ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2025, Vol. 41, No.(4):
Pg. 1194-1206

Spectral Analysis of Bioactive Compounds in Cold Methanolic Extracts of Commercial Broccoli Florets: Antioxidant Analysis and *In vitro* Cytotoxicity in A549 Lung Cancer Cells

RIHAB AKASHA[1], SIVAKUMAR S. MONI[2]*, MAALI D. ALSHAMMARI[3], ENTSAR MOHAMMED ALHAIDAN[4], AMANI SAYYAR ALRASHDI[1], BAYAN NAIF ALBASHER[1], KHADIJAHMANSOUR ALRESHIDI[1] and RAHAMAT UNISSA SYED[5]*

[1]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, University of Hai'l, 2440, Hai'l, Saudi Arabia.
[2]Department of Pharmaceutics, College of Pharmacy, Jazan University, Jazan 45142, Saudi Arabia.
[3]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Hai'l, Hai'l 81442, Saudi Arabia.
[4]College of Pharmacy, University of Hai'l, Hai'l 81442, Saudi Arabia.
*[5]Department of Pharmaceutics, College of Pharmacy, University of Hai'l, Hai'l 81442, Saudi Arabia.
*Corresponding author E-mail: ru.syed@uoh.edu.sa

http://dx.doi.org/10.13005/ojc/410415

(Received: May 24, 2025; Accepted: July 01, 2025)

ABSTRACT

The purpose of this study was to identify the bioactive components and evaluate *in vitro* antioxidant and cytotoxic activities of a cold methanolic extract of broccoli florets (CMEB). For identification of bioactive components, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography–mass-spectrometry (LC-MS) analyses were performed. The GC-MS analysis revealed the presence of several bioactive compounds including ethyl iso-allocholate, crinamine, heptadecane, and astaxanthin, among others. The LC-MS analysis in positive ionization mode identified ethyl iso-allocholate, lupeol, oleic acid, astaxanthin, and n-hexadecanoic acid, whereas in the negative mode, it identified compounds like botulin, cis-Vaccenic acid, heptadecane and phytol. The XRD analysis at 2θ showed specific diffraction peaks that corresponded to various bioactive compounds. Antioxidant tests indicated moderate activity in both DPPH and ABTS assays. Furthermore, CMEB displayed moderate cytotoxicity against A549 lung cancer cells at a concentration of 64.5µg/mL. Its moderate antioxidant activity and significant cytotoxic action against A549 lung cancer cells highlight its potential use in the development of antioxidant and anti-cancer molecules. However the results are extremely encouraging since commercially accessible florets of *Brassica oleracea L. var. italica* can be included in one's regular diet and can have a protective effect against the onset of lung cancer, while also possibly serving as an additional treatment for individuals diagnosed with lung cancer.

Keywords: Broccoli florets, GC-MS analysis, LC-MS analysis, Antioxidant activity, A549 lung cancer cells, Cytotoxic activity.

INTRODUCTION

Lung cancer, a malignant disease that originates in the cells of the lung, is primarily the result of prolonged exposure to tobacco smoke, although factors such as exposure to radon, asbestos, and certain chemicals also contribute to its development[1]. In Saudi Arabia, it is the 5th most common cancer in men and the 15th most common in women[2]. Since preventing disease is a higher priority than curing it, dietary choices are an essential aspect of maintaining good health.

Broccoli, scientifically known as *Brassica oleracea L. var. italica*, belonging to the Brassicaceae family, is of remarkable pharmaceutical importance, as it has antimicrobial properties[3,4] antioxidants, anti-cancer properties[5], immunomodulatory effects, and antidiabetic properties. It also provides hepatoprotection, cardioprotection, and anti-amnestic benefits. Syed, R. U. *et al.,* (2023) found that cruciferous vegetables such as broccoli are rich in fiber and low in calories, and they contain essential vitamins and minerals, thereby supporting various physiological functions. Research by Jacques *et al.,* suggests that an increase in the consumption of lycopene and beta-carotene may reduce the risk of heart disease[6] Additionally, a study by Murphy *et al.,* highlights the importance of an increased consumption of fruits and vegetables, which is consistent with dietary recommendations from health and nutrition experts[7]. The aim of this study is to investigate the bioactive constituents of broccoli, which is readily available in Jazan markets in Saudi Arabia. In addition, the antioxidant properties, and cytotoxic effects of a cold methanolic extract of broccoli florets (CMEB) on A549 lung cancer cells will be investigated, which will provide information about its potential in combating lung cancer.

METHODS

Materials

The broccoli was purchased from a commercial market in Jazan, Saudi Arabia. All the chemicals were purchased from Sigma Aldrich, which is supplied by Al Sada trading, Riyadh. All the chemicals used in this study are of analytical grade.

Cold maceration extraction

The broccoli samples were washed thoroughly with fresh water to remove any impurities and were then air dried. After drying, the broccoli was cut into small pieces and air-dried again in the shade for 10 days. The dried broccoli pieces were ground into a coarse powder, which was then collected and stored in an airtight container for further use. The bioactive components of the broccoli floret were extracted by method developed by R.U. Syed *et al.,* 2022[8].

Gas Chromatography Mass Spectrometry analysis

The presence of major bioactive components in the CMEB was investigated by GC-MS, (Thermo Scientific GC-MS-AS 3000 autosampler-ISQ detector). The GC-MS analysis was performed as reported by R.U.Syed *et al.,* 2022.[8]

Liquid chromatography–mass spectrometry analysis

LC-MS was used to characterize the CMEB. Liquid chromatography was performed on a Shimadzu Exion LC system (Kyoto, Japan). The LC-MS analysis was performed as reported by Menachery *et al.,* 2025[9].

X-ray diffraction analysis

X-ray diffraction (XRD) was utilized to investigate any possible crystalline structures of CMEB. The XRD analysis was performed according to the protocol developed by Rahamat *et al.,*2022[8].

Molecular docking

The CMEB revealed potential bioactive compounds that were examined through molecular docking studies. These compounds included ethyl iso-allocholate, crinamine, heptadecane, norethandrolone, botulin, cis-vaccenic acid, phytol, n-hexadecanoic acid, lupeol, oleic acid, and astaxanthin. The compounds were subjected to docking processes targeting the catalytic domain of the NAD-dependent deacetylase sirtuin-1 (SIRT1). Notably, SIRT1 is a subject of vast research in oncology; it is known for its multifaceted role in tumor-related signaling pathways. The SIRT1 catalytic domain's crystal structure, when bound to NAD and an EX527 analog (PDB: 4I5I), served as the reference for the docking studies. This protein structure was optimized by eliminating water molecules, introducing absent hydrogens, determining partial charges, and excising the ligand. A rigid 40 Å × 40 Å × 40 Å grid box, centered on the co-crystallized ligand (EX527 analog), was crafted. Docking studies were performed by using AutoDockTools-4. The authenticity of the docking outcomes was ascertained by redocking the co-crystallized ligand (EX527 analog), yielding a

binding free energy and inhibition constant (Ki) of -4.96 kcal/mol and 229.57 uM, respectively.[10-11]

Antioxidant studies: DPPH assay

The antioxidant activity of the CMEB was determined using the DPPH free-radical scavenging assay. The final concentrations of 1000, 500, 250, 125, and 62.5 g/mL, respectively, in methanol were used to make the samples. A stock solution of 20 µg/mL concentration of Trolox was prepared in methanol from which five concentrations were prepared, including 1.25, 5, 7.5,10 and 12.5 µg/mL. 100 µL of the CMEB was placed in a 96-well microtiter plate (n=6), and 100µL of freshly prepared DPPH reagent (0.1% in methanol) was added and aspirated three times. The reaction was incubated for 30 min at room temperature in the dark. At the end of the incubation period, the resulting reduction in DPPH colour intensity was measured at 540 nm[9]. The DPPH scavenging effect was calculated using the following equation:

$$\%\text{inhibition} = \frac{\text{Average absorbance of blank-average absorbance of the test}}{\text{Average absorbance of blank}} \times 100$$

ABTS assay

192 mg of ABTS (2,20-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) were dissolved in distilled water and transferred to a 50 mL volumetric flask, add the volume was completed with distilled water. 1mL of the previous solution was added to 17 µL of 140 mM potassium persulphate, and the mixture was left for 24 h in the dark. After that, 1mL of the reaction mixture was increased to 50 mL with the additional of methanol to obtain the final ABTS dilution used in the assay. 190 µL of the freshly prepared ABTS reagent was mixed with 10 µL of the CMEB in 96 wells plate (n=6), and the reaction was incubated for 30 min at room temperature in the dark. At the end of the incubation period, the reduction in ABTS color intensity was measured at 734 nm. The data are represented as means±SD according to the following equation:

$$\%\ \text{inhibition} = \frac{\text{Average absorbance of blank-average absorbance of the test}}{\text{Average absorbacne of blank}} \times 100$$

***In vitro* cytotoxicity studies**

In this study, A-549 lung cancer cells were obtained from Nawah Scientific Inc. in Mokatam, Cairo, Egypt, and were cultured in DMEM media supplemented with streptomycin, penicillin, and foetal bovine serum under controlled conditions of 37°C at 5% CO_2. The cell viability was determined by the SRB assay in which 5×10^{-3} cells were plated in 96-well plates, incubated in complete media for 24 h, and then treated with CMEB containing media for 72 hours. After exposure to the drug, the cells were fixed with 10% TCA, washed with distilled water, and stained with 0.4% w/v SRB solution. The stained cells were washed with 1% acetic acid; air dried, and then treated with 10 mM TRIS to dissolve the protein-bound SRB stain. Absorbance was measured at 540 nm using a BMG LABTECH-FLUOstar Omega micro plate reader in Ortenberg, Germany. This comprehensive approach allowed for the assessment of cell viability and drug response in the A-549 lung cancer cell line.

RESULTS

Spectral analysis

The GC-MS analysis of the CMEB identified several bioactive compounds, as illustrated in Fig. 1 and listed in Table 1. The LC-MS analysis, detailed in Table 2, confirmed the presence of compounds such as ethyl iso-allocholate, heptadecane, norethandrolone, botulin, cis-vaccenic acid, phytol, n-hexadecanoic acid, lupeol, oleic acid, and astaxanthin. These compounds are represented structurally in Fig. 2, with the LC-MS chromatogram shown in Fig. 3. Ethyl iso-allocholate was found to be a steroidal compound with significant therapeutic potential, showing diverse effects including apoptosis induction and inhibition of metastasis. Norethandrolone was detected with a retention time of 14.26 min, a probability index of 85.70%, and accounted for 2.39% of the chromatogram. Crinamine, identified at a retention time of 49.19 min and a probability index of 28.67%, comprised 6.08% of the GC-MS chromatogram but was not detected in LC-MS. Heptadecane appeared at 54.88 min with a probability index of 20.82%, making up 2.05% of the chromatogram. Betulin was noted at 72.19 min with a probability index of 16.19% and 1.24% of the chromatogram. Cis-vaccenic acid was identified at 77.68 min with a 16.63% probability index and 1.20% of the chromatogram. Phytol appeared at 75.49 min with a probability index of 68.74%, representing 1.16% of the GC-MS chromatogram. N-Hexadecanoic acid was identified at 71.54 min with a 37.80% probability index and 1.20% of the chromatogram. Lupeol was found at 117.92 min with a 34.80% probability index and 1.20% of the chromatogram. Additionally, 1-Heptatriacotanol, oleic acid, and astaxanthin were detected with varying retention times and probability indices, though 1-Heptatriacotanol was not observed in LC-MS.

Table 1: GC-MS detection of possible bioactive compounds in CMEB

Sr. No	Compound name	Molecular formula	Molecular weight	Retention time (Min)	Probability Index	Percent area of curve
1	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	91.29	40.74	23.09
2	Crinamine,1,2dihydro-6-hydroxy-11-oxo-	$C_{17}H_{19}NO_5$	317	49.19	28.67	6.08
3	Heptadecane, 9-hexyl-	$C_{23}H_{48}$	324	54.88	20.82	2.05
4	Norethandrolone	$C_{20}H_{30}O_2$	302	14.26	85.70	2.39
5	Betulin	$C_{30}H_{50}O_2$	442	72.19	16.19	1.24
6	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282	77.68	16.63	1.20
7	Phytol	$C_{20}H_{40}O$	296	75.49	68.74	1.16
8	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	71.54	37.80	1.20
9	Lupeol	$C_{30}H_{50}O$	426	117.92	34.80	1.20
10	1-Heptatriacotanol	$C_{37}H_{76}O$	536	120.37	28.45	0.24
11	Oleic Acid	$C_{18}H_{34}O_2$	282	72.45	44.08	0.24
12	Astaxanthin	$C_{40}H_{52}O_4$	596	125.77	16.19	0.20

Table 2: LC-MS detection of possible bioactive compounds in CMEB

Sr. No	Compound	Retention Time (min)	IonizationMode	CalculatedMass	Experimental Mass
1	Ethyl iso-allocholate	23.647	Positive	436.3189	437.3408
2	Heptadecane	23.44	Negative	240.2817	239.2078
3	Norethandrolone	24.77	Negative	302.2246	301.2473
4	Betulin	24.36	Negative	442.3811	441.3238
5	cis-Vaccenic acid	19.32	Negative	282.2559	281.2546
6	Phytol	20.76	Negative	296.3079	295.2350
7	n-Hexadecanoic acid	15.07	Positive	386.3036	387.3088
8	Lupeol	21.24	Positive	426.3862	427.3566
9	Oleic Acid	17.725	Positive	282.2559	283.2619
10	Astaxanthin	17.8	Positive	596.3866	597.3916

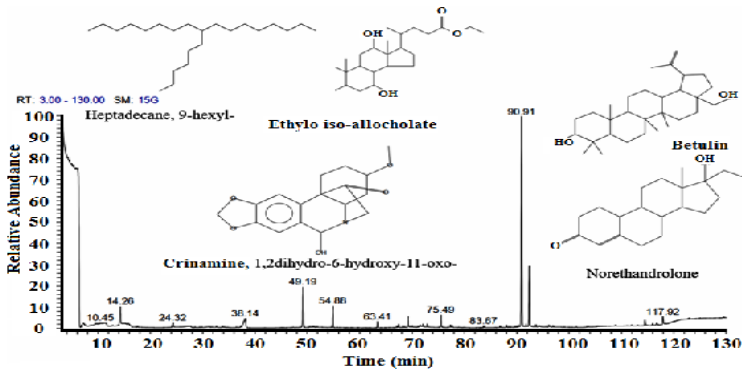


Fig. 1. GC-MS chromatogram of CMEB

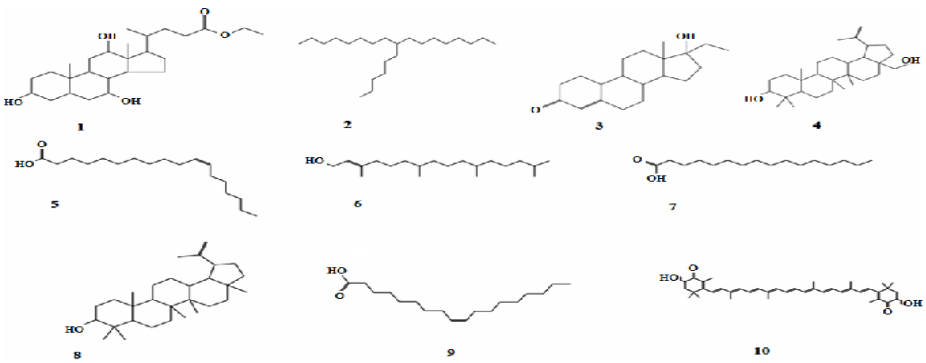
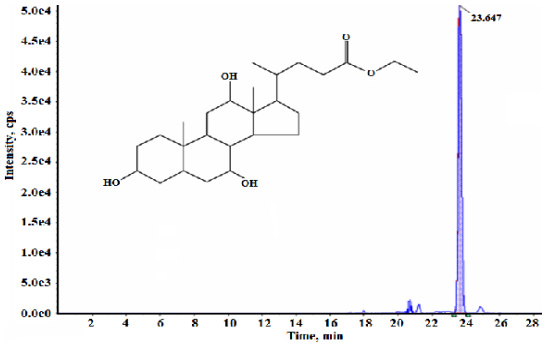
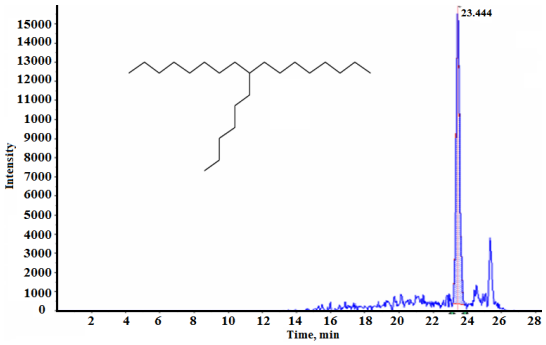


Fig. 2. The structure of bioactive compounds of the cold methanolic extract of broccoli through GC-MS and LCMS analyses. (1) Ethyl iso-allocholate; (2) Heptadecane, 9-hexyl-; (3) Norethandrolone; (4) Betulin; (5) cis-Vaccenic acid; (6) Phytol; (7 n-Hexadecanoic acid; (8) Lupeol (9) Oleic Acid; (10) Astaxanthin

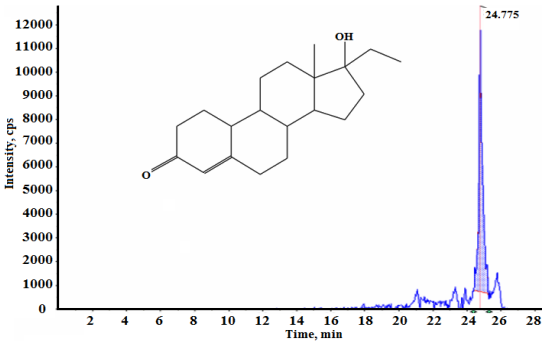
1. Ethyl iso-allocholate



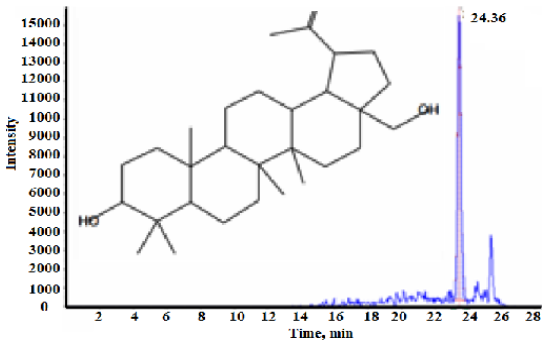
2. Heptadecane, 9-hexyl-



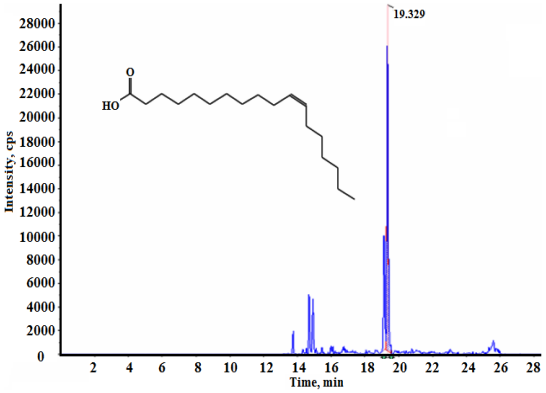
3. Norethandrolone



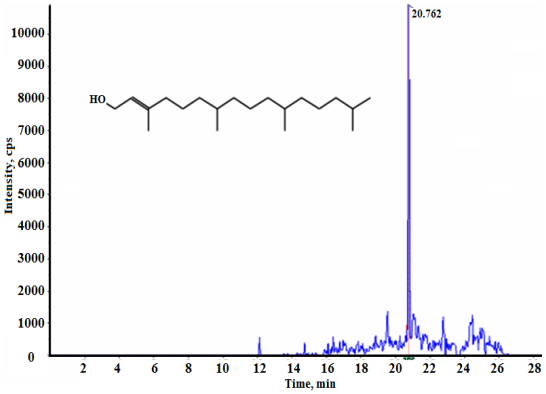
4. Betulin



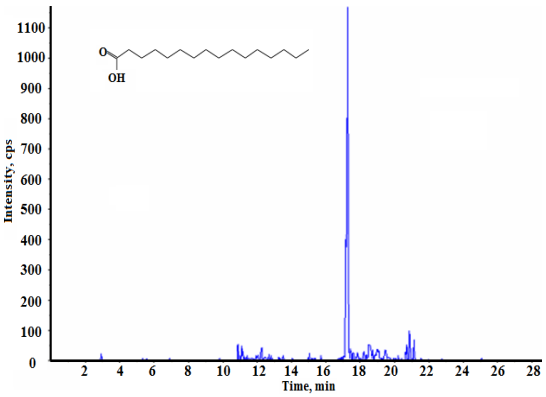
5. cis-Vaccenic acid



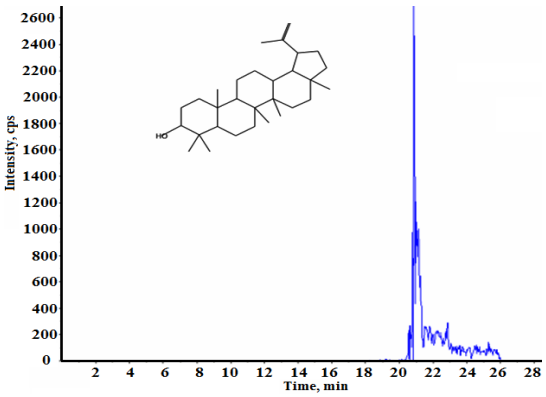
6. Phytol



7. n-Hexadecanoic acid



8. Lupeol



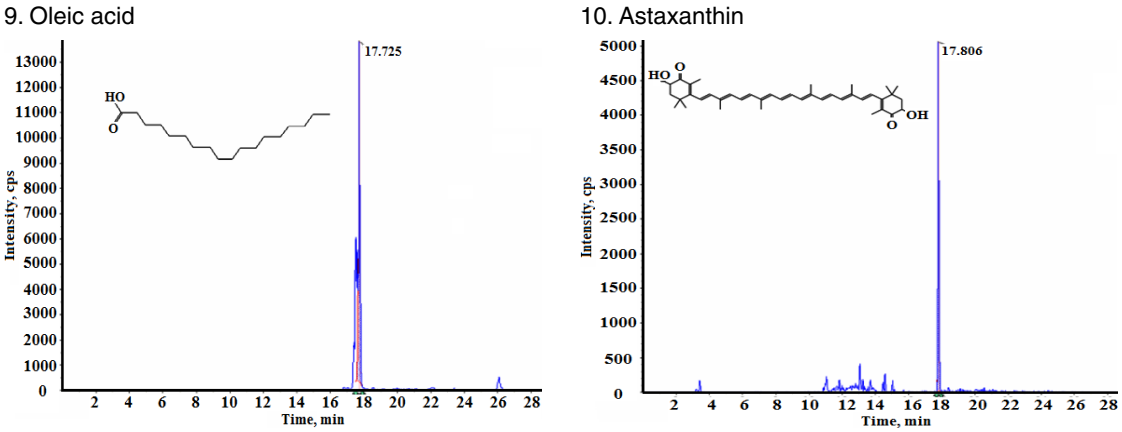


Fig. 3. LC-MS chromatogram of CMEB and the structure of bioactive compounds The structure of bioactive compounds of the cold methanolic extract of broccoli through GC-MS and LCMS analyses. (2) Heptadecane, 9-hexyl-; (3) Norethandrolone; (4) Betulin; (5) cis-Vaccenic acid; (7) n-Hexadecanoic acid; (8) Lupeol (9) Oleic Acid; (10) Astaxanthin

X-ray diffraction analysis

XRD analysis was employed to characterize the discrete structures of the dried broccoli extract. The analysis conducted after 2 h revealed distinct particles based on the diffraction peaks observed. Specifically, a cluster of peaks was detected at 65.2°, corresponding to the 2θ value, indicating strong diffraction peaks. The corresponding d-value for this 2θ value was 1.430, with an intensity of 62 counts per second per degree (cps/deg). This suggests the presence of specific biomolecules in the dried broccoli extract. The XRD peaks are consistent with the presence of mineral crystals.[12]

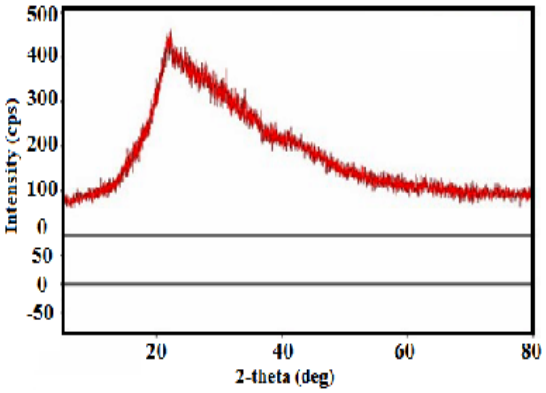


Fig. 4. XRD analysis of the cold methanolic extract of broccoli floret (The diffractogram was obtained at 2θ in the range 2°–80°)

Molecular docking

Docking the SIRT1 catalytic domain with the bioactive GC-MS detected compounds was achieved using predefined parameters. Multiple docking conformations were generated (Fig. 5). The best conformation was selected and ranked based on the inhibition constant, binding energy, and RSMD. All inhibition constants are in the micro-scale, except for heptadecane and phytol, as seen in Table 3.

Table 3: Docking analysis of possible bioactive GC-MS detected compounds of the cold methanolic extract of broccoli against SIRT1 catalytic domain

Sr. No	Docked compound	Binding energy	Docked compound
1	Ethyl iso-allocholate	-5.88	48.88 uM
2	Crinamine	-5.99	40.46 uM
3	Heptadecane	-3.53	2.58 mM
4	Norethandrolone	-5.54	87.63 uM
5	Betulin	-5.48	96.04 uM
6	cis-Vaccenic acid	-5.93	44.82 uM
7	Phytol	-3.13	5.06 mM
8	Lupeol	-6.14	31.81 uM
9	Oleic Acid	-4.96	232.36 uM
10	n-Hexadecanoic acid	-5.26	139.57 uM

Ethyl iso-allocholate exhibits a binding energy of -5.88 kcal/mol and an IC_{50} of 48.88 µM, reflecting a moderate binding affinity and inhibition potency. It binds to the target protein reasonably well but is outperformed by several other compounds in terms of potency. Crinamine shows a slightly more favorable binding energy of -5.99 kcal/mol and a lower IC_{50} of 40.46 µM, suggesting it has a stronger interaction with the protein and is more effective at inhibiting the target compared to ethyl iso-allocholate. In contrast, Heptadecane has a significantly less negative binding energy of -3.53 kcal/mol and an IC_{50} of 2.58 mM, indicating a weak interaction with the target protein and very low potency, as it requires a much higher concentration to achieve inhibition. Norethandrolone and Betulin show intermediate binding energies of -5.54 kcal/mol

and -5.48 kcal/mol, respectively, with IC_{50} values of 87.63 µM and 96.04 µM. These results suggest that both compounds have a moderate binding affinity and are effective inhibitors, though not as potent as crinamine or ethyl iso-allocholate. cis-Vaccenic acid presents a binding energy of -5.93 kcal/mol and an IC_{50} of 44.82 µM, indicating a strong binding affinity and effective inhibition similar to crinamine. However, Phytol has a binding energy of -3.13 kcal/mol and a high IC_{50} of 5.06 mM, showing weak binding and very low potency, as it requires a significantly higher concentration to inhibit the target protein.

Lupeol stands out with the most negative binding energy of -6.14 kcal/mol and the lowest IC_{50} of 31.81 µM, making it the most effective compound in the study. This strong binding affinity and high potency suggest that lupeol is highly effective in inhibiting the target protein even at low concentrations. Conversely, Oleic Acid and n-Hexadecanoic Acid display moderate binding energies of -4.96 kcal/mol and -5.26 kcal/mol, with IC_{50} values of 232.36 µM and 139.57 µM, respectively. These results indicate that while they bind to the target protein reasonably well, they are less potent compared to compounds like lupeol, requiring higher concentrations for effective inhibition. Overall, lupeol emerges as the most promising compound for further investigation due to its strong binding and potent inhibitory effects, whereas heptadecane and phytol show weaker interactions and lower efficacy.

Antioxidant studies

Antioxidants play a crucial role in protecting the body from the harmful effects of oxidative stress caused by free radicals. In this study, the antioxidant capabilities of CMEB were investigated, as shown in Table 4. The table provides a comparative analysis of the antioxidant activities of the Crude Methanolic Extract of Broccoli (CMEB) and the standard antioxidant Trolox, across two assays: DPPH and ABTS. The IC_{50} values, expressed in micrograms per milliliter (µg/mL), denote the concentration required to inhibit 50% of the radical activity.

In the DPPH assay, CMEB shows an IC_{55} value of 699.8 ± 27.99 µg/mL. This high IC_{50} value indicates that a relatively large amount of CMEB is needed to achieve 50% inhibition of the DPPH radical, suggesting that CMEB has a moderate antioxidant effect. On the other hand, Trolox, the standard, exhibits a significantly lower IC_{50} value of 7.217 ± 0.309 µg/mL. The ABTS result shows that CMEB has an IC_{50} value of 185 ± 18.37 µg/mL. This value suggests that CMEB has a considerable antioxidant effect, but it still requires a higher concentration compared to the standard to achieve 50% inhibition of ABTS radicals.

Table 4: The anti-oxidant study of cold methanolic extract of broccoli

Sample id	DPPH assay IC_{50} (µg/mL)	ABTS assay IC_{50} (µg/mL)
CMEB*	699.8 ± 27.99	185 ± 18.37
Standard Trolox	7.217 ± 0.309	2.775 ± 0.107

In-vitro cytotoxicity

In vitro cytotoxicity analysis on A549 lung cancer cells showed results at a concentration of 64.5 µg/mL (Fig. 6). Free radical scavenging activity of the crude extract could be one of the reasons the cytotoxic activity against the cancer cells. Hence the extract must be further purified to isolate bioactive compound for obtaining its maximum activity.

Sample id	DPPH assay IC_{50} (µg/mL)	ABTS assay IC_{50} (µg/mL)
CMEB*	699.8 ± 27.99	185 ± 18.37
Standard Trolox	7.217 ± 0.309	2.775 ± 0.107

*CMEB = Cold methanol extract of Broccoli

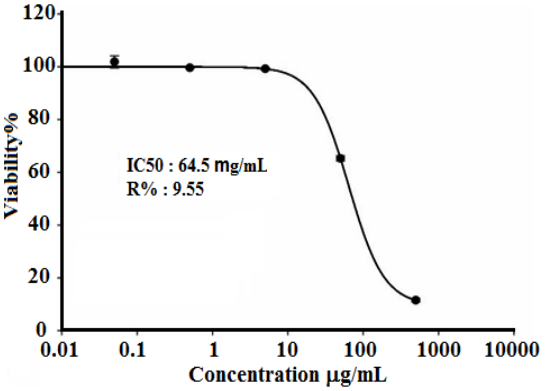


Fig. 6. The cytotoxicity effect of cold methanolic extract of broccoli on A549 lung cancer cells

DISCUSSION

Spectral analysis

CMEB extract in GC-MS and LC-MS analysis revealed a diverse array of presence bioactive of compounds each with potential therapeutic implications. Ethyl iso-allocholate, a steroidal compound having diverse therapeutic

attributes, has garnered significant attention. Thakur and Ahirwar conducted a study demonstrating that ethyl iso-allocholate, obtained from *Trigonella foenum graecum* L., induces caspase-dependent apoptosis in cancer cells[13]. Furthermore, it was found to decrease tumor growth and to inhibit metastasis and angiogenesis *in-vivo*, while also exhibiting safety for normal tissues. Significant interactions were observed between ethyl iso-allocholate and all the protein targets, with a notable emphasis on caspase-1, indicating its therapeutic potential in terms of anti-inflammatory and anti-tumor effects[14]. In a previous molecular docking study, ethyl iso-allocholate, derived from medicinal rice known as Karungkavuni, was found to inhibit dihydropteroate synthase in *Escherichia coli*. This study also highlighted the increased stability of the dihydropteroate synthase-ethyl iso-allocholate complex, as well as its excellent antibacterial properties[15]. Based on reference data from NIST's integrated library, norethandrolone, a steroid, was detected in the CMEB. It displayed a retention time of 14.26 min, had a probability index of 85.70%, and represented 2.39% of the chromatogram. Notably, norethandrolone was also detected in the LC-MS analysis using the negative ionization mode. Crinamine, an alkaloid, was detected alongside ethyl iso-allocholate in the CMEB, displaying a retention time of 49.19 min, with a probability index of 28.67% and accounting for 6.08% of the GC-MS chromatogram. However, it remained undetected in the LC-MS protocol.

A prior investigation proposed that crinamine, an alkaloid, exhibited antibacterial properties[16]. In another study, it was found that crinamine-induced DNA double-strand breaks without triggering any apoptosis. Additionally, by inhibiting the production of pro-epithelial-mesenchymal transition regulators, it effectively reduces the migration of cervical cancer cells[17]. Furthermore, crinamine derived from Crinum asiaticum demonstrated substantial cytotoxicity against DU145 and PANC1 cells[18].

Heptadecane, 9-hexyl- was identified in CMEB at 54.88 min with 20.82% probability index, occupying 2.05% in the chromatogram. Additionally, hepadecane, 9-hexyl- was also determined by the LC-MS analysis using the negative ionization mode. Betulin is a naturally occurring triterpene that is found extensively throughout the plant kingdom. In CMEB, Betulin was identified at a retention time of 72.19 min, having a 16.19% probability index, and constituting 1.24% of the chromatogram. Additionally, Betulin was confirmed through the LC-MS analysis using the negative ionization approach. Betulin displays enormous pharmacological potential, such as having anti-inflammatory activity, anti-HIV, anti-cancer, and antibacterial properties[19-22]. Cis-vaccenic acid is an omega-7 fatty acid, otherwise called as (Z)-11-octadecenoic acid. The compound was detected in CMEB at 77.68 min with 16.63 % probability index and occupying 1.20% in the chromatogram, as well as in the LC-MS analysis negative ionization mode. An earlier report suggested that cis-vaccenic acid inhibits the growth of microbes and lowers cholesterol levels[23]. According to earlier research, 11-octadecenoic acid can be a marker for broccoli consumption, and it can reduce non-alcoholic fatty liver disease (NAFLD) and enhance liver health. The same study claimed that eating broccoli regularly would normalize one's lipid profile[24].

Phytol, an acyclic monounsaturated diterpene alcohol, was identified in CMEB at 75.49 min with a probability index of 68.74% and occupying 1.16% in the GC-MS chromatogram, Moreover, phytol was discerned in the LC-MS analysis under the negative ionization mode. Phytol can be found in tocopherols such as vitamins K and E. The aromatic component can be found in cosmetic and non-cosmetic goods and is used in many fragrance compounds. Phytol has shown antinociceptive, anti-inflammatory and antiallergic effects; immunostimulant, the immune modulatory effect of innate and acquired immunity; and antimicrobial and antioxidant activities.[17,25-27]

N-Hexadecanoic acid, also known as palmitic acid, was detected in CMEB with a retention time of 71.54 minutes. It had a probability index of 37.80% and accounted for 1.20% of the GC-MS chromatogram. Furthermore, this compound was identified in the LC-MS analysis using the positive ionization mode. An earlier study suggested that palmitic acid can modulate innate immunity by coordinating the immunological activity in inflammatory tissues and controlling the activation of pattern recognition receptors in local innate immune cells. Additionally, protein palmitoylation regulates numerous cellular physiological processes[28]. A

previous study showed that palmitic acid exhibited tumorigenic properties by increasing the growth of colorectal cancer, which is dependent on beta 2-adrenergic receptors (2-AR), and it revealed beta 2-adrenergic receptor expression in the xenograft tissues of a mouse model that carried CRC[29]. Palmitic acid, on the other hand, has been reported to have anti-androgenic, nematicide, flavoring, antioxidant, and hypocholesterolemic qualities[30]. An earlier report demonstrated that palmitic acid is one of its main constituents of broccoli, *et al.,* and Olga *et al.,* reported the detection of palmitic acid in broccoli flour prepared from the dried florets, leaves, and stalks of broccoli[31,32]. A study published in 2021 found that palmitic acid substantially inhibited the growth of prostate cancer cells both *in vitro* and in-vivo[33]. Although numerous studies have indicated that broccoli has many medicinal properties, it has also been shown to have some adverse effects, such as inducing insulin resistance that leads to type 2 diabetes and promoting cancer metastasis, a more aggressive "memory" in cancer cells.[34,35]

Lupeol is a phytosterol, which is a naturally occurring pentacyclic triterpenoid identified in CMEB at 117.92 min with a probability index of 34.80% and occupying 1.20% of the GC-MS chromatogram. It has also been detected in the LC-MS analysis positive ionization mode. Lupeol has demonstrated anti-inflammatory, anti-acne, and anti-cancer properties.[26,36] In the CMEB, 1-Heptatriacotanol, oleic acid, and astaxanthin were identified, each displaying favorable retention times and probability indices. Yet, their occupancy percentages in the chromatogram were relatively low, as detailed in Table 1. Notably, the LC-MS analysis failed to detect 1-Heptatriacotanol in both positive and negative ionization modes. Conversely, both oleic acid and astaxanthin were identified in the LC-MS analysis using the positive ionization approach.

Remarkably, these molecules have good medicinal properties. 1-Heptatriacotanol has been reported as having an anti-hypercholesterolemic effect[37]. Oleic acid is a monounsaturated fatty acid that is showing good therapeutic value by reducing blood pressure, lowering cholesterol, preventing type-2 diabetes, improving brain function, and having immune modulatory effects and anti-cancer properties[38-40]. Astaxanthin was reported as an antioxidant and as having anti-cancer effects that promote healthy skin, improve cardiac health and male fertility, and reduce inflammation and pain[41-45].

X-ray diffraction analysis

The XRD results provide detailed insights into the crystalline structure of the dried broccoli extract. The distinctive diffraction peaks observed at 65.2° (2θ) with a d-value of 1.430 are indicative of the presence of mineral crystals within the extract. This cluster of peaks reflects the arrangement and spacing of atoms in the crystalline structures, allowing for the identification of specific minerals present in the extract. The intensity of 62 cps/deg associated with these peaks suggests a relatively high concentration of the mineral components, which could play a role in the extract's overall biological activity and stability. The clear and well-defined peaks observed are characteristic of crystalline materials, confirming that the dried broccoli extract contains mineral crystals with a defined and ordered structure.

Molecular docking

The docking results indicate that hydrogen bonds formed with Thr-368 and Gln-361 are essential in inhibiting the SIRT1 catalytic domain, as seen in the top two performing compounds, ethyl iso-allocate and crinamine. These results indicate that Thr-368 and Gln-361 are essential amino acids in this activity. Ethyl iso-allocate formed three hydrogen bonds with Thr-368, Glu-410, and Gln-361; similarly, crinamine formed two hydrogen bonds with Thr368 and Gln-361. The estimated free energy of binding and inhibition constant (Ki) for ethyl iso-allocholate were -5.88 kcal/mol and 48.88 uM, respectively, and for crinamine were -5.99 kcal/mol and 40.46 uM, respectively.

Both norethandrolone and betulin formed only one hydrogen bond with Ser-365 and had a similar inhibition constant (Ki) that averaged around 91 uM. Cis-Vaccenic acid formed two hydrogen bonds with Gln-421 and 361, which are identical to ethyl iso-allocholate, resulting in a similar inhibition constant at 44.82 uM. In addition, n-Hexadecanoic acid and oleic acid each formed one hydrogen bond with Gln-361. The estimated free energy of binding and inhibition constant (Ki) for n-Hexadecanoic were -26 kcal/mol and 139.57 uM, respectively, and for oleic acid were -4.96 kcal/mol and 232.36 uM, respectively. Lupeol docking results were superior to the other docked compounds, even though they lacked the hydrogen bonds. Instead, lupeol formed

multiple hydrophobic, polar, negative, and positive interactions with the surrounding residues. The estimated free energy of binding of lupeol was -6.14kcal/mol, and the inhibition constant (Ki) was 31.81 uM. The poses were visualized using the ligand interaction diagram from Maestro.

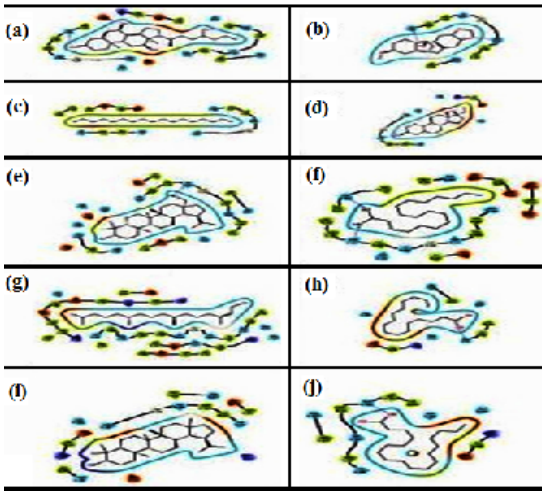


Fig. 5. 2D interaction showing hydrogen bonding in magenta arrow, hydrophobic in green, polar in cyan, negative in red and positive in purple against SIRT1 catalytic domain of (a) ethyl iso-allocholate, (b) Crinamine, (c) heptadecane, (d) norethandrolone, (e) botulin, (f) cis-vaccenic acid, (g) Phytol, (h) N-hexadecanoic acid, (i) Lupeol, (j) Oleic acid

Antioxidant studies

The antioxidant activity of CMEB was found to be comparatively lower than that of the comparator antioxidant Trolox in both DPPH and ABTS assays. Previous studies have shown that broccoli possesses commendable antioxidant properties, as demonstrated by the DPPH and ABTS assays[46-48]. The antioxidant property of CMEB in this study could possibly be related to the bioactive constituent phytol. A previous study indicated that broccoli leaves and stems are rich in total phenols, and they have remarkable antioxidant and anti-cancer properties. Another study highlighted that the ethanolic extract from selected Malaysian plants exhibited strong antioxidant properties but no cytotoxic properties[49].

In-vitro cytotoxicity

The cytotoxicity effect of a cold methanolic extract of broccoli on A549 lung cancer cells, with an IC_{50} value of 64.5 µg/mL, suggests that the extract contains bioactive compounds capable of significantly inhibiting the growth of these cancer cells, highlighting its potential as a source of anti-cancer agents. Using a cold methanolic extraction process helps retain heat-sensitive compounds, which may contribute to the extract's effectiveness.

Interestingly, broccoli florets showed lower selectivity in targeting HepG2 and SW480 cells than broccoli shoots[48]. Historical research has shown that broccoli has several therapeutic benefits, including the role it plays as an anti-cancer agent, immunomodulator, antidiabetic agent, antibacterial agent, hepatoprotectant, cardio protectant, and anti-amnesic, and also because of its antioxidant properties[4,31,50-52]. Consistent with these findings, the current research confirms the cytotoxic activity of CMEB against A549 lung cancer cells. To verify the potential of broccoli extracts in cancer treatment, additional studies would be needed to understand which specific phytochemicals are involved and their mechanisms of action against cancer cells at the molecular level, their safety profile, and their effectiveness *in-vivo*.

CONCLUSION

Preventive measures, such as dietary habits, can often be implemented through one's lifestyle. Research shows that eating broccoli, a vegetable with many medicinal properties, may be beneficial due to its content, which consists of compounds such as iso-allocholate, phytol, betulin, astaxanthin, and oleic acid, which are vital in today's fast-paced world. Broccoli has been shown to have anti-cancer and anti-proliferation effects due to its cytotoxic properties. In addition, its potential for immunomodulation and antioxidant activity cannot be ignored. Therefore, we deduce that the inclusion of broccoli in one's daily diet can potentially reduce the risk of cancer development and can serve as a complementary treatment, especially for lung cancer, which can have a significant impact on the progression of the disease.

ACKNOWLEDGMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of Interest

The authors declare that no conflict of interest is associated with this study, either financially or otherwise.

REFERENCES

1. Cruz, C. S. D.; Tanoue, L. T.; Matthay, R. A. Lung cancer: epidemiology, etiology, and prevention., *Clin. Chest Med.*, **2011**, *32*, 605–644.
2. Chaudhri, E.; Fathi, W.; Hussain, F.; Hashmi, S. K. The increasing trends in cases of the most common cancers in Saudi Arabia., *Journal of Epidemiology and Global Health.,* **2020**, *10*, 258–262.
3. Sibi, G.; Shukla, A.; Dhananjaya, K.; Ravikumar, K. R.; Mallesha, H. In vitro antibacterial activities of Broccoli (*Brassica oleracea L. var italica*) against food borne bacteria., *Journal of Applied Pharmaceutical Science.,* **2013**, *3*, 100–103.
4. Pacheco$_5$Cano, R. D.; Salcedo$_5$Hernández, R.; López$_5$Meza, J. E.; Bideshi, D. K.; Barboza$_5$Corona, J. E. Antimicrobial activity of broccoli (*Brassica oleracea var. italica*) cultivar Avenger against pathogenic bacteria, phytopathogenic filamentous fungi and yeast., *J. Appl. Microbiol.*, **2018**, *124*, 126–135.
5. Syed, R. U.; Moni, S. S.; Break, M. K. B.; Khojali, W. M.; Jafar, M.; Alshammari, M. D.; Abdelsalam, K.; Taymour, S.; Alreshidi, K. S. M.; Elhassan Taha, M. M. Broccoli: A multi-faceted vegetable for health: An in-depth review of its nutritional attributes, antimicrobial abilities, and anti-inflammatory properties., *Antibiotics.,* **2023**, *12*, 1157.
6. Jacques, P. F.; Lyass, A.; Massaro, J. M.; Vasan, R. S.; D'Agostino Sr, R. B. Relationship of lycopene intake and consumption of tomato products to incident CVD. Br., *J. Nutr.*, **2013**, *110*, 545–551.
7. Murphy, M. M.; Barraj, L. M.; Spungen, J. H.; Herman, D. R.; Randolph, R. K. Global assessment of select phytonutrient intakes by level of fruit and vegetable consumption., Br., *J. Nutr.*, **2014**, *112*, 1004–1018.
8. Syed, R. U.; Moni, S. S.; Alfaisal, R. H.; Alrashidi, R. H.; Alrashidi, N. F.; Wadeed, K. M.; Alshammary, F. N.; Habib, A. M.; Alharbi, F. M.; UR Rehman, Z. Spectral characterization of the bioactive principles and antibacterial properties of cold methanolic extract of Olea europaea from the Hai'l region of Saudi Arabia., *Arabian Journal of Chemistry.,* **2022**, *15*, 104006.
9. Menachery SJ.; Moni SS.; Khardali A.; Exploring the Bioactive Constituents, Antibacterial, Antioxidant Potentialities of Aloe officinalis Forssk Flower., *Natural Poduct Communications.*, **2025**, *20*(1), doi:10.1177/1934578X251314723
10. Zhao, X.; Allison, D.; Condon, B.; Zhang, F.; Gheyi, T.; Zhang, A.; Ashok, S.; Russell, M.; MacEwan, I.; Qian, Y. The 2.5 Å crystal structure of the SIRT1 catalytic domain bound to nicotinamide adenine dinucleotide (NAD) and an indole (EX527 analogue) reveals a novel mechanism of histone deacetylase inhibition., *J. Med. Chem.*, **2013**, *56*, 963–969.
11. Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; AutoDock, A. O. AutoDockTools4: Automated docking with selective receptor flexibility., **2009**, *30*. DOI:https://doi.org/10.1002/jcc, 21256, 2785–2791.
12. Majeed, R. S.; AlKhaffaf, A. A. A., Inhibitory effect of silver nanoparticles synthesized by aqueous extract of broccoli flowers against some species of pathogenic bacteria., **2022**, *140*(2), 2451-2466.
13. Thakur, R. S.; Ahirwar, B. A steroidal derivative from *Trigonella foenum graecum* L. that induces apoptosis *in vitro* and *in-vivo.*, *J. Food Drug Anal.*, **2019**, *27*, 231–239.
14. Sampathkumar, Y.; Elumali, S.; Halith, A. M. GCMS determination of anticancer, anti-inflammatory and anti-bacterial compounds from salt tolerance microalgae (*Lyngbya sp*. *Nostoc sp*. and *Phormidium sp*.) Isolated from Marakkanam Salt Pan, Tamil Nadu, India. Tamil Nadu, India., **2020**, *11*, 14.
15. Malathi, K.; Anbarasu, A.; Ramaiah, S. Ethyl iso-allocholate from a medicinal rice Karungkavuni inhibits dihydropteroate synthase in *Escherichia coli*: A molecular docking and dynamics study., *Indian J Pharm Sci.,* **2017**, *78*, 780–788.
16. Nair, J. J.; Wilhelm, A.; Bonnet, S. L.; van Staden, J. Antibacterial constituents of the plant family Amaryllidaceae., *Bioorg. Med. Chem. Lett.*, **2017**, *27*, 4943–4951.
17. Khumkhrong, P.; Piboonprai, K.; Chaichompoo, W.; Pimtong, W.; Khongkow, M.; Namdee, K.; Jantimaporn, A.; Japrung, D.; Asawapirom, U.; Suksamrarn, A. Crinamine induces apoptosis and inhibits proliferation, migration, and angiogenesis in cervical cancer SiHa cells., *Biomolecules.*, **2019**, *9*, 494.

18. Salehi, B.; Fokou, P. V. T.; Yamthe, L. R. T.; Tali, B. T.; Adetunji, C. O.; Rahavian, A.; Mudau, F. N.; Martorell, M.; Setzer, W. N.; Rodrigues, C. F. Phytochemicals in prostate cancer: from bioactive molecules to upcoming therapeutic agents., *Nutrients*., **2019**, *11*, 1483.
19. Schwiebs, A.; Radeke, H. H. Immuno-pharmacological activity of betulin in inflammation-associated carcinogenesis. *Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents)*., **2018**, *18*, 645–651.
20. Sharma, S.; Sharma, S.; Rai, A.; Singh, S.; Pandey, A.; Sisodia, R. Therapeutic potential of plant secondary metabolites in treatment of respiratory viral diseases: A review. *Medicinal Plants-International Journal of Phytomedicines and Related Industries*., **2022**, *14*, 30–45.
21. B$_5$benek, E.; Jastrz$_5$bska, M.; Kadela-Tomanek, M.; Chrobak, E.; Orzechowska, B.; Zwoli$_5$ska, K.; Latocha, M.; Mertas, A.; Czuba, Z.; Boryczka, S. Novel triazole hybrids of betulin: Synthesis and biological activity profile., *Molecules.,* **2017**, *22*, 1876.
22. Vinha, A. F.; Alves, R. C.; Barreira, S. V.; Costa, A. S.; Oliveira, M. B. P. Impact of boiling on phytochemicals and antioxidant activity of green vegetables consumed in the Mediterranean diet., *Food & function*., **2015**, *6*, 1157–1163.
23. Batubo, N. P.; Ogbu, O. S.; Victor, D. D. Chemical profiles and proximate analysis of n-hexane extract of Terminalia catappa kernel from Nigeria., *International Journal of Research in Medical Sciences*., **2024**, *12*, 17.
24. Eve, A. A.; Liu, X.; Wang, Y.; Miller, M. J.; Jeffery, E. H.; Madak-Erdogan, Z. Biomarkers of broccoli consumption: Implications for glutathione metabolism and liver health., *Nutrients*., **2020**, *12*, 2514.
25. Santos, C. C. d. M. P.; Salvadori, M. S.; Mota, V. G.; Costa, L. M.; de Almeida, A. A. C.; de Oliveira, G. A. L.; Costa, J. P.; de Sousa, D. P.; de Freitas, R. M.; de Almeida, R. N. Antinociceptive and antioxidant activities of phytol *in-vivo* and *in vitro* models., *Neuroscience Journal*., **2013**, *2013*, 949452.
26. Ryu, K.; Choi, J.; Chung, S.; Kim, D. Anti-scratching behavioral effect of the essential oil and phytol isolated from Artemisia princeps Pamp. in mice., *Planta Med*., **2011**, *77*, 22–26.
27. Tzeng, H.; Chyuan, I.; Chen, W. Shaping of innate immune response by fatty acid metabolite palmitate., *Cells.,* **2019**, *8*, 1633.
28. Tzeng, H.; Chyuan, I.; Chen, W. Shaping of innate immune response by fatty acid metabolite palmitate., *Cells*., **2019**, *8*, 1633.
29. Fatima, S.; Hu, X.; Huang, C.; Zhang, W.; Cai, J.; Huang, M.; Gong, R.; Chen, M.; Ho, A. H.; Su, T. High-fat diet feeding and palmitic acid increase CRC growth in 2AR-dependent manner., *Cell Death & Disease*., **2019**, *10*, 711.
30. Tyagi, T.; Agarwal, M. Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms., *Journal of Pharmacognosy and phytochemistry.,* **2017**, *6*, 195–206.
31. Bhandari, S. R.; Park, M. Y.; Chae, W. B.; Kim, D.; Kwak, J. Seasonal variation in fatty acid composition in various parts of broccoli cultivars., *Korean Journal of Agricultural Science*., **2013**, *40*, 289–296.
32. Campas-Baypoli, O. N.; Sánchez-Machado, D. I.; Bueno-Solano, C.; Núñez-Gastélum, J. A.; Reyes-Moreno, C.; López-Cervantes, J. Biochemical composition and physicochemical properties of broccoli flours., *Int. J. Food Sci. Nutr*., **2009**, *60*, 163–173.
33. Zhu, S.; Jiao, W.; Xu, Y.; Hou, L.; Li, H.; Shao, J.; Zhang, X.; Wang, R.; Kong, D. Palmitic acid inhibits prostate cancer cell proliferation and metastasis by suppressing the PI3K/Akt pathway., *Life Sci*., **2021**, *286*, 120046.
34. Korbecki, J.; Bajdak-Rusinek, K. The effect of palmitic acid on inflammatory response in macrophages: an overview of molecular mechanisms., *Inflammation Res*., **2019**, *68*, 915–932.
35. Liu, X.; Rulina, A.; Choi, M. H.; Pedersen, L.; Lepland, J.; Takle, S. T.; Madeleine, N.; Peters, S. D.; Wogsland, C. E.; Grøndal, S. M. C/EBPB-dependent adaptation to palmitic acid promotes tumor formation in hormone receptor negative breast cancer., *Nature Communications*., **2022**, *13*, 69.
36. Saleem, M. Lupeol., a novel anti-inflammatory and anti-cancer dietary triterpene., *Cancer Lett*., **2009**, *285*, 109–115.

37. Junwei, L.; Juntao, C.; Changyu, N.; Peng, W. Molecules and functions of rosewood: Pterocarpus cambodianus., *Arabian Journal of Chemistry.,* **2018**, *11*, 763–770.
38. Granado-Casas, M.; Mauricio, D. Oleic acid in the diet and what it does: Implications for diabetes and its complications., *In Bioactive food as dietary interventions for diabetes Elsevier*., **2019**, 211–229.
39. Gnoni, G. V.; Natali, F.; Geelen, M. J.; Siculella, L. Oleic acid as an inhibitor of fatty acid and cholesterol synthesis. In Olives and olive oil in health and disease prevention Elsevier: **2010**, 1365–1373.
40. Naguib, Y. M. Antioxidant activities of astaxanthin and related carotenoids., *J. Agric. Food Chem*., **2000**, *48*, 1150–1154.
41. Teo, I. T. N.; Chui, C. H.; Tang, J. C. O.; Lau, F. Y.; Cheng, G. Y. M.; Wong, R. S. M.; Kok, S. H. L.; Cheng, C. H.; Chan, A. S. C.; Ho, K. P. Antiproliferation and induction of cell death of Phaffia rhodozyma (Xanthophyllomyces dendrorhous) extract fermented by brewer malt waste on breast cancer cells., *Int. J. Mol. Med*., **2005**, *16*, 931–936.
42. Tominaga, K.; Hongo, N.; Karato, M.; Yamashita, E. Cosmetic benefits of astaxanthin on humans subjects., *Acta Biochim. Pol*., **2012**, *59*, 43–47.
43. Hussein, G.; Goto, H.; Oda, S.; Sankawa, U.; Matsumoto, K.; Watanabe, H. Antihypertensive potential and mechanism of action of astaxanthin: III. Antioxidant and histopathological effects in spontaneously hypertensive rats., *Biological and Pharmaceutical Bulletin.,* **2006**, *29*, 684–688.
44. MacDermid, J. C.; Vincent, J. I.; Gan, B. S.; Grewal, R. A blinded placebo-controlled randomized trial on the use of astaxanthin as an adjunct to splinting in the treatment of carpal tunnel syndrome., *Hand*., **2012**, *7*, 1–9.
45. Comhaire, F. H.; Garem, Y. E.; Mahmoud, A.; Eertmans, F.; Schoonjans, F. Combined conventional/antioxidant "Astaxanthin" treatment for male infertility: a double blind, randomized trial., *Asian J. Androl*., **2005**, *7*, 257–262.
46. Kim, H. Y.; Ediriweera, M. K.; Boo, K.; Kim, C. S.; Cho, S. K. Effects of cooking and processing methods on phenolic contents and antioxidant and anti-proliferative activities of broccoli florets., *Antioxidants.,* **2021**, *10*, 641.
47. Pa$_5$ko, P.; Tyszka-Czochara, M.; Galanty, A.; Gdula-Argasi$_5$ska, J.; $_5$mudzki, P.; Barto$_5$, H.; Zagrodzki, P.; Gorinstein, S. Comparative study of predominant phytochemical compounds and proapoptotic potential of broccoli sprouts and florets., *Plant Foods for Human Nutrition.,* **2018**, *73*, 95–100.
48. Sun, T.; Powers, J. R.; Tang, J. Evaluation of the antioxidant activity of asparagus, broccoli and their juices., *Food Chem*., **2007**, *105*, 101–106.
49. Ling, L. T.; Radhakrishnan, A. K.; Subramaniam, T.; Cheng, H. M.; Palanisamy, U. D. Assessment of antioxidant capacity and cytotoxicity of selected Malaysian plants., *Molecules*., **2010**, *15*, 2139–2151.
50. Sibi, G.; Shukla, A.; Dhananjaya, K.; Ravikumar, K. R.; Mallesha, H. In vitro antibacterial activities of Broccoli (*Brassica oleracea L. var italica*) against food borne bacteria., *Journal of Applied Pharmaceutical Science.,* **2013**, *3*, 100–103.
51. Vinha, A. F.; Alves, R. C.; Barreira, S. V.; Costa, A. S.; Oliveira, M. B. P. Impact of boiling on phytochemicals and antioxidant activity of green vegetables consumed in the Mediterranean diet., *Food & Function*., **2015**, *6*, 1157–1163.
52. Owis, A. I. Broccoli; the green beauty: A Review. *Journal of Pharmaceutical Sciences and Research*., **2015**, *7*, 696.