Archives of Razi Institute, Vol. 78, No. 2 (2023) 633-639

DOI: 10.22092/ARI.2022.359860.2488

Original Article

Chemical Analysis, Antioxidant, and Antibacterial Potential of Aqueous Extract of Broccoli (*Brassica oleracea*) Using GC-ms

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Received 30 August 2022; Accepted 10 September 2022
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Abstract

Antioxidant and antibacterial chemicals are key sources in medicinal plants. Alkaloids, phenolics, steroids, terpenes, flavonoids, terpenes, and volatile oils are a few of these plants' secondary metabolites. Phytochemicals, particularly the secondary metabolites produced by plants, are important for human nutrition, well-being, illness prevention, and antibacterial properties. This study aimed to ascertain the aqueous broccoli extract's chemical makeup. The phytochemical molecule that the GC-MS technique identified. The DPPH assay, which is appropriate for regular plant material screening, was performed to assess the antioxidant capacities of broccoli extract (in vitro). Subsequently looks at how well they perform against different Gram-positive and Gram-negative harmful microorganisms. GC-MS analysis of Broccoli extract revealed the existence of the9-Octadecenamide, [C18H35O], Hexadecane [c16h34] and 2, 2, 2-trifluoroethyl 2-methyltetrahydro-5-oxo-3-furancarboxylate [C23H33NO6]. There were significant changes in the extract's ascorbic acid-free radical scavenging activity at 200, 100, and 25 g/ml (P 0.05), and the activity was dose-dependent. The effectiveness of aqueous broccoli extract as a powerful, broad-spectrum antibacterial agent against tested bacteria is demonstrated by an increase in the diameter of the inhibition zone, which increases in direct proportion to the concentration of the extract and even surpasses the activity of some antibiotics. An appropriate concentration of aqueous broccoli extract strongly inhibits microbial and antioxidant growth, especially when treating external infections without any danger against resistant bacterial isolates; it is strongly advised to use aqueous broccoli extract as a cost-effective alternative antibacterial and antioxidant agent.

Keywords: Aqueous Broccoli Extract, GC-MS, Antioxidant, Antibacterial

1. Introduction

Broccoli (*Brassica oleracea*) is an edible green vegetable, a fast-growing annual plant that belongs to the family Brassicaceae. The pharmaceutical importance of broccoli has been enormously reported as an anticancer, immunomodulator, antidiabetic, antimicrobial, hepatoprotective, cardioprotective, antiamnesic, and antioxidant. Cruciferous vegetables have high fiber content, low calories, and are rich in vitamins and minerals, which are beneficial for normal human physiological functions. Though there are various reports on the benefits of broccoli as a crude vegetable, our focus was to analyze the phytochemical constituents, antioxidant and antibacterial activities,

and investigate bioactive compounds obtained by extraction (1-7).

The best method for locating the bioactive components of substances like long-chain hydrocarbons, acids, esters, steroids, alkaloids, alcohols, amino compounds, and nitro compounds is GC-MS. Gas chromatography (GC) and Mass spectroscopy (MS), along with specific detection tests, have therefore developed into sophisticated methods for analyzing a variety of compounds (8). Brassica oleracea, sometimes broccoli, is an Italian vegetable member of the Brassicaceae family (9). The plant is frequently used to cure various illnesses, including bleeding, diarrhea, poisoning, and eye disorders. It also acts as an anti-inflammatory, analgesic, and antioxidant. Our research is focused on identifying possible sources of medicinal plants using cutting-edge scientific techniques analysis like Gas Chromatography-Mass Spectrometry, that advances in biotechnology science have evaluated natural compounds quickly, faster, and more precisely than before techniques, resulting in the isolation of effective compounds with body health benefits. As a fundamental technical platform for secondary metabolite profiling in plant and non-plant species during the past few years, gas chromatography-mass spectrometry (GC-MS) has solidified its position (9, 10).

A number of regulatory mechanisms, including cell growth, death, and gene expression, are regulated by free radicals produced during aerobic metabolism. Free radicals may damage vital cell biomolecules by oxidizing membrane lipids, cell proteins, carbohydrates, DNA, and enzymes when they are produced in excess. This can undermine the antioxidant system's capacity to protect the body from free radical damage. Malonyl dialdehyde and 4-hydroxynonenal are two examples of harmful substances that are produced as a result of oxidative stress. It also affects the oxidant-antioxidant balance (redox equilibrium), which is essential for healthy cell function (11-13).

Parkinson's disease, Alzheimer's, alcohol-induced liver disease, atherosclerosis, mild cognitive impairment, ulcerative colitis, and aging are all pathologies brought on by oxidative stress (14-23). Antioxidants can break chains by quenching singlet oxygen, dissolving hydroperoxides, chelating prooxidative metal ions, and scavenging chain-starting radicals like hydroxyl, alkoxyl, or peroxyl (24). According to epidemiological research, consuming fruits and vegetables high in antioxidant-rich components can reduce the occurrence of illnesses linked to oxidative stress. Antioxidant-rich foods and minerals significantly impact prevention (25-28).

Resistance to antibiotics is a great threat in the present therapeutic era (29). Researchers worldwide are working tirelessly to find a new drug moiety with antimicrobial potential to overcome the resistance factor. Unfortunately, the new antimicrobial drug molecules under trial also do not guarantee they will overcome the resistance-related issues. Therefore, the scientific focus for the past decade has shifted to green vegetables, which are more beneficial, like day-to-day food habits (30-32).

In Iraq, no studies have been conducted on broccoli's chemical components, antioxidants, or antibacterial effects. The current experiment aimed to assess the amounts of various components, antioxidant activity, and antibacterial activity from aqueous broccoli extract.

2. Materials and Methods

2.1. Gas Chromatography-Mass Spectrometry Analysis

Shimadzu's (QP-2010) gas chromatograph mass spectrometer in Tokyo, Japan, an AOC-20i auto-sampler, and a DB-5MS capillary column, measuring 30 m x 0.25 mm i. d., 0.25 m, were used to analyze the chemical hydrolytic products. The sample injection volume was 2 l in GC grade DCM, and the column's temperature was set to 230°C for 15 minutes after being programmed to 230°C at a rate of 4°C/min. At a flow rate of 1.1 ml/min in split mode, helium was employed as the carrier gas (1:50) (33).

2.2. DPPH Radical Assay

According to the method given by 50 to the ascertained activity of aqueous broccoli extract, the

DPPH (1, 1-diphenyl, 2-picryl-hydrazine) free radical scavenging was performed. Different amounts of broccoli extract (12.5, 25, 50, and 100 g/ml) are utilized. Spectrophotometric calculations were made using the ELISA reader to determine how well samples scavenged the stable DPPH radical. When the DPPH decrease was evaluated at 517 nm, the colorimetric transition (from deep violet to bright yellow) (Figure 1). A good example of a positive control is ascorbic acid (as a reference). The equation below has been used to compute the inhibition percentage (34):

$$\text{Antioxidant activity \%} = \frac{{}^{OD}\text{control} - {}^{OD}\text{sample}}{{}^{OD}\text{control}} \times 100$$

OD= optical density

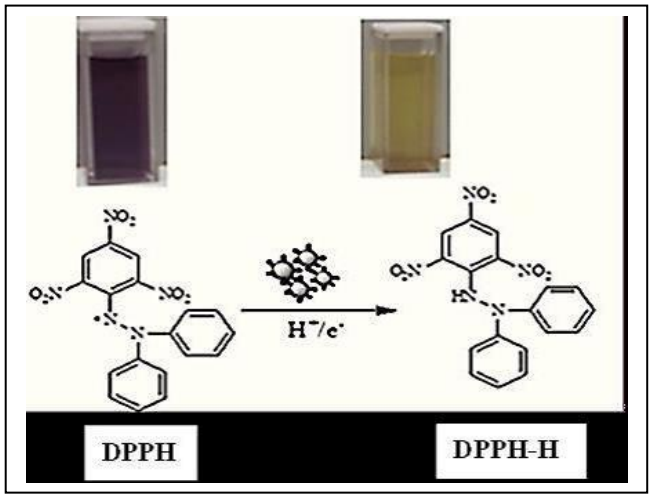

Figure 1. Radical scavenging of DPPH• to DPPH-H by Aqueous Broccoli extract

2.3. Bacterial Isolates, Solution, Media

The teaching hospital in Hillah, Iraq, provided the bacterial isolates collected from its patients. All samples underwent normal bacteriological procedures, including isolation and purification by growing on agar plates ofMacConkey's and blood agar for 24-48 hours at 37°C. By using the Biomérieux Vitek-2 compact system, all isolates were verified.

Mueller-Hinton media and Mueller-Hinton agar were obtained from Hi-Media (Mumbai, India). Different antibiotic disks ceftriaxone (CRO-3Oμg), ampicillin (AM-10μg), cephalothin (KF-30μg), chloramphenicol (C-30μg), and meropenem (MEM-10μg) were purchased from (Bioanalyse, Turkey) (34).

2.4. Antibacterial Activity of Broccoli Extract

Two G+ve bacteria (*Streptococcus mutan*ts and *Staphylococcus epidermidis*) and two G¯ve bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) were storage on nutrient agar slants when the antibacterial activity of aqueous broccoli extract was evaluated against various human pathogenic bacteria. The Clinical and Laboratory Standards Institute's guidelines for antibacterial activity were followed (35). A disk diffusion experiment is performed to examine the efficacy of broccoli extract against the study bacteria as well as the antibiotic sensitivity of the bacteria. Concentrations of aqueous broccoli extract (500, 250, 125, and 62.5 g/ml) in sterile deionized water are employed in triplicates. The isolates underwent a 15-minute at-room-temperature incubation followed by overnight incubation at 37 °C. After a specified incubation time, we visible the inhibition with a clear zone surrounding the well. Positive findings were noted when the inhibition zone's width was always measured using a digital Vernier caliper (36).

3. Results and Discussion

The National Institute of Standards and Technology database was used to interpret the GC-MS (NIST) mass spectrum. The spectrum of the known component contained in the NIST library was compared to the mass spectrum of the unknown component. The main elements were determined using absolute standards and by recording from electronic libraries. The test materials' peak area, molecular weight, chemical name, probability, molecular formula, biological activity, and peak area were determined. The relative percentage quantity of each component was computed by comparing each component's average peak area to the sum of all areas.

Figure 2 displays the GC-MS analysis findings for the aqueous broccoli extract. The constituents 9-Octadecenamide, (Z) [C18H35O] (35.217), Hexadecane [c16h34] (36.899) and 2, 2, 2-trifluoroethyl 2-methyltetrahydro-5-oxo-3-furancarboxylate [C23H33NO6](37.681) .

Figure 2 illustrates distinct components with varied retention durations based on the GC-MS spectra. To determine the kind and structure of the chemicals, the mass spectrometer examines the substances eluted at various periods. A peak with a different m/z ratio appears as the significant component breaks up into more minor compounds. These mass spectra serve as the data library's fingerprint for the molecule, making them valuable. Numerous phytochemicals that significantly contribute to the pharmacological activity of broccoli were found in the aqueous extract of the plant, according to GC-MS analysis.

3.1. DPPH Assay

The antioxidant activity of aqueous broccoli extract was determined using the DPPH test, which used ascorbic acid as a control. The extract had equivalent free radical scavenging activity to the control in the current investigation. The antioxidant activity of aqueous broccoli extract in concentrations of (200, 100, 50, and 25) µg/ml was significantly highest (75.2 percent at 200 µg/ml), while extract in concentrations of (100, 50, and 25 µg/ml) showed antioxidant activity (68.01, 53.52, and 36.04 percent) respectively, and ascorbic acid's antioxidant properties at the same concentration was (80.00, 70.40, 56.60, and 40.09 µg/ml (Figure 3).

In the doses of 200, 100, and 25 µg/ml, where there were significant differences ($P \leq 0.05$) compared to control, aqueous broccoli extract production showed a similar free radical scavenging pattern action as ascorbic acid. These findings demonstrated that the extract's DPPH free radical scavenging activity was dosage-dependent. Additionally, suspension stability can be achieved by adsorbing the quinoid chemical produced when the phenol group in phenolics is oxidized on the surface of nanoparticles (37, 38). It is generally known that phenolic chemicals may have a direct role in antioxidant activity (39). Because of their redox properties, which enable them to function as reducing agents, hydrogen donors, and singlet oxygen quenchers, plant phenolics are hypothesized to have an antioxidant effect (40).

Aqueous Broccoli extract can help with vascular changes, particularly endothelial dysfunction caused by oxidative stress (35). This syndrome can cause a decrease in NO(nitric oxide) bioavailability, impacting vascular tone control and endothelial dysfunction—the initial stage of cardiovascular disease development. As a result, a plant extract with antioxidant characteristics developed in this work might be used to treat vascular dysfunction caused by hypertension, diabetes, or atherosclerosis.

3.2. Antibacterial Activity of Broccoli Extract

Aqueous broccoli extract demonstrates broad-spectrum solid antibacterial activity when tested against microorganisms resistant to many drugs. Different antibiotics' effects on all bacterial isolates under study were compared. According to the findings in figures 4–7, not all chosen antibiotics are effectively against the chosen bacterial strains. Aqueous broccoli extract demonstrated an increase in inhibitory zone width with their concentration, even surpassing the action of several antibiotics*Pseudomonas aeruginosa*, which showed strong sensitivity (Figure 4) even at 62.5 g/ml, with a maximal zone of inhibition of 20 mm at 500 g/ml concentration against the test organisms. The least susceptible isolate to the chosen drugs is *Staphylococcus epidermidis* (Figure 6). In addition to releasing reactive oxygen species like superoxide and aiding in the destruction of biomolecules, aqueous broccoli extract causes a fast deterioration in the integrity of bacterial cell membranes (35, 41).

In Iraq, broccoli (Brassica oleracea) is a recently introduced crop. It contains chemical properties that might be used in various herbal formulations, including anti-inflammatory, antibacterial, antioxidant, analgesic, antipyretic, heart tonic, and antiasthmatic.

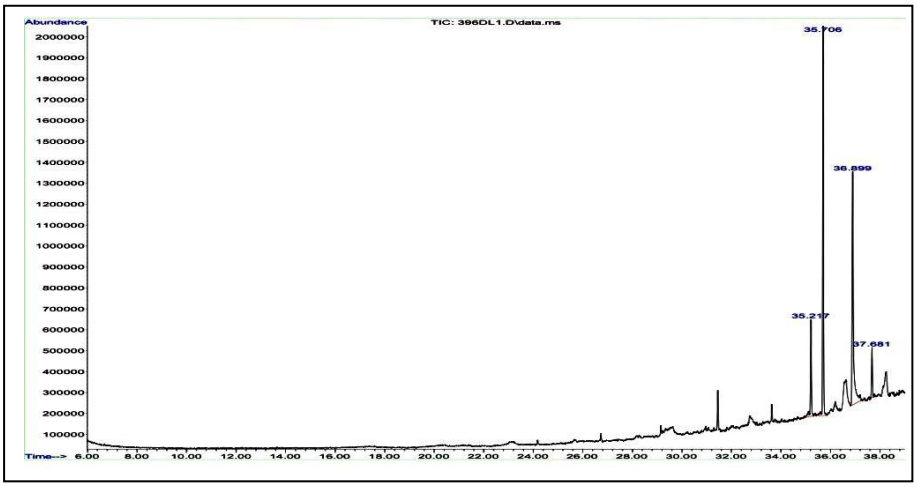

Figure 2. GC-MS analysis of aqueous Broccoli extract

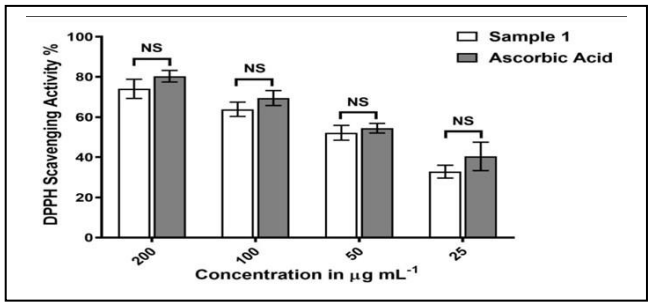

Figure 3. DPPH of water Aqueous Broccoli extract

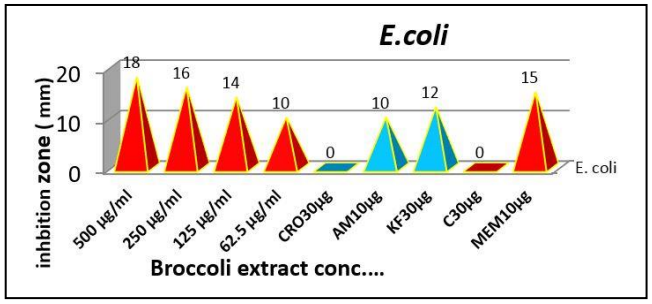

Figure 4. Antibacterial action of Broccoli extract Con. on *E. coli*

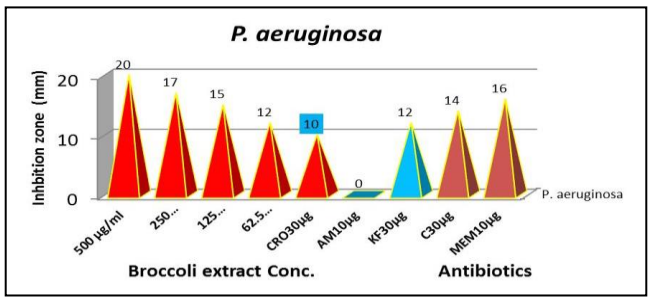

Figure 5. Antibacterial action of Broccoli extract Con. on *P. aeruginosa*

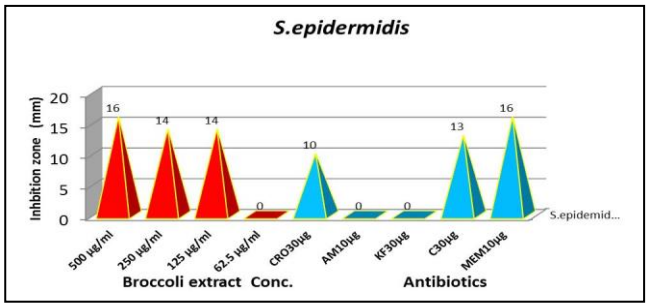

Figure 6. Antibacterial action on Broccoli extract Con. on *S. epidermidis*

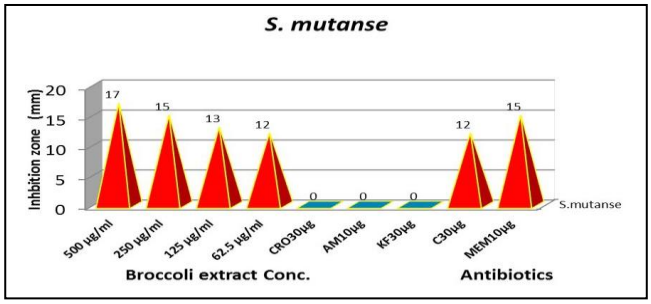

Figure 7. Antibacterial action of Broccoli extract Con. on *S. mutants*

Authors' Contribution

Study concept and design: Z. A. T.
Acquisition of data: M. M. K.
Analysis and interpretation of data: M. M. T.
Drafting of the manuscript: E. S. A.
Critical revision of the manuscript for important intellectual content: H. A. M.
Statistical analysis: C. S. A. I.
Administrative, technical, and material support: Z. A. T.

Conflict of Interest

The authors declare that they have no conflict of interest.

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