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Research Article

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Comparative Pharmacological Potential of Cumin *(Cuminum cyminum)* Seed Essential Oils: Conventional vs. Superheated Steam Distillation

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Abstract

Cuminum cyminum is a famous spice used worldwide for imparting taste to diverse food provisions, culinary and medicinal purposes, and also for appetizing purposes due to its special aromatic effect. This research work aims to estimate the yield of essential oil (EO), chemical composition, antioxidant and antimicrobial activity of *Cuminum cyminum* EOs extracted by conventional methods Hydrodistillation (HD) and Steam distillation (SD) towards advanced method Superheated steam distillation (SHSD). SHSD noticed the maximum EO yield compared to the HD and SD methods. Gas chromatography-mass spectrometry (GC-MS) analysis illustrated that Cumin aldehyde (51.87-23.54%) and 3-caren-10-ol (15.34-12.15%) were the most abundant compounds of *Cuminum cyminum* EOs. Hydrogen peroxide scavenging, ferric-reducing power assay, and DPPH free radical scavenging assay were used to evaluate the antioxidant potential. Results revealed that SHSD EO displayed the highest antioxidant potential compared to HD and SD EOs. The antimicrobial activity was determined using the resazurin microtiter plates test, microdilution broth experiment, and disc diffusion tests contradicting food pathogenic bacterial and fungal strains. SHSD EO observed the highest antimicrobial potential. The results indicated that SHSD is an efficient and excellent method for extracting EOs from *Cuminum cyminum* seeds, which also engage in biological activities.

Keywords: Superheated steam distillation; *Cuminum cyminum*; Essential oil; Antimicrobial, Antioxidant potential.

1. Introduction

Cuminum cyminum (*C. cyminum*) is a vital and famous spice recognized as ‘cumin’ belonging to the Apiaceous family [1]. *C. cyminum* is a versatile plant used worldwide for cooking, cosmetics, perfumery, and medicinal purposes [2]. The average production of *Cumin* is about 12 thousand tons/year, and it accounts for

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about 50% of seed exchange internationally [3]. *Cuminum cyminum* is an aromatic plant and medicinal spice used to add flavour to various food necessities, such as cheese, soup, bean dishes, pickles, and alcoholic beverages. It is frequently used for culinary purposes due to its unique flavour. [4].

Cuminum cyminum is a tiny, brownish boat-shaped seed plant that rises to 15-50 cm [5]. *C. Cyminum* fruit is 13 cm long, elongated, 4 to 6 mm in length, 2 mm broad, oval, thicker in the middle, compressed horizontally, and containing a single seed [6], [7]. The main bioactive component of *Cuminum cyminum* fruit essential oils is Cuminol (another name for Cumin Aldehyde), which is used for the treatment of abscesses, boils, and styles of corneal opacities and to reduce inflammation and cough [8]. It includes immunomodulatory, antidiabetic, antioxidant, antibacterial, antifungal, and anti-inflammatory capabilities. [9]. *Cuminum cyminum* EO contains cumin aldehyde, p-isopropyl benzaldehyde, cuminyl alcohol, β-foreseen, terpinene, α- and β-pinene, ρ-cymene, and ρ-mentha-1,3-dien-7-al, with percentages of 25–35%, 2.8%, 29.5%, 21%, 8.5%, 5.6%, and 1.1%, respectively [10]. It contains mineral deposits like copper, manganese, magnesium, calcium, zinc, potassium, iron, and selenium. It additionally holds exceptional amounts of vitamins, including vitamin B-6, thiamin, niacin, and riboflavin. It also includes vitamins like vitamins A, E, and C, which are necessary antioxidants [7]. In animals, *C. cyminum* EOs methanol extract strengthens the bones by enhancing the mineral calcium [10].

Foodborne diseases are caused by consuming polluted foods or beverages. Many diseases caused by various viruses and germs can contaminate foods. Several bacteria, viruses, and parasites are responsible for most foodborne illnesses. [11]. Food reaches the consumer after a lengthy industrial process in which food deterioration is possible. Food preservation is of two types: natural and synthetic. They play an important role in delaying degradation in food by microbial growth, enzyme activity, and oxidations [12]. Synthetic or chemical-based preservatives are less effective and have more significant toxicity, so natural preservatives are picked over them. Consequently, because of their significant antiviral, antifungal, and antibacterial properties, EOs can be applied as a natural preservative for food [11]. *Cumin* EOs can inhibit the growth of pathogens in food, which is responsible for disease [13].

The compounds originating from fungi, animals, plants, and other microbes are natural antioxidants [14]. Natural antioxidants reduce oxidative stress and inflammation and support disease prevention, eye health, and a healthy aging process. EOs are present in a group of natural antioxidants consisting of a multifaceted mixture of hundreds of components. Recently, more attention of public curiosity about the consumption of natural antioxidants extracted from plant materials has increased due to having no toxicity at lower concentrations [15].

Essential oils are extracted from plant material using conventional and advanced techniques. Subcritical water extraction, supercritical CO2 extraction, and superheated steam distillation (SHSD) are the most commonly used advanced extraction techniques for EO. Hydrodistillation (HD) is a conventional technique to extract essential oils and bioactive compounds from plant material. The plant material undergoes a boiling process in HD water that may cause the disintegration and decomposition of heat-sensitive, vulnerable compounds of EO. Consequently, the outcomes obtained through HD may not comprehensively illustrate the results due to these variable influences on compound extraction [16, 17]. SD is a method in which steam passes through the material to extract EO. SD is preferred over HD due to the reduction in time duration, losses of main polar molecules, energy consumption, and possible oxidation-induced modification of the natural elements of EOs. Subcritical water extraction is a green extraction approach that applies water as an extraction solution at elevated pressure and temperature. Subcritical water's polarity and dielectric value are closely associated with its pressure and temperature. It acts as an organic solvent and promotes the extraction of several substances [18]. The environment-friendly nature, efficiency, and excellent speed of subcritical water extraction are favoured over HD [19, 20]. Another innovative extraction method for extracting bioactive molecules from plant material is supercritical fluid extraction (SCFE) compared to others [20]. CO_2 is a frequently used solvent for extraction in SCFE, having advantages over other solvents such as low price, inertness, easy availability, and selectivity is high as well as its lower critical temperature, pressure, compatibility for heat-

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sensitive compounds, no toxicity, and environment-friendly nature. It is superior to others due to compound recovery, lower contamination, higher selectivity, and good speed [21].

SHSD is an advanced distillation method that uses superheated steam as the extraction medium. Steam with a temperature higher than regular steam at specific pressures is called superheated steam. Superheated steam can range from 101 °C to over 1000 °C, depending on the pressure and composition of the container used to generate it. This type of steam has several beneficial features, such as higher extraction efficiency, lower oxygen capability, and better thermal conductivity [15, 22]. The greater the temperature of superheated steam, the more quickly the plant cell breaks down, and the EOs are released. The higher temperature increases the kinetic energy of the molecules, and it disrupts the structural integrity of the plant cell walls. It makes the cell wall more permeable, which allows the essential oil to be released more quickly, and the heat can also cause a breakdown of the complex polysaccharides and the other cells, which leads to maximum extraction of EOs. SHSD is preferred over others because it is cost-effective, requires less extraction time, is efficient, is environment friendly, has maximum yield obtained, and can be used at the industrial level due to its large-scale applications. [15].

The literature review revealed that SHSD is not used to extract EO from *Cuminum cyminum*. Therefore, current research arranged to extract *C. cyminum* EOs, using SHSD to compare yields with already used extraction techniques. The chemical configuration of EOs was explored via GC-MS. Different antioxidant and antimicrobial analyses also employed the outcome of extraction approaches on antioxidant activity and antimicrobial activity.

2. Material and Methods

2.1. Collection of Plant Material

The seeds of *Cuminum cyminum* were collected from the Sahiwal region, located in the Sahiwal district of Punjab, Pakistan. Dr. Fahim Arshad (Associate Professor), Department of Botany, University of Okara, Okara, Punjab, Pakistan, further verified plant materials.

2.2. Hydrodistillation

HD was carried out using Clevenger equipment, including a heating mantle, a liter round bottom flask, a dean stark, a condenser, and a funnel for separation [23]. The flask held three hundred grams of fresh plant material and three liters of water. The volumetric flask comprising the plant material and water was heated to a boil on a heating mantle. *Cuminum cyminum* EOs and water were evaporated and condensed by the condenser. The vaporized mixture was condensed into a liquid and then poured into a funnel of separation to separate the aqueous and oily layers.

2.3. Steam Distillation

The SD equipment comprises a volumetric flask, heating mantle, condenser, biomass flask, and thermometer [24]. The C. cyminum seeds weighed approximately one hundred grams and were transferred to a biomass flask previously trimmed before the round bottom flask. Steam production occurred by utilizing a heating mantle to bring water to a boil. As steam entered the biomass flask, it reached proximity to the plant material. After evaporation and traversing a condenser, the EOs, and water vapours chilled to liquid droplets. The EOs layer was separated and captured by a separating funnel. SD process finished after three hours. Essential oils were dehydrated using anhydrous sodium sulfate and passed through microfilters to remove solid particles. The extraction process was repeated four times to confirm the reproducibility of the results.

2.4. Superheated Steam Distillation

SHSD equipment consists of a customized-shaped extraction vessel (stainless steel) coupled to the SHS producer tank, condenser, and hydrosol picked-up vessel [15]. Thermocouples continually assess the SHS temperature at the entrance and departure of the extraction vessel (stainless steel). The *Cuminum cyminum* seeds are put inside the extraction container and then exposed to superheated steam for one hour at $150^{\circ C}$ and Seventy-five PSI stress. The vaporized mixture of EOs and water is liquified using a condenser and goes toward the hydrosol collection vessel. Moisture from EOs is removed by including anhydrous sodium sulfate and stored in yellowish-brown glass vials until a similar

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evaluation. The extraction process was repeated four times to confirm the reproducibility of the results.

2.5. Antimicrobial Activity

2.5.1. Microbial Strain

The antimicrobial potential of *Cuminum cyminum* EOs was explored against *E. coli, Aspergillus flavus, Fusarium solani, Alternaria alternate, Staphylococcus aureus, Aspergillus niger, Pasteurella multicide,* and *Bacillus subtilis* in the Department of Biochemistry, University of Agriculture Faisalabad, Pakistan.

2.6. Agar Well Diffusion Method

The antimicrobial potential of EOs was examined using the Agar well diffusion method as described [25]. One mL of microbiological strain was transferred to twenty-five milliliters for a growth environment solution that adhered to a single-day tradition of holding 10^8 colony-forming gadgets. The flask's content was transferred to medium-sized Petri dishes. These contents were then allowed to solidify at room temperature before being formed using a sterilized cork borer. Agar wells filled with 10 milliliters of EOs. They have also established drugs to examine antibacterial activity. The Petri dishes were hatched for twenty-four hours at 37 ºC for microorganisms and 40 hours at 30 ºC for fungal strains. The width of inhibitory zones (mm) constituted assessed the employment of a digital Vernier caliper adhering to the incubation time.

2.7. Resazurin Micro-Titer Plate Assay

A modified assay resazurin microtiter plate assay was used to evaluate the MIC of EOs for numerous microbial strains [26]. For preparing the sample solution, 10 mL of EOs is mixed with 1mL of 10% DMSO solution. To prepare the resazurin indicator, 27 mg of resazurin was mixed with four mL of deionized water. A 100 µL of EO solution and standard antibiotic were added in the first row of the ninety-six plate. Fifty microliters of nutritional broth were added to every well except the first row of ninety-six plates; two-fold dilution serials were made so that every well comprised 50 microliters of the sample. Afterward, 10 µL of the resazurin solution and 30 µL of the iso-sensitized broth of 3.3x concentration were introduced each well. The bacterial suspension was added to each well (10 µL), aiming for an approximate concentration of 5×10^5 CFU/ml. The plates hatched at 37 ºC for twenty-four hours. After the incubation period, the MIC values were optically assessed. A bacterial growth appeared visible when the coloration was modified from purple to pink.

2.8. Micro-Dilution Broth Susceptibility Assay

The MIC value of EOs against different fungal strains was considered using the micro broth dilution susceptibility assay [27]. First, 10 mg of *Cuminum cyminum* EOs were dissolved in 1mL to prepare a 10% DMSO specimen solution of EOs using traditional and advanced extraction techniques. Then, 1mL of antibiotic fluconazole was added to 1mL of Dimethyl sulfoxide solution for preparation of fluconazole standard antibiotic solution. With the sole exception of the first row, which contained 50 µL of sabouraud and 100 µL of pattern and general, they had been poured onto the first well plate. The dextrose broth is prepared and added to every well plate. Next, two-fold serial dilutions were made so that each one contained 50µL of the specimen's solution neatly. Then, twenty liters of microbe suspensions and one hundred and thirty microliters of sabouraud solution were added to every well, and these plates were cultured for forty-eight hours at 30 °C. The minimum inhibitory concentration was visually tested when fungal growth was suppressed.

2.9. Gas Chromatography-Mass Spectroscopy analysis

Utilizing GC-MS (GCMS-QP 2010, Shimadzu, Japan) outfitted with a mass detector and capillary column—specifically, DB-5, which stretched 50 mm by 0.25 mm and had a 0.25 µm film thickness— each extraction method's volatile EO fractions were determined. Then, 1 µL of EO was diluted to a one-by-ten ratio with n-hexane as the injection quantity, which was injected into the injection port via syringe. The GC column was heated to 60 ºC for three minutes. During this time, the column's temperature amplified gradually to 240 ºC at a rate of 24 ºC per minute of heating, and it remained steady for ten minutes. Helium (He) was the supplier of gas used in this machine and has a flow rate of 1.5 mL/min. The MS transport line's temperature was maintained at 240 ºC. In electron ionization mode, MS detection employed 70 eV. N-alkane standards were run to find the indicators of retention for chemical compounds that have been found.

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The NIST library and the already-gifted MS information base were compared to these mass spectrum records and retention indices.

Co-injection of the genuine standards was carried out to verify the components found in EOs—a quotative examination of the EOs and the individual components was carried out [15].

The following expression was used to evaluate the RF values:

$$RFC = \frac{Ac}{Airs} . \frac{Cc}{Cis}$$

RF represents the response factor of the component of the EOs.

A_c = Peak area of the component of the EO

A_{is} = Peak area of the internal standard

C_c = Concentrations of the components of the EO

Cis = Internal standard

The percentage of each EO component was determined for applying the RFs as follows:

$$\text{Corrected area} = \frac{\text{peak area for the component}}{response\ factor\ for\ that\ component}$$

$$\% = \frac{Corrected\ area\ for\ the\ component}{Total\ of\ the\ corrected\ areas} \times 100$$

2.10. Antioxidant Assays

2.10.1. DPPH Free Radical-Scavenging Activity:

The DPPH scavenging activity was investigated by mixing one milliliter of 0.3M DPPH solution in 2.5 milliliters of EOs derived from plant material seeds. The samples and standard were incubated for twenty minutes at room temperature in the dark. At 518 nm, the samples' absorbance was detected. To generate the control absorbance, 1mL of methanol was added to 2.5 mL of EOs; however, DPPH was not added. 1 mL of DPPH solution was added up to 2.5 mL of gallic acid to produce the positive control [28]. The following expression was used to compute the percentage of DPPH scavenging activity:

$$\text{DPPH scavenging activity (\%)} = 100 \times \frac{Abs\ sample}{s\ control} \times 100$$

2.10.2. Reducing Power Ability (RPA)

The reducing power ability was determined by combining 1 mL of sample or standard solution of gallic acid (at 25, 50, 75, and 100 mg/L) with 2.3 mL of 0.2 M phosphate buffer and 2.5 mL of 1% potassium ferricyanide. The taster was incubated for 20 minutes at 370 degrees Celsius. From that, the mixture and 2.5 mL of 10% trichloroacetic acid were centrifuged for 10 minutes at 1000 rpm. Subsequently, 2.5 milliliters of the sample mentioned above was blended with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$, and at ten minutes of incubation, the absorbance at 700 nm was measured [29].

2.10.3. Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging activity of EOs was tested spectrophotometrically [30]. A hydrogen peroxide solution of 2 mM was made in a 0.17 M phosphate buffer with a pH of 7.4. The hydrogen peroxide solution was two mM, further enriched with 0–0.01 g/mL of ascorbic acid and varying amounts of 600 µL of solution. The sample was incubated for ten minutes, and at 230 nm, the absorbance was obtained. The hydrogen peroxide's % scavenging activity was calculated using the following formula:

$$\%age\ of\ hydrogen\ peroxide\ scavenged = (A_0 - A_1) \times 100$$

Here,

A_0 = Absorbance of the control

A_1 = Absorbance of the sample

2.11. Statistical Analysis

All of the assays were carried out in three separate experiments, and the findings were evaluated with STATISTICA 5.5 (Stat Soft Inc., Tulsa, OK, USA) software as well as (ANOVA) analysis of variance with the post hoc Tukey's HSD test. It was concluded that the value of the probability of $\rho < 0.05$ indicated a statistically significant value. The data was shown as standard deviation, and mean values are based on triplet experiments.

3. Results and Discussions

3.1. Chemical Composition

The characterization of *Cuminum cyminum* EOs was evaluated by the GC-MS, and their outcomes are displayed in **Table 1**. The GC-MS results revealed that cumin aldehyde (51.87%), 3-caren-10-ol (15.34%), γ-

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terpinene (10.23%), β-Pinene (6.83%), and α-Terpinene (4.83%) are major compounds. Monoterpene hydrocarbon (46.37%-43.14%) and oxygenated hydrocarbons (41.61%-54.03%) and sesquiterpenes hydrocarbons (0.97%-0.39%) and oxygenated sesquiterpenes (1.6%-0%) and oxygenated diterpene hydrocarbons (0.46%-0.6%) were the classes of compounds of *Cuminum cyminum* EOs. Among oxygenated monoterpenes, cumin aldehyde was the major component, making up 51.87% of the chemical composition of *Cuminum cyminum* EOs.

Table 1: GC-MS analysis of *Cuminum cyminum* seeds EOs extracted by HD, SD, and SHSD.

Components	RI[A]	Concentrations			Method of Identification
		HD	SD	SHSD	
Monoterpene Hydrocarbons					
α-Phellandrene	1005	0.18 ± 0.04 [c]	0.21± 0.06[b]	0.24± 0.1[a]	a,b
α-Pinene	933	0.46 ± 0.11[b]	0.5 ± 0.21[a]	---	a,b
β-Pinene	980	6.79 ± 0.09 [c]	6.82 ± 0.41[b]	6.83 ± 0.26 [a]	a,b
γ-terpinene	1059	7.14 ± 0.21 [c]	8.19 ± 0.02 [b]	10.23 ± 0.56 [a]	a,b
β-Myrecene	991	1.6 ± 0.08[b]	1.7 ± 0.23 [a]	----	a,b
β-Sabiene	973	0.72 ± 0.21[c]	0.75 ± 0.09 [b]	0.78 ± 0.11[a]	a,b
Terpinene-4-ol	1177	0.27 ± 0.9[b]	0.31 ± 0.05 [a]	-----	a,b
3-caren-10-ol	1147	10.24 ± 0.08 [c]	13.27 ± 0.23 [b]	15.34 ± 0.05 [a]	a,b
α-Terpinene	1018	2.75 ± 0.17[c]	3.79 ± 0.09 [b]	4.82 ± 0.51[a]	a,b
o-cymene	1011	2.61 ± 0.9 [c]	2.7 ± 0.64 [b]	2.75 ± 0.08[a]	a,b
D-limonene	1031	5.9 ± 0.23 [a]	1.5 ± 0.03 [b]	----	a,b
β-Ocimene	1050	3.07 ± 0.34 [a]	0.37 ± 0.06[b]	----	a,b
(E)-β-terpineol	1159	1.6 ± 0.71[a]	0.19 ± 0.28 [c]	0.21 ± 0.23 [b]	a,b
β-Phyllandrene	1031	0.92 ± 0.24[c]	0.95 ± 0.80 [b]	0.99 ± 0.18[a]	a,b
2-careen	1001	0.22 ± 0.07 [c]	0.28 ± 0.61[b]	0.35 ± 0.03[a]	a,b
Santolina triene	1187	1.9 ± 0.35[b]	2 ± 0.21 [a]	0.6 ± 0.51[c]	a,b
Oxygenated Monoterpene Hydrocarbons					
Cumin Aldehyde	1239	35.62 ± 0.25[c]	43.78 ± 0.34 [b]	51.87 ± 0.68 [a]	a,b
Linalool	1098	4.2 ± 0.09 [a]	0.47 ± 0.23[c]	0.49 ± 0.21[b]	a,b
Eucalyptal	1033	0.26 ± 0.67 [b]	0.29 ± 0.074 [a]	----	a,b
p-Mentha-1,4-dien-7-ol	1345	1.48 ± 0.29 [c]	1.52 ± 0.12[b]	1.67 ± 0.05 [a]	a,b
Sesquiterpenes Hydrocarbon					
(E)β-Famesene	1458	0.06 ± 0.01[b]	0.09 ± 0.17 [a]	----	a,b
γ-Muurolene	1477	0.6 ± 0.03 [b]	0.7 ± 0.05 [a]		a,b
p-Menthane, Z-	1563	0.28 ± 0.08 [c]	0.31 ± 0.14 [b]	0.39 ± 0.09 [a]	a,b
2-Decyne-1-ol	1455	0.03 ± 0.41 [b]	0.07 ± 0.12 [a]	----	a,b
Oxygenated Sesquiterpenes					
Carol	1594	1.2 ± 0.19 [b]	1.5 ± 0.02 [a]		a,b
Oxygenated Diterpene Hydrocarbon					
Cinnamic acid, methyl ester	1268	0.29 ± 0.05 [c]	0.35 ± 0.02 [b]	0.39 ± 0.06 [a]	a,b
Isopseudocumenol	1385	0.17 ± 0.13 [c]	0.18 ± 0.03 [b]	0.21 ± 0.09 [a]	a,b
Monoterpene Hydrocarbons			46.37	43.53	43.14
Oxygenated Monoterpene Hydrocarbons			41.61	46.03	54.03
Sesquiterpenes Hydrocarbon			0.97	1.17	0.39
Oxygenated Sesquiterpenes			1.6	1.6	0
Oxygenated Diterpene Hydrocarbon			0.46	0.53	0.6
Total			**91.01**	**92.86**	**98.16**

Data is shown as means of standard deviations. Different letters in superscripts represent significant differences among *Cuminum cyminum* EOs derived from different extraction methods.
RI[A] = retention indices calculated against n-alkanes. A = identification based on retention index. b = identification based on Comparison of mass spectra.

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Phellandrene (α, β), pinene (α, β), and terpinene (α, β, γ) are the major components in monoterpene hydrocarbons, and oxygenated diterpene hydrocarbons are cinnamic acid and isopsedocuminol are major constituents. Ultimately, Cumin aldehyde is the major component of the oxygenated hydrocarbons responsible for biochemical activity. The results are similar to those of previous studies, which showed that Cumin Aldehyde is the major component of *Cuminum cyminum* EOs [31, 32]. Another study noted that cumin aldehyde was the major component of *Cuminum Cuminum* EOs [33], ranging from 39.3% to 46.5% expressed by [34] and also described by [9, 35]. It was noticed that components of EOs were vastly affected by the extraction techniques. The highest yield (51.87%) of cumin aldehyde was achieved by the Superheated SD and other compounds 3-caren-10-ol, γ-terpinene, β-tinene and α-terpinene also extracted with concentration by SHSD 4.83%) 15.34%,10.23%,6.83% and 4.83% respectively. The temperature and boiling point vary in SHSD and are higher than the atmospheric pressure. It can penetrate plant material exceedingly due to its low density, so many compounds can be extracted. The risk of thermal degradation and heat-sensitive compounds can also be reduced in SHSD [36].

Overall, extraction techniques enormously affect the variation in the chemical configuration of *Cuminum cyminum* EOs.

3.2. Yield of Cuminum cyminum essential oil

Cuminum cyminum EOs were extracted using HD, SD, and SHSD methods, and the outcomes are shown in (**Figure 1**). It was noticed that the yield of EOs was affected by the extraction methods. The highest EO yield was produced from SHSD (3.34%) and the lowest by HD (2.50%). The yield of steam-distilled EOs was 2.9%, as the literature shows. Premature growth and physical and climatic conditions affect the yield of EOs [37]. SHSD obtained the highest yield due to the high steam temperature (150 ºC). It has been reported that the vaporization of *Cuminum cyminum* EOs increases with an increase in temperature, which leads to higher yield because it stimulates the extraction of volatile compounds by easily breaking the granular trichome of the cell. Superheated steam has greater penetration power, which is directly related to kinetic energy. Hence, maximum molecules colloid with each other, and a greater yield was obtained [38]. It was reported that a 2.8% yield was obtained by using the HD technique. The *Cuminum cyminum* EOs yield ranged from 1.16% to 1.98%, extracted via HD and SD [34].

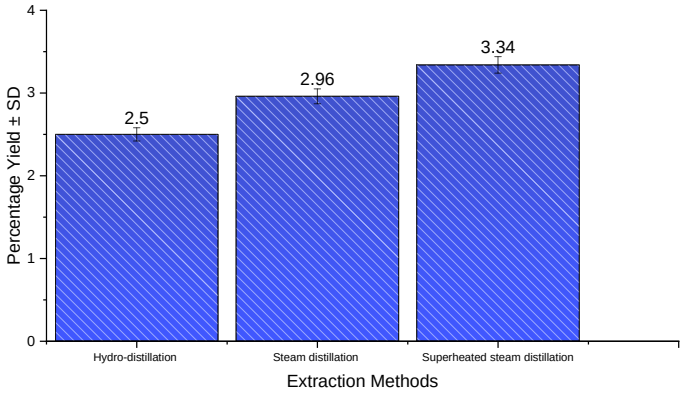

Figure 1: Comparison of *Cumin Cyminum* seed EO yield extracted by HD, SD, and SHSD.

Additionally, it has been observed that high temperatures decrease the polarity, density, surface tension, dielectric constant, hydrogen bonding, and viscosity of water molecules., increasing diffusion force, mass transfer rate, and EO efficiency increased [15]. At 150 ºC, the steam is superheated, carrying more energy and penetration power, interacting with the plant material, and extracting EOs. There is no droplet of the water molecule, which may increase the density of steam; low density containing steam interacts with *Cuminium Cyminium* seeds. Maximum heat transfer occurs, and volatile components are easily extracted, yield increasing [39]. It has been noticed that the SHSD technique has less literature about the extraction method, so it has greater significance on them. In this research, EOs obtained maximum yield by SHSD, as others did.

Overall, the Comparison demonstrated that SHSD yields substantially better results for extracting *Cuminum cyminum* EOs than traditional extraction methods.

3.3. Antioxidant and Antimicrobial Activity

DPPH-FRSA, reducing power ability assays (FRPA), and hydrogen peroxide assays were commuted to determine the antioxidant activity of *Cuminum cyminum* EOs extracted by HD, SD, and SHSD. The results are mentioned in **table 2**.

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Table 2. Antioxidant Activity of *Cuminum cyminum* Seeds EOs Extracted by HD, SD, and SHSD.

Extraction Methods	DPPH-FRSA (%)	Total Antioxidant Contents/FRPA (mg/L of Gallic acid equivalent)	Hydrogen Peroxide FRSA (%)
Hydrodistillation	72.89±0.01^{d}	97.48±0.04^{c}	53.05±0.05^{c}
Steam distillation	75.33±0.04^{c}	129.14±0.05^{b}	60.06±0.01^{b}
Superheated steam distillation	78.35±0.01^{b}	152.72±0.04^{a}	67.05±0.01^{a}
Gallic acid	81.44±0.45^{a}	-	-

Values are mean ± Standard Deviations of three separate determinations.

Different letters in superscript represent significant differences among *Cuminum cyminum* seeds essential oils extracted by different extraction methods.

The extraction procedures highlighted the variable effect in assessing the antioxidant activity of EOs of *Cuminum cyminum*. The free radical scavenging activity (FRSA) of EOs is inversely proportional to DPPH radicals. DPPH radicals decreased with increased free radical scavenging activity and vice versa. The hydrogen peroxide assay's results demonstrated that the substance had the greatest FRSA, 0.06%, originating in SHSD, and the lowest FRSA by EOs extracted through HD was 53.0 5± 0.05%. The reduction of Fe^{3+} to Fe^{2+} is responsible for the reducing power of the compound. The total antioxidant content (FRPA) was believed to be 97.48 to 152.72mg/100g. *Cuminum cyminum* EOs extracted by SHSD contributed the highest FRPA assay, 152.72mg/100g, and the lowest antioxidant content by the HD in the range of 97.48mg/100g.

The DPPH assay is a well-known method to enumerate the antioxidant activity of foods, beverages, and extracts of plants. It is simple to use at a low cost and uses a simple spectrophotometer. Free radicals are generated in the chemical body due to atoms and molecular orbitals containing unpaired electrons. When free radicals are present in the body, they can be easily scavenged by an efficient antioxidant system [2]. *Cumin* EOs have the most potent antioxidant potential, which can scavenge free radicals and neutralize oxidative stress due to cumin aldehyde being a main bioactive component. It captures and neutralizes the free radicals, reducing fat oxidation in the cell membrane and promoting cell death [13]. The DPPH-FRSA results exhibited that the highest scavenging ability of EOs extracted by SHSD was 78.35%, and the lowest by hydrodistillation was 72.89%. These outcomes showed that the effectiveness of antioxidants of *Cuminum cyminum* EOs is comparatively inferior to the ordinary gallic acid assay. The highest scavenging activity of *C. Cyminum* EOs is due to the high concentration of phenolic compounds, such as the major compound cuminol, responsible for total antioxidant activity [8].

Resazurin microtiter plate, agar well diffusion, and microdilution broth assay were utilized to assess the antimicrobial potential of HD, SD, and SHSD EOs. The outcomes of the antimicrobial activity of *Cumin Cyminum* EOs are mentioned in **Table 3**. The values of MIC and inhibition zones of *C. Cyminum* EOs were in the range of 0.031 to 10.00 (mg/L) and 33.64 to 63.46 (mm), respectively. Similarly, the positive control values ranged from 0.01 to 0.63 (mg/L) and 38.31 to 49.44 (mm), respectively. SHSD-extracted EOs have higher antibacterial activity by providing the MIC range of 0.16 to 5.00 (mg/L) and a greater inhibition zone of 39.72 to 63.46 (mm). The variable affects the antibacterial activity due to differences in the chemical composition of EOs. The antibacterial activity appeared to be significantly lower in the Gram-positive bacterial stains containing *Staphylococcus aureus* and *Bacillus subtilis* (ranged from 40.51 to 37.25 mm and 48.14 to 45.18 mm inhibition zones; 2.50 to 5.00 mg/mL and 0.63 to 2.50 mg/mL MIC values, respectively), than the Gram-negative bacterial strain containing *Pastrulla multocida* and *E. coli* (with an inhibition zone of 58.48 to 63.46 mm and 47.92 to 58.48 to 63.46 mm and 47.92 to 52.48 mm and MIC values 0.63 to 0.16 mg/mL and 5.00 and 1.25 mg/mL, respectively). [40] explained the antibacterial

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action of *Cuminum cyminum* EOs against *Staphylococcus aureus, E. coli, and Bacillus cereus* with inhibition zones 16.20 mm, 10.60 mm, and 28.00 mm, respectively. Similarly, [41] evaluated the antibacterial activity of *Cuminum cyminum* EOs against *B. cereus, S. aureus, E. coli, and S. typhi*. The literature revealed antibacterial activity against gram-positive and negative bacteria with a MIC range of 2.5 to 5.0 mg/L [42].

The outcomes of the antifungal assay of *Cuminum cyminum* EOs extracted by HD, SD, and SHSD are given in **Table 3**, which shows the antifungal activity in the inhibition zone in the range of 33.64 to 46.58 mm. The MIC values for the antifungal activity of extracted EOs were 0.031 to 5.00 mg/L. It was observed that all the EOs showed significant antifungal potential against all fungal strains. The highest antifungal activity was noticed by SHSD EOs against *Aspergillus niger*, having an inhibition zone of 46.58 mm and MIC value of 2.50 mg/L. The lowest antifungal activity of extracted EOs was observed against *Aspergillus flavus* with an inhibition zone of 36.96 mm and a MIC value of 1.25 mg/L. Previous literature illustrated that cumin aldehyde is an essential constituent of *Cumin cyminum* EOs, which is responsible for antifungal solid potential against the *Aspergillus niger, Botrytis cinerea,* and *Rhizopus stolonifer* [43]. Another research article reported that cumin aldehyde is used for antifungal activity against different fungal strains, including *Alternaria solani* and *Alternaria alternata* [44].

Previous studies revealed that antibacterial and antifungal activity depends on the cumin aldehyde, the primary bioactive component of the *Cuminum cyminum* [45]. The cell membrane of pathogens is disrupted by this component, which enhances the leakage of cellular content and eventually causes cell death. Phytochemical analysis showed that *Cuminum cyminum* EOs contains phellandrene, pinene, cymene, terpinenes, thymol, carvacrol, limonene, and phenolic compounds present in few amounts, which also strongly inhibit microbial activity [46]. Overall, the EOs extracted through SHSD exhibited the most potent antimicrobial results against the bacterial and fungal strains.

Table 3. Antimicrobial Activity of *Cuminum cyminum* Seeds EOs Extracted by HD, SD, and SHSD.

Extraction techniques	Hydrodistillation	Steam distillation	Superheated steam distillation	Positive Control
		MIC (mg/ml))		
Fusarium solani	2.50±0.03^{a}	1.25±0.01^{b}	0.63±0.04^{c}	0.16±0.00^{d}
Aspergillus niger	10.00±0.03^{a}	5.00±0.01^{b}	2.50±0.03^{c}	0.63±0.01^{d}
Alternaria alternate	1.25±0.03^{a}	0.031±0.03^{b}	0.08±0.02^{c}	0.02±0.00^{d}
Aspergillus flavus	5.00±0.02^{a}	2.50±0.03^{b}	1.25±0.04^{c}	0.32±0.03^{d}
Staphylococcus aureus	2.50 ± 0.02^{b}	5.00 ± 0.05^{a}	5.00 ± 0.03^{a}	0.078 ± 0.01^{c}
Esheriachia coli	5.00 ± 0.03^{a}	2.50 ± 0.01^{b}	1.25 ± 0.03^{c}	0.31 ± 0.01^{d}
Bacillus subtilis	0.63 ± 0.04^{c}	2.50 ± 0.06^{a}	1.25 ± 0.04^{b}	0.039 ± 0.05^{d}
Pastrulla multocida	0.63 ± 0.01^{a}	0.31 ± 0.03^{b}	0.16 ± 0.04^{c}	0.01 ± 0.00^{d}
		Inhibition zone (mm)		
Fusarium solani	37.85± 0.15^{d}	43.22± 0.04^{b}	44.96± 0.14^{a}	38.31± 0.16^{c}
Aspergillus niger	36.05± 0.12^{d}	41.72± 0.07^{b}	46.58± 0.16^{a}	39.93± 0.18^{c}
Alternaria alternata	41.13± 0.16^{b}	37.11± 0.06^{c}	41.20± 0.13^{b}	45.09± 0.15^{a}
Aspergillus flavus	37.15± 0.13^{b}	33.64± 0.09^{d}	36.96± 0.13^{c}	41.13 ± 0.14^{a}
Staphylococcus aureus	40.51 ± 0.08^{a}	37.25 ± 0.06^{c}	39.72 ± 0.05^{b}	40.00 ± 0.13^{a}
E.Coli	47.92 ± 0.09^{c}	49.54 ± 0.08^{b}	52.48 ± 0.09^{a}	45.04 ± 0.24^{d}
Bacillus subtilis	48.14 ± 0.06^{a}	45.18 ± 0.04^{c}	47.34 ± 0.03^{b}	48.14 ± 0.16^{a}
Pastrulla multocida	58.48±0.08^{c}	60.42±0.06^{b}	63.46±0.07^{a}	49.44 ± 0.18^{d}

Values are mean ± Standard Deviations of three separate determinations.
Different letters in superscript represent differences among *Cuminum cyminum* seeds essential oils extracted by different extraction methods.
Amoxicillin and Fluconazole (25 µg/disc) were positive controls for bacteria and fungi.

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4. Conclusion

The current research study is focused on the extraction of *Cuminum cyminum* EOs by using various extraction techniques and their effect on the yields, biological assays, and chemical composition. It was noticed that the extraction techniques highly affect the EO yield, antioxidant activity, antibacterial activity, antifungal activity, and chemical composition. SHSD produces the highest yield as compared to HD and SD. The GC-MS depiction of extracted EOs evaluated cumin aldehyde as the main bioactive component responsible for its antioxidant and antimicrobial activities. In conclusion, based on the study's results, superheated steam distillation is a process that shows potential for the extraction of EOs and has outstanding effectiveness in biological applications. In the future, more work is required on optimizing and experimenting with conditions for extracting EOs from *Cumnium cyminum* SHSD and their application.

Conflict of Interest

The authors declare to have no conflict of interest.

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Artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

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