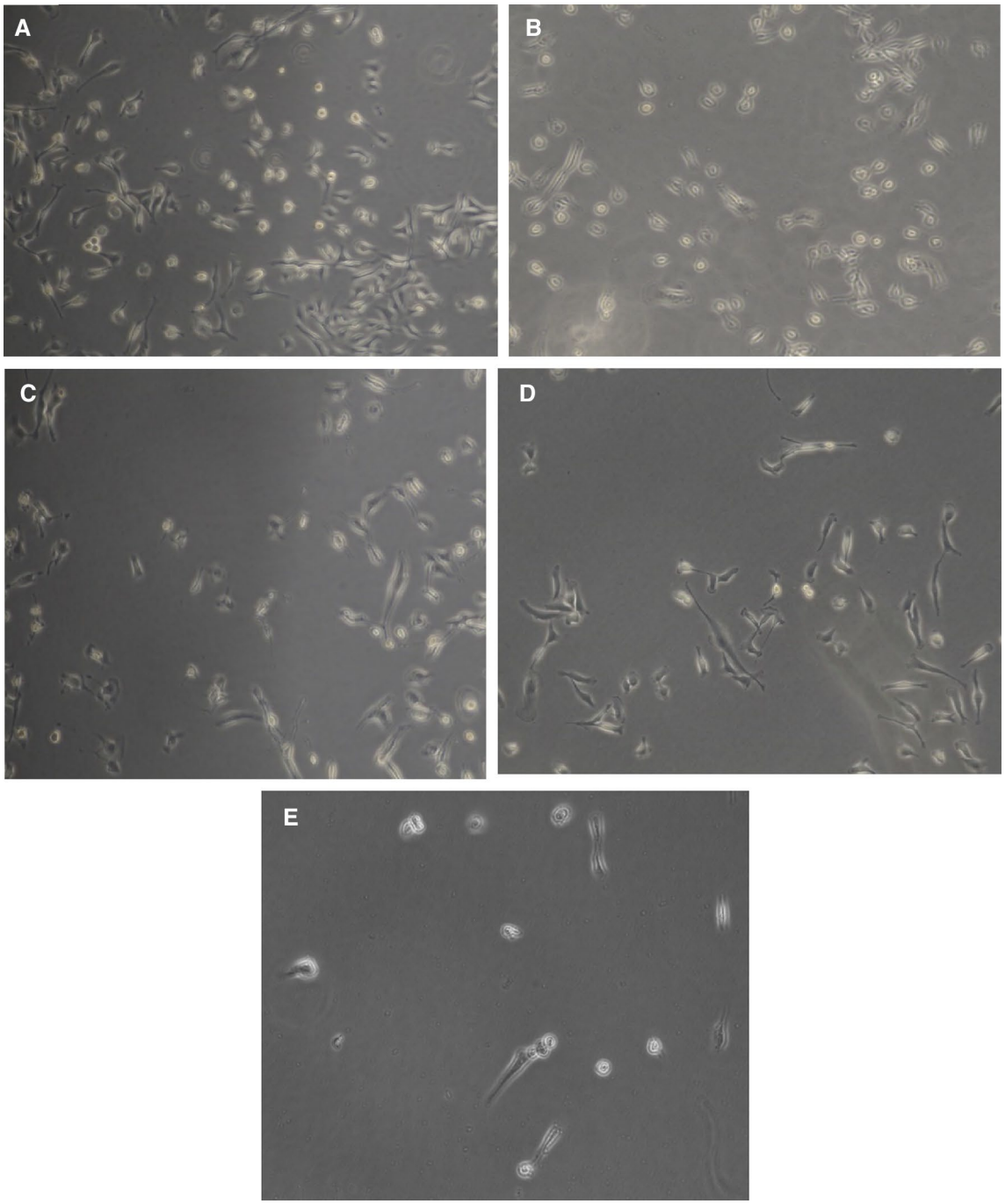


Fig. 4 The scanning electron micrographs show MDA-MB-231 cells treated with DRE. **A** Control (**B**) 0.5 mg/mL (**C**) 1.0 mg/mL (**D**, **E**) 5 mg/mL. The images validate the graphical representation of the In vitro cytotoxicity assay

discovered to possess high antioxidant capacity [28]. The results showed that at a concentration of 0.1 mg/mL, the DPPH colour was still purple due to slight amelioration of the radical. A change from purple to golden orange at 1.5 mg/mL was observed, and a concentration of 2.0 mg/mL proved that half of the DPPH radicals had been reduced. As such, the antioxidant activity of DRE has been observed to scavenge the reactive oxygen species. Previous studies have provided information about the antioxidant activity of DRE in drug and food metabolism; a synergistic combination of bioactive components causes an antagonistic effect, which might reduce or increase the efficacy of the extract or drug [4, 29, 30]. The concentration required to kill 50% of the total population is known as the IC_{50}, and the result from this study shows an IC_{50} value of 2.860 ± 0.001 mg/mL with a similar value reported by [31]. The positive control used was ascorbic acid.

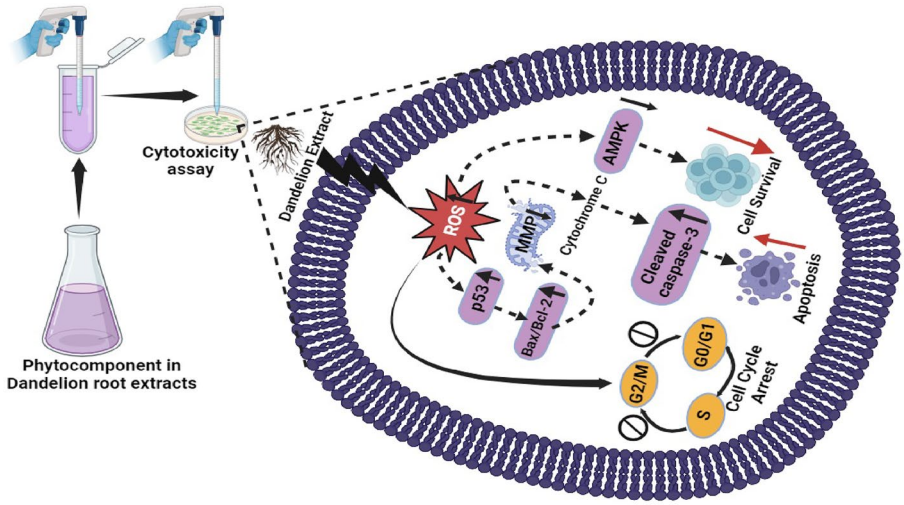


Fig. 5 The image shows the mechanistic action of DRE cytotoxic activity in MDA-MB-231 breast cancer cells. The DRE penetrates the outer membrane, causing ROS to disrupt the mitochondria, breakage of the double strand in the DNA, cleavage of the material in the peroxisome, and separation of the ribosomal membrane

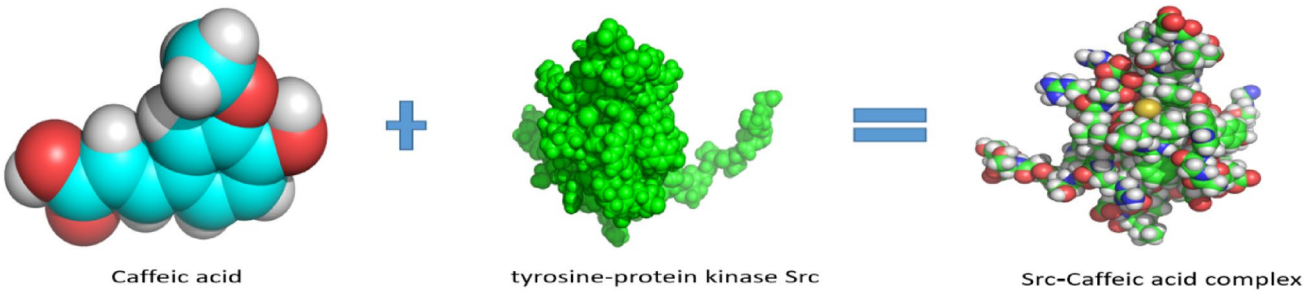


Fig. 6 The Ligand–protein interaction of caffeic acid and Src shows the binding of the two molecules using an open-source molecular graphic tool known as Pymol. This proposed reaction indicates that DRE's main component, caffeic acid, could bind to the active site of the Src gene, causing inhibition of the metabolism associated with MDA-MB-231

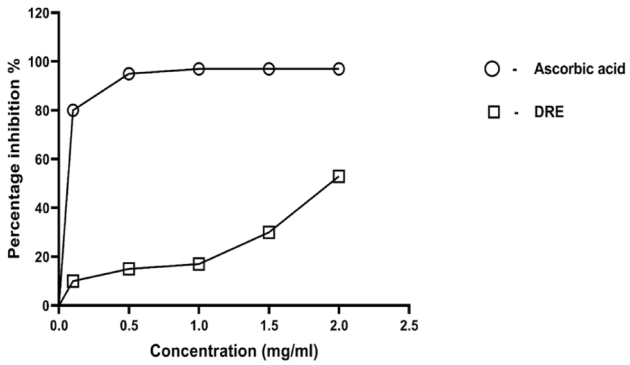


Fig. 7 Shows the antioxidant activity of DRE against DPPH. The graph displays the percentage inhibition due to the application of DRE to the stable reactive oxygen species using different concentrations in mg/mL

The graph demonstrates that ascorbic acid has more antioxidant activity than DRE at the same dose of 0.1 mg/mL to 2.0 mg/mL. At all concentrations used for the DPPH activity, there was a clear significant difference between DRE and ascorbic. At a 2.0 mg/mL concentration, Ascorbic acid has 90% inhibition compared to 50%, as shown by DRE.

5 Antimicrobial activity of DRE using disc diffusion method

The antimicrobial susceptibility assay shows the disc diffusion assay of gram-positive bacteria: *Staphylococcus aureus* and *Bacillus subtilis*, gram-negative bacteria; *Escherichia coli* and *Pseudomonas aeruginosa.* At 1–3 mg/mL of DRE concentrations, S. *aureus* did not show a sign of inhibition (Fig. 9). The concentration of the extract was increased to 4 and 5 mg/mL, also not active against the bacteria. The DRE was also not active after treatment on the *B. subtilis*. Neither the *E. coli* nor the *P.*

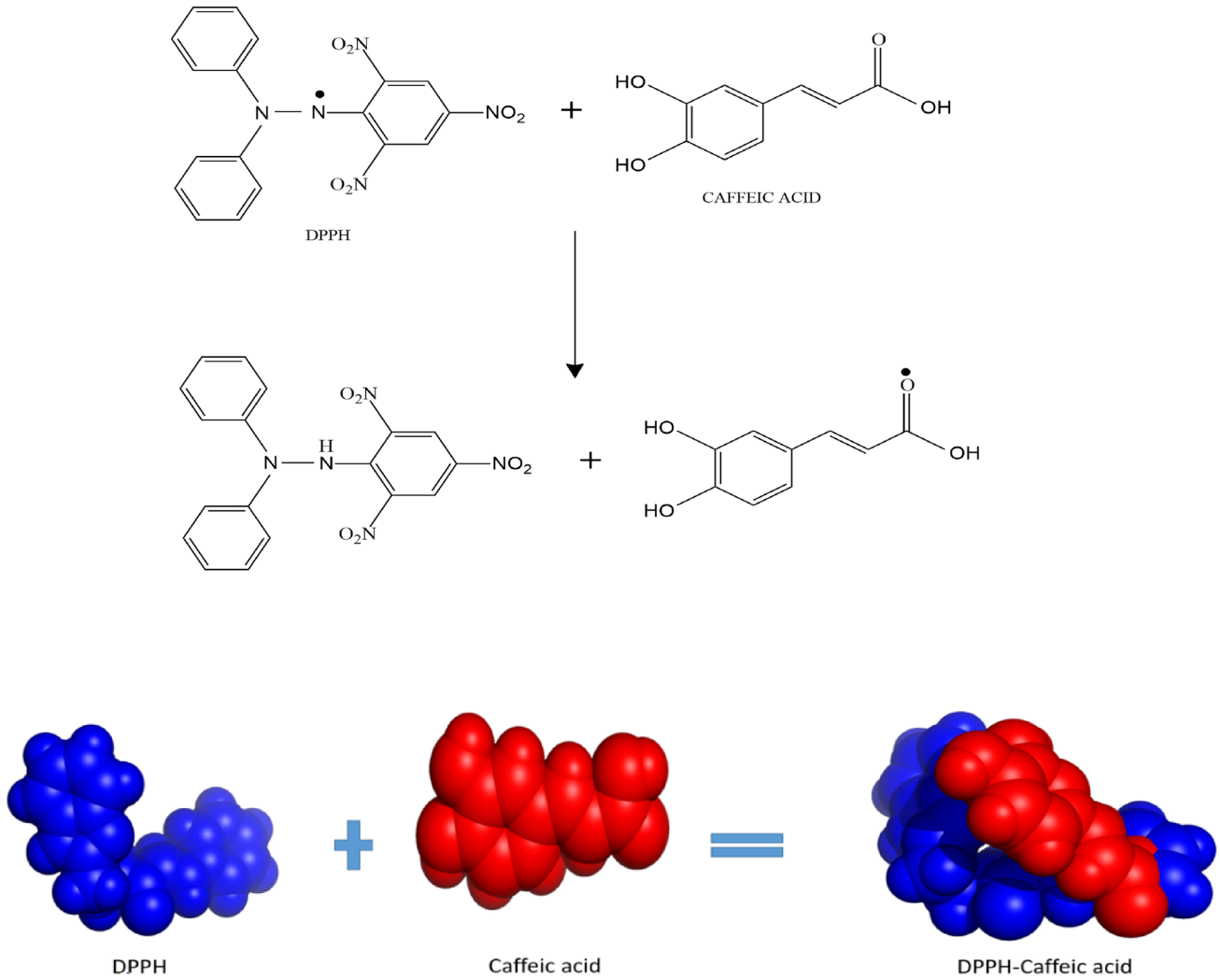


Fig. 8 Shows the proposed chemical reaction of caffeic acid on DPPH. The reaction displays the donation of hydrogen atoms and scavenging of the radical present on the Nitrogen atom of the free radical molecule

aeruginosa growth was inhibited. Gentamicin, used as the positive control, shows a high inhibition in all the bacteria, showing a diameter of 25 mm. Deionized water was used as the negative control and showed no effect on the various bacteria (Table 3).

Methanolic extract of dandelion has an inhibitory effect on *S. aureus* but was not active against *E.coli* and *B. cereus* [31]. In our study, DRE was inactive and showed no inhibitory zone against *S. aureus*, *P. autogiros*, *E. coli*, and *B. subtilis.* After incubation for 24 and 48 h, no inhibition zone was observed, which was of interest to the study. The assay was repeated three times, but the result remained the same. First, this could be attributed to the conditions or reagents used for the assay. Despite the presence of polyphenols, we could suggest that the bioactive components involved in antimicrobial activity are in a lower percentage due to environmental factors. Also, it might be due to the activation of other biological systems, such as some components in the bacteria. Woods and Nelson discovered that nitric acid synthase was activated by *Taraxacum officinale* in a mouse model, and the result shows that the dandelion plant tends to upregulate the production of Nitric oxide from rIFN-gamma-primed mouse peritoneal macrophages, which brings about the production of TO-induced TNF-alpha secretion [32].

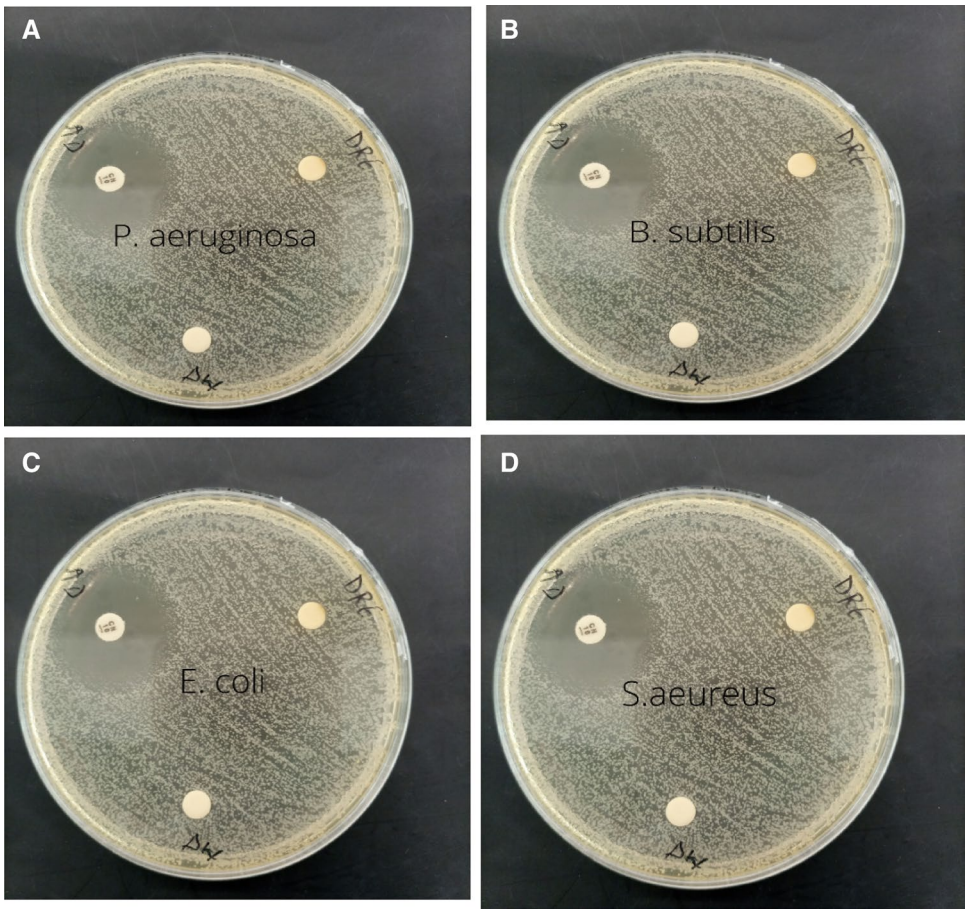


Fig. 9 Shows the antimicrobial activity of DRE on (**A**) *P. aeruginosa,* (**B**) *B. subtilis,* (**C**) *E. coli* (**D**) *S. aureus*. The disc diffusion assay was used to evaluate the effect of DRE on the above gram-positive and gram-negative bacteria inoculated on the Petri dishes. At the same time, the negative control (deionized water) and DRE were coated on the paper discs to determine the zone inhibition

Table 3 Results are presented as mean ± SEM of inhibition zone (mm) of at least triplicates (n ≥ 3)

Bacterial strains	*Bacillus cereus* (mean ± SEM)	*Escherichia coli* (mean ± SEM)	*Staphylococcus aureus* (mean ± SEM)	*Pseudomonas aeruginosa* (mean ± SEM)
Gentamycin (10 µg/disc)	25 ± 0.4	25 ± 0.7**	25 ± 0.2	25 ± 0.8**
DRE (1–3 mg/mL)	0.00**	0.00**	0.00**	0.00**
DRE (4 mg/mL)	0.00**	0.00**	0.00**	0.00**
DRE (5 mg/mL)	0.00**	0.00**	0.00**	0.00**
Distilled water	0.00**	0.00**	0.00**	0.00**

DRE Dandelion root extract, *SEM* Standard error of the mean

***P* < 0.01 vs control (standard group). (0.00) indicates no significant antibacterial activity

6 Limitations of the study

The investigation of our study on the activities of dandelion root extract (DRE) and its cytotoxic effect on MDA-MB-231 breast cancer cells has some limitations that depend on in vitro results, and these results may not necessarily translate directly to in vivo studies. The exact bioactive compound of the plant responsible for the cancer study must be further investigated and isolated. Investigating and studying the DRE extract on MDA-MB-231 breast cancer cells alone limits its applicability to other breast cancer subtypes or differentiated tumors. The effect of the DRE extract should be extensively studied also on other types of cancer cells. The DRE studies do not address possible toxicity or adverse effects in non-cancerous cell lines. The result also obtained from the antimicrobial studies must be explored

more in order to validate its effect on bacteria. Further research and study are needed to isolate the specific bioactive compounds within the DRE responsible for cancer cytotoxicity.

7 Conclusion

The DRE used for this research was confirmed to possess a variety of compounds, which were evaluated by the GC–MS device. As mentioned, the dandelion root extract contains taraxesterol, phenolic, and triterpenoid. However, the DRE with different concentrations of 0.5, 1.0, 2.5 and 5 mg/mL used in this research shows a good effect on breast cancer cells by inducing apoptosis, causing the tumor to shrink. The phytochemicals mentioned above were responsible for destroying the cancer cell membrane and the arrest of the cell cycle by activating the degradation pathway. DRE also shows good antioxidant activity that can scavenge reacting oxygen species by donating an electron to DPPH. 0.1 mg/mL of DRE did not produce more protons to the stable free radical molecule, but an increase in concentration of DRE to 0.5 mg/mL raised its ability to donate more electrons. More so, the 2.0 mg/mL produced the best antioxidant activity. The DRE was not active against *S. aureus, B. subtilis*, *P. aeruginosa* and *E. coli*. These might be due to the resistance capacity of the bacteria. The 2.5 mg/mL concentration of dandelion root extract provided the best biological activity on breast cancer cells compared to 0.5 and 1.0 mg/mL.

Acknowledgements The dandelion root extract was collected from Badagry local government area, Lagos state (Nigeria: 6° 25′ 53. 688″ N). A pharmacist at the faculty of pharmacy at Cyprus International University authenticated the plant. The root was assigned a voucher number CIU/PHAR/ASTE/001. The plant is cultivated both locally and wild within the community. No permission or license is needed to obtain the plant, as it is planted everywhere within the community or grown wild and used by everyone in the community. However local verbal permissions were given to the authors for the plant to be harvested and used for the study.

Author contribution R O S: Investigation, Writing—Original draft preparation, Methodology, Validation. P C P: Supervision, Writing—Review & Editing. I C: Resources, Writing—Review & Editing. R O S: Formal analysis, Data collection. I C: Resources, Data collection, P C P: Conceptualization, Writing—Review & Editing, Supervision, Project administration.

Data availability The Data used during the current study are available from the corresponding author on reasonable request. The data from the analysis of the cytotoxicity, characterization and the whole study will be made available upon request from the corresponding author because restrictions apply to the availability of these data and can only be used upon request.

Declarations

Competing interests The authors declare no competing interests.

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