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Research Article

Comparative Chemical Profiling and Biological Potential of Essential Oils of Petal, Choke, and Heart Parts of *Cynara scolymus* L. Head

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The essential oil and macroelemental composition of different parts of flower bud (petal, choke, and heart) of *Cynara scolymus* L. were explored and compared using gas chromatography mass spectrometry (GC-MS) and inductively coupled plasma mass spectrometry (ICP-MS). Overall, 62 organic components were detected in the flower bud based on mass spectra characteristics and retention indices. The essential oil extracted from the petals, choke, and bud showed the presence of thirty-one, twenty-one, and twenty-one compounds, respectively, with linoleic acid and palmitic acid as the major components. 21 components were identified in the oil of the petals, comprising 94.45% of the total oil, in which linoleic acid methyl ester, palmitic acid methyl ester, octadecanoic acid methyl ester, O-α-d-glucopyranoside, and heptyl oct-3-yl ester were the major constituents. Twenty-one compounds, representing 89.13% of the total oil, were detected in the choke oil. Linoleic acid methyl ester, palmitic acid methyl ester, and 2-methyl-1-hexadecanol were the main components. However, the edible heart oil contains twenty compounds, comprising 86.84% of the total oil. Cyclopropane butanoic acid, linoleic acid, methyl ester, and palmitic acid were the major constituents. The analysis executed by ICP-MS revealed the presence of significant amounts of various inorganic elements in all the three samples. The extracted essential oils were tested for antibacterial, antioxidant, and anticancer activities. The results showed that the oil extracted from the petals of *C. scolymus* flower bud displayed the highest antibacterial, antioxidant, anti-inflammatory, and anticancer effects, as compared to choke and heart oils.

1. Introduction

Cynara scolymus L. (family Asteraceae) is an herbaceous perennial thistle-like plant, commonly known as artichoke, originated in the Mediterranean regions, and is widely cultivated all over the world. The globe artichoke is an essential traditional component of Mediterranean diet and contributes remarkably to the economy of the Mediterranean agriculture, with an annual production of approximately 770,000 tons [1, 2]. There is rich ethnobotanical

documentation to reveal that artichoke has been utilized as a valued vegetable species in central Europe since the 16th century [3, 4]. Historically, globe artichoke was represented as a refined garden plant with a plethora of uses [5, 6]. It is extensively grown for its immature large inflorescences known as capitula or heads, with fleshy edible bracts and receptacle, which possess important nutrition values to their fibers, insulin, and minerals [7]. The plant is well recognized in herbal medicine and has been extensively used for therapeutic and nontherapeutic purposes [8]. The commercial extracts prepared from its foliage leaves are utilized as choleretic and hepatoprotective in food supplements [9]. Numerous pharmacological properties associated with artichoke including antioxidant [10], hepatoprotective [11], antimicrobial [12], anticancer [13], anticarcinogenic [14], hypocholesterolemic [15], anti-inflammatory [16], anti-HIV [17], and urinative [18] effects have been described in the literature. Various clinical studies have revealed that these biological activities of globe artichoke are due to the existence of high polyphenolic content in the leaves and flower heads, in particular hydroxycinnamates and flavonoids [19]. Sesquiterpenes (cynaropicrin, grosheimin), inulin, saponins, fatty acids, amino acids, minerals, and fibers were the other constituents reported from the *C. scolymus* [20]. Moreover, this plant species has potential economic importance due to the extensive industrial applications, including clotting effect (proteolytic and protease compounds) for the formation of dairy products [21], production of paper pulps due to high hemicellulose and cellulose content [22], and bioenergy and biomass production [23]. Literature studies have claimed the variability in abundance and content of chemical components due to preharvest factors, including agricultural practices, geographical location, crop management, developmental stage, and plant tissue [24–27]. A study conducted by Lombardo et al. (2010) has revealed the impact of seasonal variation in the concentration of polyphenolics in capitalism parts (floral stems, bracts, and receptacles) between spring and winter. They have verified that phenolic content was found higher in the spring as compared to other seasons and some of the identified compounds were absent in samples collected in winter [28]. Guillen-Rios et al. compared the impacts of the different manufacturing procedures on the physicochemical and sensory characteristics of the preserved artichoke hearts. The volatile oil extracted from the preserved hearts showed the presence of high content of β-selinene (46.2%) and isoamyl acetate (35.5%) [29]. A comparative study on volatile components of *C. scolymus* and *H. tuberosus* showed the presence of eight sesquiterpene hydrocarbons with β-selinene as a major component in globe artichoke samples. However, caryophyllene was not detected at all in artichoke and α-cedrene was the responsible component of the characteristic aroma of *C. scolymus* [30]. The thorough literature survey on different parts of *C. scolymus* extracts revealed that, although there are multiple studies in the literature on phytochemical and biological investigations of *C. scolymus* growing in various agroclimatic situations, there are only a few reports on essential oil isolation from *C. scolymus* [31–33]. However, no comparative study on essential oils extracted from different parts is reported. This has encouraged us to perform a comparative comprehensive chemical characterization of essential oil and inorganic constituents of different parts (petal, choke, and heart) of *C. scolymus* head.

2. Materials and Methods

2.1. Chemicals and Reagents. Nitric acid (HNO_3, 70%), tridecane (≥99%), Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), L-glutamine (≥99.5%), 1, 1-diphenyl-2-picrylhydrazyl (DPPH, 97%), and 2, 20-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid] (ABTS) (≥98%) were obtained from Sigma-Aldrich (California, USA). Penicillins (≥95%), streptomycin (≥97%), and antimycotic antibiotic were purchased from the local drug store. The isolated oil samples were diluted with analytical-grade acetone supplied from Sigma-Aldrich. Pure essential oil standard components including α-pinene, β-pinene, limonene, linalool, n-heptadecane, thymol, n-octadecane, α-bisabolol, α-terpineol, n-nonadecane, and nonanal and some essential oil-enriched fractions with p-cymene, 1,8-cineole, β-caryophyllene, α-humulene, 1-octen-3-ol, terpinen-4-ol, α-terpinolene, caryophyllene oxide, and cis-3-hexen-1-ol were available with us and were applied for co-injection and comparison analysis.

2.2. Plant Material. Fresh commercially available *Cynara scolymus* L. (artichoke) were purchased from local supermarkets in Riyadh, Saudi Arabia, in March 2019, and were exported from the Netherlands. Botanical material was identified and authenticated by a plant taxonomist Professor Dr. Mohamed Yousef at Herbarium Division, College of Pharmacy, King Saud University (KSA), Riyadh, Saudi Arabia. A voucher specimen (CS-715) has been retained in the same department. *C. scolymus* L. head was separated into petal, choke, and heart. Each part was separately cleaned, chopped, shade-dried at room temperature, and finally ground with a coffee grinder to a fine powder.

2.3. Bacterial Strain, Cell Lines, and Culture Medium. Four bacterial strains *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), and *Pseudomonas aeruginosa* (ATCC 27853) and cancer cell lines, namely RAW 264.7 mouse monocyte/macrophage, human hepatic cancer (Hep-G2) cells, breast cancer (MCF-7), and colon cancer (HT-29) cell lines, were procured from the Microbiology Department of the King Khalid Hospital Riyadh (KKH), Saudi Arabia. RAW 264.7 cells obtained from murine macrophages were cultured in Dulbecco's modified Eagle medium (DMEM) containing high-glucose media supplemented with 10% of FBS, 1% L-glutamine (200 mm), and 1% solution of the antimycotic antibiotic and incubated at 37°C with constant 5% CO_2 flow. Hep-G2, MCF-7, and HT-29 cancer cells were maintained in DMEM supplemented with 10% FBS containing penicillins (100 UmL^{-1}) and streptomycin (100 μg/mL). All the cancer cell lines were individually incubated at 37°C with a continuous supply of 5% CO_2. Experiments were carried out

with the cells in the logarithmic growth phase. After two days, cells were subcultured.

2.4. Isolation of Essential Oils and Volatile Compounds. 100 g of each air-dried, powdered aerial parts (petal, choke, and heart) of *C. scolymus* head was subjected to individual hydrodistillation in a Clevenger-type apparatus for 4 h using 1 L of distilled water by obeying the previously described method [34]. The obtained essential oil was separated from distilled water by rotavapor evaporation at ±50°C under reduced pressure. Subsequently, the extracted essential oil was dried over anhydrous sodium sulfate, transferred into dark screwed glass vials, and refrigerated at 4°C prior, to being analyzed by GS and determination of its chemical composition. The yield of obtained essential oils was determined in terms of dry yield basis, as g/100 g of dry herb. Tridecane was used as an internal standard.

2.5. Analysis of Essential Oils by Gas Chromatography (GC) and Gas Chromatography Mass Spectrometry (GC-MS). The extracted essential oils were analyzed using a GC-MS equipped with polar (DB-Wax) column. GC-MS analysis was carried out on an Agilent single quadrupole mass spectrometer assembled with an inert mass selective MSD-5975C detector (Agilent Technologies, USA) directly coupled to a GS (Agilent 7890A, Agilent Technologies, Santa Clara, USA), which was equipped with a split-splitless injector (split ratio, 10 : 1), a QuickSwap assembly, a HP-5MS fused silica capillary column (5% phenyl/95% dimethylpolysiloxane, 30 m × 0.25 mm i.d. with 0.25 μm film thickness), and an Agilent autosampler model 7693. DB-Wax fused silica capillary column (polyethylene glycol, 30 m × 0.25 mm i.d. with 0.25 μm film thickness) was used to perform the supplementary analyses of essential oil. The injector temperature of HP-5MS column was maintained at 250°C, and the oven temperature was programmed as follows: hold at 50°C (isothermal for 4 min), followed by a rise of 4oC/min up to 220°C, isothermal hold at 220°C, a second rise to 280°C at 20 oC/min rate, and finally an isothermal hold for 15 min. Conversely, the injector temperature of the DB-Wax column was maintained at 250°C and the oven temperature was programmed as follows: hold at 40°C (isothermal for 4 min), followed by a rise of 4oC/min up to 220°C and an isothermal hold for 10 min. Each acetone diluted sample (0.2 μL) was injected using the split injection mode (split flow ratio, 10 : 1). Helium was applied as the carrier gas at 1 mL/min flow rate. ChemStation data analysis software (Version E-02.00.493, Agilent) was employed to obtain the GC-TIC and mass spectra. The mass spectra data were acquired in the full-scan mode (m/z 45–600) at a 0.4 scan/s scan rate, using electron ionization mode with 70 eV ionization energy. The temperatures of the electronic impact ion source and the MS quadrupole were maintained at 230°C and 150°C, respectively. The MSD transfer line for both polar and nonpolar analyses was programmed at 280°C. The temperature of the detector was maintained at 300°C for both the polar and nonpolar analyses. The relative composition of the oil constituents was determined on the basis of peak area measured by the HP-5MS column without the correction factor.

2.6. Retention Indices. The linear indices (LRIs) of the components of oil samples were obtained by injecting a hydrocarbon mixture of n-alkanes, C8-C31 (C8-C20, 04070, Sigma-Aldrich, USA, and C20-C31, S23747, AccuStandard, USA) into both polar (DB-Wax) and nonpolar (HP-5MS) columns under the similar chromatographic conditions as described earlier in the literature. The LRIs were computed using [35].

2.7. Identification of Oil Components. The GC-MS chromatogram of essential oils is extracted from aerial parts (petal, choke, and heart) of *C. scolymus* head with identified peaks of major constituents on the HP-5MS column. The identification of different compounds of oil was performed by matching their mass spectra with those available in library entries of mass spectra databases (WILEY 9th edition, Version 2.0f NIST-08 MS Library, Flavor and Adams libraries) and by comparison of their mass spectra and linear retention indices with those already published in the literature obtained using both the polar and nonpolar columns [36, 37] and the co-injection of authentic standards available with us in our own created library.

2.8. Preparation of Sample for ICP-MS Analysis. 1 g of each dried sample was soaked individually in 50 mL of 20% nitric acid (HNO_3) solution in 250 mL Erlenmeyer conical flasks. The reaction mixture was heated for 48 h at 70–85°C with continuous stirring. During the process of heating, the volume of each flask was maintained at the same level by the intermittent addition of 20% HNO_3. After complete digestion, the extract of each flask was individually filtered using a Nalgene filter (Thermo Scientific) unit. Each filtrate was allowed to cool, transferred into a volumetric flask (100 mL), and made up to 100 mL volume by adding deionized water (Milli-Q). All the samples were analyzed by ICP-MS. All the glassware used during the sample preparation was washed with deionized water and rinsed thrice with 20% HNO_3 solution.

2.9. ICP-MS Analysis of Prepared Samples. A ELAN 9000 ICP-MS (PerkinElmer, USA) was used for the elemental analysis of the plant samples (petal, choke, and heart). The instrumental conditions applied for the analysis were as follows: nebulizer/spray chamber PFA-ST/Peltier cooled cyclonic spray chamber was used at RF power of 1500 watts, and plasma, auxiliary, and nebulizer gas flow was maintained at 15 L/min, 1 L/min, and 0.83–088 mL/min, respectively. The speed of peristaltic pump was adjusted to 0.5 mL/min, and the temperature of spray chamber was maintained at 2°C. The detector mode dual lens/outlines are enabled, and sampler/skimmer cone nickels were used. Scanning was performed in the peak hopping mode. A number of points/peaks, sweeps/readings, and readings/

replicates applied were 1, 10, and 1, respectively (Table S1). Each experiment was performed in triplicates (Table S1).

2.10. Calibration of ICP-MS and Internal Standards. The calibration of the instrument was carried out using a solution of ^{103}Rh, ^{9}Be, ^{34}Se, ^{92}U, ^{27}Co, ^{23}Na, ^{24}Mg, ^{58}Ni, ^{56}Fe, ^{204}Pb, ^{63}Cu, and ^{133}Ba 1 ppb in 1% HNO_3. The same standard solution was employed to optimize nebulizer gas flow, resolution, mass calibration, and AutoLens calibration. A multielement internal standard (20 ppb) solution was used for all analyses. 1 mL of the 10 ppm stock (BDH Chemicals) was diluted into 500 mL 1% HNO_3 to prepare a 20 ppb internal standard solution.

2.11. Antibacterial Activity. Bacterial strains (*E. coli, B. subtilis, S. aureus,* and *P. aeruginosa)* used for assay were cultured on a nutrient broth (comprised of 10 g tryptone, 10 g sodium chloride, 5 g yeast extract, and 1 L water) and nutrient agar (nutrient broth with 15 g L^{-1} agar). All the assays were individually repeated three times. The growth of normal bacteria was estimated by the growth on nutrient agar under suitable conditions (37°C, aerobic or anaerobic as required) in the absence of the test sample. Agar plates were placed in a "Gas-Pak" anaerobic jar to achieve the anaerobic conditions. The disc diffusion assay was applied to determine antibacterial activity. Freshly prepared broth cultures of bacteria were used for each assay. 15 mL of agar plates was prepared, allowed to set, and incubated at 37°C for 30 min. Each bacterial culture (1 mL) was subsequently spread uniformly over the dried surface of agar plates and incubated for 20 min at 37°C until the bacterial blanket was dried. Each isolated essential oil was individually employed at three different concentrations: 100% (undiluted), 15%, and 5% in nutrient broth. 15 μL of each essential oil or essential oil mixed with the nutrient broth was pipetted onto a sterile disc (6 mm, Oxid). The disc was then placed at the center of the agar plate and incubated for 24 h at 37°C. The zone of inhibition diameters for each dilution was recorded in mm. A sterile disc with the nutrient broth (15 μL) was applied as a control.

2.12. Antioxidant Activity. The antioxidant activity of isolated essential oils was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and 2, 20-azino-bis[3-ethylbenzothiazoline-6-sulphonic acid] (ABTS) free radical scavenging assays.

2.12.1. DPPH Radical Scavenging Assay. The antioxidant potential of isolated essential oils was investigated by measuring their ability to scavenge stable DPPH free radical by following Riwan et al.'s procedure with slight modifications [38]. 1 mL of DPPH radical (90 μM) solution was mixed with different concentrations (10 to 500 μg/mL) of each sample, and methanol (95%) was used to make up 5 mL final volume. The samples were incubated for 30 min at room temperature. After incubation, absorbance was recorded at 517 nm. Positive control was applied as ascorbic acid, and the percentage of radical scavenging activity was calculated using the following formula:

$$\text{Radical scavenging activity (\%)} = \frac{\left(A_{\text{blank}} - A_{\text{sample}}\right)}{A_{\text{blank}}} \times 100, \quad (1)$$

where A_{blank} and A_{sample} are the absorbance of the control and test samples, respectively.

2.12.2. ABTS Radical Cation Scavenging Assay. The antioxidant propensity of essential oils was determined by measuring their ability to scavenge ABTS free radical obeying the standard method reported by Sarker and Oba with minor modification [39]. In brief, ABTS and potassium persulfate were prepared in deionized water to 7 and 2.45 millimolar final concentration. 50 μg/mL of ABTS reagent was treated with various concentrations of each test sample (1 : 1, v/v), and the absorbance reading (λ_{734} nm) was taken after 1 h of reaction initiation using a UV-Vis spectrophotometer. The capacity of each sample to exert antioxidant was determined based on the absorbance of ABTS reduced solution according to abovementioned formula.

2.13. Anti-Inflammatory Activity. All the extracted oils from aerial parts (petal, choke, and heart) of *C. scolymus* were investigated for anti-inflammatory effect against RAW 264.7 cells.

2.13.1. Cell Viability by MTT Assay. 96-well plates were used to culture RAW 264.7 cells (5×10^5 cells/mL) and incubated for 24 h at room temperature. The cells were then reacted with 20 μL of different concentrations of essential oil (5.25–80 μg/mL) for 1 h, followed by the addition of 1 μg/mL of lipopolysaccharide solution and incubated for another 24 h. After 24 h of incubation, each well was treated with MTT reagents (5 mg/mL) and incubated further for 1 h at an ambient temperature. After that, the supernatant was discarded and each well was treated with 80 μL of DMSO. The quantification of formazan crystals was carried out at 540 nm absorbance using an ELISA plate reader.

2.13.2. Estimation of Nitric Oxide. The RAW 246 .7 cells have been cultured into 96-well plates and incubated for a whole day for adherence. After incubation, cells were pretreated individually with varied concentration (5.25–80 μg/mL) of each essential oil and then incubated further with or without LPS (1 μg/mL) stimulation at 37°C for 24 h. Afterwards, 80 μL of supernatant was collected and treated with an equal volume of Griess reagent (80 μL) and absorbance at 540 nm was recorded. The amount of nitrite in the culture medium was determined against the sodium nitrite standard curve.

2.14. Cytotoxicity and Cell Proliferation. MTT assay was used to determine the cytotoxicity of *C. scolymus* essential oils. The panel of cancer cells at a density of 2×10^4 cells/well was seeded into 96-well plates and assayed as the previously described method [40]. Prior to the experiment, test samples

were dissolved in 0.1% of DMSO. Cells were then treated with various concentrations (0.62–100 μg/mL) of essential oil at 37°C for 72 h in a humidified atmosphere with a continuous flow of 5% CO_2. Doxorubicin and four wells without essential oil were used as positive control and cell control, respectively. Wells were prepared in triplicate for each concentration. After 48 h of incubation, culture media carrying essential oil (different concentrations) and dead cells were poured out, leaving behind attached viable cells into the culture plate. The culture plate was then washed two times with pre-warmed phosphate buffer saline (pH 7.4). Afterwards, each well plate including blank and negative control was treated with 40 μL of MTT reagent and incubated further for 4 h under similar conditions for the reduction in MTT into formazan by the dehydrogenase enzyme present in the mitochondria of viable cells. After that, 150 μL of DMSO was added to each well to dissolve the formazan purple crystals and absorbance was noted at 570 nm using a microplate reader (ROBONIK TM P2000 ELISA Plate Reader). The percentage of viable cells was determined by applying the following equation:

$$\text{Survival rate \%} = \left(\frac{A_s - A_b}{A_c - A_b}\right) \times 100, \tag{2}$$

where A_s, A_b, and A_c represent the sample, blank, and negative control, respectively. The inhibitory concentration (IC_{50}) was calculated.

2.14.1. Isolation of RNA and Measurement of Real-Time PCR. Each cancer cell line (Hep-G2, MCF-7, and HT-29) was allowed to react with 10 μg/mL of each essential oil individually for 72 h. RNeasy® RNA Extraction Kit (Qiagen) was applied to extract total RNA, and the quality of each RNA sample (purity and concentration) was evaluated by absorbance measurements. 50 ng of total RNA was applied to each sample to generate cDNA using a cDNA Reverse Transcription Kit according to the manufacturer's instructions (Applied Biosystems, USA). Real-time PCR was cycled between 95°C/15 s and 60°C/1 min forty times after an initial denaturation for 10 min at 95°C using SYBR Green PCR Master Mix (Applied Biosystems, USA). Amplification was conducted on 7500 Fast Real-Time PCR Systems (Applied Biosystems, USA), and products were monitored routinely using dissociation curve software. The amount of the transcript was compared with the relative cut method, and the quantity of caspase-3 was normalized to the endogenous control glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [41]. The value with respect to the control was calculated by $2^{-\Delta\Delta CT}$. Real-time PCR primer sequences used for the estimation of caspase were as follows:

Caspase 3: sense: 5′-TGGTTCATCCAGTCGCTTTG-3′
Anti-sense: 5′-CATTCTGTTGCCACCTTTCG-3′

3. Results and Discussion

3.1. GC-MS Analysis of Essential Oil. A detailed characterization of chemical components of essential oil isolated from three different parts of petal, choke, and heart (aerial part) of *C. scolymus* head was exported from the Netherlands. The hydrodistillation of the petal, choke, and heart in a traditional Clevenger apparatus produced yellow, light yellow, and colorless oils in 1.23%, 0.9%, and 1.01%, v/w, respectively, based on fresh sample weight. The phytochemical components of these essential oils were analyzed by GC-MS equipped with two stationary phase columns (polar and nonpolar) led to the identification of a total of 62 different components in all the oils, of which 32 were present in the petal, 20 in the choke, and 21 in the heart essential oil of *C. scolymus* head, accounting for 94.45%, 86.84%, and 89.13% of the total oil composition of these fractions, respectively. The identified phytochemical components and their relative percentages are summarized in Table 1 and were accorded with their elution order of the HP-5MS nonpolar column (Figure 1). All the three essential oils contained high levels of oxygenated sesquiterpenes and fatty acid compounds, but vary qualitatively and quantitatively in their phytochemical composition. The oil extracted from petal was dominated by fatty acids, followed by oxygenated sesquiterpenes and sesquiterpene hydrocarbons. Linoleic acid methyl ester (31.9%) and palmitic acid methyl ester (22.2%) were the major components. Octadecanoic acid methyl ester (5.2%), O-α-d-glucopyranoside (5.01%), phthalic acid, heptyl oct-3-yl ester (4.7%), palmitic acid (4.2%), 1-hexyl-4-nitrobenzene (2.6%), D-glucopyranuronic acid (2.8%), and 4-methyl(trimethylene)silyloxyoctane (2.5%) were other components found in the oil (Figure 2).

The main components of essential oil extracted from choke were cyclopropane butanoic acid (21.3%), linoleic acid methyl ester (15.4%), palmitic acid (13.4%), hexadecanoic acid methyl ester (13.9%), and diisooctyl phthalate (4.3%). Fatty acids (55.24%), oxygenated sesquiterpenes (31.65%), and aliphatic sesquiterpenes (22.3%) were the major group of components present in the oil (Figure 3). The choke oil contains a high percentage of cyclopropane butanoic acid and cyclopropanes bearing simple functionalities and is well known for their diverse biological activities ranging from enzyme inhibitions to antibiotic, antitumor, antiviral, and neurochemical effects [42].

A total of twenty-one components were detected in the essential oil extracted from the heart part of *C. scolymus* head, and among them, linoleic acid methyl ester (25.9%) and palmitic acid methyl ester (22.2%) were present in high amounts. Other components found were 2-methyl-1-hexadecanol (9.8%), palmitic acid (8.8%), cyclopropane butanoic acid (8.9%), desulphosinigrin (5.2%), and 2-myristynoyl pantetheine (3.5%) in the oil (Figure 4). The chemical structure and EIMS fragmentation pattern of the most prevalent compounds, cyclopropane butanoic acid in the heart, linoleic acid methyl ester from the choke and palmitic acid, and methyl ester from the petal are shown in Figure 5.

A comparison between essential oils extracted from the petal, choke, and heart parts of *C. scolymus* head revealed that fatty acids and oxygenated sesquiterpenes were the main components of all the isolated oils. However, the chemical compositions of the oils obtained from the petal, choke, and

Table 1: Chemical constituents of essential oils derived from vegetable parts (petal, choke, and heart) of C. scolymus L. using GC/MS.

No.	Compound*	Rt_{Lit}	Rt_B	Rt_C	Rt_H	CS_P%	CS_C%	CS_H%	Identification[a]
1	n-Butyric acid 2-ethylhexyl ester	4.41	6.6	*t*	*t*	0.55	*t*	*t*	1, 2
2	Benzeneacetamide, α-ethyl-	5.18	*t*	*t*	6.77	*t*	*t*	2.93	1,2
3	**O-α-D-glucopyranoside,**	5.33	10.17	*t*	*t*	**5.01**	*t*	*t*	1
4	Cyclopentasiloxane, decamethyl-	6.02	—	6.21	—	—	0.44	—	1, 2
5	d-Gala-l-ido-octonic amide	6.05	6.96	*t*	*t*	1.44	*t*	*t*	1, 2
6	2-Hydroxy-5-methylbenzaldehyde	7.20	*t*	8.84	*t*	*t*	2.01	*t*	1, 2
7	D-Glucopyranuronic acid	7.48	6.8	*t*	*t*	2.88	*t*	*t*	1, 2
8	Melezitose	7.55	5.63	*t*	*t*	1.66	*t*	*t*	1, 2
9	2,6,10-Trimethyltetradecane	7.82	*t*	6.61	*t*	*t*	1.49	*t*	1, 2
10	Tetradecane	8.67	*t*	5.48	*t*	*t*	12.18	*t*	1
11	Nordazepam, TMS derivative	9.10	8.19	8.19	*t*	0.56	1.52	*t*	1, 2
12	Nordazepam, TMS derivative	9.10	—	7.91	—	—	0.51	—	1, 2
13	1-(Decylsulfonyl)-1-deoxy-d-mannitol	9.27	12.51	*t*	*t*	0.61	*t*	*t*	1, 2
14	Cyclobarbital	9.6	—	—	7.73	—	—	0.25	1
15	Acetyl-5-chloromethyl-isoxazolidin-3-one	10.40	6.47	*t*	*t*	0.61	*t*	*t*	1
16	2-Myristynoyl pantetheine	10.57	*t*	*t*	10.11	*t*	*t*	3.50	1, 2
17	(2-Phenyl-1,3-dioxolan-4-yl)methyl 9-octadecenoate	11.14	*t*	*t*	8.53	*t*	*t*	2.62	1, 2
18	Tetradecamethylcycloheptasiloxane	11.90	*t*	10.02	*t*	*t*	3.21	*t*	1, 2
19	Desulphosinigrin	12.17	*t*	*t*	10.39	*t*	*t*	5.25	1, 2
20	**Hexadecanoic acid, methyl ester**	12.25	*t*	13.50	*t*	*t*	**13.98**	*t*	1, 2
21	**Palmitic acid, methyl ester**	12.25	13.5	*t*	13.50	**22.28**	*t*	**22.07**	1, 2
22	Octadecadiynoic acid, methyl ester	13.58	—	—	12.67	—	—	0.47	1,2
23	**Octadecanoic acid, methyl ester**	13.67	15.05	*t*	*t*	**5.24**	*t*	*t*	1
24	4-(5-Pentyl-3a,4,5,7a-tetrahydro-4-indanyl)butanoicacid	13.85	—	—	9.72	—	—	0.30	1, 2
25	Arachidonic acid methyl ester	14.34	16.35	*t*	*t*	0.49	*t*	*t*	1
26	Diphenyl-1,6-dioxopyridazino[4,5 : 2′,3′]pyrrolo[4′,5′-d] pyridazine	14.41	15.53	*t*	*t*	0.22	*t*	*t*	1, 2
27	**Cyclopropanebutanoic acid**	14.80	16.51	15.05	15.04	0.19	**21.37**	**8.78**	1,2
28	Hexadecanoic acid, 14-methyl-, methyl ester	14.93	14.3	*t*	14.09	0.22	*t*	1.06	1, 2
29	4-Methyl(trimethylene)silyloxyoctane	15.05	7.06	*t*	*t*	2.51	*t*	*t*	1,2
30	Phenyl-1,3-dioxolan-4-yl)methyl 9-octadecenoate, cis-	16.08	5.82	—	—	0.45	—	—	1, 2
31	2-Trimethylsiloxy-6-hexadecenoic acid, methyl ester	16.34	8.64	*t*	*t*	1.78	*t*	*t*	1, 2
32	**Palmitic acid**	16.54	13.74	13.75	13.75	**4.24**	**13.53**	**8.84**	1, 2
33	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	16.91	16.1	*t*	*t*	0.45	*t*	*t*	1, 2
34	Diphenyl-1,6-dioxopyridazino	17.07	17.6	—	—	4.21	—	—	1, 2
35	Dasycarpidan-1-methanol, acetate (ester)	17.53	15.29	*t*	*t*	1.10	*t*	*t*	1, 2
36	Dasycarpidan-1-methanol, acetate (ester)	17.53	16.44	—	—	0.21	—	—	1, 2
37	4-Hexenoic acid, 4-methyl-6-(fluorodimethylsilyl)-6-trimethylsily-	18.16	*t*	*t*	9.59	*t*	*t*	0.93	1
38	**Linolelaidic acid, methyl ester**	18.24	*t*	*t*	14.79	*t*	*t*	**25.93**	1, 2
39	Diisooctyl phthalate	18.87	*t*	17.85	*t*	*t*	4.36	*t*	1, 2
40	Phthalic acid, bis(6-methylheptyl)ester	19.42	17.84	—	—	2.02	—	—	1, 2
41	Phthalic acid, heptyl oct-3-yl ester	19.79	*t*	*t*	17.83	*t*	*t*	1.06	1, 2
42	Docosatetraenoic acid, methyl ester	20.20	16.62	*t*	*t*	0.21	*t*	*t*	1, 2
43	**1-Hexadecanol, 2-methyl-**	20.49	*t*	*t*	5.48	*t*	*t*	**9.89**	1
44	Octadecyl vinyl, ether	20.92	5.47	—	—	**4.75**	—	—	1
45	**1-hexyl-4-nitrobenzene**	22.77	8.84	*t*	8.86	**2.60**	*t*	1.68	1
46	t-Butyl-[2-(2,2-dimethyl-6-methylene-cyclohexylmethyl)-cyclopropoxy]-dimethyl-silane	22.88	*t*	*t*	8.21	*t*	*t*	0.74	1
47	Tetradecanoic acid, 12-methyl-, methyl ester	24.62	—	—	12.36	—	—	0.40	1, 2
48	Tetradecanoic acid, 12-methyl-, methyl ester	24.62	*t*	*t*	12.43	*t*	*t*	0.95	1, 2
49	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	28.01	*t*	8.80	6.62	*t*	0.46	0.98	1
50	2,6-Dihydroxybenzoic acid, 3TMS derivative	28.07	11.66	—	—	0.40	—	—	1
51	Dodecanoic acid, 2,3-bis(acetyloxy)propyl ester	29.17	12.66	*t*	*t*	0.66	*t*	*t*	1, 2
52	Oleic acid	29.70	*t*	*t*	15.29	*t*	*t*	1.26	1, 2
53	**Linoleic acid, methyl ester**	29.86	14.8	14.80	*t*	**31.94**	**15.40**	*t*	1
54	Octamethylcyclotetrasiloxane	30.72	*t*	6.81	*t*	*t*	0.74	*t*	1
55	Dihydroxymandelic acid, 4TMS derivative	30.93	*t*	11.66	*t*	*t*	1.62	*t*	1
56	Octadecanoic acid	37.02	*t*	15.29	*t*	*t*	1.69	*t*	1, 2

Table 1: Continued.

No.	Compound*	Rt_{Lit}	Rt_B	Rt_C	Rt_H	CS_P%	CS_C%	CS_H%	Identification[a]
57	Heptasiloxane	37.78	*t*	13.08	*t*	*t*	1.20	*t*	1, 2
58	Heptasiloxane	38.65	*t*	16.62	*t*	*t*	1.10	*t*	1, 2
59	Heptasiloxane, hexadecamethyl-	38.65	*t*	15.55	*t*	*t*	1.59	*t*	1,2
60	Heptasiloxane, tetradecamethyl-	40.15	13.08	—	—	0.36	—	—	1, 2
61	Octasiloxane	40.15	*t*	14.36	*t*	*t*	1.51	*t*	1
Total identified					100	100	100		

CS_P = *C. scolymus* L. petal; CSc = *C. scolymus* L. choke; CS_H = *C. scolymus* L. heart; *t* = trace (<0.05%). Rt_{Lit} = retention time from the literature (Adams, 2007). Rt_P = determined retention time of *C. scolymus* petal; Rt_C = determined retention time of *C. scolymus* choke; Rt_H = determined retention time of *C. scolymus* heart. [a]Identification by the following: 1 = retention time (R_t) identical to literature (cf. exp. part); 2 = comparison of mass spectra (MS) with the library entries of mass spectra databases (cf. exp. part). Mean percentage is calculated from flame ionization detector (FID) data, compounds higher than 1.0% are highlighted in boldface, and their ±SD ($n = 2$) is mentioned.

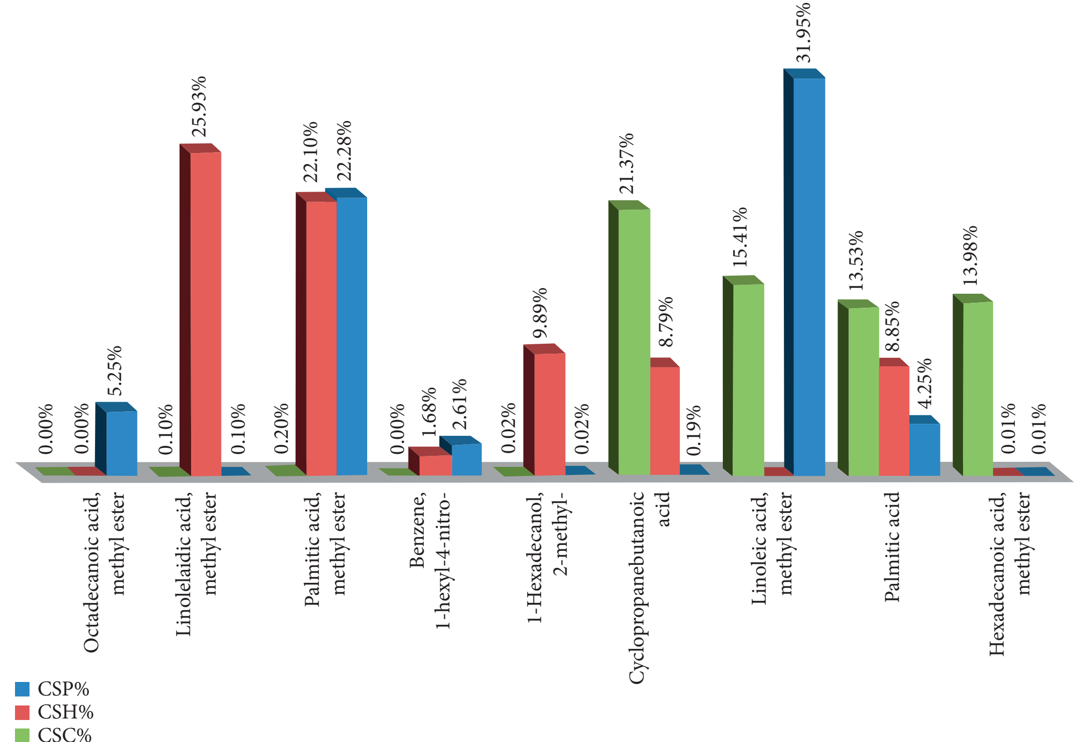

Figure 1: Chemical classes found in the oils of CSP = *C. scolymus* L. petal, CSH = C. scolymus L. heart, and CSC = C. scolymus L. choke.

heart were different. As illustrated in Table 1 and Figure 1, the contents of linoleic acid methyl ester and palmitic acid methyl ester were higher in the petal and choke oil, whereas these fatty acids were in smaller quantities in the heart oil. However, the content of cyclopropane butanoic acid was higher in heart oil, moderate in choke oil, and completely absent in petal. The fatty acid contents were higher in choke oil, followed by petal oil, whereas heart oil contains moderate amounts of fatty acids. The oil of petal also showed the presence of steroid content, which was completely missing in the heart and choke oil. The obtained results differ from those already reported in the literature for this species [30]. Previous investigations on the oils of *Cynara* genus have shown that the volatile oils extracted from *C. Scolymus* of Indian and Egyptian origin were dominated by monoterpenes, hydrocarbons, and sesquiterpene [43].

3.2. ICP-MS Analysis of the Petal, Choke, and Heart. Inorganic elements play a vital role in the survival of the biological system. In addition to the four basic elements of carbon, hydrogen, oxygen, and nitrogen involved in the preparation of several organic molecules, different inorganic elements are required for the healthy survival of organisms. Various inorganic elements are well known to be essentially required for the normal physiological functions in the human body [44]. The present investigation led to the identification of a total of 18 macroelements in all the three samples (petal, choke, and heart) of *C. scolymus* L. head, including those known to be involved in different biological functions in humans. In the petal, 17 inorganic elements were detected, whereas 15 elements were identified from choke and 18 were detected in heart part of *C. scolymus* head (Table 2). In the present analysis, three essential elements have drawn attention due to their sizable amount present in

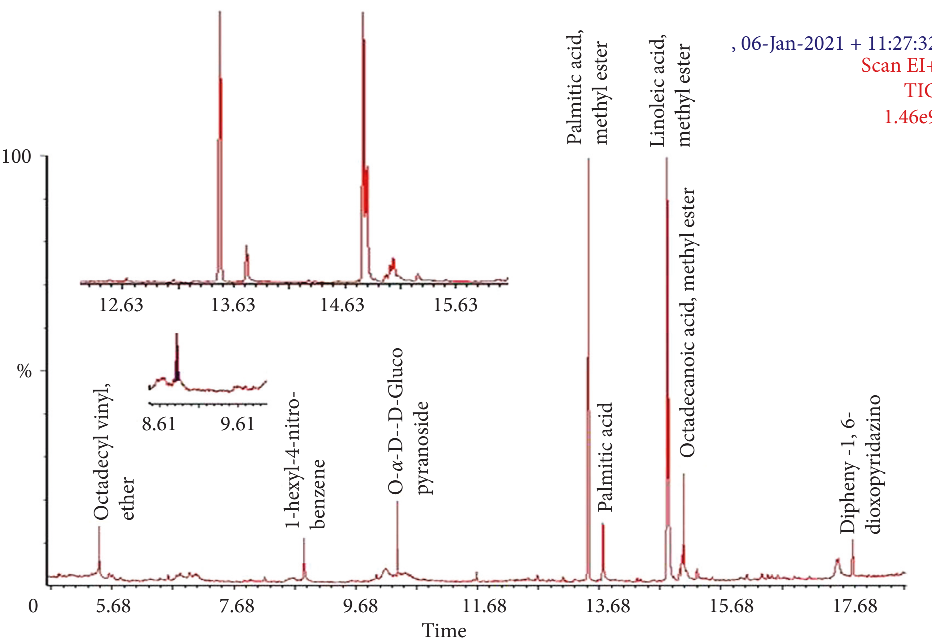

Figure 2: GC-FID chromatogram of aerial parts of essential oil of CS_P = *C. scolymus* L. petal on HP-5MS column. The numbering of the identified peaks is given according to the serial number of compounds in Table 1.

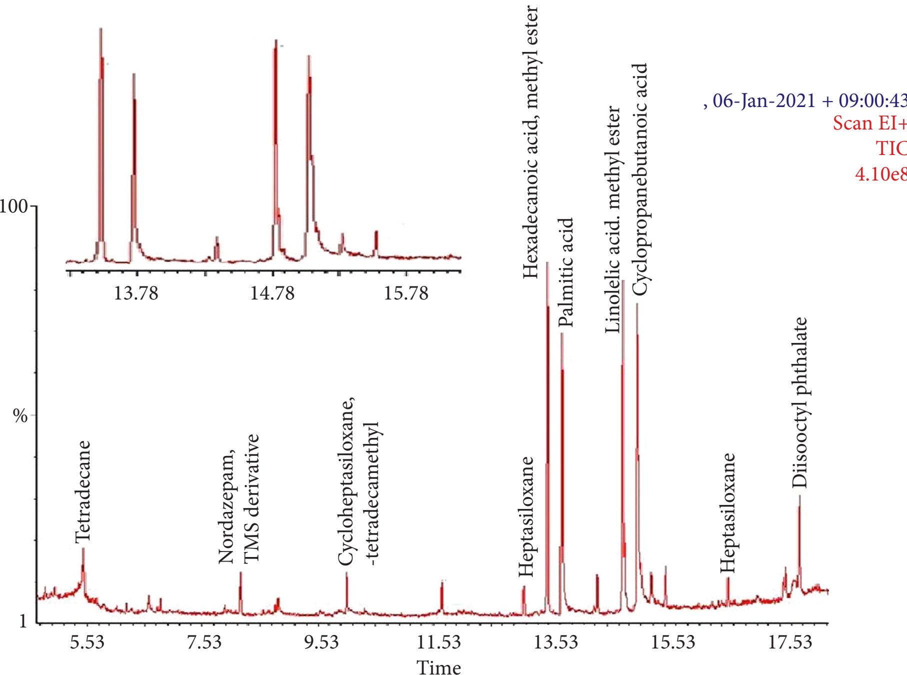

Figure 3: GC-FID chromatogram of aerial parts of essential oil of CSC = *C. scolymus* L. choke on HP-5MS column. The numbering of the identified peaks is given according to the serial number of compounds in Table 1.

all the three parts. These include potassium of 2606.322 mg/100 g, sodium of 373.5512 mg/100 g, and magnesium of 32.58552 mg/100 g. The levels of macroelements can be presented in descending order as follows: K > Na > Mg > Fe > V in all the three samples of *C. scolymus* head. The concentration of potassium (K) mineral was found highest in petal, followed by choke and hearts with mean levels of 2606.3 mg/100 g CSB, 2503.8 mg/100 g CSC, and 1904.278 mg/100 g CSH. Other mineral elements found in appreciable amount were sodium (Na), magnesium (Mg), iron (Fe), and vanadium (V) in all the three samples. These elements have been reported to be involved in various cellular functions. Sodium is mainly found in blood plasma and interstitial fluid, where Na^+ ions help in the transmission of nerve signals, regulating water in the plasma membrane and transport sugars and amino acids into the cells [45], whereas potassium is required at high levels in the cell fluids and K^+ ions participate in the activation of various enzymes, and ATP production oxidizes the glucose molecule

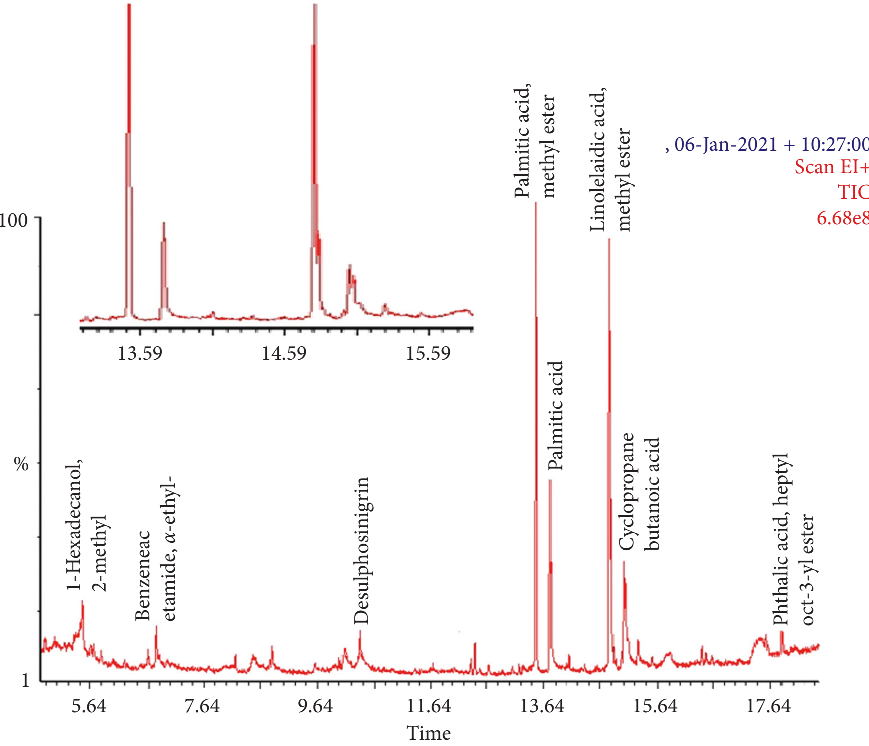

Figure 4: GC-FID chromatogram of aerial parts of essential oil of CSH = *C. scolymus* L. heart on HP-5MS column. The numbering of the identified peaks is given according to the serial number of compounds in Table 1.

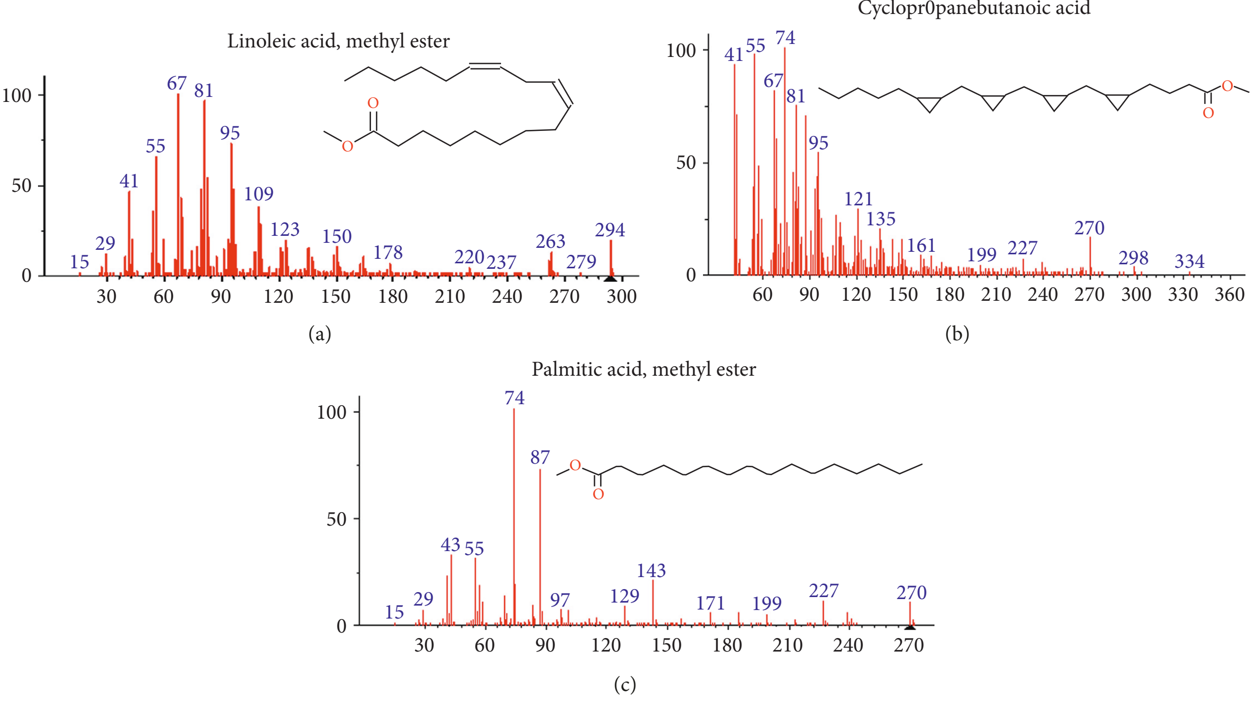

Figure 5: Chemical structure and EIMS fragmentation pattern of (a) CSP = *C. scolymus* L. petal, (b) CSC = *C. scolymus* L. choke, and (c) CSH = *C. scolymus* L. heart.

and helps in the transmission of nerve signals [46]. However, magnesium plays a vital role in neuromuscular function, intraneuronal transmission, blood coagulation, and cell membrane integrity [47].

3.3. Antibacterial Potential of Essential Oils. Microorganisms are a responsible candidate for the cause of damage to human health, food spoilage, and several other problems. They have become drug-resistant, so there is an urgent need

Table 2: Inorganic components detected from the different parts (petal, choke, and heart) of *C. scolymus* L. head.

Inorganic compounds	Petal (mgL^{-1})	Choke (mgL^{-1})	Heart (mgL^{-1})
Ag	0.000609485	0.01757032	0.001698875
Al	Not detected	0.4961	Not detected
As	0.034652145	0.038210435	0.068922025
Be	Not detected	0.015175285	Not detected
Bi	Not detected	0.00933841	Not detected
Cd	0.00169636	0.00737565	0.00456962
Cr	0.050052	0.112739	0.068151
Cu	0.013377	0.034189	0.009547
Fe	1.781358	1.230769	1.784947
K	2606.322	1904.278	2503.854
Mg	32.58552	7.435968	10.99805
Mn	0.025678	0.025904	0.023322
Mo	0.035367	0.166676	0.024306
Na	59.44024	178.4336	373.5512
Ni	0.01307	0.008614	0.016932
Se	0.030633	0.064496	0.045203
U	0.001027	0.071705	0.002912
V	0.195284	0.063255	0.307255

Table 3: Antibacterial activity of essential oil extracted from the petal, choke, and heart of *C. scolymus* L. head against four bacterial strains.

Bacterial strain	Petal essential oil		Choke essential oil		Heart essential oil		Standard drug	
	Zone of inhibition (mm)	MIC ($mgmL^{-1}$)	Zone of inhibition (mm)	MIC ($mgmL^{-1}$)	Zone of inhibition (mm)	MIC ($mgmL^{-1}$)	Zone of inhibition (mm)	MIC ($mgmL^{-1}$)
E. coli	23.2 ± 1.8	0.96 ± 0.05	12.2 ± 0.6	9.8 ± 0.40	13.4 ± 1.4	8.88 ± 0.01	25.12 ± 0.4	0.39 ± 0.07
S. aureus	20.6 ± 0.8	1.96 ± 0.02	21.6 ± 1.6	0.92 ± 0.20	10.8 ± 0.9	9.6 ± 0.003	29.8 ± 0.30	0.25 ± 0.01
B. subtilis	8.2 ± 0.6	11.4 ± 0.5	7.8 ± 1.2	12.3 ± 0.5	—	—	20.6 ± 0.60	0.48 ± 0.02
P. aeruginosa	11.3 ± 0.9	10.2 ± 0.60	18.2 ± 1.2	0.92 ± 0.20	15.3 ± 0.6	2.44 ± 0.02	22.4 ± 0.50	0.32 ± 0.03

to discover new sources against these disease-causing microorganisms. Essential oils and their active constituents play a crucial role in the growth inhibition of microorganisms [48]. In this study, three essential oils extracted from the petal, choke, and heart portions of *C. scolymus* L. were investigated against four bacterial strains. All the tested essential oils showed significant antibacterial activity against *E. coli*, *S. aureus*, and *P. aeruginosa* at 100% and 15% (Table 3), whereas no oil was able to inhibit the growth of *B. subtilis*. However, the essential oil extracted from the petal exerted the strongest antibacterial effects among the three oils and was the only oil that exhibited antibacterial effect against *S. aureus* with 8.2 ± 0.6 mm zone of inhibition at 5% concentration. The results showed that the petal essential oil displayed potent inhibitory activity against *E. coli* (23.2 ± 1.8 mm) and *S. aureus* (20.6 ± 0.8 mm) and moderate effect against *P. aeruginosa* (11.3 ± 0.9 mm) with lowest MIC values of 0.96 ± 0.005 mg/mL, 1.96 ± 0.002 mg/mL, and 10.2 ± 0.60 mg/mL, respectively. Choke essential oil expressed significant bactericidal activity against *S. aureus* and *P. aeruginosa*. The growth of *S. aureus* and *P. aeruginosa* was strongly inhibited with a large zone of inhibitions (21.6 ± 1.6 mm and 18.2 ± 1.2 mm) with MIC value (0.92 ± 0.20 mg/mL). Choke essential oil was moderately active against *E. coli* (12.2 ± 0.6 mm; MIC = 9.8 ± 0.40). However, the heart essential oil exerted an average antibacterial effect against *E. coli*, and S. and *P. aeruginosa* bacterial strains (Table 3). Rifampicin was used as a standard for the comparison of antibacterial activity. The antibacterial activity species of genus *Cynara* have been already documented in the literature. It has been suggested that the antibacterial activity of these oils can be attributed to the presence of high content of linoleic acid, methyl ester, cyclopropane butanoic acid, palmitic acid, and hexadecanoic acid, which are known for their antimicrobial activity [49, 50]. This difference in the activity of these oils may be due to the difference in the percentage of chemical components content and composition of phytoconstituents. The strong antibacterial potential of petal oil could be due to the high content of linoleic acid and palmitic acid and the synergistic effect of components of the oil.

3.4. Antioxidant Potential of Essential Oils. Free radicals are the highly reactive species produced in the human body under the influence of reactions occurring inside the human body, exposure to radiation, and environmental pollution. These radicals cause serious damage to human health and are accountable for various diseases [51]. Antioxidants are responsible for scavenging these radicals and turning them into less reactive species. Plants are considered as the best natural resources of antioxidants [52]. Antioxidant activity of plant *C. scolymus* L. essential oils was evaluated by DPPH and ABTS free radical scavenging assay. The plant oils exhibited moderate antioxidant potential at low concentration (Table 4). The

Table 4: Scavenging activities of essential oils derived from the petal, choke, and heart of *C. scolymus* L. head.

Sample concentration (μg/mL)	DPPH radical scavenging				ABTS radical cation scavenging			
	Petal essential oil	Choke essential oil	Heart essential oil	Ascorbic acid	Petal essential oil	Choke essential oil	Heart essential oil	Ascorbic acid
10	10.1 ± 4	6.7 ± 3.2	7.8 ± 0.9	80.7 ± 2.0	5.9 ± 4.1	6.2 ± 0.9	8.6 ± 0.8	80.7 ± 2.4
50	14.7 ± 1.2	17.2 ± 3.4	15.5 ± 3	85.1 ± 1.3	13.9 ± 1.1	9.8 ± 2.9	14.7 ± 1.2	81.2 ± 2.1
100	41.7 ± 2.3	20.1 ± 2.8	40.5 ± 1.9	85 ± 1.2	40. ± 3.5	35.5 ± 1.9	38.8 ± 0.5	84.2 ± 1.9
500	56.9 ± 0.7	55.1 ± 1.8	55.5 ± 1.5	88.7 ± 2.4	55.2 ± 1.3	46.1 ± 3.2	54 ± 0.8	87.2 ± 2.4
1000	76.8 ± 4.3	70.4 ± 3.9	74 ± 1.8	90.7 ± 1.4	76.7 ± 2.2	68.7 ± 2.3	73.7 ± 2.4	88.7 ± 2.1

essential oil of petal proved to be the most active with an IC_{50} values of 41.7 ± 2.3 μg/mL and 40. ± 3.5 μg/mL, followed by heart essential oil (IC_{50} = 40.5 ± 1.9 μg/mL and 38.8 ± 0.5 μg/mL) for DPPH and ABTS scavenging activities, respectively. However, the essential oil extracted from choke showed little antioxidant effect towards DPPH scavenging (IC_{50} = 20.1 ± 2.8 μg/mL) and moderate antioxidant potency towards ABTS free radical scavenging assay (35.5 ± 1.9 μg/mL). The antioxidant activities tend to increase with the increase in concentrations of the test sample. As shown in Figure 6, the petal essential oil showed higher DPPH and ABTS radical scavenging activity (IC_{50} = 353.5 and 388.1 μg/mL) compared with choke essential oil (IC_{50} = 315.3 and 339.6 μg/mL) and ascorbic acid (IC_{50} = 381.2 and 578.6 μg/mL), respectively, at higher concentration.

Natural antioxidants help in controlling the formation of free radicals and activated oxygen species or they can inhibit their reaction with biological structures [53]. These antioxidants include antioxidative enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, and small nonenzymatic antioxidant molecules, such as glutathione and vitamins C and E [54]. Many herbs and spices (rosemary, thyme, oregano, sage, basil, pepper, clove, cinnamon, nutmeg, and saffron) and plant extracts (tea, grapeseed, and lemon balm) contain antioxidant components [55, 56].

Despite playing a key role in cellular processes, free radicals pose a threat to cells by damaging DNA, proteins, and cellular membranes, leading to the onset of many diseases including cancer. Thus, by decreasing free radicals and oxidative stress, antioxidants play a role in ameliorating DNA damage, reducing the rate of abnormal cell division, and decreasing mutagenesis [57]. As a result, many antioxidant-rich plants possess anticancer activity [58].

3.5. Anti-Inflammatory Activity of Essential Oils. The measurement of viability of RAW 264.7 cells after treatment with *C. Scolymus* essential oil extracted from the petal, choke, and heart was conducted by MTT assay. The results revealed that the RAW 264.7 cells treated with different concentrations (5.25–40 μg/mL range) of essential oil have not shown much decrease in the viability after 24 h, as compared to the control (untreated RAW 264.7 cell). However, a decrease of 73.59%, 75.03%, and 84.23% was observed in cell viability after treatment with 80 μg/mL of *C. Scolymus* essential oil isolated from the petal, choke, and heart, respectively (Figure 7(a)). Hence, the concentrations below 40 μg/mL were chosen for the anti-inflammatory assay. The anti-inflammatory response of *C. scolymus* essential oils was evaluated on LPS-stimulated murine macrophage RAW 264.7 cells. LPS was applied to stimulate the nitric oxide (NO) release from macrophage cells. NO serves as a mediator for various pathological reactions, especially in case of acute inflammatory responses [59]. The concentration of nitrite was determined after LPS (1 μg/mL) treatment alone and in the presence of each *C. scolymus* essential oil (5.25, 10.5, 20, and 40 μg/mL). As depicted in Figure 7(b), all the three *C. scolymus* essential oil-treated RAW 264.7 cells exhibited the inhibition of LPS-induced production of nitrite in a concentration-dependent manner. The essential oils showed 92%, 87%, and 68% of inhibition of LPS-induced NO generation in RAW 264.7 cells at 50 μg/mL concentrations of a petal, choke, and heart essential oil, respectively. The inhibitory action of linoleic acid and palmitic acid in rat paw edema by reducing the release levels of pro-inflammatory cytokines (IL-6 and TNFα) and prostaglandin E2 has been reported in the literature [60].

3.6. Cytotoxicity and Apoptotic Potential of Essential Oil. The adverse side effects of conventional medication along with increased resistance to chemotherapy of mammalian cancer cells have initiated the search for alternative and new anticancer therapeutic agents from natural sources. In this study, the cytotoxicity of *C. scolymus* essential oils extracted from the petal, choke, and heart was assessed on three different cancer cell (Hep-G2, MCF-7, and HT-29) lines by MTT assay. Each cancer cell line was treated individually with increasing concentration (0.62–100 μg/mL) of essential oil for 72 h, and the results revealed that the essential oil inhibited the proliferation of all the treated cancer cells in a concentration-dependent manner. The respective calculated IC_{50} values are presented in Table 5. Based on standard criteria established by the National Cancer Institute (NCI) Natural Product Screening Program, a crude natural product extract is considered to possess potential if the *in vitro* cytotoxicity evaluation reported an IC_{50} value < 20 μg/mL following incubation for 48–72 h [61]. The results revealed that all the three isolated essential oils exhibited potent cytotoxic potential towards all the investigated cancer cell lines. *C. scolymus* essential oil extracted from petal exhibited IC_{50} values of 0.79 ± 0.45, 2.85 ± 0.39, and 5.74 ± 0.03 μg/mL against Hep-G2, MCF-7, and HT-29, respectively. Meanwhile, IC_{50} values of 1.52 ± 0.22, 3.89 ± 1.10, and 6.23 ± 0.56 μg/mL were recorded for *C. scolymus* heart essential oil on the abovementioned cancer cell lines,

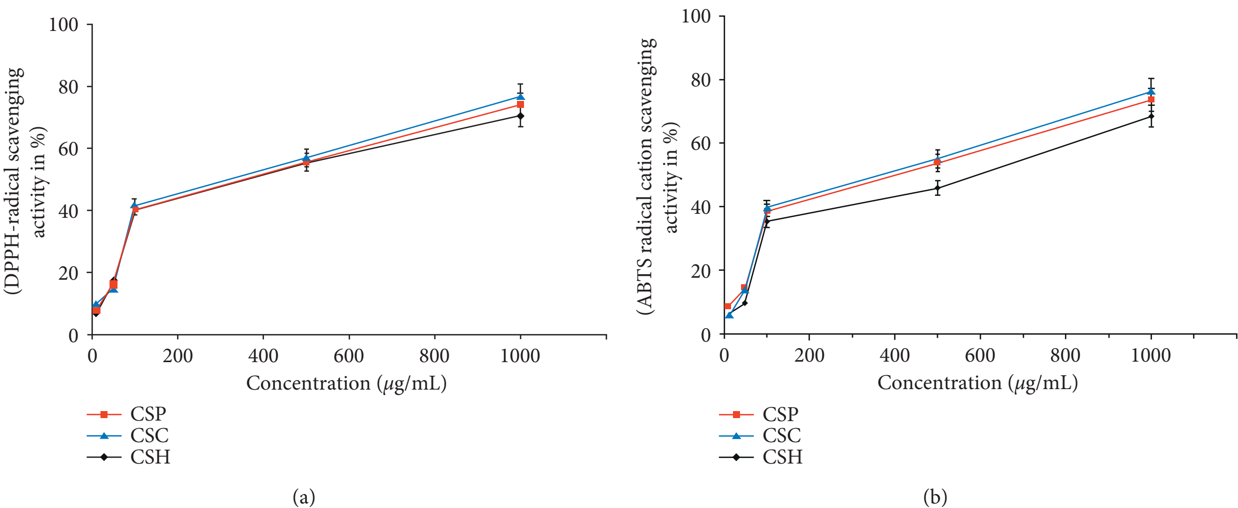

Figure 6: Antioxidant activities of (a) CSP = *C. scolymus* L. petal, (b) CSC = *C. scolymus* L. choke, and (c) CSH = *C. scolymus* L. heart, (a) DPPH scavenging activity; (b) ABTS scavenging activity. Data results are presented as means ± standard deviation (SD).

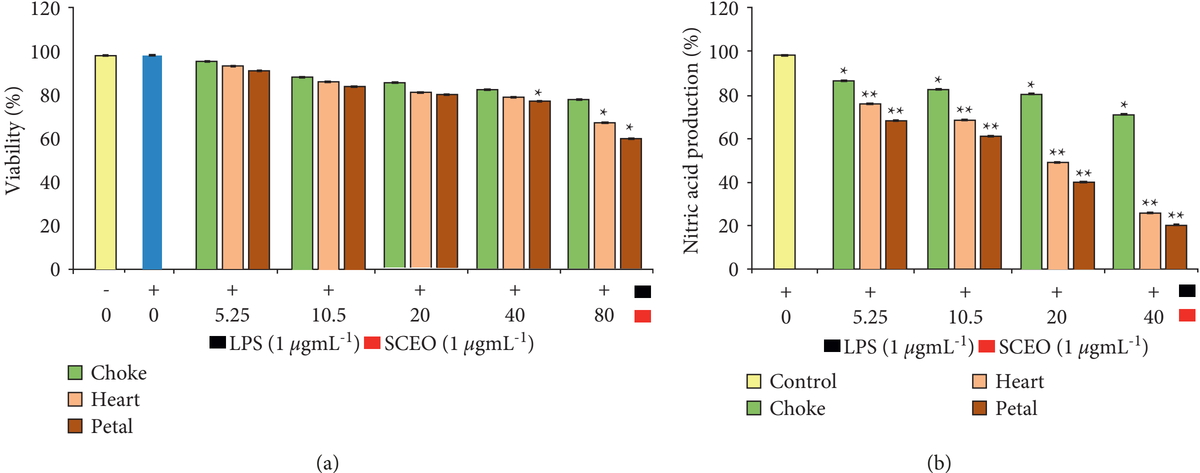

Figure 7: Effect of petal, choke, and heart essential oils of *C. scolymus* on (a) cell viability and (b) nitric acid production on LPS-stimulated RAW 264.7 macrophages. Data are expressed as mean ± SD of three independent experiments ($n = 3$). *and ***represent significant statistical difference ($P < 0.05$) relative to the control group as performed by Dunnett's multiple comparison test.

Table 5: Cytotoxic effects (IC_{50}) of essential oils derived from the petal, choke, and heart of *C. scolymus* L. head on the growth of different cell lines.

Sample	IC_{50} (μgmL^{-1})		
	Hep-G2	HT-29	MCF-7
Petal	0.79 ± 0.45	5.74 ± 0.03	2.85 ± 0.39
Choke	4.25 ± 0.44	7.98 ± 1.20	1.32 ± 0.24
Heart	1.52 ± 0.22	6.23 ± 0.56	3.89 ± 1.10
Doxorubicin	0.65 ± 0.01	0.56 ± 0.03	0.68 ± 0.05

The data are represented as ± mean of three independent experiments.

respectively. However, an essential oil extracted from choke of *C. scolymus* flower displayed IC_{50} values of 4.25 ± 0.44, 1.32 ± 0.24, and 7.98 ± 1.20 on Hep-G2, MCF-7, and HT-29, respectively. The highest cytotoxic effect was attained by *C. scolymus* petal essential oil (IC_{50} = 0.79 μg/mL), followed by *C. scolymus* heart essential oil (IC_{50} = 1.52 μg/mL) against Hep-G2 cancer cells. However, *C. Scolymus* choke essential oil exerted pronounced cytotoxic activity towards the MCF-7 (IC_{50} = 1.32 μg/mL) cancer cell line. All the essential oils showed a significant decrease in cell viability compared with the control group. Notably, the cytotoxic potential of the tested essential oils was found to be comparable with that of the reference drug doxorubicin (1.98 μg/mL). These results showed that essential oils extracted from different parts of *C. Scolymus* flower (petal, choke, and heart) possess a significant cytotoxic effect on Hep-G2, MCF-7, and HT-29 cancer cells, which can be attributed to their major phytoconstituents. Subsequently, the underlying mechanism for the obtained effects was further investigated.

The caspase-3 activity was performed to determine the capability of the essential oils to activate the apoptotic cascade. Caspases serve as the key mediators in programmed

Table 6: Caspase-3 activity in Hep-G2, HT-29, and MCF-7 cells after incubation with a petal, choke, and heart of *C. scolymus* L. head.

Sample	Hep-G2	HT-29	MCF-7
Petal	1.20 ± 0.32	1.82 ± 0.52	2.27 ± 0.10
Choke	2.87 ± 0.13	6.89 ± 0.36	3.45 ± 0.50
Heart	1.24 ± 0.12	1.67 ± 0.50	2.32 ± 0.36
Control	1.0	1.0	1.0
Doxorubicin	12.63 ± 0.12	5.14 ± 0.14	23.34 ± 0.33

The data are represented as ± mean of three independent experiments.

apoptosis (cell death), a complex bioprocess that helps to remove and kill the undesirable cells [62]. The caspase-3 activation leads to various downstream incidences that eventually result in cell demise [63]. In this study, a significant elevation ($P < 0.01$) in the caspase-3 gene expression of Hep-G2, MCF-7, and HT-29 cells was observed after treatment with petal and heart essential oil (Table 6). The cytotoxic effects of these oils can be attributed to their chemical constituent composition, which has possibly triggered the apoptosis through the caspase pathway as indicated by the induction of gene expression of caspase-3 [64]. However, *C. Scolymus* choke essential oil did not show any significant increase in caspase-3 gene expression. Moreover, the small molecular weight and lipophilic nature of its chemical constituents impart an added advantage of the essential oil, facilitating its absorption and permitting easy movement across the plasma membranes, thus increasing the interaction of essential oil interaction with intracellular proteins in intra-organelle site [65]. In several cases, the evaluation of individual component does not replicate the treatment effect with the whole essential oil. Therefore, it is unconvincing to assign the bioactivity of essential oil to an individual constituent but to phytoconstituents as a whole [66]. The synergistic effect of different constituents, including monoterpenes and abundant volatile components in the phytocomplex, may be the reason for the cytotoxic effect of essential oil. The pronounced cytotoxic effects of the essential oils extracted from petal and heart could be attributed to linoleic acid and palmitic acid, major components of both these oils, whereas choke essential oil displayed strong cytotoxic activity against MFC-7 cancer cells that could be possibly due to the high content of cyclopropane butanoic acid (21.3%) as reported in previous studies [67]. In turn, previous studies have shown that linoleic acid and palmitic acid present in the oil contribute to the cytotoxic effects against various cancer cell lines and were comparable to that of standard drug doxorubicin [68, 69]. Additionally, minor components would contribute also could be of great importance and might be contributing synergistically with major components of *C. Scolymus* essential oils (petal, choke, and heart).

4. Conclusion

This study reports the first detailed chemical characterization of the essential oil and inorganic constituents of the petal, choke, and heart parts of *C. scolymus* head using GC-MS and ICP techniques. The results have revealed that oil composition of petal, choke, and heart of *C. scolymus* head differed significantly from each other. The essential oil composition of petal, choke, and heart was dominated by fatty acids and oxygenated sesquiterpenes. The concentration of linoleic acid methyl ester and palmitic acid methyl ester is high in petal, followed by choke oil, whereas heart oil contains the highest content of cyclopropane butanoic acid. Thus, the existence of high content of linoleic acid methyl ester, palmitic acid methyl ester, and cyclopropane butanoic acid in petal, choke, and heart, respectively, could be the responsible factors for potential biological and industrial applications. All the isolated essential oils were evaluated for antibacterial, antioxidant, anti-inflammatory, and cytotoxicity property. All the oils displayed remarkable antibacterial, antioxidant, anti-inflammatory, and cytotoxic activity. Besides this, all the three samples showed the presence of important macroelements with K, Na, and Mg in high quantity. Thus, this study proposes the role of the organic components in the medicinal effects together with its inorganic constituents. It is also suggested that the petal, choke, and heart can be used as a source of many inorganic elements in case of deficiency. To the best of our knowledge, this is the first in-depth and comparative exploration of the essential oils obtained from petal, choke, and heart of *C. scolymus* globe. The significant differences in the constituents of the essential oils found in these three parts provide compelling evidence that petal, choke, and heart must be treated as three different constituents once analyzed.

Data Availability

The data used to support the findings of this research study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

S. R. M. A. preformed the experimental work and prepared the figures; H. A. A. supervisor designed and developed the idea, the research, and helped in writing manuscript. N. M. A. M. carried out the data interpretation, A. E. edited and revised the manuscript; S. R. A. preformed the GC-MS analysis; J. A. M. carried out ICP analysis; O. M. N. performed the biological studies; and M.A. designed the study and prepared the manuscript. All authors reviewed the manuscript.

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Supplementary Materials

The instrumental setting details applied for the elemental analysis are given in Table S1. (*Supplementary Materials*)

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