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# EXTRACTION CONDITIONS FOR DIFFERENT PARTS OF MELOCAN (*SMILAX EXCELSA* L.) USING SUPERCRITICAL CARBON DIOXIDE

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## ABSTRACT
Melocan, which is very important in both traditional Turkish cuisine and traditional medicine, especially in the Black Sea region of Türkiye, is a climbing, thorny and perennial plant that grows naturally in the bushy woodlands. In the study, different melocan parts (sprout, fruit and leaf) have been extracted under two different pressure levels (250 and 350 bar), temperatures (30 and 50 °C), durations (60 and 90 minutes), and two different levels of ethanol concentration (10% and 20%) through supercritical carbon dioxide (Sc-$CO_2$) extraction method, which is an environmentally-friendly method. It has been observed that the melocan leaf which has been extracted through Sc-$CO_2$ at 350 bar pressure and 50 °C which has been modified with 20% ethanol for 90 mins has the richest total phenolic substance content (TPC) as 2265.1 mg gallic acid equivalent (GAE)/kg dry weight and total antioxidant capacity (TAC) as 2876.7 mg trolox equivalent (TE)/kg dry weight.
**Keywords:** Supercritical carbon dioxide, co-solvent, phenolic substances, antioxidant capacity, *Smilax excelsa*

# MELOCAN'IN (*SMILAX EXCELSA* L.) FARKLI KISIMLARI İÇİN SÜPERKRİTİK KARBON DİOKSİT EKSTRAKSİYON KOŞULLARI

## ÖZ
Özellikle Türkiye'nin Karadeniz bölgesinde hem geleneksel mutfak kültüründe hem de geleneksel tıpta oldukça önemli yere sahip olan melocan, ormanlık arazilerin çalılık bölgelerinde kendiliğinden yetişen tırmanıcı, dikenli ve çok yıllık bir bitkidir. Bu çalışmada çevre dostu bir yöntem olan süperkritik karbon dioksit (Sc-$CO_2$) ekstraksiyon yöntemi ile melocan bitkisinin farklı kısımları (filiz, meyve ve yaprak); iki farklı basınç (250 ve 350 bar), sıcaklık (30 ve 50 °C), süre (60 ve 90 dakika (dk)) ve iki farklı etanol konsantrasyonunda (%10 ve %20) ekstrakte edilmiştir. 350 bar basınç ve 50 °C'de, %20 etanol ile 90 dk Sc-$CO_2$ ekstraksiyonuna tabii tutulan melocan yaprağının; 2265.1 mg gallik asit eşdeğeri (GAE)/kg kuru ağırlık olarak toplam fenolik madde (TFM) ve 2876.7 mg troloks eşdeğeri

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(TE)/kg kuru ağırlık olarak toplam antioksidan kapasite (TAK) açısından en iyi sonucu verdiği belirlenmiştir.
**Anahtar kelimeler:** Süperkritik karbon dioksit, yardımcı çözücü, toplam fenolik madde, antioksidan kapasite, *Smilax excelsa*

## INTRODUCTION

Dietary habits have an important effect on human health. Some dietary ingredients and habits are beneficial for our health, whereas others are known to have potentially toxic effects and cause health problems (Estévez et al., 2017). Free radicals, derived from oxygen, sulfur, and nitrogen, are highly unstable and active molecules that contain one or more unpaired electrons and go into chemical reactions with other molecules (Lü et al., 2010; Carocho and Ferreira, 2013). Free radicals, derived from normal essential metabolic processes in the human body or external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals cause oxidative damage in living systems (Bagchi and Puri, 1998). In the human body, oxygen radicals, which can damage DNA, RNA, proteins, and lipids, cause several diseases, including cancer, diabetes, cardiovascular diseases, and ageing (Ozsoy et al., 2008; Lü et al., 2010). Oxidative damage caused by these reactive oxygen species is called oxidative stress (Sies, 1986). Oxidative stress can also lead to the development of neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases (Foy et al., 1999).

Antioxidants are vital substances that possess the ability to repair the oxidative damage caused by free radicals in the human body (Silva et al., 2005). Antioxidants are divided into natural and synthetic ones based on their occurrence (Aziz et al., 2019). In the past, synthetic antioxidants were generally used to repair oxidative damage. Recent studies show synthetic antioxidants have lower antioxidant activity, may show toxicity, have carcinogenic effects, and require higher costs than natural antioxidants. Therefore, there is a growing trend towards natural antioxidants that can be taken in through food (Uzombah, 2022). Natural antioxidants, most of which are phenolic compounds, are found in many foods and medicinal plants (Ho, 1992; Xu et al., 2017). Phenolic compounds, which include phenolic acids, flavonoids, tannins, stilbenes, curcuminoids, coumarins, lignans, quinones, and so on, are secondary metabolites isolated from plants (Huang et al., 2010). Phenolic compounds offer protection against the development of cancer, cardiovascular diseases, diabetes, osteoporosis and degenerative diseases (Albuquerque et al., 2021).

*Smilax*, commonly called sarsaparilla, is the genus of the Smilacaceae family. The genus *Smilax* species, characterized as climbers with long, thin, thorny stems, grow in temperate, tropical, and subtropical zones worldwide. Traditionally, plants from the *Smilax* genus are used for the treatment of some diseases such as syphilis, acute bacilli dysentery, acute and chronic nephritis, eczema, dermatitis, cystitis, mercury-silver poisoning, breast cancer, stomach pain, and swelling (Yeşilada et al., 1999; Ivanova et al., 2010). *Smilax excelsa L.* is one of the two species growing in Turkey also known as *Anatolian sarsaparilla, melocan, diken otu, melvocan, kircan, citirgi, melevcen, siraca, silcan,* and *mamula* (Yıldız et al., 2018). The leaves of the melocan plant are cordate, and its fruits have a spherical shape (Tanker et al., 1993). The shoots of melocan start to give young shoots in spring, which can be consumed as a vegetable. Known as a medicinal and economic plant, melocan is also used for treating stomach pain, breast cancer, and bloating in folk medicine (Yeşilada et al., 1999; Özbucak et al., 2007). The different parts of melocan are shown in Figure 1. Researchers found that the leaves and shoots of melocan possess flavonoids and anthocyanins, which are the main chemical constituents responsible for antioxidant activity (Ozsoy et al., 2008).

For phenolic compounds to be used both in industrial applications and in scientific research, they must first be extracted from the food. There is no standard and unique method for extracting phenolic compounds (Ignat et al., 2011). One of the traditional extraction methods, Soxhelet extraction has major disadvantages such as the need for large amounts of raw materials, long processing time required, application at high temperatures, and waste problems (Castro and Priego-Capote, 2010). Therefore, new techniques that minimize the use of organic solvents, shorten the extraction time, and are environmentally friendly have emerged. Alternative extraction techniques developed for this purpose are ultrasound-assisted, microwave-assisted, and supercritical fluid extraction (Jahromi, 2019).

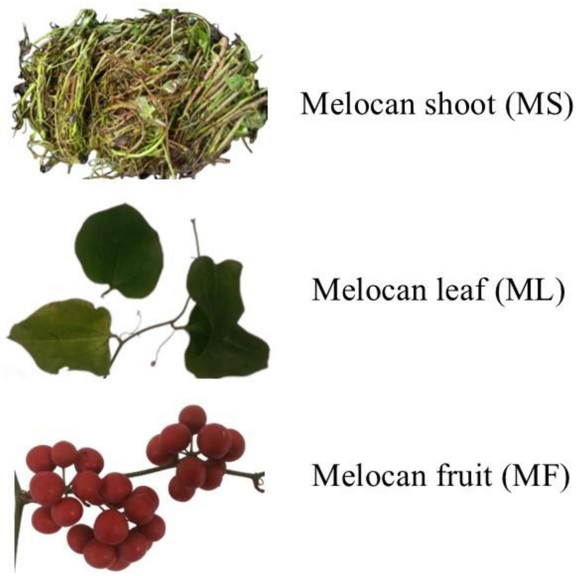


Figure 1. The different parts of the Melocan

Supercritical fluids have solvating powers like liquid organic solvents but with higher diffusivity, lower viscosity, and lower surface tension. The most widely used supercritical fluid is supercritical carbon dioxide ($CO_2$) due to its harmless effect on the environment, low toxicity, non-flammability, and compatibility with processed foodstuffs (Ignat et al., 2011). $CO_2$ is not a suitable solvent for polar compounds due to its non-polar feature. It can be modified with polar organic co-solvents such as ethanol to increase its solubility in polar compounds (Sajadian et al., 2023).

Factors affecting the successful performance of the supercritical carbon dioxide extraction process are sample type, sample preparation method, type of fluid, choice of co-solvent, method of introducing fluid into the system, pressure, temperature, flow rate, and time (Arumugham et al., 2021). Supercritical $CO_2$ extraction method has many advantages compared with traditional extraction methods (Li et al., 2021). The advantages of this method are being environmentally friendly, safe (GRAS), inexpensive, and easy to remove, as well as working in mild conditions, and having manageable solvent power and fast diffusion rate (Wang et al., 2021). Temperatures used in various studies to extract phenolic compounds from plant sources using the Sc-$CO_2$ extraction method varied between 40-60 °C, and pressures varied between 200-500 bar. The most important advantage of this method is that low extraction temperatures can be used to preserve the structure of phenolic compounds (Da Silva et al., 2016). In many studies where phenolic compounds from plant sources were obtained by the Sc-$CO_2$ extraction method, 250 bar and 350 bar pressure parameters were used (Bimakr et al., 2011; Bitencourt et al., 2014; Monroy et al., 2016a; Monroy et al., 2016b).

In this study, it was aimed to extract melocan leaf (ML), shoots (MS) and fruits (MF) by supercritical carbon dioxide extraction and to determine the total phenolic content, total antioxidant capacity. For this purpose, extractions were performed in three different plant parts (leaf, shoot and fruit), two different pressures (250 bar and 350 bar), two different ethanol concentrations (10% and 20%), two different temperatures (30 °C and 50 °C) and two different times (60 min and 90 min).

## MATERIALS AND METHODS

### Sample Preparation and Extraction

The melocan fruit was obtained in October 2018. The shoots and leaves were obtained from Tokat-Niksar and Ordu-Ünye in May 2018. The leaves (until the dry matter content was 93.1±0.19%), fruits (until the dry matter content was 94.5±0.07%), and shoots (until the dry matter content was 93.8±0.01%) were dried at room temperature. Dried samples (ash content of dried leaves, fruits, and shoots are 6.68±0.04%, 4.36±0.03%, and 7.85±0.04%) were packaged and stored until experimentation (Şahin, 2019). The samples were ground and passed through a 2 mm sieve before being used in the extraction, and the part under the sieve was kept in the refrigerator at +4 °C to be used in the extraction. Melocan parts were extracted using Sc-$CO_2$ extractor (SFE-100-2-FMC10, Thar Instruments, PA, USA) at two different pressures (250 bar and 350 bar), two different ethanol concentrations (10% and 20%), two different temperatures (30 °C and 50 °C) and two different times (60 min and 90 min). Extracts were stored at -18 °C.

### Experimental Design

The Sc-$CO_2$ was performed according to general full factorial design to determine the desirable condition for ML, MS, and MF. Full factorial design consists of all possible combinations of the selected factors and levels (Jankovic et al., 2021). In the present study, a general full factorial design was employed to evaluate the most critical parameters affecting the TPC and TAC values of ML, MS, and MF extracts. Five factors were investigated, including plant parts (Part), pressure (P), temperature (T), extraction time (ti), and ethanol concentration (EtOH) at different levels (Table 1). These conditions were chosen by taking into account the Sc-$CO_2$ extraction conditions applied to other plants in the literature because there is no study in the literature in which melocan parts were subjected to the Sc-$CO_2$ extraction method (Hamburger et al., 2004; Bimakr et al., 2011; Da Porto et al., 2014;Da Silva et al., 2016; ; Monroy et al., 2016a; Monroy et al., 2016b). Since we consider five parameters, the total run with full factorial design is $3*2^4$ (48 runs), which is the number of experiments (Table 2). The TPC and TAC values of extracts were determined using two parallels. The dependence of each experimental response (TPC and TAC) on these factors was modeled by applying the equation given in Equation 1 (Box et al., 1978).

$$y = \beta_0 + \sum_{i=1}^{n}(\beta_i x_i) + \sum_{i=1}^{n}\sum_{j=i+1}^{n}(\beta_{ij} x_i x_j) + \varepsilon$$

Equation 1.

In this equation, $\beta_0$ is the constant term, $\beta_i$ and $\beta_{ij}$ are the regression coefficients, ε is the error, $x_i$ and $x_j$ are the independent variables, and n is the independent variable number.

### Total Phenolic Content (TPC) of Extracts

The amount of TPC of the extracts was determined by the Folin-Ciocalteu method with some modifications (Shahidi and Naczk, 2003). 0.5 mL of extract solution (5-fold diluted with ethanol-Sigma, USA) was mixed with 2.5 mL of 0.2 N Folin–Ciocalteu's phenol reagent (Sigma, USA). After 5 min in a dark place, 2 mL of 7.5% sodium carbonate (Sigma, USA) was added to the mixture and kept in the dark for 1 hour before the absorbance was measured spectrophotometrically (G10S UV-VIS, Thermo Fisher Scientific) at 760 nm. Results were calculated as mg GAE/kg dry weight using a calibration curve (y=0.0096x-0.052, $R^2$=0.9957) generated with the gallic acid standard (Sigma, USA).

Table 1. Variables for the experimental procedure

| Factors | Levels | Values | | |
|---|---|---|---|---|
| Part | 3 | ML | MS | MF |
| P (bar) | 2 | 250 | | 350 |
| T (°C) | 2 | 30 | | 50 |
| ti (min) | 2 | 60 | | 90 |
| EtOH (%) | 2 | 10 | | 20 |

**Total Antioxidant Capacity (TAC) of Extracts**

The TAC values of the extracts were measured by the DPPH (2,2-diphenyl-1-picrylhydrazil) method (Akdeniz et al., 2018). 0.1 mL of extract solution (20-fold diluted with methanol-Sigma, USA) was mixed with 3.9 mL 25 ppm DPPH (Sigma, USA) solution. After 1 hour in a dark place, absorbance was measured spectrophotometrically (G10S UV-VIS, Thermo Fisher Scientific) at 517 nm. Results were calculated as mg TE/g dry weight using a calibration curve ($y=0.0019x+0.0116$, $R^2=0.9903$) generated with the trolox standard (Sigma, USA).

**Statistical Analysis**

The results were given as mean±standard error. The experimental design (DOE) for data analysis and full factorial runs was obtained with the Minitab 21 statistical program. The analysis of variance (ANOVA) was obtained, and confidence levels of 95% ($P<0.05$) were considered statistically significant effects of the factors and their interaction. Response Optimizer (Minitab 21) was used to determine which melocan part and experiment gave the maximum TPC and TAC values.

## RESULTS AND DISCUSSION

### The Effects of the Main Factors and their Interactions on TPC Values

The general full factorial design experiments with the determined factors and their respective responses are presented in Table 2. In the experiments obtained, the amounts of TPC varied from 229.37 mg GAE/kg dry weight to 2265.06 mg GAE/kg dry weight. The best conditions obtained for Sc-$CO_2$ extraction of phenolics from melocan was ML, 350 bar, 50 °C, 90 min, and with a 20% modifier of ethanol, and the TPC value of the extracts obtained under these conditions was measured as 2265.06 mg GAE/kg dry weight. The worst conditions for phenolics extraction were MS, 250 bar, 30 °C, 60 min, and with a 10% modifier of ethanol, and the TPC value of the extracts obtained under these conditions was measured as 229.37 mg GAE/kg dry weight. Although TPC and TAC values have not been analyzed in melocan parts extracts obtained with this method before, these values have been analyzed in melocan parts extracts obtained with other methods. Ozsoy et al. (2008) reported that TPC value of melocan leaf ethanolic extract was 30100 mg GAE/kg on dry weight basis. Al Yassine et al. (2023) reported that the TPC value on dry weight basis, in the melocan stem and leaf was 11580 and 2938.9 mg GAE/kg for the water and 70 % ethanolic extracts, respectively. Dehghan et al. (2016) reported that the highest TPC value on dry weight mass of the methanolic extracts of melocan leaf was 239000 mg GAE/kg, while that value was 226700 mg GAE/kg on the methanolic extract melocan stem. In the same study, the highest TPC value was found to be 19300 mg GAE/kg on dry weight mass of the melocan leaf hexane extract, and 7100 mg GAE/kg on the melocan stem hexane extract. According to Sarıaltın et al. (2023), the maximum TPC value was observed in the methanolic extract of melocan as 402.94 mg GAE/g of crude extract. Topdas et al. (2021) suggested that the TPC value of fresh and dried melocan shoots' water extracts varied from 18390 mg GAE/kg dry weight to 34860 mg GAE/kg dry weight. Studies have shown that the TPC values in melocan extracts are generally higher than the ones found in this study. According to Şahin (2019), TPC values of melocan leaves, fruits and shoots were found to be 55980, 55250, and 37130 mg GAE/kg dry weight, respectively, with traditional extraction method, 57300, 57120, and 38120 mg GAE/g dry weight with ultrasonic-assisted extraction method, and 67270, 66230, and 50350 mg GAE/kg dry weight with microwave-assisted extraction method. Studies have shown that the TPC values in melocan extracts are generally higher than the ones found in this study.

Table 2. Experimental design for extraction and TPC, TAC results

| Exp. | Part | P | T | ti | EtOH | TPC (mg GAE/kg dry weight) | TAC (mg TE/kg dry weight) |
|---|---|---|---|---|---|---|---|
| ***1*** | ML | 350 | 30 | 90 | 20 | 1012.86±51.56 | 2139.94±90.61 |
| ***2*** | MF | 350 | 30 | 90 | 20 | 671.48±26.50 | 1824.19±46.63 |
| ***3*** | MF | 250 | 30 | 90 | 20 | 618.39±22.08 | 1761.07±44.63 |
| ***4*** | MS | 350 | 30 | 90 | 10 | 333.23±33.34 | 521.00±44.93 |
| ***5*** | MS | 350 | 30 | 60 | 20 | 435.07±4.45 | 822.09±59.93 |
| ***6*** | ML | 350 | 50 | 60 | 10 | 980.31±12.65 | 604.06±90.24 |
| ***7*** | MF | 350 | 50 | 90 | 10 | 726.28±14.34 | 1258.15±22.30 |
| ***8*** | MF | 250 | 30 | 60 | 20 | 425.57±13.18 | 1084.01±29.60 |
| ***9*** | MS | 250 | 30 | 90 | 10 | 380.39±17.78 | 727.49±22.46 |
| ***10*** | ML | 350 | 30 | 60 | 10 | 625.59±44.12 | 810.13±15.11 |
| ***11*** | MF | 250 | 30 | 60 | 10 | 317.80±16.21 | 808.66±89.34 |
| ***12*** | MS | 250 | 50 | 90 | 20 | 490.14±17.89 | 2550.0±45.19 |
| ***13*** | ML | 250 | 50 | 60 | 20 | 1482.97±14.94 | 828.27±60.38 |
| ***14*** | ML | 350 | 30 | 90 | 10 | 648.45±1.11 | 761.18±22.52 |
| ***15*** | MF | 250 | 50 | 90 | 10 | 872.94±5.52 | 1037.42±22.30 |
| ***16*** | ML | 350 | 50 | 90 | 10 | 746.94±42.34 | 777.10±45.04 |
| ***17*** | MF | 350 | 30 | 90 | 10 | 492.25±9.93 | 1100.49±22.30 |
| ***18*** | MS | 350 | 50 | 90 | 20 | 670.20±8.90 | 1329.07±44.97 |
| ***19*** | MF | 250 | 50 | 90 | 20 | 1102.48±48.59 | 2076.67±44.63 |
| ***20*** | MF | 350 | 50 | 90 | 20 | 1378.88±28.71 | 2076.67±44.63 |
| ***21*** | MS | 350 | 50 | 60 | 20 | 528.91±8.94 | 847.50±30.11 |
| ***22*** | MS | 250 | 30 | 90 | 20 | 522.31±8.90 | 1901.40±44.97 |
| ***23*** | ML | 250 | 30 | 60 | 20 | 1068.92±2.99 | 977.71±30.19 |
| ***24*** | MF | 250 | 50 | 60 | 20 | 1274.73±13.24 | 1573.12±59.48 |
| ***25*** | ML | 250 | 50 | 90 | 20 | 883.60±11.10 | 1674.78±89.72 |
| ***26*** | ML | 350 | 50 | 60 | 20 | 985.18±41.41 | 629.88±89.68 |
| ***27*** | ML | 250 | 30 | 60 | 10 | 277.61±18.86 | 217.35±59.57 |
| ***28*** | MS | 250 | 50 | 60 | 10 | 256.66±0.74 | 910.16±15.00 |
| ***29*** | ML | 250 | 30 | 90 | 20 | 939.94±15.69 | 1178.89±90.61 |
| ***30*** | MS | 350 | 50 | 60 | 10 | 463.99±28.21 | 485.84±105.01 |
| ***31*** | ML | 250 | 30 | 90 | 10 | 534.49±34.71 | 364.88±45.26 |
| ***32*** | MF | 250 | 30 | 90 | 10 | 454.02±11.03 | 1068.96±66.89 |
| ***33*** | ML | 350 | 50 | 90 | 20 | 2265.06±2.24 | 2876.74±45.30 |
| ***34*** | MF | 350 | 30 | 60 | 10 | 541.30±8.10 | 966.60±14.89 |
| ***35*** | MF | 350 | 50 | 60 | 20 | 1079.10±22.07 | 1657.24±59.48 |
| ***36*** | MS | 350 | 50 | 90 | 10 | 483.35±32.23 | 600.42±67.39 |
| ***37*** | ML | 350 | 30 | 60 | 20 | 618.00±11.89 | 760.45±90.12 |
| ***38*** | MS | 350 | 30 | 90 | 20 | 657.74±4.47 | 1016.17±45.19 |
| ***39*** | MS | 250 | 30 | 60 | 20 | 238.89±4.43 | 1724.60±29.82 |
| ***40*** | MF | 250 | 50 | 60 | 10 | 385.01±0.74 | 882.36±14.89 |
| ***41*** | MS | 250 | 30 | 60 | 10 | 229.37±9.65 | 782.87±15.00 |
| ***42*** | ML | 250 | 50 | 90 | 10 | 722.15±44.79 | 508.91±22.63 |
| ***43*** | MS | 250 | 50 | 60 | 20 | 445.55±28.17 | 1817.93±29.96 |
| ***44*** | ML | 250 | 50 | 60 | 10 | 701.53±42.36 | 1189.34±41.43 |
| ***45*** | MS | 250 | 50 | 90 | 10 | 367.81±13.34 | 1235.77±22.46 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***46*** | MF | 350 | 50 | 60 | 10 | 420.96±4.42 | 745.48±29.78 |
| ***47*** | MS | 350 | 30 | 60 | 10 | 281.86±28.95 | 464.63±15.00 |
| ***48*** | MF | 350 | 30 | 60 | 20 | 671.19±45.62 | 1383.84±89.23 |

The statistical significance of all five factors and their possible two-, three-, four-, and five-way interactions for the TPC values were evaluated for their F- and p-values. If a factor had a higher F-value, its statistical significance was greater. The coefficient of determination ($R^2$) and adjusted $R^2$ were also supplied to check the adequacy and fitness of the model (Maran et al., 2015). $R^2$ refers that the percentage of variation in the response that is explained by the model rather than random error. Adjusted $R^2$ is adjusted for the number of predictors in the model relative to the number of observations. $R^2$ should be >80% in a well fitted model, and adjusted $R^2$ should not be less than 90% to evaluate the model adequacy (Koocheki et al., 2009; Mitić et al., 2019). There is no study in the literature in which melocan parts were subjected to the Sc-$CO_2$ extraction method. Therefore, the extraction conditions of other plants will be considered when comparing our results. The factorial design's ANOVA results are presented in Table 3. As can be seen in Table 3, the results indicated that the effects of "Part", "P", "T", "ti" and "EtOH" on TPC values of extracts are statistically significant ($P<0.05$). The most important factor was EtOH, followed by the Part, T, ti, and P. In our study, according to the ANOVA results, the regression model was suitable for explaining the behavior due to the $R^2$ value of 99.75%and adjusted $R^2$ value of 99.55% in reduced model, as seen in Table 4. The closer the $R^2$ value is to 1, the more significant the good model is and the less significant the lack-of-fit is (Noordin et al., 2004). The lack of fit ($P=0.058$) suggested that it is an adequate model to accurately predict the TPC. This represents a better precision and reliability in the experiments that were conducted. Wang et al. (2011) also concluded that all the four parameters- pressure, temperature, time, and modifier - significantly affected the TPC of the *Ampelopsis grossedentata* stem extracts, which were obtained by the Sc-$CO_2$ extraction method. Rahmawati et al. (2015) also suggest that pressure and temperature significantly affected the TPC of the *Mimosa pudica* Linn extracts, obtained through the Sc-$CO_2$ extraction method. Many researchers have reported that extraction conditions such as temperature, pressure, time, and ethanol concentration have a significant effect on the TPC value of the extracts (Maran et al., 2015; Uwineza et al., 2021).

Table 3. Full Model ANOVA Results for TPC values

| Source | DF | Seq SS | Contribution | Adj. SS | Adj. MS | F-Value | P-Value |
|---|---|---|---|---|---|---|---|
| Model | 47 | 13830720 | 99.80% | 13830720 | 294271 | 499.74 | 0.000 |
| *Linear* | 6 | 9210216 | 66.46% | 9210216 | 1535036 | 2606.83 | 0.000 |
| Part | 2 | 3765845 | 27.17% | 3765845 | 1882922 | 3197.62 | 0.000 |
| P | 1 | 309373 | 2.23% | 309373 | 309373 | 525.38 | 0.000 |
| T | 1 | 1880485 | 13.57% | 1880485 | 1880485 | 3193.48 | 0.000 |
| ti | 1 | 437215 | 3.15% | 437215 | 437215 | 742.49 | 0.000 |
| EtOH | 1 | 2817299 | 20.33% | 2817299 | 2817299 | 4784.40 | 0.000 |
| *2-Way Interactions* | 14 | 1503258 | 10.85% | 1503258 | 107376 | 182.35 | 0.000 |
| Part*P | 2 | 34330 | 0.25% | 34330 | 17165 | 29.15 | 0.000 |
| Part*T | 2 | 486976 | 3.51% | 486976 | 243488 | 413.50 | 0.000 |
| Part*ti | 2 | 2766 | 0.02% | 2766 | 1383 | 2.35 | 0.106 |
| Part*EtOH | 2 | 513292 | 3.70% | 513292 | 256646 | 435.84 | 0.000 |
| P*T | 1 | 24209 | 0.17% | 24209 | 24209 | 41.11 | 0.000 |
| P*ti | 1 | 116372 | 0.84% | 116372 | 116372 | 197.63 | 0.000 |
| P*EtOH | 1 | 2310 | 0.02% | 2310 | 2310 | 3.92 | 0.053 |
| T*ti | 1 | 1212 | 0.01% | 1212 | 1212 | 2.06 | 0.158 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| T*EtOH | 1 | 302600 | 2.18% | 302600 | 302600 | 513.88 | 0.000 |
| ti*EtOH | 1 | 19191 | 0.14% | 19191 | 19191 | 32.59 | 0.000 |
| *3-Way Interactions* | 16 | 1825096 | 13.17% | 1825096 | 114068 | 193.71 | 0.000 |
| Part*P*T | 2 | 179290 | 1.29% | 179290 | 89645 | 152.24 | 0.000 |
| Part*P*ti | 2 | 346066 | 2.50% | 346066 | 173033 | 293.85 | 0.000 |
| Part*P*EtOH | 2 | 21556 | 0.16% | 21556 | 10778 | 18.30 | 0.000 |
| Part*T*ti | 2 | 70044 | 0.51% | 70044 | 35022 | 59.48 | 0.000 |
| Part*T*EtOH | 2 | 229220 | 1.65% | 229220 | 114610 | 194.63 | 0.000 |
| Part*ti*EtOH | 2 | 132760 | 0.96% | 132760 | 66380 | 112.73 | 0.000 |
| P*T*ti | 1 | 195837 | 1.41% | 195837 | 195837 | 332.58 | 0.000 |
| P*T*EtOH | 1 | 58888 | 0.42% | 58888 | 58888 | 100.01 | 0.000 |
| P*ti*EtOH | 1 | 590904 | 4.26% | 590904 | 590904 | 1003.49 | 0.000 |
| T*ti*EtOH | 1 | 531 | 0.00% | 531 | 531 | 0.90 | 0.347 |
| ***4-Way Interactions*** | 9 | 1197399 | 8.64% | 1197399 | 133044 | 225.94 | 0.000 |
| Part*P*T*ti | 2 | 86150 | 0.62% | 86150 | 43075 | 73.15 | 0.000 |
| Part*P*T*EtOH | 2 | 214762 | 1.55% | 214762 | 107381 | 182.36 | 0.000 |
| Part*P*ti*EtOH | 2 | 512747 | 3.70% | 512747 | 256374 | 435.38 | 0.000 |
| Part*T*ti*EtOH | 2 | 184470 | 1.33% | 184470 | 92235 | 156.64 | 0.000 |
| P*T*ti*EtOH | 1 | 199269 | 1.44% | 199269 | 199269 | 338.40 | 0.000 |
| ***5-Way Interactions*** | 2 | 94751 | 0.68% | 94751 | 47376 | 80.45 | 0.000 |
| Part*P*T*ti*EtOH | 2 | 94751 | 0.68% | 94751 | 47376 | 80.45 | 0.000 |
| Error | 48 | 28265 | 0.20% | 28265 | 589 | | |
| Total | 95 | 13858985 | 100.00% | | | | |
| Model Summary | Std. Dev. | | $R^2$ | | $R^2$(Adj.) | | $R^2$(Pred.) |
| | 24.27 | | 99.80% | | 99.60% | | 99.18% |

Table 4. Reduced Model ANOVA Results for TPC values

| Source | DF | Seq SS | Contribution | Adj SS | Adj MS | F-Value | P-Value |
|---|---|---|---|---|---|---|---|
| Model | 42 | 13823901 | 99.75% | 13823901 | 329141 | 497.23 | 0.000 |
| Linear | 6 | 9210216 | 66.46% | 9210216 | 1535036 | 2318.95 | 0.000 |
| 2-Way Interactions | 10 | 1496971 | 10.80% | 1496971 | 149697 | 226.14 | 0.000 |
| 3-Way Interactions | 15 | 1824564 | 13.17% | 1824564 | 121638 | 183.76 | 0.000 |
| 4-Way Interactions | 9 | 1197399 | 8.64% | 1197399 | 133044 | 200.99 | 0.000 |
| 5-Way Interactions | 2 | 94751 | 0.68% | 94751 | 47376 | 71.57 | 0.000 |
| Error | 53 | 35084 | 0.25% | 35084 | 662 | | |
| Lack-of-Fit | 5 | 6819 | 0.05% | 6819 | 1364 | 2.32 | 0.058 |
| Pure Error | 48 | 28265 | 0.20% | 28265 | 589 | | |
| Total | 95 | 13858985 | 100.00% | | | | |
| Model Summary | Std. Dev. | | $R^2$ | | $R^2$(Adj.) | | $R^2$(Pred.) |
| | 25.7285 | | 99.75% | | 99.55% | | 99.17% |

Except for Part*ti, P*EtOH, and T*ti, all two-way interactions of these factors significantly affected the TPC values ($P<0.05$). While the most important interaction was T*EtOH, the least important one was Part*P. Except for T*ti*EtOH, all three-way interactions of these factors had a significant effect ($P<0.05$). According to the F-value, the most important difference was the P*ti*EtOH interaction between all three-way interactions. The least significant difference was in the Part*P*EtOH (18.30) three-way interaction. All four-way interactions had a statistically significant effect on TPC values ($P<0.05$). Part*P*T*ti*EtOH interaction of all factors had a statistically significant effect on TPC values ($P<0.05$). If we consider all factors and their interactions that have statistical significance, EtOH is the one with the most significant level of difference due to its high f-value.

**The Effects of the Main Factors and their Interactions on TAC Values**

As seen in Table 2, the amounts of TAC obtained in the experiments varied from 217.4 mg TE/kg dry weight to 2876.7 mg TE/kg dry weight. While the experiment factors with the highest TAC value were ML, 350 bar, 50 °C, 90 min, and with a 20% modifier of ethanol; the lowest TAC value was seen in the extract obtained under conditions ML, 250 bar, 30°C, 60 min, and a 10% modifier of ethanol. According to Al Yassine et al. (2023), the TAC value of melocan (mix of leaf and stem) was higher in the water extract (1928 mg TE/kg) than in the 70 % ethanol extract (1547 mg TE/kg) on dry weight basis. In contrast to our study, TAC value of ethanolic extract was lower than the water extract in the same study. In our study, higher TAC values were observed in leaf samples in the best conditions. Our findings indicated that the antioxidant activity of melocan was related to the phenolic compounds significantly.

The factorial design's ANOVA results are in Table 5. It is necessary for $R^2$ and adjusted $R^2$ to be more than 80% and 90%, respectively, for the adequacy of the model (Koocheki et al., 2009; Mitić et al., 2019). The closer the $R^2$ value is to 1, the more significant the good model is and the less significant the lack-of-fit is (Noordin et al., 2004). When all interactions were included in the model, the total variation in the experimental design was completely explained by the model, so there was no Lack of fit value. In our study, $R^2$ and adjusted $R^2$ values were found as 99.58% and 99.17% for TAC values of the extracts. This shows that the prediction capacity of the model proposed is high. ANOVA results indicate that the effects of "Part," "P," "T," "ti," and "EtOH" on TAC values of the extracts are statistically significant ($P<0.05$). The most important difference among these factors is "EtOH" due to its high f-value of 4504.88. F-values of "Part", "P", "T", and "ti" factors are 287.06, 84.64, 360.80, and 1217.17, respectively. Among these factors, "ti" is the one that has the most important effect on the TAC values of the extracts after "EtOH." This is followed by "T," "Part," and "P." Previous studies in which other plants were extracted by the Sc-$CO_2$ extraction method will be examined, and extraction parameters' effects on antioxidant capacity will be compared with our study. Uwineza et al. (2021) also found the effect of the "T" factor on *Lamium album* extracts TAC value obtained through Sc-$CO_2$. Ghafoor et al. (2012) suggested that the effects of "T" and "P" factors on antioxidant compounds from grape seeds are statistically significant. Mandana et al. (2011) concluded that all three independent variables (pressure, temperature, and ethanol flow rate) had a significant effect on the antioxidant activity of spearmint (*Mentha spicata* L.) leaves extracts.

The effect of all two-way interactions of the independent variables on the TAC values of extracts was found to be statistically significant ($P<0.05$). Part*P is the most important effect on TAC values between other two-way interactions, and this is due to the high f-value of 764.28. This is followed by the ti*EtOH two-way interaction, which has an f-value of 747.82. The interaction with the lowest f-value of 4.21 is Part*T. All three-way interactions of these factors significantly affected the TAC values of the extracts ($P<0.05$). According to the F-value (233.34), the most important effect on TAC values is the P*ti*EtOH interaction, just as TPC values. The least significant difference was found in the Part*P*T (5.45) three-way interaction. All four-way interactions of these factors also significantly affected the TAC values of extracts ($P<0.05$). Among these, Part*P*ti*EtOH has a high f-value (75.78). This is followed by Part*T*ti*EtOH (74.86), Part*P*T*ti (38.75), Part*P*T*EtOH (37.28), and P*T*ti*EtOH (17.57). Part*P*T*ti*EtOH interaction of all factors had a statistically significant effect on TAC values ($P<0.05$) and its f-value is 8.47. If we consider all factors and their interactions that have statistical significance, EtOH is the one with the most significant level of difference due to its high f-value.

Table 5. Full Model ANOVA Results for TAC Values

| Source | DF | Seq SS | Contribution | Adj. SS | Adj. MS | F-Value | P-Value |
|---|---|---|---|---|---|---|---|
| Model | 47 | 32783306 | 99.58% | 32783306 | 697517 | 241.18 | 0.000 |
| *Linear* | 6 | 19653747 | 59.70% | 19653747 | 3275624 | 1132.60 | 0.000 |
| Part | 2 | 1660427 | 5.04% | 1660427 | 830214 | 287.06 | 0.000 |
| P | 1 | 244776 | 0.74% | 244776 | 244776 | 84.64 | 0.000 |
| T | 1 | 1043480 | 3.17% | 1043480 | 1043480 | 360.80 | 0.000 |
| ti | 1 | 3676375 | 11.17% | 3676375 | 3676375 | 1271.17 | 0.000 |
| EtOH | 1 | 13028688 | 39.57% | 13028688 | 13028688 | 4504.88 | 0.000 |
| *2-Way Interactions* | 14 | 7910345 | 24.03% | 7910345 | 565025 | 195.37 | 0.000 |
| Part*P | 2 | 4420817 | 13.43% | 4420817 | 2210409 | 764.28 | 0.000 |
| Part*T | 2 | 24379 | 0.07% | 24379 | 12190 | 4.21 | 0.021 |
| Part*ti | 2 | 313627 | 0.95% | 313627 | 156813 | 54.22 | 0.000 |
| Part*EtOH | 2 | 32361 | 0.10% | 32361 | 16180 | 5.59 | 0.007 |
| P*T | 1 | 233923 | 0.71% | 233923 | 233923 | 80.88 | 0.000 |
| P*ti | 1 | 329824 | 1.00% | 329824 | 329824 | 114.04 | 0.000 |
| P*EtOH | 1 | 54680 | 0.17% | 54680 | 54680 | 18.91 | 0.000 |
| T*ti | 1 | 214293 | 0.65% | 214293 | 214293 | 74.10 | 0.000 |
| T*EtOH | 1 | 123659 | 0.38% | 123659 | 123659 | 42.76 | 0.000 |
| ti*EtOH | 1 | 2162782 | 6.57% | 2162782 | 2162782 | 747.82 | 0.000 |
| *3-Way Interactions* | 16 | 3808368 | 11.57% | 3808368 | 238023 | 82.30 | 0.000 |
| Part*P*T | 2 | 31535 | 0.10% | 31535 | 15768 | 5.45 | 0.007 |
| Part*P*ti | 2 | 993681 | 3.02% | 993681 | 496841 | 171.79 | 0.000 |
| Part*P*EtOH | 2 | 810462 | 2.46% | 810462 | 405231 | 140.12 | 0.000 |
| Part*T*ti | 2 | 94692 | 0.29% | 94692 | 47346 | 16.37 | 0.000 |
| Part*T*EtOH | 2 | 119672 | 0.36% | 119672 | 59836 | 20.69 | 0.000 |
| Part*ti*EtOH | 2 | 1349703 | 4.10% | 1349703 | 674852 | 233.34 | 0.000 |
| P*T*ti | 1 | 72384 | 0.22% | 72384 | 72384 | 25.03 | 0.000 |
| P*T*EtOH | 1 | 96781 | 0.29% | 96781 | 96781 | 33.46 | 0.000 |
| P*ti*EtOH | 1 | 63623 | 0.19% | 63623 | 63623 | 22.00 | 0.000 |
| T*ti*EtOH | 1 | 175834 | 0.53% | 175834 | 175834 | 60.80 | 0.000 |
| *4-Way Interactions* | 9 | 1361873 | 4.14% | 1361873 | 151319 | 52.32 | 0.000 |
| Part*P*T*ti | 2 | 224116 | 0.68% | 224116 | 112058 | 38.75 | 0.000 |
| Part*P*T*EtOH | 2 | 215644 | 0.66% | 215644 | 107822 | 37.28 | 0.000 |
| Part*P*ti*EtOH | 2 | 438311 | 1.33% | 438311 | 219155 | 75.78 | 0.000 |
| Part*T*ti*EtOH | 2 | 432988 | 1.32% | 432988 | 216494 | 74.86 | 0.000 |
| P*T*ti*EtOH | 1 | 50814 | 0.15% | 50814 | 50814 | 17.57 | 0.000 |
| *5-Way Interactions* | 2 | 48974 | 0.15% | 48974 | 24487 | 8.47 | 0.001 |
| Part*P*T*ti*EtOH | 2 | 48974 | 0.15% | 48974 | 24487 | 8.47 | 0.001 |
| Error | 48 | 138822 | 0.42% | 138822 | 2892 | | |
| Total | 95 | 32922128 | 100.00% | | | | |
| Model Summary | Std. Dev. | $R^2$ | | $R^2$(Adj.) | | $R^2$(Pred.) | |
| | 53.78 | 99.58% | | 99.17% | | 98.31% | |

**Response Optimizer Results**

Extraction conditions that give the maximum TPC and TAC values are seen in Table 6. In both results, the melocan part with the highest value was found to be the leaf, and the extraction parameters were 350 bar pressure, 50 °C temperature, 90 minutes time, and 20% ethanol concentration.

Table 6. Solution of Response Optimization

| Solution | Part | P | T | ti | EtOH | TAC(mg TE/kg dry weight) Fit | TPC(mg GAE/kg dry weight) Fit | Composite Desirability |
|---|---|---|---|---|---|---|---|---|
| 1 | ML | 350 | 50 | 90 | 20 | 2876.74 | 2265.06 | 0.993738 |

## CONCLUSION

Overall, phenolic substances of melocan parts have been extracted through Sc-$CO_2$ extraction method and their TPC and TAC values have been measured for the first time in this study. The extraction parameters on the TPC and TAC values of the Sc-$CO_2$ extracts from melocan parts have been investigated in this study to find the best extraction conditions. The highest TPC (2265.06 mg GAE/kg dry weight) and TAC (2876.74 mg TE/kg dry weight) of Sc-$CO_2$ extracts from ML were obtained at 350 bar, 50 °C, 90 min, and 20% ethanol concentration. It was concluded that ML is a good source of antioxidants when extracted with the Sc-$CO_2$ extraction method. In further studies, extracts of ML obtained under these conditions could be used as a source of natural antioxidants for enhancing food quality and extending shelf life of food.

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## COMPETING INTERESTS

The authors state no conflict of interest.

## AUTHOR CONTRIBUTIONS

Esra Bostancı Selbeş: Formal analysis, data curation, methodology, conceptualization, writing-original draft, visualization, Özlem Şahin: Formal analysis, data curation, methodology, conceptualization, Halil Vural: technical support, writing-review & editing.

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