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Optimization of Different Extraction Methods of irradiated Chamomile on Antioxidant Activity and Chamazulene Content Using Response Surface Methodology

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ABSTRACT

This study successfully determined the optimal supercritical fluid (CO_2) extraction (SFE) parameters for recovering high-potency antioxidants from irradiated chamomile (*Matricaria recutita* L.). Utilizing a Box–Behnken response surface design, the effects of pressure, temperature, and extraction time were modeled to maximize radical-scavenging activity. Determinations of antioxidant activities of chamomile oil samples were accomplished using 2, 2-Diphenyl-1-picrylhydrazyl **(**DPPH) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) methods The optimization identified distinct coordinates for peak performance: 257.17 bar, 50°C, and 180 min for DPPH (IC_{50} = 162.15 µg/mL), and 272.72 bar, 50°C, and 131.51 min for ABTS (IC_{50} = 85.55 µg/mL). Validation trials confirmed the model's high predictive accuracy. While SFE-derived oils and powders exhibited robust antioxidant activity, they remained slightly below synthetic standards. Furthermore, Gas Chromatography / Mass Spectroscopy (GC/MS) analysis identified key bioactives - including α-bisabolol oxides, (E)-β-farnesene, and chamazulene - in accordance with European Pharmacopoeia standards, noting that maximum chamazulene recovery (7.12%) required a 4-hour steam distillation.

Keywords: Chamomile, Gamma irradiation, Response Surface Methodology, Antioxidants, DPPH, ABTS.

1. Introduction

Matricaria chamomilla L. possesses a broad spectrum of biological activities, including antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. These diverse functionalities extend its utility beyond the medicinal and veterinary sectors into areas such as food preservation, phytosanitary management, and industrial applications like anti-corrosive agents. The chemical profile of chamomile essential oil is highly complex; however, it is primarily characterized by its terpenoid content. Key bioactive constituents identified in the literature include bisabolol (and its oxides A and B), bisabolone oxide A, chamazulene, and beta-farnesene (El Mihyaoui *et al.,* 2022) and (Szőke *et al*. 2004).

Chamazulene, an aromatic bicyclic sesquiterpene (1,4-dimethyl-7-ethylazulene), is recognized as a premier bioactive constituent of chamomile due to its potent anti-inflammatory and radical-scavenging activities (Capuzzo *et al*., 2014). This blue-pigmented compound is not naturally present in the plant but is generated through the thermal degradation of matricin during the steam distillation of the essential oil (Alver *et al*., 2009; Salihović *et al.,* 2016). Despite its therapeutic importance, established literature on its isolation remains limited. Existing purification protocols are characterized by their complexity, often requiring multiple stages and various antioxidant assays such as DPPH and ABTS—to monitor the separation process (Capuzzo *et al*., 2014).

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Response Surface Methodology (RSM) encompasses a suite of mathematical and statistical tools designed for the refinement and optimization of complex processes. This approach is particularly effective at deciphering how multiple independent factors, and the synergy between them, influence a specific outcome (del Valle, 2015). Research by Gontard *et al*. (1992) and (Zaharia, Trofin *et al*. 2020) highlights the utility of RSM in extraction science, where it allows for the systematic assessment of variables to maximize both the yield and the overall quality of target compounds.

In a comparative study by Fırat *et al*. (2018), the *in vitro* antioxidant potential of chamomile essential oil (EO) was evaluated alongside its primary constituents, including alpha-bisabolol oxide A, (E)-beta-farnesene, chamazulene, and alpha-bisabolol. Utilizing the spectrophotometric DPPH radical-scavenging microdilution assay, researchers determined that chamazulene exhibited the most potent inhibitory activity. This was followed in descending order of efficacy by α-bisabolol oxide A, the whole chamomile essential oil, and (E)-β-farnesene.

Supercritical CO_2 extraction (SC-CO_2) has emerged as a superior alternative to traditional steam or hydro-distillation, offering significant operational benefits. By utilizing low temperatures and shorter processing durations, (SC-CO_2) prevents the thermal degradation of sensitive terpenes (Conde-Hernándeza *et al.,* 2017). Furthermore, it is highly regarded in the pharmaceutical, cosmetic, and food sectors as a "green" solvent; since the CO_2 can be entirely removed without additional energy intensive steps, it ensures a residue-free final product (Lucal *et al.,* 2023).

Research by Reverchon and Senatore, (1994) demonstrated the utility of supercritical CO_2 for generating chamomile extracts, which were subsequently refined into essential oil and cuticular wax through fractional separation. Operating at pressures between 15 and 20 MPa and temperatures ranging from 308 to 323 K, the authors confirmed that SFE simultaneously recovers both oil and wax components. Subsequent studies by Branislav *et al.* (1997) at a constant temperature of 313 K showed that increasing the pressure from 8 to 24 MPa directly correlated with higher extract quantities, reaching a peak yield of 0.44% (m/m). Furthermore, (Scalia *et al*., 1999) conducted a comparative analysis between SFE and traditional techniques - including Soxhlet extraction, maceration, and steam distillation - concluding that SFE is significantly more effective for isolating the primary bioactive constituents of chamomile flower heads.

The present study investigates the use of supercritical carbon dioxide (SC-CO_2) for the recovery of irradiated chamomile extracts. The primary objectives involve the application of a theoretical extraction model and the identification of optimal processing parameters, specifically temperature, time and pressure. Furthermore, this research evaluates how different extraction methodologies influence the resulting chamazulene concentration and overall antioxidant capacity of the extracts

2. Materials and Methods

2.1. Plant material

Fresh Egyptian chamomile (*Matricaria chamomilla*) specimens were procured from local commercial suppliers. The flowers were manually separated from the biomass, achieving a yield of 14–15% (w/w). To preserve the integrity of the bioactive compounds, the floral material was subjected to immediate processing following separation.

2.2. Chemicals and Reagents

All solvents utilized for the isolation of compounds were of HPLC grade and obtained from Sigma-Aldrich (Steinheim, Germany). The chemical reagents, including 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, 98% purity), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and the synthetic antioxidant tert-butylhydroquinone (TBHQ, 99.9% purity), were also sourced from Sigma-Aldrich. Absolute ethanol and methanol used in the study were of analytical grade from the same supplier.

2.3. Extraction methods of essential oils

2.3.1. Steam distillation

Essential oils were extracted from 250 g samples via 4-hour hydro-distillation in a Clevenger apparatus. Following extraction, the characteristic blue oils were dried over anhydrous sodium sulfate. To prevent photodegradation and thermal alterations, the samples were kept in sealed brown glass vials and refrigerated at 4°C until the analytical phase.

2.3.2. Super critical fluid CO_2 extraction

2.3.2.1. Experimental Process

Supercritical Fluid Extraction (SFE) was employed to recover oleoresins from chamomile (*Matricaria chamomilla*). To determine the most efficient extraction parameters, Response Surface Methodology (RSM) was implemented via a Box–Behnken experimental design. This approach allowed for the simultaneous optimization of pressure, temperature, and extraction duration. All statistical modeling and data processing were conducted using Minitab software (version 18.1; Minitab Inc., State College, PA, USA), leading to the establishment and subsequent validation of the optimal processing conditions (Setyani *et al*., 2023).

2.3.2.2. Chamomile Preparation

Egyptian chamomile (*Matricaria chamomilla*) was subjected to a 48-hour dehydration process using a solar dryer maintained at 40°C. Following the drying phase, the plant material was pulverized to an 80-mesh particle size using a specialized grinder (DA280-S; Daesung Artlon, Paju, Republic of Korea). To ensure sample stability, the ground chamomile was preserved in a desiccator under controlled conditions, specifically at a temperature of 23°C and a relative humidity of 14% (Saleh *et al.,* 2024).

2.4. Irradiation treatment

Fine powder and oil fractions of chamomile were subjected to gamma irradiation at doses of 0, 5, 10, and 15 kGy. The treatment was conducted using a ^{60}Co Russian Gamma chamber with a constant dose rate of 665.6 Gy/h, located at the Cyclotron Project of the Nuclear Research Center (Egyptian Atomic Energy Authority, Egypt). Following the irradiation procedure, all processed samples were refrigerated at 4°C to preserve their chemical profile for subsequent experimental analysis.

2.5. Response Surface Methodology Design

2.5.1. Experimental Design

Process optimization for the SFE method was performed using Minitab software (State College, PA, USA), implementing a Response Surface Methodology (RSM) through a Box–Behnken experimental design (Baldwin *et al*., 2012). Based on preliminary trials, three independent variables were established and coded into three levels (−1, 0, 1): extraction duration (60–180 min), temperature (50–80°C), and pressure (120–400 bar). Following the design matrix, extractions were executed using a laboratory-scale supercritical fluid extractor.

2.5.2. Result Analysis

The antioxidant potential, quantified as IC50 values (µg/mL) for both DPPH and ABTS assays, served as the primary dependent variables in the regression analysis. Following the Box–Behnken design, fifteen distinct experimental runs were conducted (Table 1). The resulting response models demonstrated high reliability, with coefficients of determination (R^2) exceeding 0.95. These values, being near unity, suggest a robust fit between the experimental data and the mathematical models. Furthermore, three-dimensional surface plots were employed to evaluate the interactive effects of the independent variables on each antioxidant response.

2.6. Pilot Supercritical Fluid CO_2 Extraction (SFE) Method

A total of 14 kg of air-dried chamomile, pulverized to a 1 mm particle size, was loaded into a 30 L pilot-scale Supercritical Fluid Extraction (SFE) unit (Applied Separations, Inc., Allentown, PA, USA). The sample was evenly distributed between two 15 L extraction vessels. The specific operational parameters for pressure, temperature, and duration are detailed in Table 1. Following the extraction process, the recovered material was maintained at ambient temperature for approximately 2 hours to allow for the complete evaporation of residual CO_2. The final extract weight was then recorded for subsequent analytical procedures.

Table 1: Experimental values of DPPH and ABTS (IC_{50}) as antioxidant indicators based on extraction pressure, temperature, and time using the Box–Behnken design

	Experiment No.	Pressure (Bar)	Temperature (°C)	Time (min.)	DPPH (IC_{50} µg/mL)	ABTS (IC_{50} µg/mL)
Irradiated Chamomile Oil	**(1) 5 kGy**	120	50	120	260.03	143.64
	(2) 10kGy	120	65	60	274.70	201.03
	(3) 15kGy	120	80	120	252.61	140.86
Irradiated Chamomile Powder	**(4) 5kGy**	400	65	60	250.53	265.19
	(5) 10kGy	400	50	120	236.05	126.75
	(6) 15kGy	400	80	120	219.73	247.44
Control (0 kGy)	**(7) 0 kGy**	120	65	180	226.29	220.04
	(8) 0 kGy	400	65	180	236.21	236.76
	(9) 0 kGy	260	50	60	222.54	154.27
	(10) 0 kGy	260	80	60	187.92	155.02
	(11) 0 kGy	260	50	180	160.09	118.02
	(12) 0 kGy	260	80	180	192.44	135.69
	(13) 0 kGy	260	65	120	192.08	133.94
	(14) 0 kGy	260	65	120	191.22	133.32
	(15) 0 kGy	260	65	120	191.35	133.88
Optimal condition						
	DPPH	257.17	50	180	162.15	--
	ABTS	272.72	50	131.51	--	85.55
	TBHQ	--	--	--	73.22	91.32

TBHQ = tert-butylhydroquinone

2.7. Antioxidant activity analysis

Determinations of antioxidant activities of chamomile samples were accomplished using DPPH and ABTS methods as follows:

2.7.1. The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant potential of chamomile was determined across a concentration gradient (50, 100, 150, and 200 µg/mL) using the DPPH radical-scavenging method (Viuda-Martos *et al*., 2010). Briefly, each concentration was added to 4 mL of a 0.004% methanolic DPPH solution. Following a 30-minute incubation period at ambient temperature, the absorbance was measured at 517 nm against a methanol blank. The inhibition percentage (%) was calculated using the following relationship:

$$\boldsymbol{DPPH\ scavenging\ activity}\ (\%) = \frac{\boldsymbol{A_{blank} - A_{sample}}}{\boldsymbol{A_{blank}}} \times \mathbf{100}$$

Where, A_{blank} denotes the absorbance of the control (reagents only), while A_{sample} represents the absorbance of the chamomile extract. All analyses were performed in triplicate to ensure reproducibility. The IC_{50} value - defined as the extract concentration required to achieve 50% inhibition of the DPPH• radical - was determined by plotting the inhibition percentages against the corresponding extract concentrations. Under this analytical framework, lower IC_{50} values (µg / mL or ppm) signify a more potent antioxidant capacity.

2.7.2. The 2,2´-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity

The ABTS radical scavenging activity of the chamomile extracts was determined following the protocols of Mohafrash and Mossa, (2020); El Kar *et al.* (2011). To generate the ABTS• cation, a 7 mM ABTS solution (0.0384 g in 10 mL distilled water) was reacted with 2.45 mM potassium persulfate (0.0066 g in 10 mL distilled water) in equal proportions. The mixture was incubated in the dark at ambient temperature for 12 – 16 hours to ensure radical stability. Before use, the stock was diluted with distilled water to achieve a working absorbance of 0.700 ± 0.02 at 734 nm. Various sample concentrations (50 – 200 µg/mL) were then mixed with 4 mL of the ABTS working solution.

After a 30-minute dark incubation, the absorbance was recorded at 734 nm against a sample-free control.

$$\textbf{\textit{ABTS scavenging activity}}\ (\%) = \frac{\boldsymbol{A_{blank} - A_{sample}}}{\boldsymbol{A_{blank}}} \times \mathbf{100}$$

In this calculation, A_{blank} represents the control absorbance, while A_{sample} denotes the absorbance of the chamomile extract. To ensure statistical reliability, all assays were performed in triplicate. The resulting antioxidant values are expressed as the mean ± standard deviation (SD) in µg/mL. These data were subsequently compared against the performance of tertiary butylhydroquinone (TBHQ), which served as a synthetic antioxidant reference standard.

2.8. Gas Chromatography–Mass Spectrometry (GC/MS) analysis

Chromatographic analysis was conducted at the Central Laboratory of Chromatography (Radioisotopes Application Division, Nuclear Research Center, Egyptian Atomic Energy Authority) using an Agilent Technologies 8890GC system. The unit was equipped with an automated liquid sampler and coupled to an Agilent 5977B series Mass Selective Detector (MSD). Separation was achieved on a DB-5ms capillary column (60 m x 250 µm x 0.25 µm). The oven temperature program commenced at 50°C (2 min), 5°C/min / 110°C (2 min), and further ramped by 5°C/min to a final temperature of 240°C (5 min). Helium served as the carrier gas at a constant flow rate of 1 mL/min. The temperature of injector was set at 250 °C, transfer line temperature was set as 250 °C and ion source at 250 °C. Injection mode was split ratio 50:1 and solvent delay was 4 min. Mass spectra were acquired in electron impact (EI) mode at 70 eV, covering a scan range of 50–550 amu. Data processing was performed using MSD Mass-Hunter software, with compound identification accomplished by matching mass spectra against the National Institute of Standards and Technology (NIST20) library.

2.9. Statistical Processing

All experimental analyses were performed in triplicate to ensure data reproducibility. Statistical evaluation was carried out using Minitab software, where a one-way analysis of variance (ANOVA) was utilized to determine significant differences between treatment groups. Statistical significance was established at a 95% confidence interval ($p < 0.05$).

3. Results and Discussions

3.1. Experimental Design Based on Response Surface Analysis

3.1.1. Effects of Box–Behnken independent variables and correlation with applied models

Previous researches has established that terpene solubility in supercritical fluids is a critical factor influencing overall extraction yields (Hong *et al*., 2024). To maximize the recovery of chamomile oleoresins and enhance their corresponding antioxidant properties, this study utilized a Box–Behnken response surface design to evaluate 15 distinct experimental conditions. As detailed in Table 1, the antioxidant capacities (quantified via DPPH and ABTS assays) were analyzed as a function of three primary independent variables: extraction time (60–180 min), temperature (50–80°C), and pressure (120–400 bar).

3.1.1.1. DPPH-IC_{50} value

In the DPPH assay, the Half Maximal Inhibitory Concentration (IC_{50}) serves as a critical indicator of antioxidant potency, where a lower numerical value (µg/mL) represents superior radical-scavenging activity. The most effective antioxidant performance was achieved in Experiment 11 (180 min; 50°C; 260 bar), yielding an IC_{50} of 160.09 µg/mL. In contrast, the lowest activity (highest IC_{50} value) was recorded in Experiment 2 (60 min; 65°C; 120 bar) at 274.70 µg/mL. These results indicate that extraction parameters significantly influence antioxidant capacity, with IC_{50} values fluctuating between 160.09 and 274.70 µg/mL across the 15 experimental runs. To quantify the specific impact of each independent variable, the response data were processed via Minitab software to generate the following regression equation:

DPPH-IC$_{50}$ = *550.4 – 1.532 Pres. – 0.55 Temp. – 1.859 time + 0.002723 (Pres. x Pres) – 0.0125 (Temp. x Temp.) + 0.00056 (time x time) – 0.00106 (Pres. x Temp.) + 0.001015 (Pres. x time) + 0.01860 (Temp. x time)*

To optimize antioxidant capacity, a second-order polynomial model was generated via Response Surface Methodology (RSM) to express DPPH IC_{50} as a function of pressure, temperature, and time. This model incorporates the linear, quadratic, and interactive impacts of these parameters. Through regression analysis, the coefficients were calculated to align predicted outcomes with experimental observations, resulting in an optimized equation for radical scavenging activity. Regarding the irradiation treatment, irradiated chamomile oil shows relatively high IC_{50} values for DPPH (ranging from 252.61 to 274.70 µg/mL). The highest IC_{50} for DPPH (274.70 µg/mL) occurred in the oil at 10 kGy (Exp. 2), indicating that specific combinations of irradiation and extraction parameters might slightly diminish radical scavenging efficiency.

On the other hand, Box–Behnken design (response surface plots) highlights how physical variables dictate extraction efficiency as shown in Fig. 1. As a function of temperature and time, the lowest IC_{50} for DPPH among the control samples (160.09 µg/mL) was achieved at 260 Bar, 50°C, and 180 minutes (Exp. 11). This indicates that lower temperatures combined with longer extraction times may preserve heat-sensitive antioxidant compounds. While, increasing pressure from 120 to 260 Bar generally improved (lowered) IC_{50} values in the control group. For example, at 260 Bar, DPPH values frequently stayed below 200 µg/mL, whereas at 120 Bar, they exceeded 220 µg/mL. Thus, the light green areas (< 180 – 200 µg/mL) indicate the "sweet spot" of the extraction process. According to the earlier experimental data, this often occurs at moderate pressures (~260 bar) and lower temperatures (~50°C). The dark green regions indicate conditions where the antioxidant activity is poor. This could be due to incomplete extraction at low pressure or the thermal degradation of chamomile compounds at excessively high temperatures.

3.1.1.2. ABTS-IC$_{50}$ value

In the ABTS assay, the half maximal inhibitory concentration (IC_{50}) serves as a primary metric for antioxidant potential, where lower concentrations IC_{50} (µg/mL) signify a more potent radical-scavenging effect. The most effective antioxidant activity was recorded in Experiment 11 (180 min; 50 °C; 260 bar), achieving an IC_{50} of 118.02 µg/mL. Conversely, the least potent extract was produced in Experiment 4 (180 min; 50°C; 400 bar), resulting in an IC_{50} of 265.19 µg/mL. These findings demonstrate that supercritical fluid extraction (SFE) conditions significantly modulate the antioxidant capacity of chamomile, with values spanning a range of 118.02 to 265.19 µg/mL. To evaluate the specific influence of each processing variable, the following regression equation was derived using Minitab software:

ABTS-IC$_{50}$ = *78 – 2.238 Pres. + 13.81 Temp. – 2.511 time + 0.003086 (Pres. x Pres.) – 0.1312 (Temp. x Temp.) + 0.01016 (time x time) + 0.01470 (Pres. x Temp.) – 0.00141(Pres. x time) + 0.00470 (Temp. x time)*

The IC_{50} values for ABTS (measured in µg/mL) were modeled as a function of the independent variables: extraction time (t), Temperature (T), and Pressure (P). Using Response Surface Methodology (RSM), a second-order polynomial equation was developed to fit the experimental results. This mathematical model accounts for linear effects, quadratic curvatures, and the interactions between the three primary extraction factors. To ensure the highest accuracy in predicting radical scavenging activity, regression analysis was applied to determine the coefficients by minimizing the deviation between observed data and the model's predictions.

The radical-scavenging capacity of irradiated chamomile oil was characterized by relatively high IC_{50} values for the ABTS activity, ranging from 140.86 to 201.03 µg/mL. These results suggest that the essential oil fraction may possess lower overall antioxidant potency than the powdered form or specific control samples. In contrast, the irradiated chamomile powder exhibited superior performance; for instance, at a 10 kGy dose (Experiment 5), an ABTS-IC_{50} of 126.75 µg/mL was achieved, indicating a more robust scavenging activity. As illustrated in the 3D response surface plots (Fig. 2), the optimization objective was to identify the minimum point on the surface, representing the

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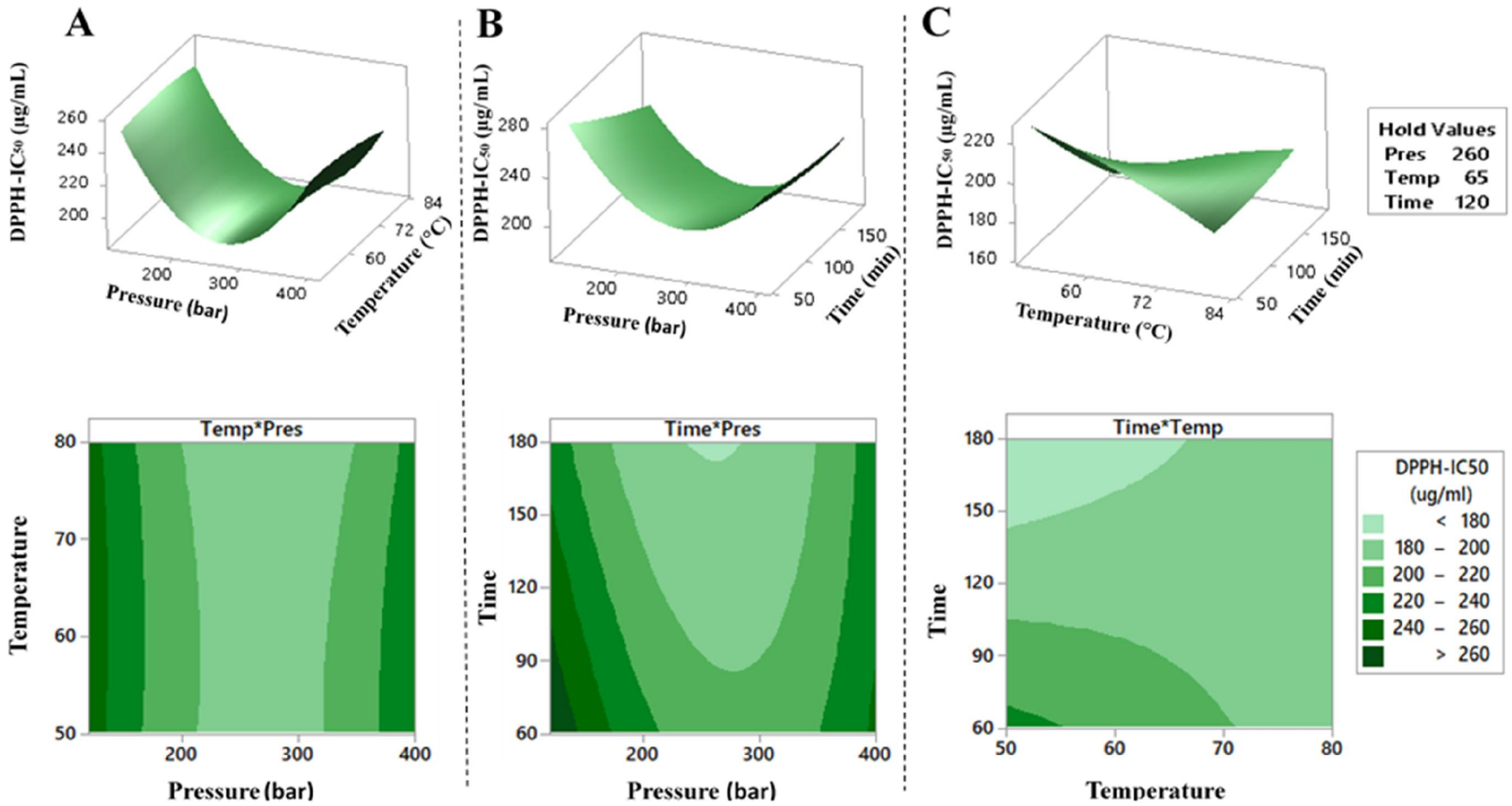

Fig. 1. Response surface plots for DPPH-IC_{50} Value at constant values of extraction time, temperature, and pressure: (A) DPPH-IC_{50} Value as a function of extraction pressure and temperature, with time fixed at 120 min; (B) DPPH-IC_{50} Value as a function of extraction time and pressure, with extraction temperature fixed at 65 ˚C; and (C) DPPH-IC_{50} Value as a function of extraction time and temperature, with extraction pressure fixed at 260 Bar.

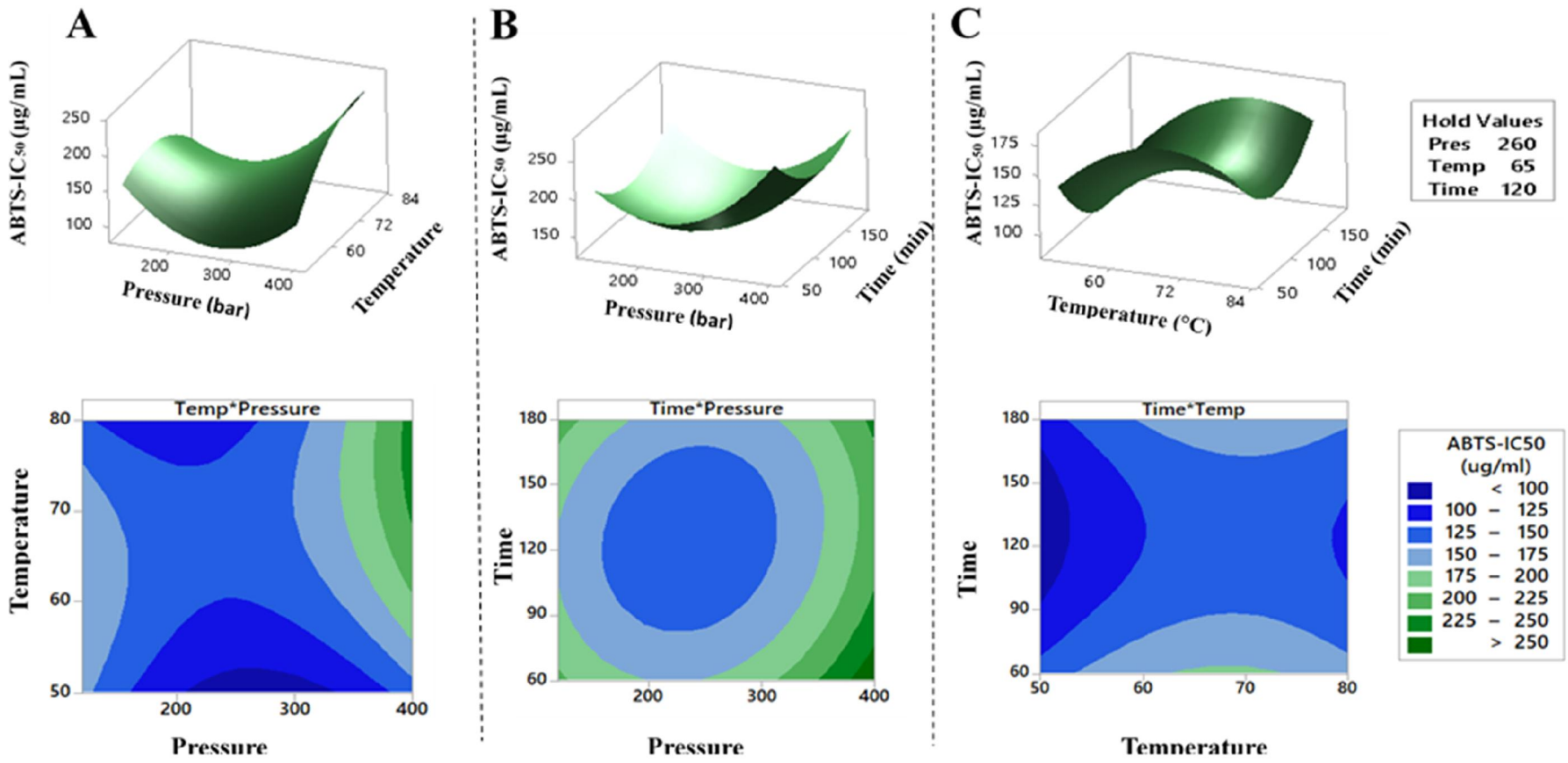

Fig. 2: Response surface plots for ABTS-IC50 Value at constant values of extraction time, temperature, and pressure: (A) ABTS-IC50 Value as a function of extraction pressure and temperature, with time fixed at 120 min; (B) ABTS-IC50 Value as a function of extraction time and pressure, with extraction temperature fixed at 65 ◦C; and (C) ABTS-IC50 Value as a function of extraction time and temperature, with extraction pressure fixed at 260 Bar**.**

maximum antioxidant activity. The experimental data identified the absolute optimal parameters for ABTS as 272.72 bar, 50°C, and 131.51 minutes, which produced a notably low IC_{50} of 85.55 µg/mL.

While the antioxidant trends for ABTS generally correlated with those observed in the DPPH assay, the ABTS method demonstrated superior sensitivity to the diverse lipophilic and hydrophilic constituents of the chamomile extract. Optimization of the ABTS response required a slightly higher pressure (272.72 bar) compared to DPPH (257.17 bar), yet achieved peak potency in a significantly shorter duration (131.51 min vs. 180 min). Most notably, the optimized chamomile extract (85.55 µg/mL) displayed higher radical-scavenging activity than the synthetic antioxidant TBHQ (91.32 µg/mL). This high efficacy is visually evidenced by the distinct "wells" or optimal response zones within the 3D surface models.

3.2. Optimization process for Antioxidant Activity

3.2.1. DPPH-IC_{50} and ABTS-IC_{50} Values from extraction Process Optimization

This study successfully optimized the supercritical fluid extraction (SFE) parameters for chamomile antioxidants using Response Surface Methodology (RSM), evaluating the complex interactions between pressure, temperature, and time. The optimized coordinates are illustrated in Figure 3 for both DPPH (Fig. 3A) and ABTS (Fig. 3B) assays. For the DPPH response, the model identified optimal conditions at 257.52 bar, 50°C, and a 180-minute extraction duration, yielding a predicted DPPH-IC_{50} of 162.15 µg/mL. In contrast, the ABTS optimum was achieved at a higher pressure of 272.72 bar and a shorter time of 131.51 minutes at 50°C, resulting in a significantly lower predicted ABTS-IC_{50} of 85.55 µg/mL.

The high degree of congruence between the predicted and experimental outcomes underscores the precision and reliability of the response surface model for practical applications (Fernández-Ponce *et al*., 2016). Model validation was achieved by benchmarking the predicted extraction yields against empirical data. The marginal discrepancy between the theoretical and observed yields was statistically insignificant, confirming the robustness of the optimization framework. Consequently, this predictive model serves as a highly effective tool for process engineers seeking to maximize extraction efficiencies in chamomile processing.

The second-degree polynomial equations derived for each response variable within the RSM framework demonstrated high statistical significance. These results confirm that the optimized response surface model is an appropriate and effective tool for explaining the extraction phenomena. As noted by Tyśkiewicz *et al.* (2019), the significance of these polynomial equations highlights the critical role of quadratic terms in modeling; they are essential for capturing the non-linear, complex interactions between processing variables that simpler linear models might overlook.

The SFE process offers several distinct advantages, notably high extraction efficiency and the preservation of potent antioxidant activity. By eliminating the need for toxic organic solvents, this technique aligns with "green" chemistry and sustainable industrial practices. It is particularly suitable for high-value applications where solvent-free extracts are required to meet safety and environmental standards. However, the method is constrained by high capital expenditure for equipment and substantial energy requirements to maintain supercritical pressures and temperatures, which can affect its cost-effectiveness at an industrial scale. Future research may explore hybrid extraction systems, combining SFE with other intensification technologies to further optimize yield and process economics.

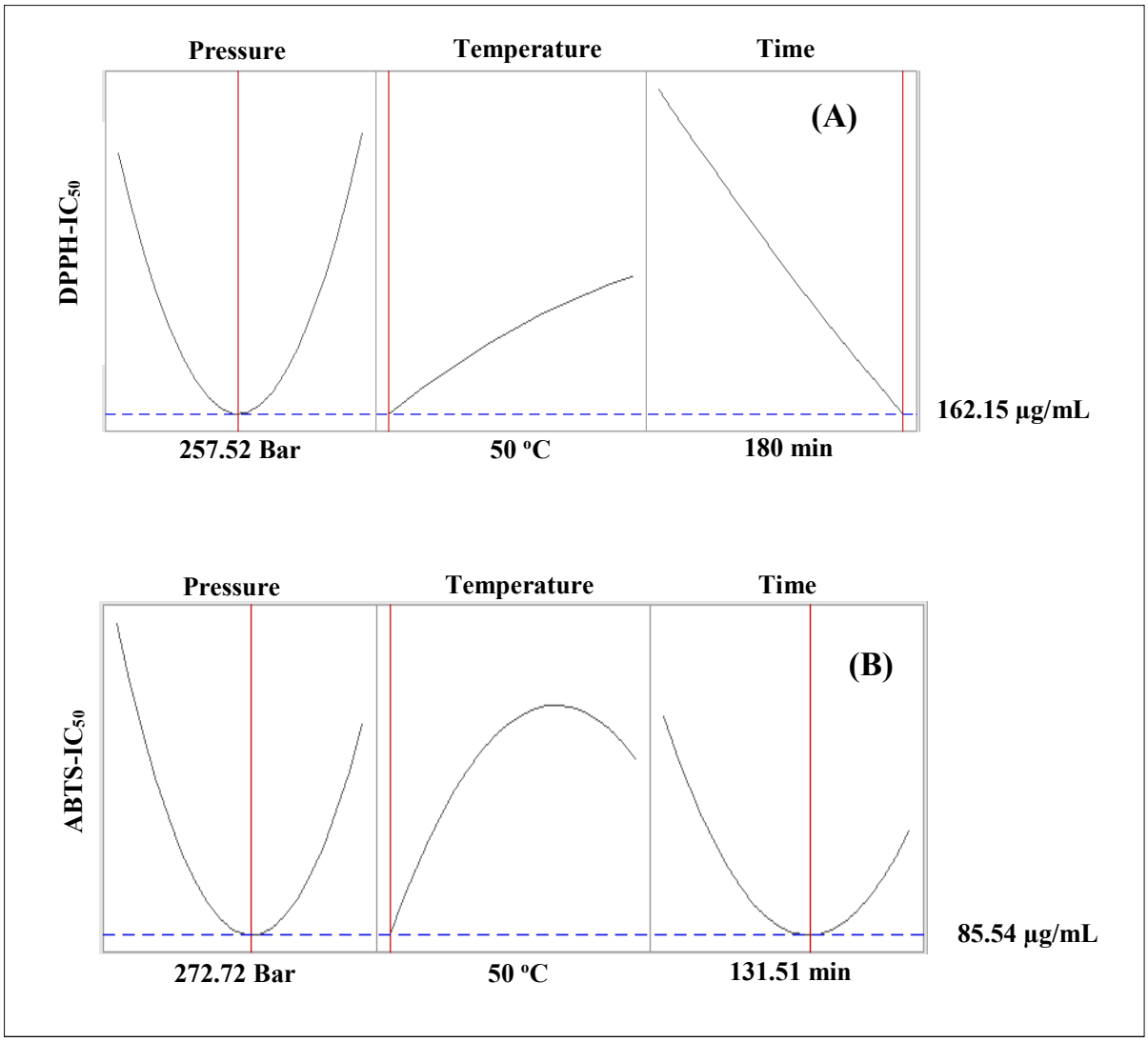

Fig. 3: Response surface optimization graph of; A) DPPH-IC_{50} and B) ABTS-IC_{50} Values

3.3. Gas Chromatography–Mass Spectrometry (GC/MS) Analysis of Essential Oils

The volatile profile of the chamomile essential oil is detailed in Table 2, with the corresponding representative chromatogram illustrated in Figure 4. A total of twenty-four distinct compounds were identified within the distilled oil control. Across all experimental treatments, the chemical composition was dominated by several key sesquiterpenes and coumarins, specifically α-bisabolol oxides A and B, *cis*-en-yne-dicycloether, bisabolone oxide, β-trans-farnesene, α-bisabolol, and chamazulene. These findings align with the characteristic chemical markers established for chamomile in previous literature (Szőke *et al*., 2004).

Chamazulene is an artifact rather than a direct secondary metabolite; it is generated through the thermal degradation of matricin during the extraction process. The presence of this compound imparts a characteristic blue hue to the resulting essential oil, the intensity of which is directly proportional to the chamazulene concentration. Low concentrations yield a pale blue tint, while higher levels result in a deep, saturated blue coloration. In comparison, Roman chamomile (*Chamaemelum nobile* L.) typically contains chamazulene in a relatively low range of 0 to 4.4% (Ciko *et al.,* 2016) and (Karadağ *et al.*, 2023).

Based on the analysis in Fig. 4 and data tabulated in Table 2, there is a clear and interesting trend regarding Chamazulene and its precursor, 3,6-Dihydrochamazulene, as the extraction time (which implies increasing cumulative heat exposure or "increasing temperature") progresses. Chamazulene

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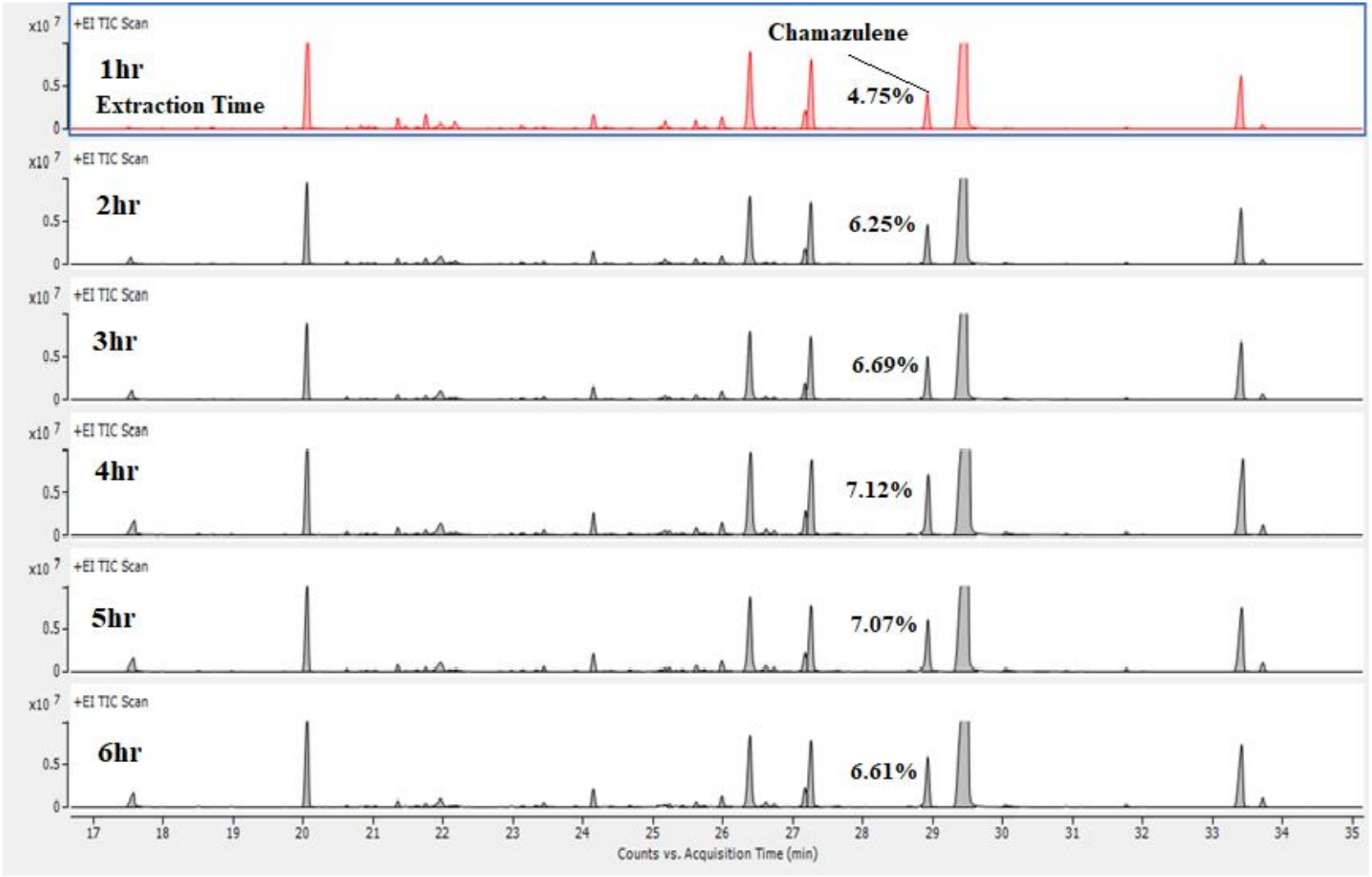

Fig. 4. GC/MS chromatogram of different interval time extraction (Hydro-distillation) of chamomile essential oil

content starts at 4.75% (after one hour extraction) and steadily increases to a peak of 7.12% at the 4-hour mark. This is the classic behavior of chamomile distillation, where Matricin (a colorless sesquiterpene lactone naturally present in the plant) is thermally degraded into Chamazulene (the compound responsible for the blue color of the oil) (Karadağ *et al*., 2023). On the other hand, 3,6-Dihydrochamazulene shows peaking earlier (at 3 hours with 3.32%) and then declining significantly to 1.51% by the 6th hour. Chemically, dihydrochamazulenes are intermediates in the formation of Chamazulene. The data reflects this relationship: as the intermediate (dihydrochamazulene) is consumed or converted, the final product (Chamazulene) continues to rise before stabilizing. After 4 hours, the Chamazulene content begins to slightly decrease (from 7.12% to 6.61%). This suggests that after a certain point of heat exposure, the rate of new Chamazulene formation from precursors slows down, and the compound may begin to undergo minor thermal degradation or evaporate at a rate higher than its formation. Our findings were in similar like that obtained by Gawde *et al.* (2014), who found that the increasing in extraction time, maximizing chamazulene content of chamomile.

Table 2. GC/MS of chamomile essential oil extracted by hydro distillation at different interval times

			Extraction time					
No	**RT (min)**	**Compounds**	**1 hr**	**2 hr**	**3hr**	**4hr**	**5hr**	**6hr**
1	**17.53**	**n-Decanoic acid**	ND	1.31	1.82	2.42	2.4	2.46
2	**20.06**	**(E)-β-Farnesene**	13.91	10.85	9.95	9.54	10.14	10.79
3	**20.82**	**9-epi-β-Caryophyllene**	0.43	ND	ND	ND	ND	ND
4	**20.94**	**β-Himachalene**	0.39	ND	ND	ND	ND	ND
5	**21.35**	**Germacrene D**	1.12	0.76	0.65	0.68	0.64	0.77
6	**21.45**	**α-Farnesene**	0.38	ND	ND	ND	ND	ND
7	**21.63**	**β-Selinene**	0.36	ND	ND	ND	ND	ND
8	**21.75**	**β-Cyclogermacrane**	1.69	0.89	0.59	0.53	0.44	0.54
9	**21.96**	**3,6-Dihydrochamazulene**	2.33	3.31	3.32	3.25	1.99	1.51
10	**22.16**	**γ-Cadinene**	1.18	0.73	0.85	0.69	ND	ND
11	**23.12**	**E-Farnesene epoxide**	0.55	0.81	ND	ND	ND	ND
12	**23.44**	**Dendrasaline**	ND	0.38	0.75	0.5	0.47	0.88
13	**24.15**	**Espatulenol**	1.74	1.65	1.73	2.05	2.02	2.01
14	**25.61**	**.[(2,2,3,3-Tetramethylcyclopropylidene)methyl] benzene**	0.92	0.83	0.73	0.77	0.64	0.81
15	**25.98**	**tau.-Cadinol**	1.46	1.33	1.62	1.37	1.29	1.29
16	**26.38**	**(-)-α-Bisabolol oxide B**	12.34	11.7	11.34	10.77	11.38	11.18
17	**27.17**	**α-Bisabolol**	2.22	2.12	1.97	2.28	2.54	2.12
18	**27.26**	**α-Bisabolone oxide A**	10.55	9.78	9.89	9.79	9.16	9
19	**26.73**	**β-Santalol**	ND	ND	ND	0.38	0.4	0.43
20	**28.92**	**Chamazulene**	4.75	6.25	6.69	7.12	7.07	6.61
21	**29.47**	**α-Bisabolol oxide A**	34.81	36.93	36.99	35.55	37.9	38.33
22	**33.41**	**cis-ene-yne-Dicycloether**	8.26	9.63	10.26	10.86	10.09	9.74
23	**33.71**	**1,6-Dioxaspiro[4.4]non-3-ene, 2-(2,4-hexadiyn-1-ylidene)-**	0.61	0.74	0.85	1.05	1.02	1.07
24	**35.39**	**n-Hexadecanoic acid**	ND	ND	ND	0.4	0.41	0.46

4. Conclusion

Irradiated chamomile calyces were processed using a pilot-scale supercritical fluid CO_2 extraction (SFE) system across varying pressure, temperature, and time intervals. Response Surface Methodology (RSM) was implemented to determine the ideal SFE parameters for maximizing antioxidant capacity. Based on DPPH and ABTS assays, the optimal extraction conditions were

identified as 257.17 bar, 50°C, and 180 min for DPPH, and 272.72 bar, 50°C, and 131.51 min for ABTS. In contrast to the SFE process, the chamazulene yield in steam-distilled essential oil was found to be temperature-dependent, increasing significantly with higher thermal exposure.

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