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DETERMINATION OF PHYTOCHEMICALS, ANTIOXIDANT, ANTIBACTERIAL ACTIVITIES AND ANALYSIS BY FOURIER-TRANSFORM INFRARED SPECTROSCOPY (FTIR) FOR THE FRESH ETHANOLIC EXTRACT OF BRASSICA OLERACEA

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ABSTRACT

Introduction: Broccoli (*Brassica oleracea var. italica*) is a widely consumed cruciferous vegetable due to its high nutritional value contributed by present phytochemicals such as flavonoids, phenolic compounds, glucosinolates and terpenoids. These phytochemical compounds have antioxidant and antibacterial properties, making broccoli a potential source of natural bioactive agents for pharmaceutical and nutraceutical applications. This research focuses on evaluating the phytochemical composition, antioxidant activity, antibacterial activity and functional groups of ethanolic broccoli extract using Fourier Transform Infrared (FTIR) spectroscopy. **Objectives:** The objectives of this study were to determine the phytochemicals composition, evaluate the antioxidant activity, examine the antibacterial activity and perform functional groups characterization of fresh broccoli extract using FTIR spectroscopy. **Method:** Fresh *Brassica oleracea var. italica* was extracted by maceration using 95% ethanol. Qualitative phytochemical screening was performed to detect the presence of primary and major secondary metabolites. Antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay with ascorbic acid as the standard reference. Antibacterial activity was evaluated against *Bacillus subtilis* and *Escherichia coli* using broth dilution to determine minimum inhibitory concentration (MIC), followed by minimum bactericidal concentration (MBC) determination through subculturing on Mueller-Hinton agar. FTIR spectroscopy analysis was performed to identify the functional groups associated with the phytochemical constituents. **Result:** Phytochemicals found in broccoli extract include flavonoids, phenolic compounds, tannins, glycosides and terpenoids. Besides that, the broccoli extract exhibited concentration-dependent antioxidant activity, with the percentage of DPPH radical scavenging activity increasing from 6.11% at 100µg/ml to 86.93% at 1000µg/ml. The average IC50 value of broccoli extract was 434.45 µg/ml, compared with 1.63µg/ml for ascorbic acid, showing lower antioxidant capacity than the standard. Furthermore, the broccoli extract showed negative result towards *Bacillus subtilis* and *Escherichia coli*. No complete inhibition of bacterial growth against either *B. subtilis* and *E. coli* at concentration up to 400 mg/ml and bacterial colonies remained visible in MBC test. Additionally, FTIR analysis identified key absorption peaks corresponding to hydroxyl, alkane, aromatic ring and sulphur containing functional groups, supporting the presence of flavonoids, phenolic compounds, tannins, glycosides and terpenoids. **Conclusion:** It is concluded that the ethanolic extract of broccoli contained several useful phytochemicals and exhibited measurable concentration-dependent antioxidant activity, which is contributed by its flavonoid and phenolic constituents. However, antibacterial activity against *B. subtilis* and *E. coli* could not be confirmed under the conditions of this study. FTIR analysis further supported the phytochemical findings by confirming the presence of corresponding functional groups. Overall, broccoli extract showed potential as a natural source of antioxidants while further optimization of extraction methods and phytochemical characterization is recommended to improve its biological applications.

KEYWORDS: *Brassica oleracea var. italica*, Phytochemicals, Antioxidant, Antibacterial, FTIR spectroscopy.

Description of Broccoli (*Brassica oleracea* var. *italica*)

Broccoli, scientifically known as *Brassica oleracea* var. *italica*, is a nutritious cruciferous vegetable which belongs to the *Brassicaceae* family. It originated from the eastern Mediterranean region and was introduced to England and America in the 1700s. Broccoli can grow 60-90 cm tall, can reach a diameter of 60 cm and a weight of 700 g (Li et al., 2022). It is made up of flowerhead, florets, stems and trunk as shown in Figure 1.

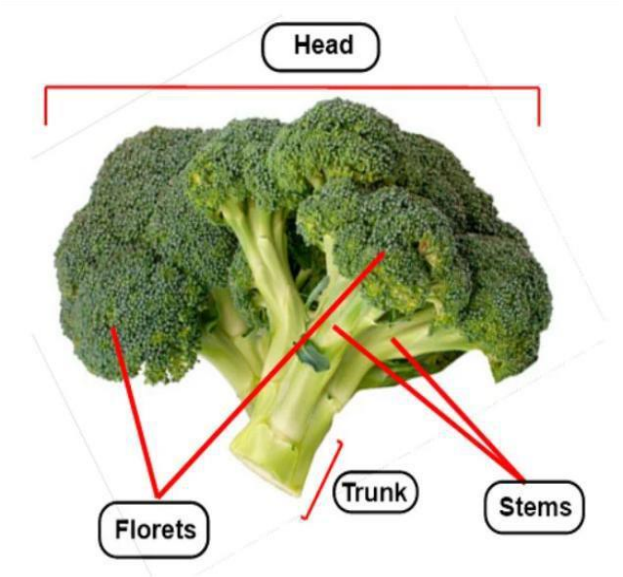


Figure 1: **Anatomy of broccoli showing head, florets, stems and trunk. Image adapted from (Andrés et al., 2025).**

Broccoli has become increasingly popular due to its high nutritional content and multiple health benefits. It contains several important nutrients such as vitamin A, vitamin C, vitamin K, potassium, calcium, iron, dietary fiber and various phytochemical compounds (Andrés et al., 2025). Traditionally, broccoli was well-known for its high vitamins, minerals and dietary fiber. However, recent research emphasized the antioxidant potential of broccoli due to its high bioactive components, which includes a wide range of phenolic compounds, carotenoids and glucosinolates. These phytochemicals significantly contribute to their potential protective effects against chronic diseases, including inflammation and oxidative stress.

Phytochemical screening

Phytochemicals are defined as naturally occurring bioactive compounds that are found in plants which are responsible for the medicinal activity of plants (Rao et al., 2023). In general, phytochemicals are classified into primary metabolites and secondary metabolites. Primary metabolites are compounds vital for basic metabolic processes, growth and development of the plant. They include carbohydrates, proteins and lipids. On the other hand, secondary metabolites are synthesized from the primary metabolites, which contribute to the flavors, aromas, colors and specific therapeutic properties of plants, including antioxidant, antibacterial, anti-inflammatory and anticancer effects. Major classes of secondary metabolites include phenolics, alkaloids, terpenoids and flavonoids (Babulal et al., 2025).

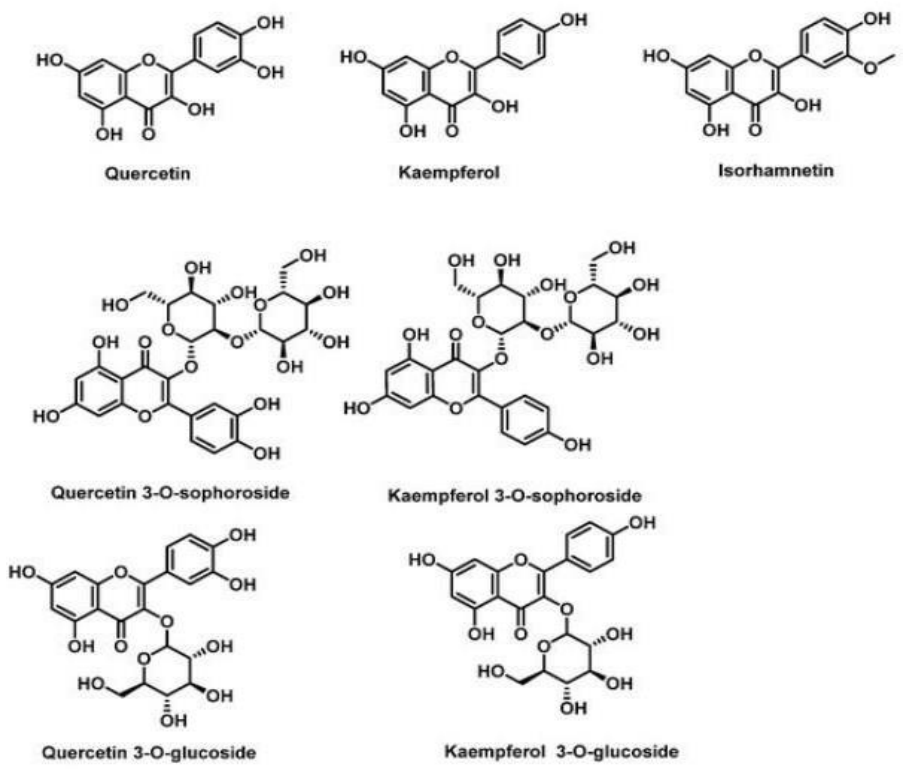


Figure 2: Flavonoids in broccoli. Image adapted from (Andrés et al., 2025).

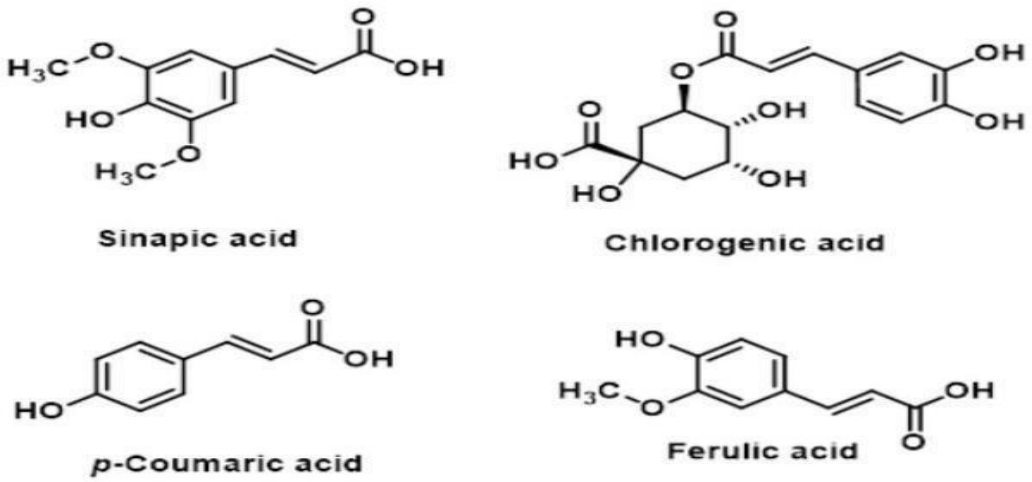


Figure 3: Common hydroxycinnamic acids in broccoli. Image adapted from (Andrés et al., 2025). Antioxidant activity.

Antioxidant activity refers to the ability of phytochemical compounds in a plant extract to neutralize the free radicals. Study of (Syed et al., 2023) states that the antioxidant properties of broccoli is contributed by several antioxidants, such as vitamin C, vitamin E, β-carotene and various flavonoids. These antioxidants protect the cells and tissues from oxidative damage caused by harmful free radicals that potentially lead to cancer development. To detect whether broccoli possesses antioxidant activities, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay can be carried out (Uvaraj et al., 2024). Broccoli's antioxidant properties make it a valuable natural compound that can protect against oxidative stress. Figures 2 and 3 explains all this.

Antibacterial activity

Antibacterial activity refers to the ability to inhibit the growth and proliferation of harmful bacteria. Broccoli has been shown to inhibit the growth of several pathogenic bacteria, including Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Pseudomonas aeruginosa*). Antibacterial activity of broccoli is mainly attributed to its isothiocyanates bioactive compounds, where sulforaphane (SFN), the hydrolysis product of glucoraphanin, being the most extensively studied. SFN has become potential antibiotics-independent candidate molecule in combating infectious diseases caused by multidrug-resistant (MDR) bacteria (Pötschke et al.,2025). These findings show that broccoli extracts could be the potential alternative natural antibacterial agent.

Fourier -transform infrared Spectroscopy (FTIR) analysis

In phytochemical research, an increase in the application of advanced analytical instruments to study the phytochemicals of natural substances was observed. One of the latest techniques include Fourier Transform Infrared Spectroscopy (FTIR), which is a widely used spectral technique to identify the internal molecular structures of phytochemicals (Gong et al., 2024). FTIR spectroscopy operates on the principles of atomic vibration and rotation. When a beam of infrared light with continuous wavelength is directed on a sample molecule, the molecule will absorb light at different wavelengths and result in absorbance spectrum. By analyzing the unique spectral fingerprint of the chemical bonds, the functional groups that present in the plant extracts can be identified (Gong et al., 2024). Analysis by FTIR brings a lot of benefits because this analytical method is rapid, efficient, non-destructive, highly sensitive, minimal sample preparation and versatility for different type of samples such as solid, liquid or gas (Tkachenko & Niedzielski, 2022). FTIR provides both qualitative and quantitative information, thereby identification and quantification of materials can be done simultaneously.

METHODOLOGY

Requirements Chemicals

1. 95% Ethanol (John Kollin Corporation, USA), 2. Dragendorff's reagent (R&M Chemicals, UK), 3. 10 % Lead acetate solution, 4. Ferric chloride (EMD M.C. Germany),5. 5% alcoholic α-naphthol, Sulphuric acid (95-99%) (EMD M.C. Germany), 6. Fehling's A and Fehling's B reagent (R&M Chemicals, UK), 7. Ninhydrin solution, Chloroform (EMD M.C. Germany, Broccoli extracts, 8. Distilled water, 9. 2,2-Diphenyl-1-picryhydrazyl (DPPH) reagent (Cisco Research Laboratories Pvt. Ltd. India. 10. L (+) - Ascorbic acid (HmbG Chemicals).

Equipment

1. Digital analytical weighing balance,
2. Rotary evaporator,
3. Water bath,
4. Glassware & Apparatus,
5. UV-Visible spectrophotometer.

II. Antibacterial Activity Screening

Chemicals

1. Nutrient Agar
2. Nutrient Broth
3. Mueller Hinton Agar

4. Mueller Hinton Broth
5. Broccoli extracts
6. Distilled water
7. Ciprofloxacin 0.5 mg

Equipment

1. Laminar airflow cabinet
2. Autoclave machine
3. Incubator

Fourier-transform infrared (FTIR) spectroscopy Chemicals

1. Broccoli extracts
2. 70% Ethanol
3. Isopropyl alcohol

Equipment

1. FTIR spectrophotometer (Perking Elmer)
2. Computer with FTIR software

Collection and Preparation of fresh broccoli extract

Two broccoli which weighed 650g were purchased from a local shop (fresh market) in the city of Sungai Petani, Malaysia on 7th April 2026. The broccoli was brought to the laboratory under hygienic conditions. Then, the florets and the stems were cut into small pieces using a clean knife. The florets together with the stems were collected in a basket and washed with distilled water to remove dirt and unwanted impurities. The broccoli's florets and the stems were weighed and the value was recorded as shown in Figure 4.

Figure 4: *Brassica oleracea* var. *italica* freshly bought.

Maceration extraction

1. A 2L conical flask was prepared and washed with distilled water and ethanol.
2. The cut broccoli was transferred into the conical flask.
3. 95% ethanol was prepared and poured carefully into the conical flask until all the broccoli was fully immersed.
4. The conical flask was sealed with 2 sheets of aluminum foil wrapped around the mouth to cover the opening.
5. The mixture was left to macerate at room temperature by shaking manually 10 minutes daily for 7 days.
6. After 7 days, the mixture was poured out from the 2L conical flask, filtered using a clean muslin cloth and the

solution remaining in the broccoli florets was squished by compressing the muslin cloth. The solution was collected in a 1L beaker.

7. The extract was then evaporated by using a rotary evaporator under reduced pressure at a temperature of 55°C for 120 rotations per minute (rpm).
8. When there were no further changes observed, the extract was removed and transferred into a China dish.
9. The extract was further dried by placing on the heated water bath at 60°C until a semi-solid state was formed.
10. The final extract, covered with poked aluminum foil, was stored in the laboratory fridge at 4°C until further analysis.

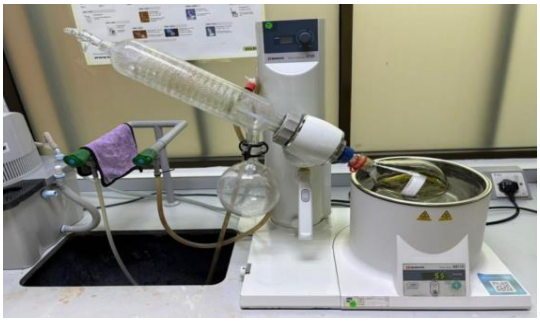


Figure 5: Evaporation of solvent using rotary evaporator.

Figure 6: Dried extract collected.

Antioxidant Activity Determination DPPH Free Radical Scavenging Assay

Preparation of different concentration of extract

1. A 1 mg/ml extract stock solution was prepared by dissolving 100 mg extract obtained from maceration in 100 ml 95% ethanol.
2. Different concentration of extract stock solution, which are 100, 200, 400, 600, 800 and 1000 µg/ml, were prepared by mixing different volume of stock solution with different volume of ethanol as shown in the table below.
3. The total volume of each dilution should be 10 ml.
4. The required volume of extract stock solution ethanol for each concentration was pipetted into a 10 ml volumetric flask and stored for further use.

Preparation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution

0.3 mM of DPPH reagent was prepared by dissolving 11.83 mg in 100 ml of ethanol.

Procedure of DPPH assay

1. 2.5 ml of different concentration of broccoli extract was added into 6 separate test tubes.
2. 1 ml of 0.3mM alcoholic DPPH reagent was added into each test tube.
3. All the test tubes were covered with aluminum foil as DPPH is a photosensitive reagent.
4. The above steps was performed in triplicate for each concentration of broccoli extract.

5. Control solution was prepared by replacing extract with ethanol and 1 ml of 0.3mM alcoholic DPPH reagent was added.
6. A blank solution was prepared with only 3 ml of 95% ethanol.
7. All three set of test tubes were allowed to be incubated in a dark cupboard for 30 minutes.
8. The absorbance of different concentrations of extract was measured by using UV-Visible spectrophotometer at 517 nm (Uvaraj et al., 2024).
9. The absorbance value of each different concentrations of extract, control and blank was recorded.
10. The percentage of radical scavenging activity of different concentration of extract was calculated by using the formula (Venugopal et al., 2025):

DPPH radical scavenging activity (%) = [(A0-A1) / A0] x 100 A0 = absorbance of the control

A1 = absorbance of the sample extract

Preparation of different concentration of standard ascorbic acid

Table 1: Shows all the concentrations verses volume of ethanol.

Concentration (µg/ml)	Volume of stock solution (ml)	Volume of ethanol (ml)
100	1.0	9.0
200	2.0	8.0
400	4.0	6.0
600	6.0	4.0
800	8.0	2.0
1000	10.0	0.0

1. The standard ascorbic acid solution was prepared by dissolving 10 mg of ascorbic acid in 100 ml of 95% ethanol to obtain a 100µg/ml stock solution.
2. Six concentrations of 1, 2, 4, 6, 8 and 10 µg/ml were prepared from the stock solution. The total volume of each dilution was 10 ml.
3. The required volume of ascorbic acid stock solution and ethanol were shown below.
4. The required volume of ascorbic acid solution and ethanol for each concentration was pipetted into a 10 ml volumetric flask and stored for further use.
5. 2.5 ml of different concentration of ascorbic acid solution was added into 6 separate test tubes.
6. 1 ml of 0.3mM alcoholic DPPH reagent was added into each test tube.
7. All the test tubes were covered with aluminum foil.
8. Control solution was prepared by replacing extract with ethanol and 1 ml of 0.3mM alcoholic DPPH reagent was added.
9. A blank solution was prepared with only 3 ml of 95% ethanol.
10. Test tubes were allowed to be incubated in a dark cupboard for 30 minutes.
11. The absorbance of different concentrations of ascorbic acid solution was measured by using UV-Visible spectrophotometer at 517 nm.
12. The absorbance value of each different concentrations of ascorbic acid, control and blank was recorded.
13. The percentage of radical scavenging activity of different concentration of extract was calculated by using the formula:

DPPH radical scavenging activity (%) = [(A0-A1) / A0] x 100 A0 = absorbance of the control

A1 = absorbance of the ascorbic acid solution

Table 2: Formulae for different dilution of ascorbic acid.

Concentration (µg/ml)	Volume of stock solution (ml)	Volume of ethanol (ml)
1	0.10	9.90
2	0.20	9.80
4	0.40	9.60
6	0.60	9.40
8	0.80	9.20
10	1.00	9.00

Antibacterial Activity Determination

Preparation of Mother Plate of *Bacillus subtilis* and *Escherichia coli*

1. Nutrient agar was prepared by dissolving 28 g of nutrient agar powder in 1000 ml of distilled water.
2. The mixture was stirred until completely dissolved and sterilized by autoclaving.
3. After autoclave, the nutrient agar mixture was allowed to cool.
4. The mixture was poured into Petri plates and left undisturbed until solidified.
5. Pure cultures of Bacillus subtilis and Escherichia coli were aseptically inoculated onto separate nutrient agar plates using a sterile inoculating loop.
6. Each bacterial strain was streaked on the agar surface using the quadrant streak method to obtain isolated colonies.
7. The inoculated plates were incubated at 37 °C for 24 hours to allow sufficient bacterial growth.

Subculturing of *Bacillus subtilis* and *Escherichia coli*

1. A single isolated colony of *Bacillus subtilis* from the mother plate was picked with a sterile inoculating loop and streaked onto a fresh nutrient agar plate.
2. The same procedure was repeated for *Escherichia coli*.
3. The subculture plates were incubated at 37 °C for 24 hours to obtain fresh and pure bacterial colonies.

Broth dilution method

The minimum inhibitory concentration (MIC) of broccoli extract against bacteria was determined using the broth dilution method.

1. Mueller-Hinton Broth (MHB) was used as the culture medium.
2. A bacterial suspension of *Bacillus subtilis* was prepared from fresh colonies cultured on nutrient agar and adjusted to a turbidity equivalent to the 0.5 McFarland standard (around 1-2 x 10^8 CFU/ml).
3. A stock solution of broccoli extract was prepared and diluted to obtain final test concentrations of 25, 50, 100, 200 and 400 mg/ml.
4. Five sterile test tubes were prepared. For each test tube, 1 ml of the bacterial suspension added, and the remaining volume of broccoli extract stock solution and Mueller-Hinton Broth were added based on each concentration as shown in the table below. Final volume of each test tube was 10 ml.
5. Two control test tubes were prepared. The positive control consisted of ciprofloxacin and bacterial suspension in MHB while the negative control consisted of distilled water and bacterial suspension in MHB.
6. All test tubes were mixed and incubated at 37°C for 24 hours.
7. After incubation, test tubes were examined visually for bacterial growth based on turbidity.

8. The lowest concentration of broccoli extract with no visible bacterial growth was determined by MIC.
9. The experiment was conducted in triplicate and the results were recorded.
10. Steps 1-9 were repeated to test the MIC of broccoli extract against *Escherichia coli.*

Table 3: Volume of extract stock solution and MHB at different concentration Minimum Bactericidal Concentration (MBC) Test.

Concentration (mg/ml)	Extract Stock Solution (ml)	Mueller-Hinton Broth (ml)	Bacterial suspension (ml)
25	0.06	8.94	1.00
50	0.13	8.87	1.00
100	0.25	8.75	1.00
200	0.50	8.50	1.00
400	1.00	8.00	1.00

The MBC of broccoli extract against *Bacillus subtilis* and *Escherichia coli* was determined following the MIC test.

1. After incubation of the broth dilution tubes at 37°C for 24 hours, all tubes which showed no visible bacterial growth were chosen for MBC test.
2. Sterile Mueller-Hinton Agar (MHA) plates were divided into several regions and labelled according to the corresponding broccoli extract concentrations.
3. A loopful of culture from each clear MIC tube was streaked onto the designated region of the MHA plate by using a sterile inoculating loop. The inoculating loop was sterilized using Bunsen burner between each streak to prevent cross-contamination.
4. After streaking, the MHA plates were sealed with parafilm and incubated at 37°C for 24 hours.
5. After incubation, the regions were examined for the presence or absence of bacterial growth.
6. This test was performed in triplicate for both bacterial strains.
7. The results were recorded as bacterial growth (+) or no bacterial growth (-) and the MBC value was determined from the lowest concentration of broccoli extract that exhibited complete absence of colony formation.

Fourier-transform infrared (FTIR) spectroscopy analysis

Brassica oleracea var. *italica* extract was analyzed by FTIR spectrometer (Perkin Elmer Spectrum Two^{TM}). The FTIR spectrometer and the infrared source were turned on and allowed them to warm up for 15-30 minutes. The crystal surface was cleaned with isopropyl alcohol using lint-free wipes before starting sample analysis. A background scan was run with the Attenuated Total Reflectance (ATR) anvil left open to measure the “black” air. Then, a single drop of the broccoli extract was placed onto the crystal using a spatula. The pressure adjuster knob was screwed down until the pressure tip made a light contact with the sample. The knob was continued screwing until the force gauge reached 95 lbs. A sample scan was run to acquire the spectrum. Spectral data was obtained across the range of 4000-400 cm^{-1} with a spectral resolution of 4 cm^{-1}. The recorded spectra were analyzed by comparing the peaks to the reference spectra to identify the specific functional groups present in the broccoli extract.

Figure 7: FTIR Spectrometer (Perkin Elmer Spectrum Two™)

RESULTS

Phytochemical Screening

Table 4: Results of phytochemicals present in ethanolic broccoli extract.

No	Phytochemical screening test	Results
1	Alkaloids	+
2	Flavonoids	+
3	Phenolic compounds and Tannins	+
4	Carbohydrates	+
5	Glycosides	+
6	Proteins and amino acids	+
7	Saponins	-
8	Terpenoids	+
9	Anthraquinones	-

+ = Present - = Absent

Antioxidant activity

DPPH Radical Scavenging Activity

The following triplicate results showed the absorbance and the percentage of radical scavenging activity of maceration extract along with graphs of IC50 calculated. Tables 5 and 6 explains absorbance values.

Table 5: Different concentrations of broccoli extract with its absorbance value (Trial 1).

Sample Concentration (µg/ml)	Absorbance (WL517nm)
Control	0.714
100	0.648
200	0.595
400	0.276
600	0.171
800	0.109
1000	0.102

Table 6: Different concentrations of broccoli extract with its percentage of radical scavenging activity (Trial 1).

Sample Concentration (µg/ml)	Percentage of radical scavenging activity (%)
100	9.24
200	16.67
400	61.34
600	76.05
800	84.73
1000	85.71

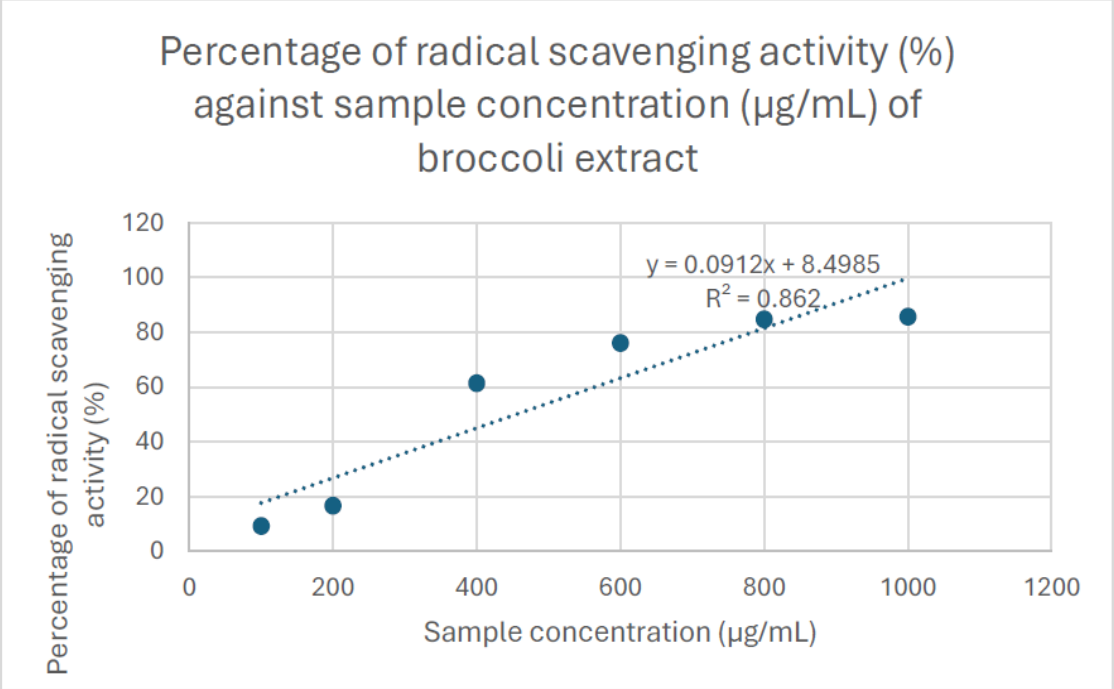


Figure 8: Percentage of radical scavenging activity (%) against sample concentration (µg/ml) of broccoli extract (Trial 1)

IC50

Y = mx+c

50 = 0.0912x + 8.4985

0.0912x = 41.5015

x = 455.06 µg/ml

Table 7: Different concentrations of broccoli extract with its absorbance value (Trial 2).

Sample Concentration (µg/ml)	Absorbance (WL517nm)
Control	0.714
100	0.703
200	0.477
400	0.248
600	0.193
800	0.112
1000	0.095

Table 8: Different concentrations of broccoli extract with its percentage of radical scavenging activity (Trial 2).

Sample Concentration (µg/ml)	Percentage of radical scavenging activity (%)
100	1.54
200	33.19
400	65.27
600	72.97
800	84.31
1000	86.69

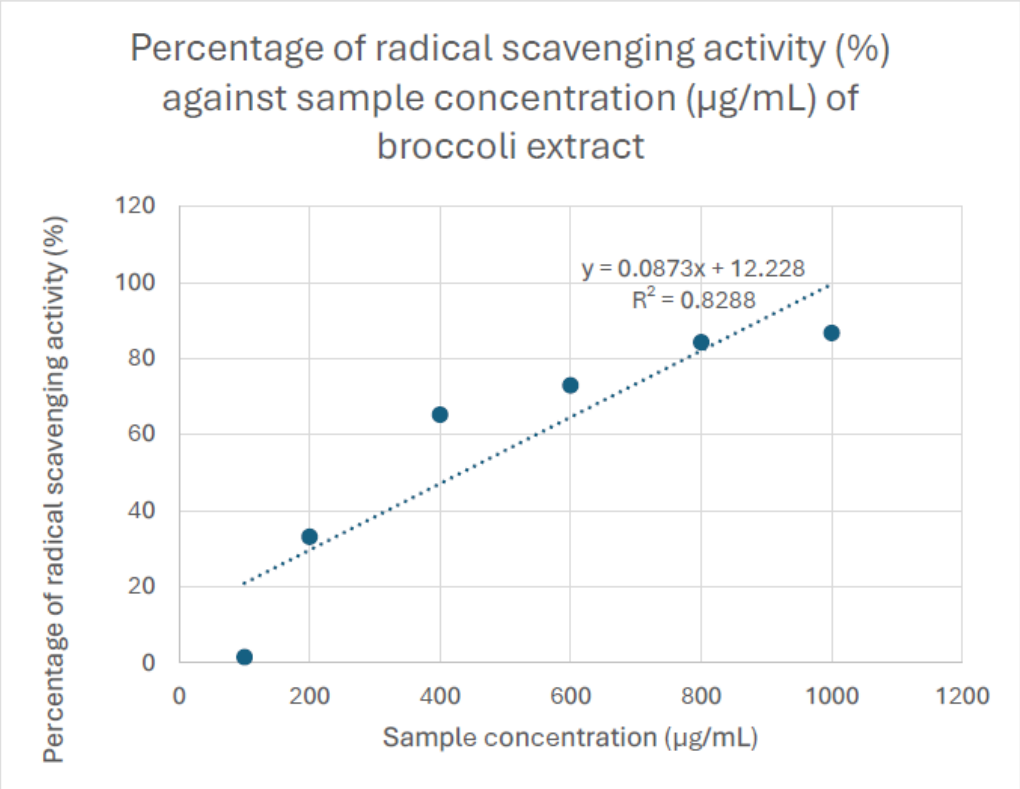


Figure 9: Percentage of radical scavenging activity (%) against sample concentration (µg/ml) of broccoli extract (Trial 2)

IC50

Y = mx+c

50 = 0.0873x + 12.228

0.0873x = 37.772

x = 432.67 µg/ml

Table 9: Different concentrations of broccoli extract with its absorbance value (Trial 3).

Sample Concentration (µg/ml)	Absorbance (WL517nm)
Control	0.714
100	0.660
200	0.446
400	0.279
600	0.197
800	0.112
1000	0.083

Table 10: Different concentrations of broccoli extract with its percentage of radical scavenging activity (Trial 3)

Sample Concentration (µg/ml)	Percentage of radical scavenging activity (%)
100	7.56
200	37.54
400	60.92
600	72.41
800	84.31
1000	88.38

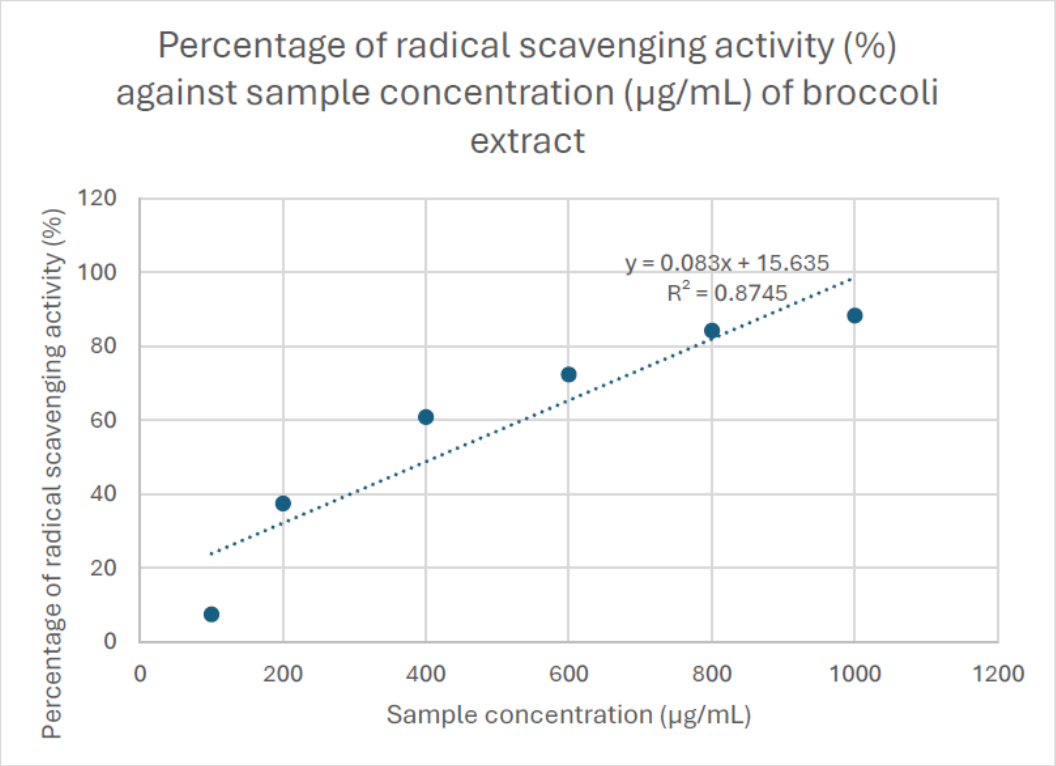


Figure 10: Percentage of radical scavenging activity (%) against sample concentration (µg/ml) of broccoli extract (Trial 3).

IC50

Y = mx+c

50 = 0.083x + 15.635

0.083x = 34.365

x = 414.04 µg/ml

Table 11: Average absorbance value of three trials.

Sample Concentration (µg/ml)	Absorbance (WL517nm)
Control	0.714
100	0.670
200	0.506
400	0.268
600	0.187
800	0.111
1000	0.093

Table 12: Average percentage of radical scavenging activity of three trials.

Sample Concentration (µg/ml)	Percentage of radical scavenging activity (%)
100	6.11
200	29.13
400	62.51
600	73.81
800	84.45
1000	86.93

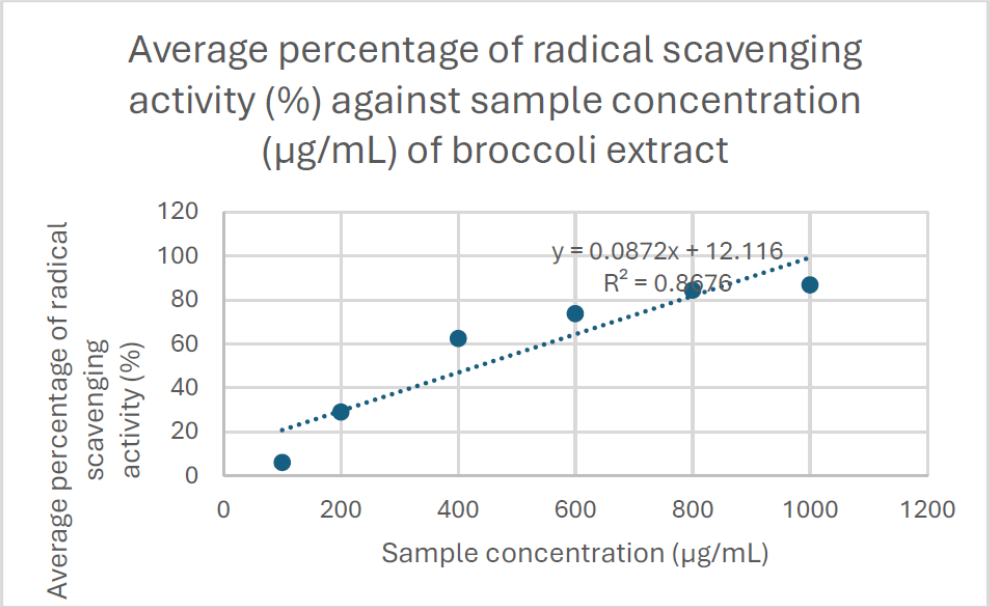


Figure 11: Average percentage of radical scavenging activity (%) against sample concentration (µg/ml) of broccoli extract.

IC50

Y = mx+c

50 = 0.0872x + 12.116

0.0872x = 37.884

x = 434.45 µg/ml

Table 13: Each concentration of ascorbic acid solution with its absorbance value.

Sample Concentration (µg/ml)	Absorbance (WL517nm)
Control	0.526
1	0.288
2	0.245
4	0.109
6	0.027
8	0.023
10	0.023

Table 14: Each concentration of ascorbic acid solution with its percentage of radical scavenging activity.

Sample Concentration (µg/ml)	Percentage of radical scavenging activity (%)
1	45.25
2	53.42
4	79.28
6	94.87
8	95.63
10	95.63

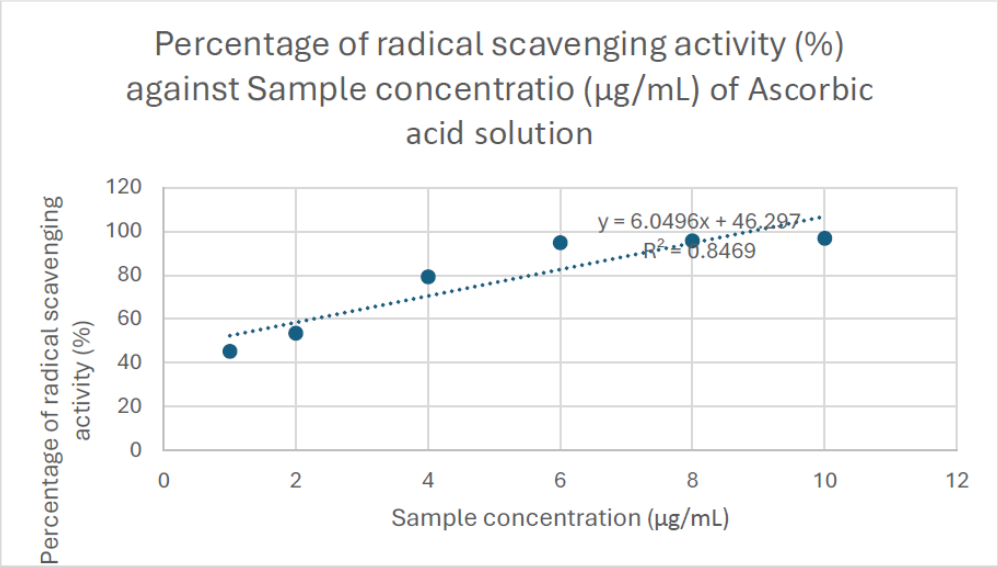


Figure 12: Percentage of radical scavenging activity (%) against sample concentration (µg/ml) of ascorbic acid solution.

IC50

Y = mx+c

50 = 6.0496x + 46.297

6.0496x = 3.703

x = 1.63 µg/ml

Table 15: Comparison of IC50 of broccoli extract vs ascorbic acid solution.

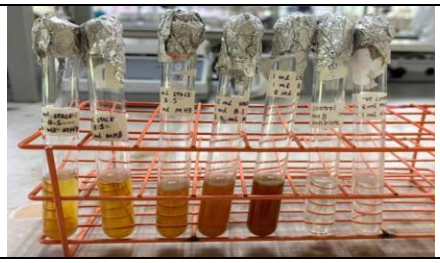
Sample type	IC50 (µg/ml)
Broccoli extract	434.45
Ascorbic acid	1.63

Antibacterial activity determination

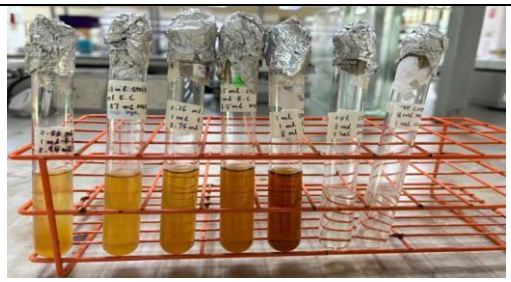
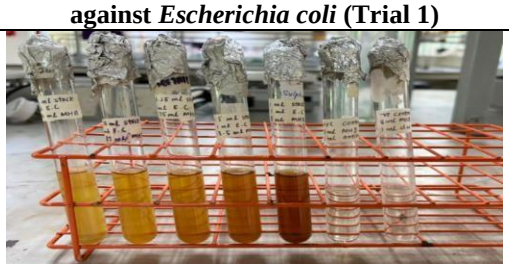
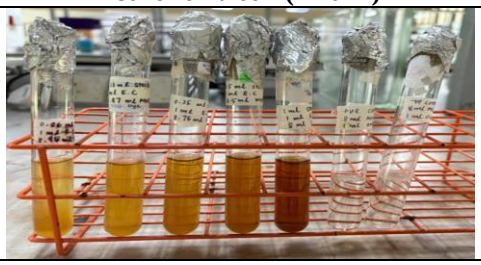
Minimum Inhibitory Concentration (MIC) test

Bacillus subtilis

Trial	Observation	Result
1	 Figure MIC result of broccoli extract against *Bacillus subtilis* (Trial 1)	Visible turbidity was observed in all the five tubes of different concentration, indicating bacterial growth.
2	 Figure MIC result of broccoli extract against *Bacillus subtilis* (Trial 2)	Visible turbidity was observed in all the five tubes of different concentration, indicating bacterial growth.

3		Visible turbidity was observed in all the five tubes of different concentration, indicating bacterial growth.

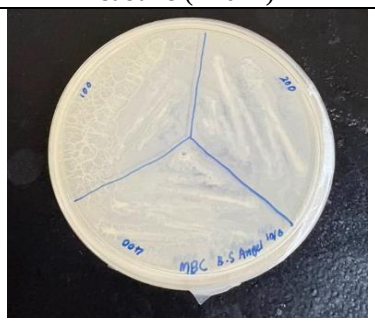
Escherichia coli

Trial	Observation	Result
1	 **Figure MIC result of broccoli extract against *Escherichia coli* (Trial 1)**	Visible turbidity was observed in all the five tubes of different concentration, indicating bacterial growth.
2	 **Figure MIC result of broccoli extract against *Escherichia coli* (Trial 2)**	Visible turbidity was observed in all the five tubes of different concentration, indicating bacterial growth.
3		Visible turbidity was observed in all the five tubes of different concentration, indicating bacterial growth.

Minimum Bactericidal Concentration (MBC) test

Bacillus subtilis

Trial	Observation	Result
1	 Figure MBC result of broccoli extract against *Bacillus subtilis* (Trial 1)	Bacterial colonies were observed, indicating that no bactericidal effect against *Bacillus subtilis* was achieved and the MBC was higher than 400 mg/mL.

2	 Figure MBC result of broccoli extract against *Bacillus subtilis* (Trial 2)	Bacterial colonies were observed, indicating that no bactericidal effect against *Bacillus subtilis* was achieved and the MBC was higher than 400 mg/mL.
3	 Figure MBC result of broccoli extract against *Bacillus subtilis* (Trial 3)	Bacterial colonies were observed, indicating that no bactericidal effect against *Bacillus subtilis* was achieved and the MBC was higher than 400 mg/mL.

Escherichia coli

Trial	Observation	Result
1	 Figure 4.19 MBC result of broccoli extract against *Escherichia coli* (Trial 1)	Bacterial colonies were observed, indicating that no bactericidal effect against *Escherichia coli* was achieved and the MBC was higher than 400 mg/mL.
2	 Figure 4.20 MBC result of broccoli extract against *Escherichia coli* (Trial 2)	Bacterial colonies were observed, indicating that no bactericidal effect against *Escherichia coli* was achieved and the MBC was higher than 400 mg/mL.

3	 Figure 4.21 MBC result of broccoli extract against *Escherichia coli* (Trial 3)	Bacterial colonies were observed, indicating that no bactericidal effect against *Escherichia coli* was achieved and the MBC was higher than 400 mg/mL.

FOURIER FTIR Spectroscopy Analysis

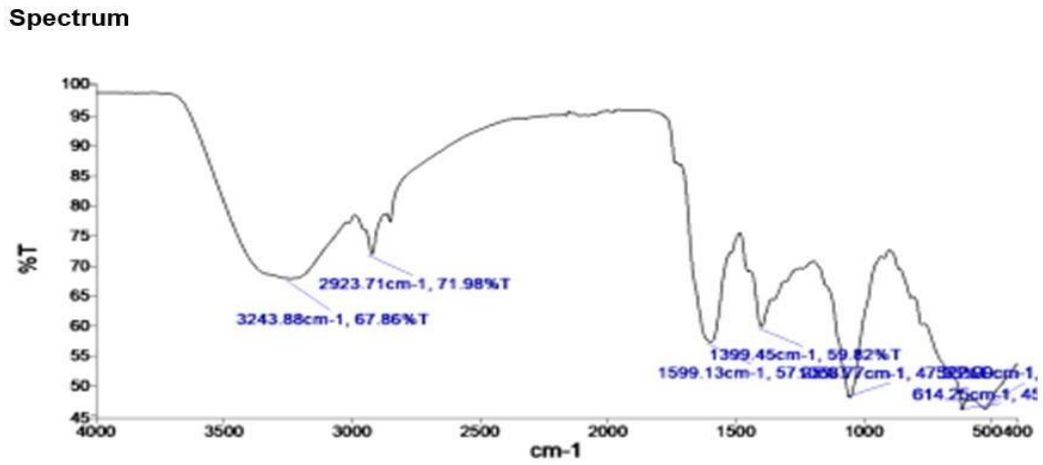


Figure 13: FTIR spectrum of *Brassica oleracea* var. *italica.*

Based on the FTIR spectrum of *Brassica oleracea* var. *italica*, the major absorption peaks appeared at 3243.88, 2923.71, 1599.13, 1399.45, 1077.87 and 614.26 cm^{-1}. These peaks indicated the presence of various phytochemical functional groups found in broccoli.

Table 16: FTIR spectral peak values and functional groups of *Brassica oleracea* var. *italica.*

Spectrum No.	Wave Number (cm^{-1})	Wave Number Range (cm^{-1})	Functional Group	Predicted Compound
1	3243.88	3200-3550	O-H stretching (hydroxyl group)	Phenolic compounds, flavonoids and carbohydrates
2	2923.71	2850-3000	C-H stretching (alkane group)	Terpenoids, fatty acids and lipids
3	1599.13	1400-1600	Aromatic rings	Phenolic compounds and flavonoids
4	1399.45	1300-1400	O-H bending (hydroxyl group)	Phenolic compounds and organic acids
5	1077.87	1000-1300	C-O stretching (alcohols)	Glycosides and carbohydrates
6	614.26	600-700	C-S stretching (thioesters)	Sulphur-containing compounds

DISCUSSION

The present research investigated the phytochemical composition, antioxidant activity, antibacterial activity and FTIR profile of the ethanolic broccoli extract (*Brassica oleracea* var. *italica*). Qualitative phytochemical screening, DPPH antioxidant assay, MIC and MBC test and FTIR analysis were the main approaches used in this study.

Phytochemical screening

Qualitative phytochemical screening of the broccoli extract showed positive test results for flavonoids, phenolic compounds, tannins, glycosides and terpenoids, while test for saponins and anthraquinones showed negative result. These findings were consistent with previous studies reported that broccoli is rich in phenolic compounds, flavonoids, glucosinolate-derived glycosides and other secondary metabolites (Idris et al., 2024). The presence of flavonoids and phenolic compounds is especially important because these compounds are the effective free radical scavengers. Phenolic compounds contain hydroxyl groups that can donate hydrogen atoms to neutralize the reactive oxygen species and reduce oxidative stress. Additionally, flavonoids can stabilize the free radicals through electron delocalization mechanisms. The presence of glycosides was attributed to glucosinolates, which are sulphur-containing compounds in broccoli. Studies had shown that glucoraphanin, glucoerucin and some other glucosinolates are among the major phytochemicals present in broccoli. On the other hand, the absence of anthraquinones supported the known phytochemical profile of broccoli. Anthraquinones are not typically reported as phytochemicals of cruciferous vegetables. However, they are commonly found in plants such as senna and aloe species.

Antioxidant activity

The antioxidant potential of broccoli extract was evaluated by using DPPH radical scavenging assay and compared with ascorbic acid as the standard reference. The result revealed that the ethanolic broccoli extract exhibited concentration-dependent free radical scavenging activity, by increasing from 6.11% at 100 µg/ml to 86.93% at 1000 µg/ml. As the sample concentration increased, the percentage of radical scavenging activity also increased. The average IC50 value of broccoli extract was 434.45 µg/ml while the IC50 value of ascorbic acid was 1.63 µg/ml. IC50 is defined as the concentration required for the sample to scavenge 50% of the DPPH free radicals. A lower IC50 value indicates stronger antioxidant activity. Therefore, the results suggested that ascorbic acid has a higher antioxidant capacity than broccoli extract.

The large difference between the IC50 values can be explained by the nature of the tested sample. Ascorbic acid is a pure antioxidant compound which possesses the ability to donate electrons and hydrogen atoms to neutralize free radicals. However, broccoli extract is a crude plant extract comprising of a complex mixture of active and inactive constituents. Although the broccoli extract contains phytochemical compounds which have antioxidant capability such as phenolics, flavonoids, glycosides and glucosinolate derivatives, these compounds are present at much lower concentration than pure ascorbic acid. This explained that a higher concentration of broccoli extract is required to achieve the same 50% radical scavenging effect as the standard ascorbic acid.

The antioxidant activity should not be evaluated by directly comparing the range of concentration used for the sample and the standard. As the antioxidant potency of pure standards and crude plant extracts differed greatly, different concentration ranges were employed in DPPH assay. Broccoli extract was tested at 100 to 1000 µg/ml while ascorbic acid was tested at 1 to 10 µg/ml. Ascorbic acid exhibited very strong antioxidant activity, so it reached 50% DPPH inhibition at a very low concentration. In contrast, broccoli extracts required a relatively higher range to produce

measurable radical scavenging activity as extract contained both antioxidant and non-antioxidant compounds.

From the result, when broccoli extract concentration increased, DPPH solution gradually changed from deep purple to pale yellow. This result was consistent with (Akar et al., 2017). The color change was due to the higher concentration of broccoli extract containing more antioxidant molecules, such as phenolic compounds and flavonoids, which donated hydrogen atoms or electrons to neutralize more DPPH radicals. The more DPPH radicals being neutralized, the deep purple color faded.

These findings showed that broccoli extract possessed antioxidant activity. Although its antioxidant activity is weaker than ascorbic acid, the concentration-dependent increase in percentage of radical scavenging activity confirmed that broccoli contained phytochemicals that act as natural antioxidants.

Antibacterial activity

The antibacterial activity of broccoli extract was evaluated against *Bacillus subtilis* and *Escherichia coli* using MIC and MBC assays. The concentrations tested were 25, 50, 100, 200 and 400 mg/ml. The results showed that complete inhibition of bacterial growth was not achieved at the concentrations tested. Bacterial colonies were observed in MBC test, indicating that the broccoli extract did not exhibit bactericidal activity against *Bacillus subtilis* and *Escherichia coli* and the MBC value was greater than 400 mg/ml.

Previous study in Taiwan reported MIC values of 0.78-1.56 mg/mL for broccoli extract against *Bacillus subtilis* and *Escherichia coli* (Le, Sakulsataporn, et al., 2020). However, no complete inhibition was observed in this study at concentrations up to 400 mg/ml. This may be explained by the difference in geographical origin of the broccoli. Environmental factors associated with geographical location can affect biosynthesis of secondary metabolites such as glucosinolates and sulforaphane. Since these phytochemicals are responsible for the antibacterial activity of broccoli, variations in their concentrations may cause differences in antibacterial efficacy between studies. As a result, broccoli grown in different regions may exhibit different levels of antibacterial activity. Additionally, degradation of sulforaphane, the compound that contributed to the antibacterial properties during extraction and drying may reduce the antibacterial activity. Furthermore, crude broccoli extracts contain carbohydrates, proteins and other non-active compounds that may dilute the concentration of active antibacterial compounds. Although the antibacterial activity could not be confirmed under the present experimental conditions, the result does not completely exclude the antibacterial properties of broccoli extract. It is just that the concentration of active antibacterial compounds present in the broccoli extract was insufficient under the experimental conditions used.

FTIR analysis

FTIR analysis showed characteristic absorption bands at 3243.88, 2923.71, 1599.13, 1399.45. 1077.87 and 614.26 cm^{-1}. These bands indicated the presence of hydroxyl, aliphatic, aromatic, glycosidic and sulphur-containing functional groups. These findings supported the phytochemical screening results and provided additional evidence regarding the chemical composition of the broccoli extract.

A broad peak was observed at 3243.88 cm^{-1}. This corresponded to O-H stretching vibrations, indicating the presence of hydroxyl groups that were commonly found in phenolic compounds, flavonoids and carbohydrates. The small peak at 2923.71 cm^{-1} was attributed to the aliphatic C-H stretching vibrations. This suggested the presence of terpenoids, lipids

and other hydrocarbon-containing compounds. Next, a sharp peak at 1599.13 cm^{-1} reflected an aromatic C=C stretching vibrations. This sharp peak indicated the presence of phenolic compounds and flavonoids. Furthermore, the appearance of an absorption band at 1399.45 cm^{-1} was assigned to O-H bending, which may be related to phenolic compounds and organic acids. Additionally, a strong peak observed at 1077.87 cm^{-1} corresponded to C-O stretching vibrations. This peak suggested the presence of glycosides, alcohols and carbohydrate derivatives. Lastly, the peak observed at 614.26 cm^{-1} corresponded to C-S stretching vibrations. This could indicate the presence of sulphur-containing compounds such as glucosinolates.

CONCLUSION

This research study successfully evaluated the phytochemical composition, antioxidant activity, antibacterial activity and functional groups present in the broccoli extract using FTIR analysis method. Qualitative phytochemical screening indicated that broccoli contained a wide range of phytochemicals including flavonoids, phenolic compounds, tannins, glycosides and terpenoids. Presence of these phytochemical compounds showed that broccoli is a valuable natural source of biologically active compounds that contribute to our health benefits. DPPH free radical scavenging assay also demonstrated that broccoli extract exhibited concentration-dependent antioxidant activity with an average IC50 value of 434.45 µg/ml.

The antibacterial activity of the broccoli extract against *Bacillus subtilis* and *Escherichia coli* could not be demonstrated under the experimental conditions employed. In the MIC test, no complete inhibition of bacterial growth was observed while bacterial growth remained evident in MBC test. This concluded that the tested concentrations of the broccoli extract were not enough to produce bacteriostatic or bactericidal effects.

Last but not least, FTIR analysis further confirmed the presence of hydroxyl, aliphatic, aromatic, glycosidic and sulphur-containing functional groups in the broccoli extract. The absorption bands obtained from the FTIR spectrum corresponded to the functional groups associated with phenolic compounds, flavonoids, terpenoids, glycosides and glucosinolate-related compounds.

To conclude, this study achieved its objectives by identifying the phytochemical compounds, evaluating the antioxidant and antibacterial activities and determining the major functional groups present in the broccoli extract. These results contributed to the growing knowledge about the bioactive properties of broccoli and provided a foundation for future studies which aimed at optimizing the extraction methods and evaluating their biological activities using advanced analytical techniques.

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