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Research Article

ANTICANCER EFFECTS OF BROCCOLI EXTRACT ON BREAST CANCER CELL LINES: A PHYTOCHEMICAL PERSPECTIVE

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ABSTRACT

Objectives: The study aims to evaluate the phenolic content, antioxidant activity, GCMS and LCMS phytochemical profiles, as well as the therapeutic properties of broccoli extract on the breast cancer cells (MCF-7) and the non-cancerous mouse fibroblast cell line, 3T3-L1.

Methods: We studied the antibreast cancer effect of the broccoli extract *in vitro* using human breast cancer cell line MCF-7 and mouse fibroblast cell line 3T3-L1 using the cell cytotoxicity assay, and the percentage of cells that are viable was calculated at varying concentrations and different time incubations, i.e., 24 and 48 h. Furthermore, biochemical estimation was done to check the total phenolic content using Folin–Ciocalteu's method and antioxidant assays using 2,2-diphenyl-1-picrylhydrazyl and ABTS assays. To check the bioactive profile of the broccoli extract, gas chromatography–mass spectrometry, liquid chromatography–mass spectrometry (LCMS), and high-performance liquid chromatography (HPLC) analyses were done. As sulforaphane is the major bioactive compound of the broccoli, its presence was confirmed by LCMS and HPLC.

Results: Broccoli extract was found to be rich in polyphenols and showed a potent number of antioxidants, which states that it reduces the reactive oxygen species production and hence reduces the oxidative stress. It also showed that it decreased cell viability, and the outcomes depended on the dose and time.

Conclusion: Based on the reduction of breast cancer cells' viability, broccoli extract was found to exhibit anticancer effect on MCF-7 cell lines. It has shown that the broccoli extract is rich in antioxidants and highly abundant in polyphenols and anticancer in nature. Further *in vivo* studies will be done to investigate the anticancer efficacy of the broccoli extract in mice models. This encouraging discovery emphasizes the necessity to investigate broccoli extract's potential application as a breast cancer treatment.

Keywords: Breast cancer, *Brassica oleracea*, Antioxidants, Anticancer, MCF-7, Fibroblast, Cell lines, Liquid chromatography–mass spectrometry/mass spectrometry, Gas chromatography–mass spectrometry.

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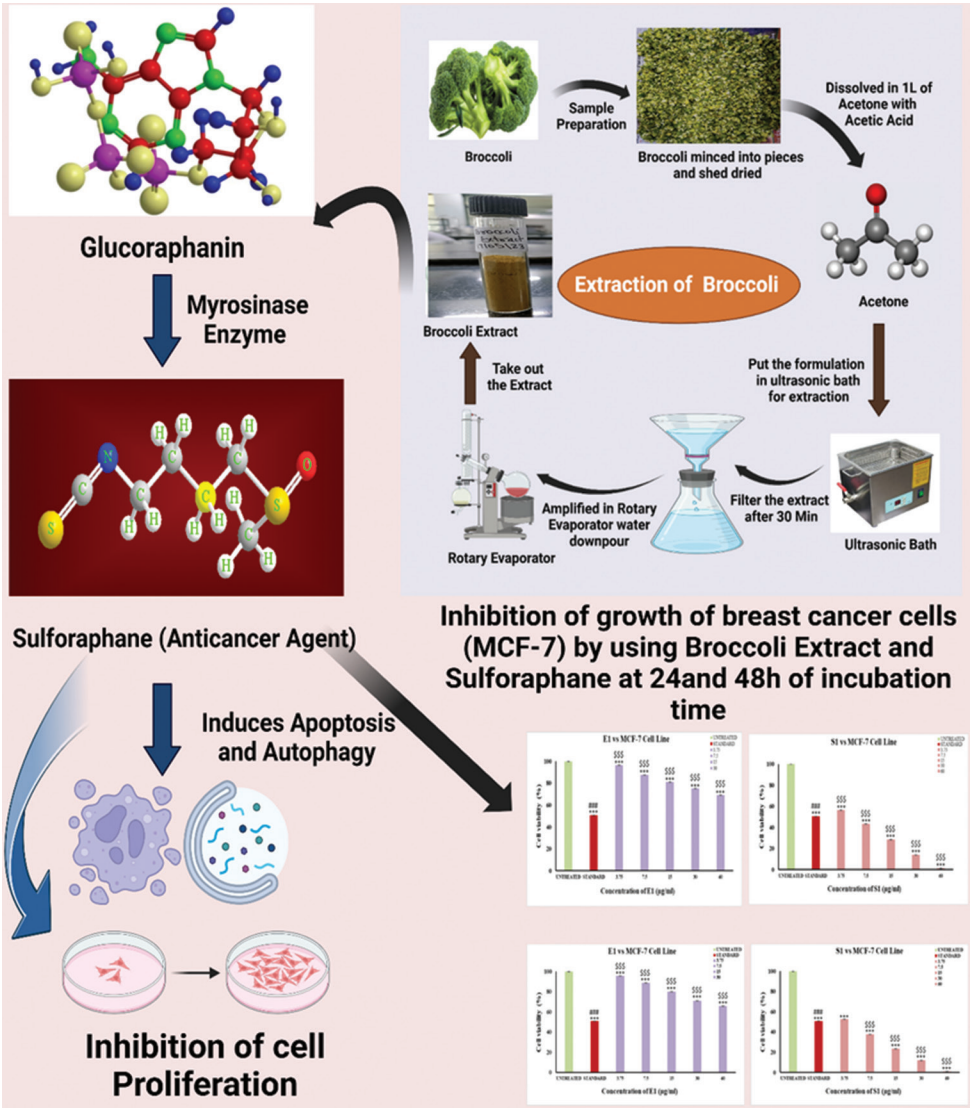
INTRODUCTION

Breast cancer is among the top two cancer-related causes of death for women globally and the most common cancer overall. In 2022, there were projected 51,400 cases of ductile carcinoma *in situ* and 2.8 million new occurrences of invasive breast cancer, with 43,250 deaths anticipated as a result, according to the most recent figures [1]. According to the projections made by the International Agency for Research on Cancer/World Health Organization, there will be one million annual deaths and over 3 million new cases of breast cancer by 2040 [2]. With 1.57 million cases officially reported in 2012, breast cancer ranks among the most serious illnesses that impact women. As of right now, the primary cause of fatalities for women is breast cancer [3]. Numerous internal and external variables can contribute to the development and occurrence of breast cancer. Its prevalence is associated with poor lifestyle choices, variables related to the environment, and sociopsychological factors. Research indicates that between 5% and 10% of cases of breast cancer are linked to family history and genetic abnormalities, while 20% to 30% of cases are related to potentially modifiable variables [4]. Breast cancer patients can receive a variety of traditional treatment options, including hormonal therapies, monoclonal antibody therapies, immunotherapy, small molecular inhibitors, radiotherapy, and chemoradiotherapies (such as adjuvant chemotherapies as well as neoadjuvant therapy) [5,6]. However, these therapeutic modalities have risks and side effects. Therefore, to minimize the drawbacks associated with breast cancer patients, including growing resistance to traditional therapies, side effects, and the toxicities of current treatment modalities, new techniques and methods are required [7].

It is well recognised that therapeutic plant extracts are now receiving significant attention. Currently, 70% of anticancer chemicals are derived from medicinal plants, making them the primary source for the development of anticancer medications. Regarding natural resources, phytochemicals are crucial for anticancer medication. From towns to urban areas and from developing to industrialized nations, they are non-toxic, safe, and easily accessible sources [8]. There are different types of new chemical compounds found in plants, including lignins, glycosides, steroids, terpenoids, and alkaloids. Many well-known medications are developed using lead compounds from natural items. In addition, herbal medications have less or no adverse effects compared to other synthetic drug groups, and they may be used in combination with target therapy [9]. The well-known green vegetable "broccoli" (*Brassica oleracea* var. *italica*) is a staple of the Mediterranean diet because of its many health advantages. The potential anticancer effect of broccoli is one of the main bioactive effects that have been identified. Broccoli is one of the vegetables in the Brassicaceae family that is becoming more and more popular since numerous epidemiological studies have shown that vegetables high in certain phytochemicals are linked to a lower incidence of breast cancer and other cancers [10]. Because of their potential to prevent cancer, glucosinolates are one of the most significant phytochemicals

GRAPHICAL ABSTRACT



among all the bioactive components of broccoli. It is also important to note that there are already more than 200 glucosinolates known to exist. Sulforaphane is a phytoconstituent belonging to the isothiocyanate group and an organosulfur compound that is common in broccoli [11].

There have been few studies on broccoli's ability to prevent cancer in people to date. The majority of research on the anti-cancer effects of broccoli extract focused on the characteristics of sulforaphane, a substance presents in cruciferous vegetables [12]. Chemopreventive agents have been included in combination treatment strategies to achieve a therapeutic harmony between individual drugs when limiting systemic toxicity induced by these therapies. This avoids the complications related to standard cancer treatments, such as chemotherapy. Therefore, this study investigated the potential preventative effects of broccoli (*B. oleracea* var. *italica*) extracts obtained from different phenological stages in conjunction with its bioactive component SFN. More *in vivo* research will be conducted to investigate the cancer-fighting abilities of broccoli extract.

METHODS

Chemicals and reagents

Products from Sigma-Aldrich included fetal bovine serum, MTT reagent 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, and Dulbecco's Modified Eagle's medium (DMEM) media. Fibroblast cells were used as the control group, and the selection of MCF-7 breast cancer cells was based on the preservation of multiple characteristics common to the mammary epithelium. Both cell line cultures were purchased from the National Centre for Cell Science (NCCS) Pune, India. Gallic acid, Folin reagent, Ascorbic acid, ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid, Hydrogen peroxide, DPPH (2,2'-diphenyl-1-picrylhydrazyl), Potassium persulfate was purchased from Sigma Aldrich, Sulforaphane was purchased from Sigma Aldrich.

Collection, authentication, and extraction of plant samples

Samples of broccoli (*B. oleracea*) were bought in January 2023 at a market close to Punjab, India. Prior to examination, the broccoli samples were kept in a dry, cool environment. The samples of broccoli were thoroughly cleaned with sterile distilled water. Broccoli stems and florets were divided. Only florets were used for the further process. The plant was identified and a voucher number (NIScPR/RHMD/Consult/2023/4406-07) was provided by Mr. R.S. Jayasomu, Chief Scientist and Head RHMD, and Dr. Sunita Garg, prior Chief Scientist and Head RHMD, CSIR-NIScPR, at the CSIR-National Institute of Science Communication and Policy Research, Raw Materials herbarium and Museum in New Delhi, India. The broccoli leaves underwent separation, cleaning, shed drying, and chopping. Three liters of acetone was mixed

with 5 kg of minced broccoli along with 5 mL of acetic acid per liter of acetone. The extraction process was placed in an ultrasonic bath for 30 min at 25±2°C. Following this, the extract underwent filtering, and the leftover material was cleaned with acetone. At 40°C, the extracted material was concentrated using a rotating evaporator in a water bath [13]. The broccoli extract yield was 31.25±0.2 g. The following formula was used to determine the extract's yield in percentage:

$$\% \text{ Yield} = \frac{\text{Mass of extract (g)}}{\text{Mass of sample (g)}} \times 100$$

Total phenolic content (TPC) determination

The amount of phenolic content in an extract was determined using the Folin–Ciocalteu's procedure. Initially, 200 µL of the extract was mixed (1 mg/mL) with 3 mL of water. Then, 0.5 mL of the Folin–Ciocalteu's reagent was added and left it for a duration of 3 min. Next, 2 mL of 20% sodium carbonate was added. After an hour of the combination standing in the dark, the absorbance was measured at 650 nm. The overall concentration of phenolic chemicals was determined using a calibration curve. The findings were presented as milligrams of gallic acid equivalent/gram of dry weight [14].

Antioxidant analysis assays

DPPH assay

The extracts' ability to scavenge free radicals was accessed using the DPPH radical scavenging test. The decolourisation rate of a 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution in methanol was assessed to determine the ability of the plant extracts to donate hydrogen atoms. The purple-violet hue produced by DPPH in a methanol solution fade to yellow tones when antioxidants are present. In a methanol solution, 3 mL of 0.1 mM DPPH was mixed with 1.6 mL of the extracts in methanol at various concentrations (100, 200, 300, 400, and 500 µg/mL). After fully vortexing the reaction mixture, it was allowed to sit at room temperature in the dark for half an hour. At 517 nm, the mixture's absorbance was determined using spectrophotometry. The reference was L-ascorbic acid [15]. The DPPH radical scavenging activity % was calculated using the following formula:

$$\% \text{ Inhibition of DPPH and radical scavenging activity } = \frac{(A_c - A_s)}{A_c} \times 100$$

Where *As* stands for the extractives'/standard's absorbance and *Ac* for the control's absorbance. The IC_{50} was then calculated by graphing the percentage of inhibition against concentration. Three runs of the experiment were made at each concentration.

ABTS radical scavenging activity

To assess antioxidant capacity, the ABTS free radical scavenging assay was used. After combining 2.45 mM of potassium persulfate (K2S2O8) with ABTS to achieve a 7 mM concentration, the reagent was kept at room temperature in the dark for 12–16 h. The test solutions were then mixed with 2 mL of diluted ABTS solution (mixed to a total volume of 1 mL with distilled water) at various concentrations (100, 200, 300, 400, and 500 µg/mL). After vortexing the reaction mixture for 10 s, it was allowed to sit at room temperature in the absence of light. After 6 min, the absorbance was taken, and the percentage of scavenging radicals was computed using a blank that contained no antioxidant. The classic antioxidant component, ascorbic acid, was produced fresh and used in varying concentrations [16]. To determine the proportion of ABTS radical scavenging activity, the following formula was applied:

$$\% \text{ Inhibition of ABTS and radical scavenging activity } = \frac{(A_c - A_s)}{A_c} \times 100$$

Where A_c represents the intensity of absorbance of the control and A_s is of the extractives/standard. The IC_{50} was then calculated by graphing the percentage of inhibition against concentration. Three runs of the experiment were made at each concentration.

Identification of bioactive compounds in broccoli extract

Gas chromatography–mass spectroscopy (GC-MS) analysis of the extract

This analysis was carried out at Lovely Professional University in Phagwara, Punjab, India, using gas chromatography and mass spectrometry by using this Agilent analytical equipment [GCMS-TQ8040 NX] Gas Chromatograph Mass Spectrometer, purchased from Shimadzu Corp. 00018, serial number 0217556, and a capillary column measuring 30m, 0.25mmID and 0.25µm df were used to analyze the broccoli extract bioactive compounds. The column oven temperature was 50°C, the injection volume was 1 µl, and the injection temperature was 250°C with a flow rate of 1.02 mL/min and a total run time of 33.50 min. The solvent was used high-performance liquid chromatography (HPLC) grade DMSO [17].

Liquid chromatography–mass spectroscopy (LC-MS) of the broccoli extract

At Punjab University, LC-MS analysis was carried out (Chandigarh, India) as outlined by Ali Redha *et al.* [18]. Chromatographic separation was performed using an ultra-performance liquid chromatography mass spectrometry system (Waters, SYNAPT-XS HDMS, UK) with a model number DBA064 system. The system used a Waters column C18 Waters, Acquity BEH 2.1*100mm 1.7µm kept at 40°C and two mobile phases (A and B). The mobile phase A included 0.1% formic acid in LC-MS grade water, whereas the mobile phase B contained 0.1% formic acid in acetonitrile. The samples were separated under the subsequent conditions over a 45-min run time at the rate of flow of 0.2 mL/min along with an injection volume of 5 µL: 95% of A to 5% of B at 6 min, 10% of A to 90% of B at 9 min, and 95% of A and 5% of B at 13 min, and additionally, they took 1 min to equilibrate. Mass measurements were conducted utilising positive electrospray ionisation modes (ESI+) along with the subsequent parameters: The source temperature is set at 120 °C, with a capillary voltage of 3.22 keV. The cone voltage is maintained at 50 V, while the desolvation and cone gas flow rates are 950 L h-1 and 50 L h-1, respectively.

HPLC of the broccoli extract

The broccoli extract was analyzed using the HPLC method described by Park [19], using a Shimadzu system consisting of an SPD-M20A photodiode array detector. The Shimadzu Column oven CTO-10ASvp was utilised, maintaining a column oven temperature of 30°C. Mobile phase A was made with formic acid (0.1%) and distilled water, and mobile phase B was made with formic acid (0.1%) and acetonitrile to create the mobile solvent system. The slope profile changed throughout the next five to twenty minutes. The flow rate was maintained (1.0 mL/min) throughout the analysis of the sample. For sample injection, 20 µL of the broccoli extract solution was injected at 5 mg/mL. Furthermore, a standard sample consisting of 100 ppm sulforaphane (Sigma-Aldrich) dissolved in methanol was injected. The wavelength for the detector was changed to 254 nm. By correlating the retention periods of the peaks identified in the extract analysis with those in the standard analysis, the chemicals were successfully identified.

Cell culture

The human MCF-7 breast cancer cell line and the 3T3-L1 mouse fibroblast cell line were obtained from the National Centre for Cell Science in Pune. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS) (Himedia) at 37°C and 5% CO_2, with the addition of 1 ml of antibiotics (penicillin and streptomycin) (Himedia).

Cell viability testing

The method previously outlined by Almawash [20] and Khedkar and Khan [21] was used to measure cell viability. To put it briefly, 96-well microtiter plates were seeded with MCF-7 cells, and the plates were then incubated for 24 and 48 h. After the adhesion of cells, the cells were twice washed in phosphate-buffered saline before being

reintroduced with fresh media containing the specified amounts of broccoli extract. After the treatments were completed, the cells were cleaned with phosphate-buffered saline and treated with a solution of MTT in the medium for 4 h. Dimethyl sulfoxide was employed for dissolving formazan crystals generated in living cells. Before assessing the cell viability of the plates using an ELISA plate reader (Bio rad model) calibrated at 595 nm, the plates were incubated for another 24 h. The formula used for the calculation of the cell viability is:

$$\text{Cell viability } (\%) = \frac{\text{Abs of treated cells}}{\text{Abs of control cells}} \times 100$$

Statistical analysis

The data were shown as the standard deviation plus the means of the samples' (n=3) triple analysis. GraphPad Prism (v, 5.01), a program for statistical analysis application, was used to do a one-way analysis of variance (ANOVA) using Tukey's multiple comparison the data was subjected to a statistical test ($p<0.05$). Substantial variations between the types or in comparison to the control were identified using $p < 0.05$.

RESULTS

TPC

Using the gallic acid curve as a standard (Fig. 1), the amount of TPC of the plant extract was calculated and expressed as the equivalent of gallic acid (GAE)/g. The TPC of *B. oleracea* var. *italica* was determined by utilizing the standard gallic acid calibration curve. The extract's phenolic concentration was determined to be 82.907 µg GAE/g. Phenolic chemicals were present in the *B. oleracea* var. *italica* extract, as confirmed by the phytochemical screening assay. *B. oleracea* var. *italica* had a significant phenolic content (Fig. 2), which could account for the extract's anti-inflammatory and antioxidant properties.

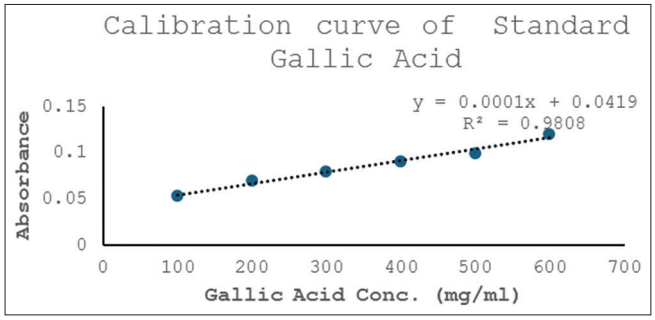


Fig. 1: Calibration curve of standard gallic acid

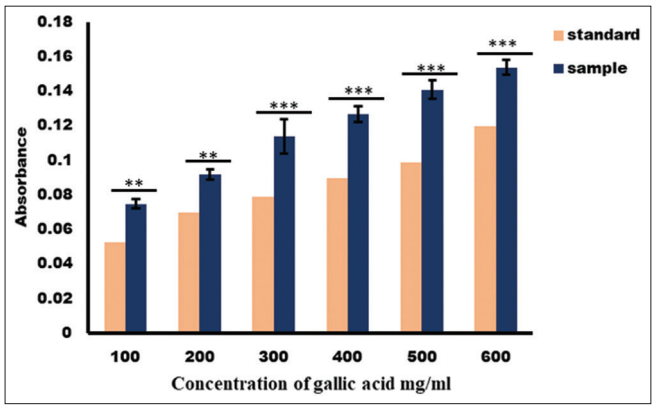


Fig. 2: Indicates the broccoli extract's total phenolic content; the results are shown as the mean±SEM of three different experiments. ANOVA was used to show the significant variations, with the significant values **p<0.01, ***p<0.001, the experiment was conducted in triplicates (n=3)

Antioxidant activity

DPPH radical scavenging activity

The antioxidant activity of the broccoli extract was evaluated employing the DPPH assay, in which ascorbic acid was used as a control. The extract's capacity to scavenge free radicals in the current investigation was equivalent to that of the control group. The antioxidant capacity of the broccoli extract at various concentrations (500, 400, 300, 200, and 100 µg/mL) was pointedly highest (79.2% at 500 µg/mL), while extract in concentration of (400, 300, 200, and 100 µg/mL) exhibited antioxidant activity (69.01, 63.52, 52.04, and 36.04%) individually, and the antioxidant characteristics of ascorbic acid at the same dose were (80.00, 70.40, 69.60, 59.40 and 40.09 µg/mL) (Fig. 3). Similar to ascorbic acid, the synthesis of broccoli extract demonstrated a free radical scavenging activity that displayed significant variations at each dosage ($p\leq0.05$) when compared to the control. These findings confirmed that the extract's ability to scavenge free radicals (DPPH) was dose related. The equation below was used to calculate the percentage inhibition:

$$\%\ \text{Inhibition of DPPH and activity of radical scavenging} = \frac{(A_c - A_s)}{A_c} \times 100$$

Where A_c denotes the value of control, i.e., DPPH, and A_s is the value of sample.

ABTS radical scavenging activity

The Trolox Equivalent Antioxidant Capacity (TEAC) technique, also known as the ABTS method, was used to evaluate the antioxidant potential of extracts derived from residual broccoli. The anti-radical efficacy of the extracts was assessed by comparing them to the stable free radical ABTS+. The activity of the samples was monitored spectrophotometrically on ABTS+ at 414 nm. ABTS scavenging activity was also dose dependent and the same concentrations were taken, and the activity of antioxidants was checked (Fig. 4). However, the interpretations showed that ABTS assay is more efficient in reflecting the antioxidant properties for the highly pigmented materials. Percentage inhibition was measured by the following equation:

$$\%\ \text{Inhibition of ABTS and activity of radical scavenging} = \frac{(A_c - A_s)}{A_c} \times 100$$

Where A_c is value of control, i.e., ABTS and A_s is the value of sample.

GCMS analysis of the prepared extract

The National Institute of Standards & Technology (NIST) database was used to analyse the GC-MS mass spectrum. It was decided to compare

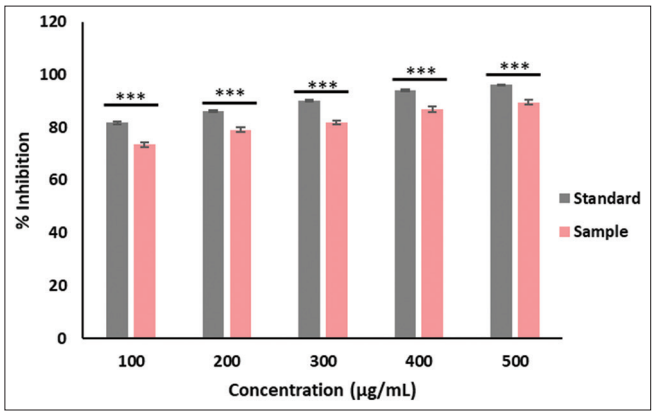


Fig. 3: The radical scavenging activity using DPPH assay of the broccoli. The results are shown as the mean±SEM of the extract's three independent experiments and ANOVA was used to achieve the analysis of variance, the experiment was conducted in triplicates (n=3). The statistical significance was determined as ***p<0.001

the mass spectra of the unidentified component with the spectrum of the previously identified element found in the NIST collection. Using exact criteria and data from electronic libraries, the essential components were identified. Regarding the test materials, the following data were gathered: molecular mass, peak area, chemical name, probability, chemical formula, and biological activity. By relating each component's median peak region to the overall area, the associated percentage or amount was found. Based on the GC-MS spectrum, Fig. 5 shows different components with different retention times. To ascertain the chemical composition and arrangement of the molecules, the mass spectrometer is used to analyze the compounds that elute at various times. The important component disintegrates into more minor molecules, producing a peak having variable m/z ratio. The mass spectra serve as the fingerprint of the molecule in the data store because of their value. GC-MS study revealed several phytochemicals in the plant's aqueous extract that considerably enhance the pharmacological action of broccoli. Table 1 represents the phytochemicals pharmacological activity.

Liquid chromatography–mass spectrometry (LCMS) analysis of the broccoli extract

The LC-MS mass spectrum was interpreted using the Waters database as shown in Fig. 6. It was decided to compare the mass spectra of the unidentified component with the spectrum of the previously identified component found in the Waters collection. Using exact criteria and data from electronic libraries, the essential components were identified. Regarding the test materials, the following data were gathered: molecular mass, peak area, chemical name, probability, chemical formula, and biological activity, which are presented in Table 2. Each component's relative percentage amount was calculated by summing its average peak areas across all areas.

HPLC analysis of the broccoli extract

Sulforaphane was regarded as a reference compound in the HPLC analysis of the broccoli extract because it is one of the principal compounds in broccoli. The HPLC chromatograms for the standard and extract were acquired at 254 nm as shown in Figs. 7 and 8. The main peak of the sulforaphane was shown at the retention time of 19.65 min.

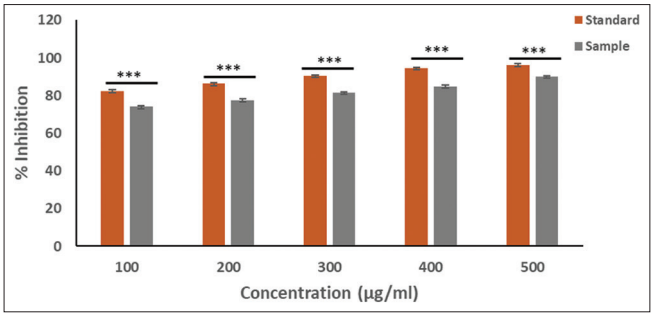


Fig. 4: The activity of radical scavenging using ABTS assay of broccoli. The findings are shown as the mean±SEM of three separate trials for the extract obtained using ANOVA, the experiment was conducted in triplicates (n=3). The statistical significance was determined as ***p<0.001

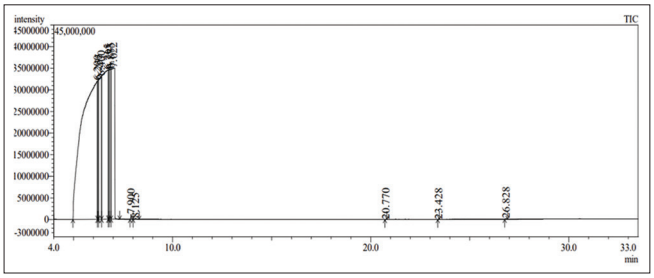


Fig. 5: GCMS chromatogram of the broccoli extract

The broccoli extract also showed the peak of sulforaphane at the same retention time of 19.65 min. The extract's sulforaphane content was 0.05±0.00%.

Cytotoxicity assay

The *in vitro* cytotoxic impact of the broccoli extract was quantified using the MTT test. The percentage of cell viability for MCF-7 and mouse fibroblast cell lines (3T3-L1) was determined. In a manner that is dependent on the concentration, the broccoli extract has the power to significantly reduce the viability of other cancerous cells. Although there was no discernible decrease in the viability of fibroblast cells, breast cancer cells exhibited a decrease in viability. Cell viability was assessed at two distinct incubation intervals (24 and 48 h), and the results showed that, at 1000 g/mL, MCF-7 and fibroblast cells had viability percentages of 75% and 85% at 24 h and 67% and 83% at 48 h, respectively. Cell viability remains unchanged at lower doses; a notable decline was seen only at values of 15 µg/ml and above as

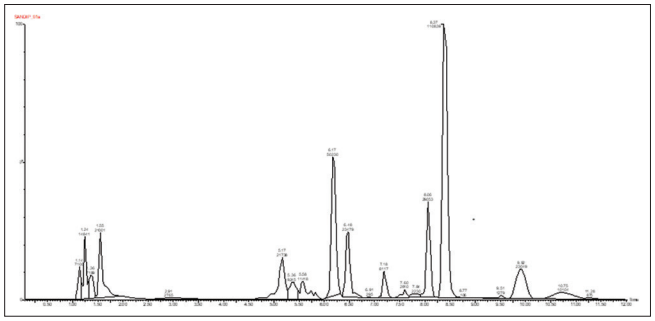


Fig. 6: Liquid chromatography–mass spectrometry chromatogram of the broccoli extract

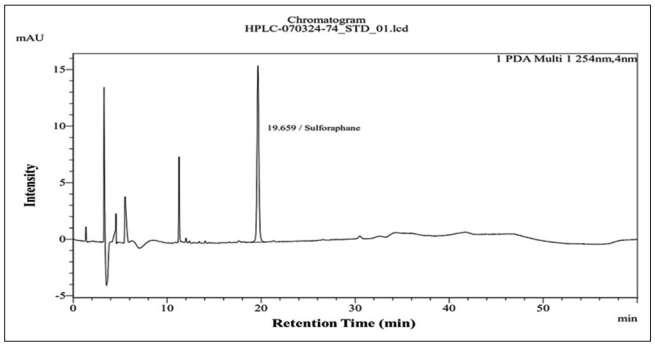


Fig. 7: The high-performance liquid chromatography chromatogram of sulforaphane standard

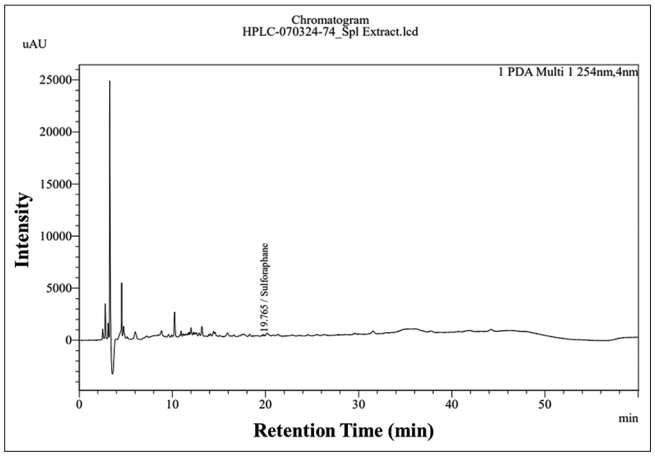


Fig. 8: The high-performance liquid chromatography chromatogram of broccoli extract

Table 1: Illustrates the phytochemical constituents found in the broccoli extract along with their pharmacological properties as determined through GCMS analysis. Two diverse compounds were identified through GCMS analysis, and these compounds have not been documented until now

Peak	Name of compound	R. time (Min.)	Area (%)	Molecular formula	Molecular weight (Da)	Biological activity
1.	Dimethylsulfoxonium formylmethylide	6.200	49.27	$C_4H_8O_2S$	120	Antioxidant and antimicrobial (Sheela *et al.*, 2021)
2.	1-Ethanol, 2-(ethylsulfinyl)-	6.255	3.01	$C_4H_{10}O_2S$	122	Miscellaneous compound
3.	Methane, (methylsulfinyl) (methylthio)-	6.400	8.65	$C_3H_8OS_2$	124	Antibacterial (Dhurgham, 2021)
4.	Dimethyl disulfide	6.715	19.39	$C_2H_6S_2$	94	Antioxidant, antibacterial (Ghoran *et al.*, 2018)
5.	Dimethyl sulfide	6.795	2.95	C_2H_6S	62	Volatile compound
6.	Disulfide, methyl (methylthio) methyl	6.875	5.32	$C_3H_8S_3$	140	Antioxidant, antibacterial (Ghoran *et al.*, 2018)
7.	Dimethyl sulfone	7.900	11.32	$C_2H_6O_2S$	94	Anticancer (Caron *et al.*, 2013)
8.	Benzene diazonium, 2-hydroxy-, hydroxide, inner salt	8.125	0.06	$C_6H_4N_2O$	120	Antimicrobial and antioxidant (Dhurgham, 2021)
9.	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	20.770	0.03	$C_{16}H_{22}O_4$	278	Anticancer, anti-inflammatory, and antioxidant (Sudha *et al.*, 2023)
10.	Dibutyl phthalate	20.770	0.00	$C_{16}H_{22}O_4$	278	Anticancer (Poonkodi *et al.*, 2017)
11.	1-Isobutylsulfanylmethyl-2,8,9-trioxa-5-aza-1-sila-bicyclo [3.3.3] undecane	23.428	0.00	$C_{11}H_{23}NO_3SSi$	277	Miscellaneous compound (Daji *et al.*, 2023).
12.	Bis (2-ethylhexyl) phthalate	26.828	0.01	$C_{24}H_{38}O_4$	390	Anticancer and anti-inflammatory (Tsai *et al.*, 2023 and Hsieh *et al.*, 2022)
13.	Phthalic acid, di (2-propylpentyl) ester	26.828	0.00	$C_{24}H_{38}O_4$	390	Anticancer (Ukwubile *et al.*, 2020)

Table 2: The phytochemicals present in broccoli extract identified by LCMS analysis

S. No.	Compound	Molecular/ Chemical formula	Particular molecular mass (Da)	Observed m/z	Mass error/ppm	Retention time (min)
1	Di (1,3-thiazol-2-yl)- sulfide	$C_6H_4N_2S_3$	199.95	222.94	4.63	1.14
2	(4Z)-2-(4-Chlorophenyl)-4-(3,5-dichloro-2-methoxybenzylidene)-5-methyl-2,4-dihydro-3H-pyrazol-3-one	$C_{18}H_{13}Cl_3N_2O_2$	394	395.01	−1.27	1.24
3	8-Amino-7-[2-hydroxy-3-(4-methoxyphenoxy) propyl]-1,3-dimethyl-3,7-dihydro-1H-purine-2,6-dione	$C_{17}H_{21}N_5O_5$	375.1	376.191	−2.88	1.36
4	Triethyl citrate	$C_{12}H_{20}O_7$	278.28	318.15	3.32	1.55
5	Gluconasturtiin (GNS)	$C_{15}H_{21}NO_9S_2$	422.05	422.05	− 3.3	2.91
6	Epomediol	$C_{10}H_{18}O_3$	186.28	225.08	0.89	5.17
7	Trimethylsilyl (3E)-3-methyl-5-[(trimethylsilyl) oxy]-3,5-hexadienoate	$C_{13}H_{26}O_3Si_2$	296.50	287.14	-1.25	5.36
8	2-(1,4,4-Trimethylcyclohex-2-en-1-yl) ethyl p-toluene sulfonate	$C_{18}H_{26}O_3S$	322.5	323.16	2.62	5.58
9	Glucoraphanin (GRA)	$C_{12}H_{23}NO_{10}S_3$	436.04	436.03	−4.1	5.73
10	4-(2-([tert-Butyl (diphenyl) silyl] oxy) ethyl)-2-methyl-4,5-hexadien-3-ol	$C_{25}H_{34}O_2Si$	394.23	436.25	−3.91	6.17
11	2,4-Dimethyl-1-([4-(4-propylcyclohexyl) phenyl] ethynyl) benzene	$C_{25}H_{30}$	330.23	369.19	1.162	6.48
12	({4-Methoxy-3-[(trimethylsilyl) oxy] benzyl} oxy) (trimethyl) silane	$C_{14}H_{26}O_3Si_2$	298.52	321.13	−3.69	6.91
13	Sulforaphane	$C_6H_{11}NOS_2$	177.28	177.21	0.10	7.18
14	2,3,4,6-Tetrafluoroaniline	$C_6H_3F_4N$	165.02	207.05	4.17	7.60
15	1-O-(indol-3-ylacetyl)-beta-D-glucose	$C_{16}H_{19}NO_7$	337.32	338.12	2.48	7.84
16	Ethyltributoxysilane	$C_{14}H_{32}O_3Si$	276.48	277.21	0.44	8.06
17	Leaf salicylate	$C_{13}H_{16}O_3$	220.36	243.09	−1.25	
	2-(2-Isobutoxyethoxy) ethyl acetate	$C_{10}H_{20}O_4$	204.26	243.09	−2.09	8.37
18	Pipofezine	$C_{16}H_{19}N_5O$	297.36	339.19	2.13	8.77
19	Gibberellin A24	$C_{20}H_{26}O_5$	364.4	347.18	1.56	
	Sphinganine	$C_{18}H_{39}NO_2$	301.29	302.30	4.13	9.51
20	Phytosphingosine	$C_{18}H_{39}NO_3$	317.5	317.29	3.85	9.92
21	N, N′-Bis (dimethyl-t-butyl silyl) ethylenediamine	$C_{14}H_{36}N_2Si_2$	288.62	289.24	−4.75	10.75

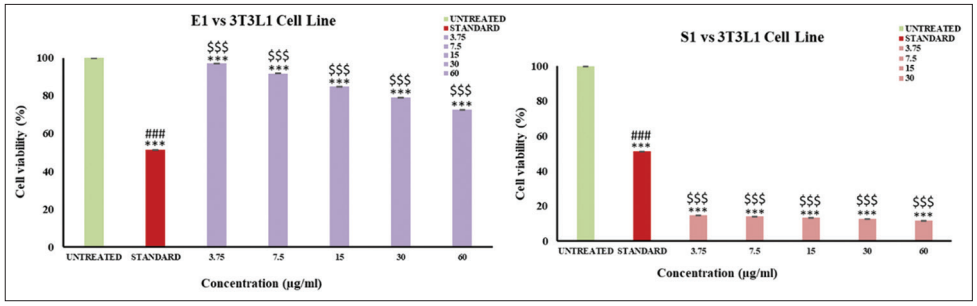


Fig. 9: The % cell viability at varying concentrations of broccoli extract and test compound sulforaphane at 24 h of incubation in comparison with the standard (Capsaicin) and untreated group, where E1 represents Broccoli extract and S1 represents test compound Sulforaphane, ***p<0.001, ANOVA and the comparison of the untreated group with the standard and extract are represented by the symbols ***p<0.001, respectively, in the results. The mean±standard deviation of the three independent experiments is displayed. The untreated and conventional treatments are compared using the values ###p<0.001 and $$$p<0.001 is showing the comparison of extract with the standard

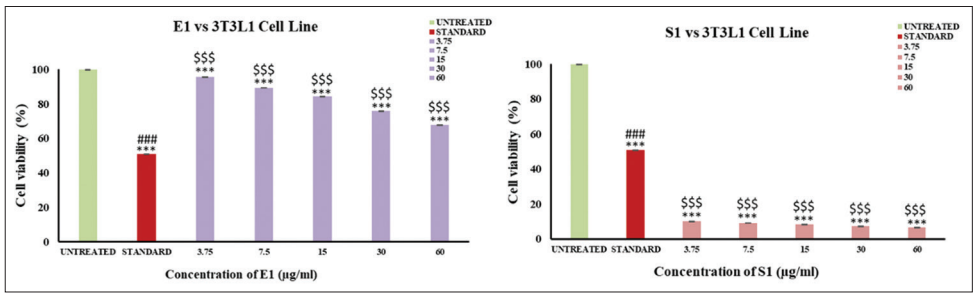


Fig. 10: The % cell viability at varying concentrations of broccoli extract and test compound sulforaphane at 48 h of incubation in comparison with the standard (Capsaicin) and untreated group, where E1 represents Broccoli extract and S1 represents test compound Sulforaphane, the three independent experiments' means ± standard deviations are displayed, with ***p<0.001, ANOVA denoting the comparison of the untreated group with the standard and extract, respectively. The statistical significance of Untreated with the standard is displayed by the numbers ###p<0.001 and $$$p<0.001 is showing the comparison of Extract with the standard

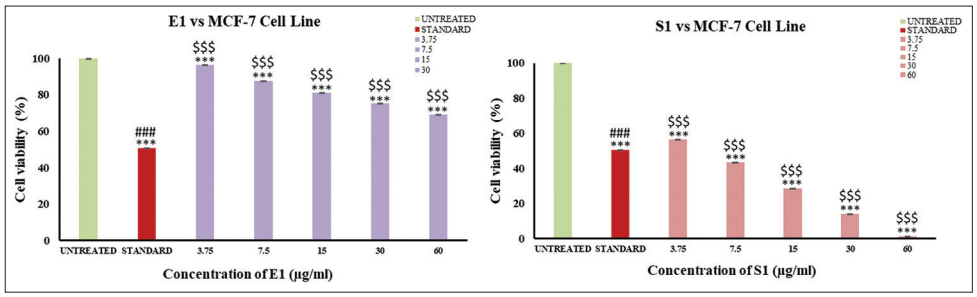


Fig. 11: The % cell viability at varying concentrations of broccoli extract and test compound sulforaphane at 24hr of incubation in comparison with the standard (cisplatin) and untreated group, where E1 represents Broccoli extract and S1 represents test compound Sulforaphane, the three independent experiments' means ± standard deviations are displayed, with ***p<0.001, ANOVA denoting the comparison of the untreated group with the standard and extract, respectively. The statistical significance of Untreated with the standard is displayed by the numbers ###p<0.001 and $$$p<0.001 is showing the comparison of extract with the standard

shown in Figs. 9-12. Sulforaphane was used as the test compound to measure cell vitality, and the results showed that while sulforaphane also reduced cell viability in mouse fibroblast cells, it only reduced cell viability in MCF-7 cells to 28% and 25% over the 24 and 48-h incubation periods, respectively.

DISCUSSION

According to gene expression profiling research, breast cancer is a diverse disease with several biologic subgroups. The expression of the estrogen receptor (ER), progesterone receptor, and human epidermal growth factor receptor 2 is used in clinical settings to identify these subtypes [22]. Surgery, hormonal therapy, radiation, chemotherapy, and immunotherapy are just a few of the breast cancer treatment techniques that can have severe adverse effects.

Consequently, there is an increasing necessity to find natural sources of safe and effective anticancer chemicals.

Broccoli, or *B. oleracea,* is one of the most popular vegetables. It has a high content of soluble fiber and a variety of nutrients, such as Vitamin A and C [23]. *B. oleracea*, commonly known as broccoli, is a rapidly growing annual plant in the Brassicaceae family that is used as an edible green vegetable. The medicinal properties of broccoli as an immunomodulator, antidiabetic, antibacterial, hepatoprotective, cardioprotective, anti-amnesic, antioxidant, and particularly in

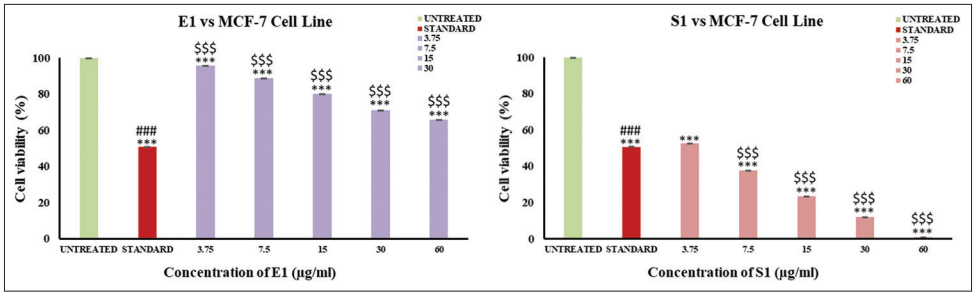


Fig. 12: The % cell viability at varying concentrations of broccoli extract and test compound sulforaphane at 48 h of incubation in comparison with the standard (cisplatin) and untreated group, where E1 represents broccoli extract and S1 represents test compound sulforaphane, the three independent experiments' means ± standard deviations are displayed, with ***p<0.001, using ANOVA, and analysis of variance denoting the comparison of the untreated group with the standard and extract, respectively. The statistical significance of Untreated with the standard is displayed by the numbers $^{###}$p<0.001and $^{$$$}$p<0.001 is showing the comparison of extract with the standard

anticancer applications have been well established [24]. Because broccoli (*B. oleracea*) naturally has a high quantity of phytochemicals that are bioactive such glucosinolates, phenolic components, Vitamin C, and mineral elements, it has been advertised as a food that promotes health. Therefore, eating a significant amount of broccoli contributes to the prevention of chronic illnesses like cancer, cardiovascular disease, and carcinogenic pathologies. It also helps prevent breast and prostate cancer [15]. According to reports, 25% of all cancers in women worldwide are breast cancers. This makes them the most common cancer in this population [25]. This study corroborates the findings of inquiry [26] and demonstrates that broccoli extract exhibits cytotoxic properties that inhibit the proliferation of MCF-7 cells. In addition, the antitumor activity of the broccoli extract was evaluated against two widely used cancer cell lines, MCF-7 and HeLa, respectively. The broccoli extracts significantly reduced the viability of these quickly proliferating cells, indicating that the extract does, in fact, have potent antitumor properties [27].

The *B. oleracea* extract's high phenolic component content was verified by the phytochemical screening assay. According to studies, cruciferous veggies may contain phenolic compounds [28]. The results of this study done by Jang *et al.* [29] showed that broccoli sprouts have a great potential to be an important antioxidant source. Broccoli sprouts are also rather simple to grow and have a short harvest period of 2 weeks. As a result, broccoli sprouts could be a fantastic natural antioxidant source. The study showed that the broccoli extract had high number of antioxidants in it when compared to the previous studies and both DPPH and ABTS assays showed similar results. The extract's ability to reduce stable DPPH radicals was evaluated using the DPPH test, which was based on the quantity of residual DPPH (%) shortly after 30 min. The high quantity of polyphenol chemicals in broccoli extract indicates that it has potent antioxidant properties, according to the research [30]. This finding suggests that broccoli may be used as a natural source of antioxidants or nutraceuticals to supplement industrially produced ones. In comparison to the DPPH radical, the extracts demonstrated greater scavenging activity against superoxide. Furthermore, florets and sprouts are a good source of anticancer chemicals, according to an *in vitro* antiproliferative research against prostate cancer cells (PC-3) [31].

As sulforaphane is the major active component of the broccoli, it was taken as a test compound. To validate the presence of Sulforaphane in broccoli extract, HPLC and LCMS analyses were done, as has been established in earlier research. The HPLC test verified the presence of sulforaphane in the broccoli extract, as did the LCMS experiments. The HPLC analysis previously reported by [33] was carried out using the standard test substance Sulforaphane, and it was verified at a retention period of 19.56 min. In LCMS, the sulforaphane was detected in the broccoli extract at the retention time of 7.18 min as previously confirmed by the study of Farag and Motaal [33]. Further to check the profile of bioactive compounds in broccoli extract, GC-MS analysis was done. There are 13 significant components described in broccoli extract, two of which are unknown chemicals that have not been documented before and have a role in anticancer action. Previous research have revealed the analysis of the broccoli extract using GCMS study [33].

After the phytochemical screening and biochemical estimation, the broccoli extract was tested for the *in vitro* studies using the breast cancer cell lines and fibroblast cell line, i.e., MCF-7 and 3T3L1. Numerous studies have shown the anticancerous effect of broccoli extract [34]. The *in vitro* studies were dose and time dependent; varying concentrations with the varying time were considered for the cell viability testing. The time incubation was 24 and 48 h for the varying concentrations of the broccoli extract and the test compound sulforaphane.The optimal concentration of broccoli extract and sulforaphane was determined to be 60 µg/ml, with a significant effect observed after 24 hours of incubation against the breast cancer cell line. Both the extract and sulforaphane inhibit cell growth in MCF-7 breast cancer cells, consistent with the findings of Gasmi *et al.* [10]. Numerous investigations have demonstrated the anticancer effects of SFN by influencing the various biological processes that tumor cells engage in. In breast cancer cell lines, SFN functions as an HDAC inhibitor and reduces the expression of ER, EGFR, and HER-2 proteins. SFN also triggers apoptosis and cell cycle halt [35]. The study emphasises critical aspects of tumour development and recurrence, including CRC stem cells and tumor-initiating cells, alongside their metastatic potential. The findings from [35] present strong evidence supporting the chemotherapeutic effectiveness of ITC-enriched extracts in the treatment of colorectal cancer (CRC). By avoiding the drawbacks of existing therapies and the use of conventional bioactive compounds chemically produced under hazardous procedures, this study reveals an exciting starting point to open the door for new therapeutic strategies resorting to the use of natural extracts for CRC treatment.

The outcomes showed that the two broccoli cultivars have distinct glucosinolate hydrolytic products. Broccoli extracts demonstrated the ability to scavenge free radicals and had a specific *in vitro* cytotoxic impact on the human colon cancer cell line (COLO-205). Furthermore, it has been observed to enhance the expression of thioredoxin reductase 1 in human MCF-7 cells, indicating a potential function in preserving redox in cell homeostasis [36].

The invasive potent of broccoli extracts on a human breast carcinoma cell line MCF-7 was demonstrated. Sulforaphane and glucoraphanin were found to be the two main ITC in the plant extract, according to LC-MS analysis. This result was consistent with the earlier findings [37]. In this study, the dose- and time-dependent cytotoxic actions of broccoli extract and sulforaphane are shown in Figs. 11 and 12.

CONCLUSION

The findings of this study demonstrate that broccoli extract significantly reduces the viability of MCF-7 breast cancer cells, indicating its

potential anticancer properties. This effect is likely attributed to the extract's rich composition of antioxidants and polyphenols, which are known to play a pivotal role in combating oxidative stress and inhibiting cancer cell proliferation. The promising in vitro results suggest that broccoli extract possesses bioactive compounds with strong anticancer potential. To further validate these findings, upcoming in vivo studies using a murine model will be essential in evaluating the extract's therapeutic efficacy, bioavailability, and safety profile within a living system. This encouraging discovery highlights the potential of broccoli extract as a natural, plant-based candidate for breast cancer treatment and underscores the importance of continued research into its clinical applications.

AUTHORS' CONTRIBUTION

Principal author: Kajal Parashar (lab work and drafting of the manuscript); Corresponding author: Minhaj Ahmad Khan (research inputs, critical review, and proofreading) (Address for correspondence: minhaj.15324@lpu.co.in), Mohammad Rashid Khan: (Proofreading)

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AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article. Contact the corresponding author to request data from this study.

DECLARATIONS

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

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COMPETING INTERESTS

None declared.

REFERENCES

1. Xu J, Gao F, Liu W, Guan X. Cell-cell communication characteristics in breast cancer metastasis. Cell Commun Signal. 2024 Jan 19;22(1):55. doi: 10.1186/s12964-023-01418-4, PMID 38243240
2. Dongsar TT, Dongsar TS, Abourehab MA, Gupta N, Kesharwani P. Emerging application of magnetic nanoparticles for breast cancer therapy. Eur Polym J. 2023 Apr 3;187:111898. doi: 10.1016/j.eurpolymj.2023.111898
3. Safiri S, Noori M, Nejadghaderi SA, Sullman MJ, Bragazzi NL, Almasi-Hashiani A, *et al.* Burden of female breast cancer in the Middle East and North Africa region, 1990-2019. Arch Public Health. 2022 Jul 11;80(1):168. doi: 10.1186/s13690-022-00918-y, PMID 35818086
4. Obeagu EI, Obeagu GU. Breast cancer: A review of risk factors and diagnosis. Med (Baltim). 2024 Jan 19;103(3):e36905. doi: 10.1097/MD.0000000000036905, PMID 38241592
5. Aizaz M, Khan M, Khan FI, Munir A, Ahmad S, Obeagu EI. Burden of breast cancer: developing countries perspective. Int J Innov Appl Res. 2023;11(1):31-7.
6. Sohel M, Aktar S, Biswas P, Amin MA, Hossain MA, Ahmed N, *et al.* Exploring the anti-cancer potential of dietary phytochemicals for the patients with breast cancer: A comprehensive review. Cancer Med. 2023 Jul;12(13):14556-83. doi: 10.1002/cam4.5984, PMID 37132286
7. Maashi MS, Al-Mualm M, Al-Awsi GR, Opulencia MJ, Al-Gazally ME, Abdullaev B, *et al.* Apigenin alleviates resistance to doxorubicin in breast cancer cells by acting on the JAK/STAT signaling pathway. Mol Biol Rep. 2022 Sep;49(9):8777-84. doi: 10.1007/s11033-022-07727-0, PMID 35804214
8. Gu HF, Mao XY, Du M. Metabolism, absorption, and anti-cancer effects of sulforaphane: An update. Crit Rev Food Sci Nutr. 2022 May 3;62(13):3437-52. doi: 10.1080/10408398.2020.1865871, PMID 33393366
9. Dura G, Hare J. Antiulcer activity of methanolic extract of *Gracilaria dura* (ag.). J Agric (red seaweed) in hare island, Thoothukudi, Tamil Nadu, India. Int J Adv Pharm. 2017;7:34-37.
10. Gasmi A, Gasmi Benahmed A, Shanaida M, Chirumbolo S, Menzel A, Anzar W, *et al.* Anticancer activity of broccoli, its organosulfur and polyphenolic compounds. Crit Rev Food Sci Nutr.2024;64(22):8054-72. doi: 10.1080/10408398.2023.2195493, PMID 37129118
11. Zhang Y, Lu Q, Li N, Xu M, Miyamoto T, Liu J. Sulforaphane suppresses metastasis of triple-negative breast cancer cells by targeting the RAF/MEK/ERK pathway. NPJ Breast Cancer. 2022 Mar 24;8(1):40. doi: 10.1038/s41523-022-00402-4, PMID 35332167
12. Kaiser AE, Baniasadi M, Giansiracusa D, Giansiracusa M, Garcia M, Fryda Z, *et al.* Sulforaphane: A broccoli bioactive phytocompound with cancer preventive potential. Cancers (Basel). 2021 Jan;13(19):4796. doi: 10.3390/cancers13194796, PMID 34638282
13. Borja-Martínez M, Lozano-Sánchez J, Borrás-Linares I, Pedreño MA, Sabater-Jara AB. Revalorization of broccoli by-products for cosmetic uses using supercritical fluid extraction. Antioxidants (Basel). 2020 Nov 27;9(12):1195. doi: 10.3390/antiox9121195, PMID 33261112
14. Nadzri MN, Majeed S, Sami F, Tabish M, Ansari MT. Preparation and evaluation of hydrogels containing methanolic extract of *Brassica oleracea* var. Italica. J Glob Pharm Technol. 2019;11(Suppl 1):140-6.
15. Hwang JH, Lim SB. Antioxidant and anticancer activities of broccoli by-products from different cultivars and maturity stages at harvest. Prev Nutr Food Sci. 2015 Mar;20(1):8-14. doi: 10.3746/pnf.2015.20.1.8, PMID 25866744
16. Xu Y, Xiao Y, Lagnika C, Song J, Li D, Liu C, *et al.* A comparative study of drying methods on physical characteristics, nutritional properties and antioxidant capacity of broccoli. Dry Technol. 2020 Jul 1;38(10):1378-88. doi: 10.1080/07373937.2019.1656642
17. Abukhabta S, Khalil Ghawi S, Karatzas KA, Charalampopoulos D, McDougall G, Allwood JW, *et al.* Sulforaphane-enriched extracts from glucoraphanin-rich broccoli exert antimicrobial activity against gut pathogens *in vitro* and innovative cooking methods increase *in vivo* intestinal delivery of sulforaphane. Eur J Nutr. 2021 Apr;60(3):1263-76. doi: 10.1007/s00394-020-02322-0, PMID 32651764
18. Ali Redha A, Hong HT, Torquati L, Nash GR, Gidley MJ, Cozzolino D. Development of liquid chromatography-mass spectrometry (LC-MS) method for quantification of broccoli sulforaphane. Food Anal Methods. 2024 Jan;17(1):1-6. doi: 10.1007/s12161-023-02544-y
19. Park HJ. Anti-inflammatory properties of broccoli sprout extract in a lipopolysaccharide-induced testicular dysfunction. J Anim Reprod Biotechnol. 2014;38(1):17-25. doi: 10.12750/JARB.38.1.17
20. Almawash S, El Hamd MA, Osman SK. Polymerized β-cyclodextrin based injectable hydrogel for sustained release of 5-fluorouracil/methotrexate mixture in breast cancer management: *In vitro* and *in vivo* analytical validations. Pharmaceutics. 2022;14(4):817. doi: 10.3390/pharmaceutics14040817, PMID 35456651
21. Khedkar S, Khan MA. An *in vitro* study elucidating the synergistic effects of aqueous cinnamon extract and an Anti-TNF-α biotherapeutic: Implications for a complementary and alternative therapy for non-responders. BMC Complement Med Ther. 2024 Mar 23;24(1):131. doi: 10.1186/s12906-024-04438-w, PMID 38521924
22. Mondal P, Kundu S, Banerjee U. Expression of vimentin and CD34 in triple negative breast cancer in a Tertiary Care Hospital in Kolkata. Int J Curr Pharm Rev Res. 2024;16(12):25-31.
23. Kamiyama M, Shibamoto T. Increase of bioactive sulforaphane and its related compounds with sulfur compounds in broccoli (*Brassica oleracea* var. italica) sprouts during cultivation. J Agric Food Chem. 2013;61(12):3113-9.
24. Al Talebi Z, Karhib MM, Taki MM, Salaam Abood E, Mubarak HA, Sahar Ahmed Ibrahim C. Chemical analysis, antioxidant, and antibacterial potential of aqueous extract of broccoli (*Brassica oleracea*) using GC-MS. Arch Razi Inst. 2023;78(2):593-9. doi: 10.22092/ARI.2022.359860.2488, PMID 37396738
25. Azubuike SO, Muirhead C, Hayes L, McNally R. Rising global burden of breast cancer: The case of sub-Saharan Africa (with emphasis on Nigeria) and implications for regional development: A review. World J Surg Oncol. 2018 Dec;16(1):63.
26. Ahmed ZS, Li X, Li F, Cheaito HA, Patel K, Mosallam EM, *et al.* Computational and biochemical studies of isothiocyanates as inhibitors

of proteasomal cysteine deubiquitinases in human cancer cells. J Cell Biochem. 2018 Nov;119(11):9006-16. doi: 10.1002/jcb.27157, PMID 30015387.
27. Shivale NI, Shah S, Sampat K, Deshpande K. Broad range activity of sulforaphane extracted from broccoli. Bionano Front. 2014;7:82-5.
28. Hwang JH, Lim SB. Antioxidant and anti-inflammatory activities of broccoli florets in LPS-stimulated RAW 264.7 cells. Prev Nutr Food Sci. 2014 Jun;19(2):89-97. doi: 10.3746/pnf.2014.19.2.089, PMID 25054107
29. Jang HW, Moon JK, Shibamoto T. Analysis and antioxidant activity of extracts from broccoli (*Brassica oleracea* L.) sprouts. J Agric Food Chem. 2015;63(4):1169-74. doi: 10.1021/jf504929m, PMID 25574973
30. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total phenolic content, flavonoid content, and antioxidant potential of wild vegetables from Western Nepal. Plants (Basel). 2019 Apr 11;8(4):96. doi: 10.3390/plants8040096, PMID 30978964
31. Chaudhary A, Sharma U, Vig AP, Singh B, Arora S. Free radical scavenging, antiproliferative activities and profiling of variations in the level of phytochemicals in different parts of broccoli (*Brassica oleracea* italica). Food Chem. 2014;148:373-80. doi: 10.1016/j.foodchem.2013.10.042, PMID 24262571
32. Liang H, Yuan QP, Dong HR, Liu YM. Determination of sulforaphane in broccoli and cabbage by high-performance liquid chromatography. J Food Compos Anal. 2006 Aug 1;19(5):473-6. doi: 10.1016/j.jfca.2005.11.005
33. Farag MA, Motaal AA. Sulforaphane composition, cytotoxic and antioxidant activity of crucifer vegetables. J Adv Res. 2010 Jan 1;1(1):65-70. doi: 10.1016/j.jare.2010.02.005
34. Çobanoğlu G, Nisari M, Nisari M, Uçar S, Kerek G, İnanç N. Does broccoli (*Brassica oleracea* var. *italica*) extract have potential therapeutic targets on human breast cancer (MDA-MB-231) using AgNOR detection method? Cumhuriyet Med J. 2023 Sep 30;45(3):44-56. doi: 10.7197/cmj.1297877
35. Pereira LP, Silva P, Duarte M, Rodrigues L, Duarte CM, Albuquerque C, *et al.* Targeting colorectal cancer proliferation, stemness and metastatic potential using Brassicaceae extracts enriched in isothiocyanates: A 3D cell model-based study. Nutrients. 2017;9(4):368. doi: 10.3390/nu9040368, PMID 28394276
36. Chaudhary A, Rampal G, Sharma U, Thind TS, Singh B, Vig AP, *et al.* Anticancer, antioxidant activities and GC-MS analysis of glucosinolates in two cultivars of broccoli. Med Chem Drug Discov. 2012;2(2):30-7.
37. Rose P, Huang Q, Ong CN, Whiteman M. Broccoli and watercress suppress matrix metalloproteinase-9 activity and invasiveness of human MDA-MB-231 breast cancer cells. Toxicol Appl Pharmacol. 2005 Dec 1;209(2):105-13. doi: 10.1016/j.taap.2005.04.010, PMID 15953625