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Impact of extraction techniques on phytochemical profile and antimicrobial activity of garlic essential oil

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Abstract

Fresh garlic and its essential oil serve as natural antioxidants, taste enhancers, and antimicrobials in the food industry. So, the aim of the work was to study the impact of different extraction methods of garlic essential oil (GEO) on phytochemical profile and antimicrobial activity. In this study 3 techniques for oil extraction such as conventional hydrodistillation (HD), supercritical fluid (SCF), and ultrasound-assisted (UAE) were used. The phytochemical, antioxidant activity, total phenolic, antimicrobial activity, and minimum inhibitory concentration (MIC) of GEO were evaluated. Fresh raw garlic pulp contains 65 % moisture, 27.5 % carbohydrate, 3 % protein, 2 % fiber, 2 ash and 0.5 fatty acids. Higher yield of oil extracted by SCF with ratio < 42% compared to HD and UAE. Total phenolic contents were 24.6%, 12.9%, and 5.3% for the SCF, HD, and ultrasound-assisted methods, respectively. A potential DPPH radical scavenging activity is showed in GEOs with IC50 values of 37.91, 218.7 and 262.54 µl/mL for SCF, HD, and UAE processes respectively. The results demonstrated that GEO obtained by HD was higher than those of SCF and UAE extracts was active against all tested strains i.e. *Salmonella typhi, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus. Aspergillus niger, Aspergillus flavus, Candida albicans, and Saccharomyces cerevisiae.* Results concluded that the GEO obtained using SCF demonstrated significantly ($P \leq 0.05$) higher yield, higher quantities of bioactive compounds (TPC) and higher antioxidant. On the other hand, GEO made by SCF and UAE had lower antimicrobial and minimum inhibitory concentrations (MIC) than extracts made by HD.

Keywords: garlic, essential oil, antioxidant, Antimicrobial.

1. Introduction

Garlic (*Allium sativum*) is now grown all over the world, and recognized as a preventive and medicinal treatment in many cultures' [1]. Garlic was utilized in ancient times to treat a variety of illnesses, including diarrhea, constipation, asthma, fever, and infections, according to historical documents found in the Bible, Chinese, Egyptian, Greek, Indian, Israeli, and Babylonian literature [2]. Garlic is known for having a high concentration of γ-glutamyl-S-allyl-L-cysteines. Garlic's alliinase enzyme is triggered when it is chewed or crushed, turning alliin into allicin, an organosulfur compound (OSC). Diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS) are among the sulfur compounds that are produced as a result of allicin's breakdown [3].

Various preparation techniques have been used to extract and ultimately identify the constituents of garlic essential oil, which mostly include organosulfur compounds [4]. The predominant extraction technique at now is steam extraction or hydro-distillation. Nevertheless, the use of high temperatures in this method might result in the deterioration of chemically unstable particles and the partial breakdown of substances that are susceptible to water according to Francisco and Sivik, [5]. At present, supercritical extraction (SFE) has garnered growing interest, especially in the food, pharmaceutical, and perfume sectors [6]. Also, they reported the carbon dioxide (CO2) is the mostly employed fluid in solid-state electrolysis (SFE). It has various distinctive features and physicochemical qualities, including non-toxic, non-flammable, cost-effective, odorless, and having a low critical pressure (7.38 MPa) and temperature (31.1 C). Mason and Lorimer [7] investigated the utilising an ultrasonic cleaning bath for essential oil extraction has been found to minimise the risk of thermal deterioration and enhance extraction by substantially decreasing extraction durations. Ultrasonic mechanical action enhances the penetration of solvent into cellular materials by cavitation effects, thereby facilitating the discharge of cell contents into the bulk media.

Garlic oil (GO) has been demonstrated to contain more than thirty organic sulfur-containing compounds, including diallyl trisulfide, diallyl sulphide, also, diallyl disulfide, and has been associated with a number of antioxidant advantages [8,9]. According to published studies, GO protects against experimental acetaminophen hepatotoxicity, hepatocarcinogenesis and hyperglycemia in both humans and animals [10, 11]. Additionally, it has been discovered that in pancreatic cancer cell lines, GO induces apoptosis and suppresses growth. Garlic essential oil is primarily made up of oil-soluble substances such as DAS, DADS, vinyldithiins, and other OSCs; it is devoid of allicin and water-soluble substances. Compressed entire raw garlic cloves are combined with vegetable oil to form an oil macerate of garlic, which is used as a seasoning in food [12]. Allicin is absent from this oil macerate, but it does contain alliin and sulfur compounds that are soluble in oil, such as sulfides, ajoene, and dithiins [13]. This molecule is created when the non-proteinogenic amino acid alliin combines with the enzyme alliinase when garlic cloves are crushed. But GEO's antibacterial properties come from the several diallyl sulfide derivatives that make up its

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makeup and are extracted using steam or water distillation [14]. The distillation heat produces these compounds as a result of the alteration of allicin [15,16]. The effectiveness of GEO as an antibiotic against certain food pathogens is dependent on its chemical composition, specifically the diallyl sulfide derivatives [17].

Garlic formulations, particularly aged garlic extract (AGE), have shown considerable promise as antioxidants. Raw garlic homogenate has been shown to possess antioxidant nevertheless; greater quantities have been demonstrated to be harmful to the cardiovascular system, liver, and kidneys [18].

This important characteristic depends on the garlic's provenance and cultivar. Variations in the ratio of these diallyl sulfide derivatives and their consequent effect on the antibacterial activity of various cultivars from various geographical origins have been reported. [19-22]. The Allium vegetables, namely garlic (Allium sativum L.), have a wide range of antibacterial, antifungal, antiparasitic, and antiviral properties against various types of bacteria, including multidrug-resistant enterotoxicogenic strains of Escherichia coli, Candida albicans, and major human intestinal protozoan parasites such Entamoeba histolytica and Giardia lamblia [23]. Gram-negative If treatment is not administered, dangerous bacteria known as Pseudomonas aeruginosa can cause fatal illnesses such wound infections, pneumonia, stomach, and respiratory infections [24, 25]. Gram-positive One dangerous bacteria is Staphylococcus aureus. Finding novel ways to fight these bacteria is essential because it has become resistant to antibiotics over time [26 - 28]. Plant extracts, oils, powders, and their derivatives have been identified as natural preservatives with antibacterial capabilities that are effective and comparable to synthetic preservatives [29]. Sulfur content, antimicrobial qualities, and other phenolic compounds have made allium species well studied plants [30]. This research aimed to describe the chemical composition, antioxidant activity, and antibacterial activity of garlic essential oil cultivated in Egypt, extracted using supercritical CO2 extraction, HD, and ultrasound-assisted techniques.

2. Materials and Methods

2-1 Raw materials and chemicals

Balady (*Allium sativum*) bulbs were purchased from (Horticulture institute, Agricultural Research Centre, Giza, Egypt) at the season (April 2023). The Folin–Ciocalteu (FC) reagent was acquired from Merck (Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (APTS) were acquired from Sigma Aldrich (St. Louis, MO, USA). All remaining chemicals and reagents were of analytical grade.

2.2. Microorganisms

The antimicrobial activity of garlic essential oil was assayed against four species of pathogenic bacteria, two Gram-positive bacteria *Staphylococcus aureus* (ATCC 6538) and *Bacillus cereus* (EMCC 1080) and three Gram negative bacteria *Pseudomonas aeruginosa* (NRRL B-272)*, Escherichia coli 0157 H7* (Rockvill, Maryland, USA 51659), and *Salmonella typhi* (ATCC 25566). Four fungal species were used in assay, *Aspergillus flavus* (NRRL 3357), *Aspergillus niger* (ITEM 10027), *Saccharomyces cerevisiae* (FTC 500)*, and Candida albicans* (ATTC 10231). All bacterial strains were maintained on tryptic soy agar, (TSA) powder (Merk, Germany) slants at 37°C, allowed to grow for 24 h then refrigerated (4°C) till use. Mold and yeast strains were maintained on potato dextrose agar, PDA (Sisco Research Laboratory, India) slants at 28°C allowed to grow for 5 days then refrigerated (4°C) till use.

2.3. Extraction methods of GEO:

2.3.1. Hydrodistillation HD:

Garlic puree was crushed using a blender (Lab Stomacher Blender 400-BA 7021, Seward Medical, UK) for three minutes. The puree was mixed with water (crushed garlic: water, 1:6; *w/v*) was transferred into 5-L round-bottom flasks and further distilled water was added to make it appropriate for HD. Clevenger-style equipment was used to hydrodistill the essential oil for three hours. Since garlic essential oil (GEO) is denser than water, it was extracted from the side arm of the Clevenger apparatus, dried over anhydrous sodium sulphate, and stored in dark brown vials at 4^{o}C until needed **[31]**.

2.3.2. Supercritical fluid (SCF) CO2:

Garlic essential oil (GEO) was extracted using a laboratory-scale unit at the National Research Centre (Speed TM SFE-2/2, Applied Separations, developed in collaboration with the USDA, USA). A stainless steel extraction cell with an approximate volume of 20 mL was thermostatically controlled in the oven constructed in our laboratory, and the backpressure of the entire system was managed using an LF-540 Pressure Tech valve (USDA1 - USA). For each extraction, one hundred grams of finely powdered sample was placed in the extraction cell, with a little quantity of glass wool positioned at both the top and bottom of the cell to prevent system clogging. Ethanol 85% was provided by a Jasco PU2080 HPLC pump (Jasco Inc., Easton, PA) and combined at high pressure with supercritical CO2 as a solvent modifier. The compressed mixture of CO2 and solvent modifier was sent into the heater before entering the extraction cell. Dynamic extractions were conducted with high-purity CO2 at a constant flow rate of 10 mL min^{-1}, combined with the solvent modifier at a flow rate of 0.5 mL min−1. Under optimal conditions, the extraction cell was positioned in the oven at a temperature of 40±1°C and a pressure of 100 psi, while 85% ethanol was continuously mixed with GEO before to entering the cell. The extraction duration was 120 minutes for all tests [32].

2.3.3. Ultrasound-assisted (UAE):

Ultrasound-assisted hydrodistillation extraction was performed utilizing an ultrasonic and Clevenger-type apparatus, with a schematic representation of the apparatus illustrated in Figure (1). Was executed according to the previously defined methodology with minor modifications [33]. Freshly crushed garlic (0.3 kg) was submerged in filtered water within a 5 L round-bottom flask. The mixtures underwent ultrasonic treatment with an ultrasonic cell pulverizer at 40 kHz for 40 minutes, utilizing a power of 600 W, at a temperature of 30±1°C, with a pulsing cycle of 10 seconds on-time and off-time. Following the ultrasonic treatment, the resulting mixture was immediately submitted to hydrodistillation for the extraction of garlic oil utilizing a Clevenger apparatus.

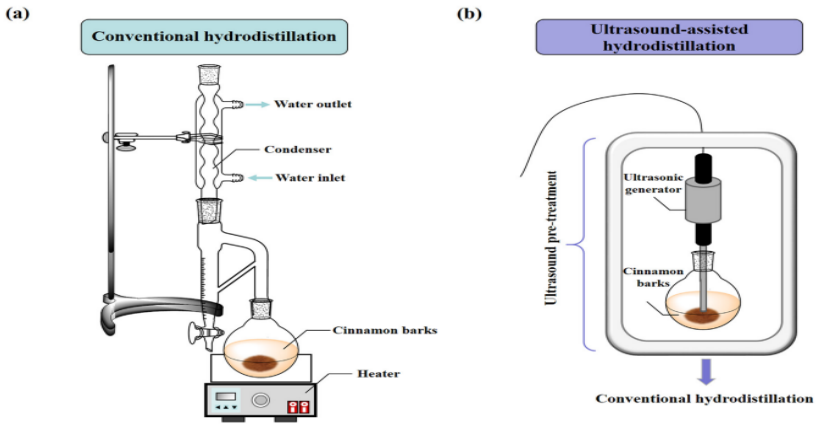

Fig. (1). Schematic depiction of the experimental apparatus for the extraction of essential oil from crushed garlic: (a) Traditional hydrodistillation; (b) Ultrasound-enhanced hydrodistillation.

2.4. Determination of yield

The extract from the trap was gathered, and the yield was calculated and expressed as a percentage (w/w) in accordance with Eq. 1 (Furtado et al. 2018). The oil was preserved in amber-sealed vials at –4°C until required for subsequent examination.

Yield of Essential oil % = [Essential.oil (g) / Sample.weight (g)] X 100 (1)

2.5. Chemical composition analytical Methods:

The proximate composition of each sample was determined based on the standard method of the Association of Official Analytical Chemists According to AOAC **[34]**. The analysis carried out on the garlic bulb samples were moisture content, ash, fat, crude fibre and crude protein (Kjeldahl method). Carbohydrate was determined by difference.

2.6. Antioxidant activity

2.6.1. DPPH scavenging activity:

The free radical scavenging capacity of extracts was assessed using the stable DPPH method as described by Iman et al. [35]. The final concentration of DPPH was 200 µM, and the total reaction volume was 3.0 mL. The absorbance was recorded at 517 nm using pure methanol as a blank after 60 minutes of incubation in the dark. The percent inhibition of the DPPH free radical was determined using the following equation:

Inhibition (%) = $100 \times [(A_{control}-A_{sample})/A_{control}]$

Where: $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound). A_{sample} is the absorbance with the test compound.

The IC_{50} value is the substrate concentration that induces a 50% reduction in DPPH activity, derived from linear regression analyses of inhibition percentages and concentrations.

2.6.2. ABTS scavenging activity:

The stock solutions of ABTS reagent were prepared according to the method of Iman et al. [35] by mixing equal volumes of a 7 mM aqueous ABTS* solution with 2.45 mM potassium persulfate, permitting the reaction to occur for 16 hours at a comfortable temperature (25°C) in the dark. The operational solution was prepared by diluting 1 mL of ABTS* solution with 60 mL of a 50:50 (v/v) ethanol-water mixture to attain an absorbance of 1.0 ± 0.02 units at 734 nm, as determined by the spectrophotometer. 50 microliters of extracts were allowed to react with 4.95 milliliters of the ABTS* solution for one hour in

the absence of light. The wavelength of absorbance was recorded at 734 nm using the spectrophotometer. The percentage inhibition of the ABTS* free radical was calculated using the subsequent equation:
Inhibition (%) = 100 × [($A_{control}$-A_{sample})/$A_{control}$]
Where: $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound). A_{sample} is the absorbance with the test compound.

2.7. Determination of total phenolic content:

The quantification of the total phenolic compounds in the extracts was conducted using the Folin–Ciocalteu (FC) reagent, using a slightly modified method of the technique developed by Najafi et al.[36]. The reaction mixture consisted of 50 microliters of a 0.5% dilution of garlic oil, 2.5 mL of newly made 0.2 M FC reagent, and 2 mL of sodium carbonate solution. The combination was then placed in a dark environment at room temperature for 30 minutes to allow the reaction to fully occur. The resultant solution's absorbance was quantified at a wavelength of 760 nm using a UV–Vis spectrophotometer (model 8453 Hewlett Packard, Agilent Technologies, USA). The total phenolic component content was quantified in milligrams of Gallic acid equivalents (GAE) per gram of garlic essential oil, utilizing a gallic acid standard curve. The measurements were conducted in three replicates.

2.8. Antimicrobial activity

2.8.1. Disc diffusion method

A complete loop of each bacterial species from the 24-hour incubated nutrient agar slant was inoculated into a tube containing 5 ml of tryptic soy broth. The broth culture was incubated at 35 degrees Celsius for 2 to 6 hours until it attained a turbidity corresponding to 0.5 McFarland BaSO4 standards. The bioactivity of crude garlic oil extracts was assessed against various bacterial species employing the disc diffusion method of the Kirby-Bauer technique [37, 38]. Bacterial cultures were uniformly distributed on nutritional agar plates utilizing cotton swabs saturated with tryptic soy broth. Each extract and fraction was prepared at a concentration of 10 mg/ml by dissolving 10 mg in 1 ml of dimethyl sulfoxide (DMSO). Six millimeter sterilized discs composed of Whatman No. 1 filter paper were infused with either extracts or fractions and subsequently dried meticulously under sterile conditions. The discs were placed on the seeded plates with sterile forceps. DMSO served as the negative control, whereas Ampicillin (10 mL-1) functioned as the positive control. Following a 24-hour incubation of the inoculated plates at 37°C, the inhibition zones were measured by including the diameter of the paper disc. The fungal strains were cultivated on potato dextrose agar (PDA) and maintained at 25°C for five days. The suspension of each fungus was prepared at a concentration of 2 x 10^8 colony-forming units per millilitre in a 0.01% Tween 80 solution, utilizing the 0.5 McFarland standards for comparison. Yeast extract sucrose medium (YES) Petri plates were inoculated with 50 cc of each fungal culture and uniformly spread using a sterile L-glass rod. Sterilized 6 mm discs were impregnated with either extracts or fractions (10 mg/ml) and desiccated under sterile conditions before to placement on inoculated plates using sterile forceps. DMSO served as the negative control, while Ampicillin (10 µg) functioned as the positive control. Following a 48-hour incubation of the inoculation plates at 25°C, the antifungal activity was assessed by measuring the zone of inhibition (mm) [37]. The average outcome was calculated using a minimum of three replicates for each assay.

2.8.2. Minimum inhibitory concentration (MIC) method:

The MIC was determined using the tube dilution technique [39]. The bacterial species culture, grown for 24 hours, was diluted in 10 mL of tryptic soy broth (TSB) based on the 0.5 McFarland standard to reach an inoculum of 10^8 CFU/mL. Nine culture tubes were constructed with varying amounts of garlic oil extract (100, 50, 25, 12.5, 6.25, 3.125, 1.65, 0.82 mg ml^{-1} in DMSO). Each tube contained 100 ml of bacterial cell suspension and was maintained at 37°C for 24 hours. The augmentation of the inoculum in the liquid medium is indicated by the turbidity of the solution, and the least quantity of the extract that inhibited the growth of the test organism was deemed the minimum inhibitory concentration (MIC). Fungal minimum inhibitory concentration (MIC) was determined using the method described by Perrucci *et al* [40]. Garlic oil extract was dissolved in various quantities and blended with 0.1% Tween 80 before being combined with melting PDA at 45°C. The mixture was then put onto a 6 cm Petri plate. Each plate was infected at the centre with 3 ml of fungal solution containing 10^8 colony-forming units per ml and equivalent to a 0.5 McFarland standard. The plates were kept at 25 ^{0}C for 24 to 48 h. Mycelial growth was observed and the Minimum Inhibitory Concentration (MIC) was obtained at the conclusion of the incubation period.

2.9. Statistical analysis

All experiments were performed in triplicate to compute the mean and standard deviation. A one-way analysis of variance (ANOVA) was employed to determine significant differences among treatments, then followed by Duncan's multiple range tests [41].

3. Results and discussions

3.1. Chemical composition of garlic

Garlic, used as a spice and flavouring agent, has essential nutritional components. This plant has an abundance of carbohydrates, proteins, fats, minerals, water, and vitamins. The plant has significant therapeutic potential and is used to treat many human

ailments [42]. **Table (1)** displays the typical constituents of a fresh garlic bulb. The content of water (64.8), crude protein (3.05%), ash (2.11%), crude fat (0.56%), crude fiber (1.95%), and carbohydrates (NFE) (27.53%), in garlic cloves was determined by the results. The results obtained are in line with the findings of **Divya *et al.* [43]**, who stated that fresh garlic has a composition of roughly 63% water, 28% carbs, 3.2% protein, and 1.5% fibres.

Table (1). Chemical composition of garlic bulb

Component	Percentage %
Moisture	64.8±1.26
Carbohydrate	27.53±0.76
Crude protein	3.05±0.09
Ash	2.11±0.03
Crude fat	0.56±0.02
Crude Fibres	1.95±0.17

Furthermore, Kimura *et al*. [44] found that garlic contains roughly 65% water, 28% carbs, 3.2% protein, and 1.5% fibres. Garlic's chemical makeup was examined by Eissa *et al*. [45] and they discovered that it is 62.2% water, 3.01% protein, 2.28% ash, 1.16% crude fibres, 0.56% crude fat and 27.81% carbohydrates.

3.2. Extraction yield

Figure (2) shows the yields of garlic essential oil (GEO) acquired using various extraction processes. The best extraction of essential oil was obtained SCF at the higher pressure (300 bar and the temperatures 40 C) it recorded 1.2% compared to 0.24% and 0.7% for HD and ultrasound-assisted extraction techniques, respectively. The extracted essential oils from each experiment shared comparable organoleptic properties. They appeared like moving liquids, were yellow, and smelled strongly of acid. The results obtained are comparable to those of Benmeziane *et al*. [46], who reported essential oil yields of 0.3% for local garlic and 0.5% for Chinese garlic, respectively, using the HD method. Conde-Hernandez *et al* [47] studied the yields of rosemary essential oils extracted using the HD and steam distillation (SD) techniques. The yield was 0.35% (w/w) after extracting 25 g of powdered rosemary using the HD technique. The superior yield (2.35%) was achieved with the SD approach utilizing 50 g of the whole sample. It is important to mention that the season, variety, and other factors may influence the concentration of essential oils in plants. Also, they reported the sample with the lowest yield was achieved at a pressure of 10.34 MPa and 50 °C, while the optimal performance of the system for achieving increased rosemary oil content was seen in the sample treated at 17.24 MPa and 40 °C.

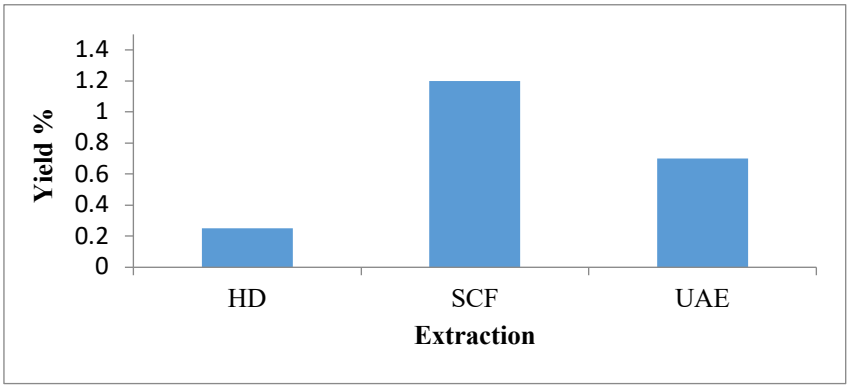

Fig. (2). GEOs yield obtained by hydrodistillation (HD), supercritical fluid (SCF), and ultrasound-assist extraction (UAE).

Similarly, Carvalho *et al*. [48] reported the yields of up to 4% (w/w) at 24.78 MPa and 40 °C after 250 minutes of extraction. On the contrary, Bagheri *et al* [49] reported that hydro-distillation using water as a solvent for *P. nigrum* L. essential oil yielded a greater output (2.88%), but SC-CO2 extraction (30 MPa, 50 °C, and 80 min) generated a lower output (2.16%). The hydro-distilled essential oil of *P. nigrum* L. is characterized by a complete recovery of essential oil from the seeds. Similar results were reported by Ferreira et al.,[50]. Were they stated the yield of *P. nigrum* L. essential oil extracted using SC-CO2 under optimal circumstances was substantially lower (2.05%) compared to the yield obtained using steam distillation (3.46%). This may be elucidated by the distinction that water, as a polar solvent, has differing extraction capabilities compared to CO2, which is a non-polar solvent. This finding may be attributable to a modest escaping proportion of the recovered volatile components in conjunction with CO2 from the container. An alternative reason for this may be because during hydro-distillation, the duration of exposure to water as a solvent is comparatively prolonged, resulting in the extraction of essential oil from the glands.

3.3. Total phenol content of garlic oil (GO)

Based on yield profile, chemical composition, antioxidant activity, and total phenolic content, a comparison was made between the essential oils extracted from garlic bulbs using various techniques. The bioactive components (total phenolic content) of garlic oil that has undergone multiple processing methods, such as HD, SCF, and UAE, are summarized in **Table (2).** Garlic oil TPC values in the samples subjected to SCF, HD, and UAE are 24.6, 12.92, and 5.31 mg/g, correspondingly.

Table (2). Total phenol content for the extracts of garlic

Treatment	Total phenols (mg GAE /g)
HD	12.92±0.25^{b}
SCF	24.6±0.56^{a}
UAE	5.31±0.34^{c}

hydrodistillation (HD), supercritical fluid (SCF), and ultrasound-assist extraction (UAE).*Means (±SD) followed by different superscripts (within columns) is significantly different (p≤0.05).

The extraction samples obtained using SCF demonstrated significantly ($P < 0.05$) higher quantities of bioactive compounds (TPC) in comparison to those obtained through HD and UAE extraction processes. This result is in harmony with that observed by Ekeleme-Egedigwe *et al* [51] who reported that garlic oil extracts had phenol content 90.1 ± 8.6 mg GAE/100. Similarly, author indicated garlic oil extract containing on 5.61 mg GAE/g **[52].** Phenolic compounds serve as reliable indications of antioxidant activity due to their prominent redox potential, which facilitates their ability to function as hydrogen donors or radical scavengers [53,54]. These entities have also been documented to have a significant impact on the antibacterial efficacy of the essential oil **[55].** On the other hand, **Bozin *et al*, [56]** proved that garlic extract had phenol content ranged from 0.05 to 0.98 mg GAE/g. They reported the reduction in overall phenolic levels is likely due to the higher concentration of sulphur compounds and terpenoid species found in the essential oil of fully grown garlic bulbs.

3.4. Antioxidant activity

3.4.1. DPPH Radical Scavenging Activity

In the DPPH test, antioxidants were able to change the purple-colored, stable radical DPPH into the yellow diphenylpricryhydrazine. According to Molyneux, [57], the antioxidant can supply hydrogen atoms to the mechanism that transforms the DPPH radical into its reduced form, DPPH. These essential oils' main ingredients, sulfur components, are responsible for their remarkable antioxidant qualities.3. The moderate breakdown of components such diettrisulfides is responsible for the little drop in scanning activity observed in both ultrasonography-based techniques. Garlic's antioxidant properties are largely attributed to diallyl polysulfides.

As shown in **Table 3**, using SCF CO_2 to extract an essential oil gave a better antioxidant effect (IC_{50} 37.91 µl/ml) than hydro-distillation (218.70 µl/ml) or ultrasound-assisted extraction (262.54 µl/ml). This may result from the heat deterioration, hydrolysis, and solubilization of some components in water, which might alter their antioxidant activity. Moreover, the water used in hydro-distillation may render some antioxidants unstable or destroy them via enzyme activity in the moist plant material. Lawrence *et al*. [57], for instance, observed that the EO of garlic growing in the northern Indian plains had an IC_{50} value of 0.5 mg/mL. Another oil produced by HD has an IC_{50} value of 37.91 mg/mL that has been reported. **Mnayer and colleagues [52]**.

Table (3). Antioxidant activity of garlic oil measured by DPPH method

Essential oil	DPPH (inhibition %)					
	40 µl/ml	**60 µl/ml**	**80 µl/ml**	**100 µl/ml**	**120 µl/ml**	**IC_{50}**
HD	12.159	16.143	18.865	22.442	25.48	218.70
SCF	13.359	17.975	20.578	26.175	29.783	37.91
UAS	12.46	16.342	20.355	23.421	25.565	262.54
Trolox	-	-	-	-	-	14.88

hydrodistillation (HD), supercritical fluid (SCF), and ultrasound-assist extraction (UAE).

In contrast to the results of Ndoye et al. **[59],** who found an IC_{50} value of 0.19 µg/mL, the EO demonstrated an IC_{50} of 124.60 µg/mL [60]. This, at an IC_{50} value of 0.19 µg/mL, differs from the results of Ndoye et al. [45]. When compared to Trolox, which has an IC_{50} of 14.88 µg/mL, the GEO in our investigation demonstrated an IC_{50} of 37.91 µg/mL for oil acquired by SCF, 218.70 µg/mL for oil obtained by HD, and 262.54 µg/mL for oil obtained by ultrasound-assisted. Obtained results are similar to those reported by Abbas *et al*, [61**]** illustrated the scavenging activity of *Eucalyptus camaldulensis* essential oil (EO) extracted using SCF extraction (SFE) at a temperature of 45°C, with a liquid carbon dioxide flow rate of 10 mL/min and a pressure of 100 bar, is marginally superior (IC_{50}= 19.89 µg/mL and inhalation 65.3%) compared to EO produced via HD (HD) (IC_{50}=16.21 µg/mL and inhalation 53.2%). Generally, SCFs with elevated densities have enhanced solvating capabilities, hence facilitating more selective extraction of bioactive constituents compared to HD methods [62]. Hu *et al*. [63] elucidated that pressure enhanced the antioxidant efficacy of extracts derived from natural sources. Solati *et al*. [64] found that the antioxidant

activity of *Nigella sativa* L. oil extracted using SC-CO2 increases with rising pressure. The confirmation of this notable antioxidant activity is partially attributed to the sulfur components, which are the primary constituents of these essential oils [65]. Nevertheless, both techniques using HD and ultrasound-assist technology exhibit a little reduction in scanning activity. This might be attributed to the incremental deterioration of certain components, such as Diallyl polysulfides according to Benkeblia and Lanzotti, [66].

3.4.2. APTS Radical Scavenging Activity

However, when measured using ABTS, the antioxidant activity of garlic extract obtained by HD, SCF extraction, and ultrasound-assisted was shown to be higher than that of trolox (IC_{50} values were 80.91, 75.04, and 236.55 µg/mL, respectively, versus 14.56 µg/mL for Trolox) as shown in **Table (4)**. Given the high value in comparison to the other findings, diallyl trisulfide, diallyl disulfide, and methylallyl di- and trisulfides may be the cause. Dialyl sulfide was the primary ingredient in the garlic extract obtained by HD, but when tested as inhibitors of the controlled autoxidation of isopropylbenzene or styrene, dialyl disulfide and allyl methyl sulfide showed no antioxidant activity, suggesting that they oxidize in tandem with the oxidizable substrate [67].

Table (4). Antioxidant activity of garlic oil measured by ABTS method

Essential oil	ABTS (inhibition %)					
	40 µl/ml	60 µl/ml	80 µl/ml	100 µl/ml	120 µl/ml	IC_{50}
HD	32.726	41.549	50.043	59.394	64.42	80.91
SCF	30.443	42.855	54.433	64.53	70.291	75.04
UAE	9.262	12.895	18.129	22.179	25.34	236.55
Trolox	-	-	-	-	-	14.56

hydrodistillation (HD), supercritical fluid (SCF), and ultrasound-assist extraction (UAE).

Dialyl trisulfide was the main component that we found, while other sulfur volatiles have been found to be prevalent in other garlic essential oils. However, according to Boubechiche *et al.* [68], some methods of extracting essential oils may have an impact on their antioxidant activity. We could also attribute the variations we saw in our study to variables like height, climate, and chemo type variations**.** Pingret *et al*, [69] noted that although ultrasonography has been shown to be successful in modifying traditional equipment as highlighted in many research. Also, previous studies have shown alterations in organoleptic properties [70].

3.5. Antimicrobial activity of garlic oil extracts

The antibacterial efficacy of garlic oil extracted using various techniques at different concentration against five types of harmful bacteria is displayed in Table (5). The extracts obtained from the HD, supercritical, and ultrasound-assisted processes exhibited antibacterial efficacy against all harmful microorganisms that were tested. With 14.67, 14.56, 13.5, 13.2, and 9.67 mm inhibition zones against *S. aureuse, B. cerus, E. coli, PS,* and *S. typhi*, respectively, the HD extract exhibited the strongest antibacterial activity. The antibacterial activity of the supercritical extract was also strongest against *Salmonella typhi, B. cerus, E. coli, PS,* and *S. aureuse,* with respective inhibition zones of 12, 11.67, 11.56, 9 and 8 mm. The maximum antibacterial activity against *E. Coli, B. cerus, S. aureuse, PS,* and *S. typhi* was also shown by the ultrasonication extract, which recorded 10, 9.78, 9.33, 9.33, and 8 mm inhibitory zones, respectively. It was generally observed that at lower extract concentrations, the antibacterial properties of garlic oil extracts against the pathogens were diminished. The antibacterial activity of hydro-distilled garlic oil extracts was higher than those of supercritical and ultrasound-assisted extracts.

Table (5). Antibacterial activity of garlic oil extracts at different concentration (40%).

			Inhibition zone mm (Mean ± *S.E)					
Bacteria	**-cont**	**+cont**	**HD**		**SCF**		**UAE**	
			20%	**40%**	**20%**	**40%**	**20%**	**40%**
S. aueruse	—	29.67±0.58	14.67±0.58^{a}	14.00±1.0^{c}	11.67±0.58^{b}	13.67±0.58b	9.33±0.58^{b}	12.67±0.58^{a}
Salmonella typhi	—	35.33±1.53	9.67±0.58^{d}	12.00±0.0^{d}	8.00±0.00^{d}	7.50±0.5d	8.00±0.00^{c}	10.67±0.58^{c}
E. coli	—	34.33±1.15	13.5±0.5^{b}	14.67±0.58^{b}	9.00±0.00^{c}	13.00±1.0c	10.00±0.5^{a}	11.67±0.58^{b}
PS	—	25.67±2.08	13.0±2.65^{c}	15.50±2.18^{a}	12.00±1.0^{a}	14.33±0.58a	9.33±1.15^{b}	11.33±2.02^{b}
B. ceruse	—	26.22±3.03	14.56±0.51^{a}	15.83±1.61^{a}	11.56±0.51^{b}	13.89±0.19b	9.78±0.39^{a}	12.56±0.1^{a}

***Means (±SD) followed by different superscripts (within columns) is significantly different (p≤0.05). hydrodistillation (HD), supercritical fluid (SCF), and ultrasound-assisted extraction (UAE).**

The antibacterial activity of garlic oil extracts (Table 5) is consistent with the findings of **Xainhiaxang *et al.* [71]**, who investigated the antibacterial qualities of garlic oil to prevent the growth of some foodborne pathogens, namely *Salmonella enterica*, *Staphylococcus aureus*, and *Escherichia coli*. SCF extraction or HD were used to create the plant extracts. The agar disc diffusion technique was then used to test the extracts' antibacterial capabilities against three different pathogens on microbiological media. The efficacy of the garlic oil extract produced by SCF extraction and HD to inhibit every pathogen

tested However, as compared to the garlic oil extract obtained using SCF extraction, the extract obtained through HD shown a higher capacity to eradicate infections. Oonmetta-aree *et al*. [72] measured the breadth of the inhibitory zone in a prior study to determine how susceptible bacteria were to garlic oil extracts.

Researchers noted that a microbe would show resistance at 9 mm in the inhibition zone diameter, intermediate susceptibility between 10 and 13 mm in the inhibition zone diameter, and susceptibility at 14 mm in the inhibition zone diameter. These categories allowed for the determination that S. aueruse was susceptible to the hydrodistillation extract of garlic.
Variations in the microbial strains and oil extraction conditions may have an impact on discrepancies in the results. Allicin, an oxygenated sulfur compound that can inhibit several bacterial enzymes containing thiol groups, may be the cause of the garlic hydrodistillation extract's effectiveness against pathogenic bacteria [73]. The capacity of antibacterial chemicals to penetrate bacterial cell walls and target the cytoplasmic membrane.

3.6. Minimum inhibitory concentration (MIC) values of garlic oil

Table (7) displays the minimum inhibitory concentration (MIC) values of garlic oil extracts against four bacterial strains *(B. cereus, E. coli, P. aeruginosa, and Salmonella*)and four fungal and yeast strains (*A. flavus, A. niger, S. cerevisiae, and Candida*) that were obtained using HD, SCF, and ultrasound-assisted methods. Table (8) analysis shows that the extraction methods used had an impact on the antibacterial activity of garlic oil extracts. When the HD method was used, the antibacterial activity of the garlic oil extracts was shown to be superior overall. On the other hand, extracts of garlic oil made by ultrasound-assisted and SCF extraction had lower minimum inhibitory concentrations (MIC) than extracts made by HD.
The minimal inhibitory concentration (MIC) of garlic oil determined by UAE, HD, and SCF extraction methods is shown in **Table (6).**

Garlic oil hydrodestillation extract showed the strongest effects against Candida and Pseudomonas aeruginosa, with minimum inhibitory concentrations (MIC) of 0.05 mg ml^{-1} and 12.5 mg ml^{-1}, respectively. Salmonella typhi and S. cerevisiae showed the least amount of activity, with minimum inhibitory concentrations (MIC) of 50 and 0.2 mg ml^{-1}, respectively. Zhao and Zhang [74] noted that differences in the chemical composition of the extracts made using various processing methods could have an impact on the study's findings. According to Bagheri et al, [54], the garlic essential oil produced by HD and supercritical carbon dioxide extraction showed similar chemical groups with different relative abundances.

Table (6). Minimum Inhibitory Concentrations (%) of garlic oil

Microorganism	**MIC values mg. ml $^{-1}$**		
Bacteria	**HD**	**SCF**	**USA**
B. cereus	6.25	50	50
E. coli	50	50	50
P. aeruginosa	12.5	25	50
Salmonilla	50	100	100
Fungi	**HD**	**SCF**	**USA**
A. flavus	0.1	0.2	0.1
A. niger	0.1	0.1	0.2
S. cerevisiae	0.2	0.4	0.1
Candida	0.05	0.05	0.05

hydrodistillation (HD), supercritical fluid (SCF), and ultrasound-assist extraction (UAE).

leading to cytoplasmic leakage and/or coagulation, may account for the susceptibility of Gram positive bacteria, like *Staph aureus,* to herb and spice extracts [75].

4. Conclusion

Results concluded that the extraction samples obtained using SCF demonstrated significantly ($P < 0.05$) higher quantities of bioactive compounds (TPC) in comparison to those obtained through HD and ultrasound-assisted extraction processes. When compared to Trolox, which has an IC_{50} of 14.88 µg/mL, the EO in our investigation demonstrated an IC_{50} of an oil acquired by SCF< an oil obtained by HD, and < an oil obtained by ultrasound-assist. The efficacy of the garlic oil extract produced by SCF extraction and HD to inhibit every pathogen tested However, as compared to the garlic oil extract obtained using SCF extraction, the extract obtained through HD shown a higher capacity to eradicate infections. On the other hand, extracts of garlic oil made by ultrasound-assisted and SCF extraction had lower minimum inhibitory concentrations (MIC) than extracts made by HD. Garlic oil hydrodestillation extract showed the strongest effects against *Candida* and *Pseudomonas aeruginosa*, with minimum inhibitory concentrations (MIC). *Salmonella typhi* and *S. cerevisiae* showed the least amount of activity, with minimum inhibitory concentrations (MIC).

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