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Research Article

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Unlocking the Antioxidant Potential of *Calendula Officinalis*: A Comparative Study of Extraction Methods

Aynısefa Bitkisinin Antioksidan Potansiyelinin Ortaya Çıkarılması: Ekstraksiyon Yöntemlerinin Karşılaştırılması

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ABSTRACT

Aim: *Calendula officinalis* which is also known as marigold is a species of plant in the daisy family that has antioxidant, anti-inflammatory, cytotoxic, and antitumor properties [1]. In this study the antioxidant activity of *calendula officinalis* flowers extract was investigated using three different extraction techniques; soxhlet, maceration, and ultrasonic methods. Methods: In this study, soxhlet, maceration and ultrasonic extraction methods were utilized. Ethanol was used as solvent to obtain *calendula officinalis* extracts. The antioxidant activity of the extracts with different concentrations are investigated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution. Results: DPPH radical scavenging activity for the extract obtained using soxhlet method was found to be the highest of all three methods. Increasing the *calendula officinalis* extract concentration, was observed to increase it the antioxidant activity as expected. Conclusions: In conclusion, *calendula officinalis* flowers demonstrate remarkable antioxidant efficacy and soxhlet method can be considered as a better method compared to the other extraction methods utilizing ethanol as the solvent. Further researches for the application of the extract can be studied with different solvents and operating temperatures.

Key Words

Calendula officinalis, extraction, antioxidant, radical scavenging, antioxidant activity.

ÖZ

Amaç: Aynı zamanda Marigold olarak da bilinen *Calendula officinalis*, antioxidant, anti-inflamatuar, sitotoksik ve anti-tümör özelliklere sahip olan papatyagiller familyasındaki bir bitki türüdür [1]. Bu çalışmada, *Calendula officinalis* çiçek ekstresinin antioksidan aktivitesi üç farklı ekstraksiyon tekniği kullanılarak araştırılmıştır; soxhlet, maserasyon ve ultrasonik yöntemler. Yöntemler: Bu çalışmada, soxhlet, maserasyon ve ultrasonik ekstraksiyon yöntemleri kullanılmıştır. *Calendula officinalis* ekstraktlarını elde etmek için çözücü olarak etanol kullanılmıştır. Farklı konsantrasyonlardaki ekstraktların antioksidan aktivitesi 2,2-difenil-1-pikrilhidrazil (DPPH) çözeltisi kullanılarak araştırılmıştır. Ölçüm Sonuçları: Soxhlet yöntemi kullanılarak elde edilen ekstrakt için DPPH radikal temizleme aktivitesi üç yöntem arasında en yüksek bulunmuştur. *Calendula officinalis* ekstrakt konsantrasyonunun artmasıyla, beklenildiği gibi antioksidan aktivitesinin arttığı gözlenmiştir. Sonuçlar: Sonuç olarak, *Calendula officinalis* çiçekleri dikkate değer antioksidan etkinlik sergilemektedir ve etanolü çözücü olarak kullanan diğer ekstraksiyon yöntemleriyle karşılaştırıldığında soxhlet yöntemi daha iyi bir yöntem olarak düşünülebilir. Ekstraktın uygulanması için farklı çözücüler ve işletme sıcaklıklarıyla ilgili daha fazla araştırma yapılabileceği sonucuna varılmıştır.

Anahtar Kelimeler

Aynısefa, ekstraksiyon, antioksidan, radikal temizleme, antioksidan aktivite.

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INTRODUCTION

Antioxidants play a pivotal role in mitigating cellular damage in humans caused by free radicals, which are molecules characterized by the presence of a free, unpaired valence electron. Numerous plant-based antioxidants exist that effectively neutralize these free radicals, thereby safeguarding the human body [2]. Unlike antioxidant supplements, which are typically isolated and consist of a single substance, plant-based antioxidants are often more efficacious due to their synergistic interactions with other plant chemicals. Therefore, sources of antioxidants derived from plants are superior for scavenging radicals, with *calendula officinalis* standing out as one of the most notable examples [2-6]. Free radicals within the body can lead to various diseases such as inflammation, diabetes, and cancer [7-10]. Numerous studies have focused on the exploration of non-toxic drugs capable of scavenging free radicals, often derived from plant extracts. Several plant extracts and products, including turmeric tuber [11] and emblica officinalis [12], have exhibited antioxidant potential. Additionally, there is substantial research on the pharmacological activities of the *Calendula officinalis* plant. Literature reports highlight its anti-inflammatory activity [13], wound-healing properties [14,15], and antioxidant activity [16] as fundamental pharmacological attributes. This study aims to demonstrate the antioxidant activity of the *Calendula officinalis* plant through various extraction methods. Among the most utilized plant extraction techniques in the literature are Soxhlet, maceration, and ultrasonic extraction methods. Soxhlet extraction involves placing the dried and fragmented sample in a thimble, which is then inserted into a distillation flask containing the solvent for analyte extraction from solid materials [17-18]. In ultrasonic-assisted extraction, energy is transferred to the solvent and solid particle mixture through waves, resulting in forceful extraction [18-19]. Maceration, another widely employed extraction method, utilizes solvents such as methanol, ethanol, or acetone along with plant material placed in a maceration bath and agitated at varying time intervals [18-19].

This study comprises two primary phases: the first involves the extraction of *Calendula officinalis* using various extraction procedures, while the second entails measuring the DPPH radical scavenging activity of these extracts. *Calendula officinalis* flowers are subjected to extraction utilizing three commonly employed methods in the literature: Soxhlet, maceration, and ultrasonic extraction. Optimal conditions for each extraction method are determined based on prior studies. In the subsequent phase of the study, the DPPH radical scavenging activity of each extract is investigated across varying concentrations. The objective is to ascertain the most effective extraction method for *Calendula officinalis* in terms of antioxidant properties and to observe an augmentation in activity with increasing extract concentration.

This study seeks to enrich existing literature by assessing and contrasting the antioxidant potential of *Calendula officinalis* extracts obtained through Soxhlet, maceration, and ultrasonic extraction methods employing ethanol as the solvent. The significance of this study lies in its comparative analysis of extraction methods and the identification of the optimal technique to harness the antioxidant benefits of *Calendula officinalis* flowers. This endeavor addresses the gap in knowledge regarding the relative efficacy of different extraction methods reported in the literature.

MATERIALS and METHODS

Herbal material:

The dried flowers of *Calendula officinalis* were purchased from Herbal Vital.

Chemicals:

Ethanol, methanol and Dimethyl Sulfoxide (DMSO) were purchased from Merck (Merck KGaA, Darmstadt, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma Aldrich (Sigma-Aldrich Pty Ltd, an affiliate of Merck KGaA, Darmstadt, Germany)

Methods

The presented study encompasses the extraction of *Calendula officinalis* flowers using three distinct procedures: Soxhlet, maceration, and ultrasonic extraction. Concurrently, the antioxidant activities of the extracts are evaluated utilizing a UV-visible spectrophotometer (GENESYS 150).

Figure 1. Dried and crumbled *Calendula officinalis* flower tops.

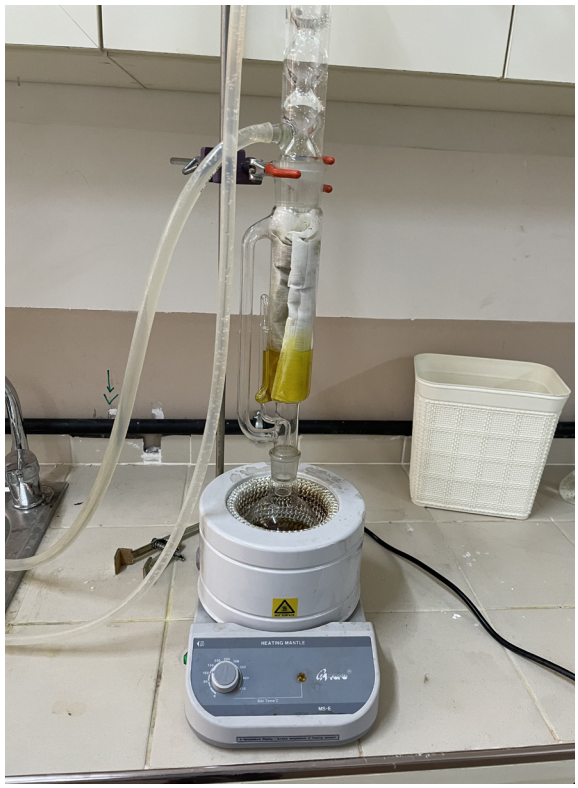

Figure 2. Soxhlet extraction of *Calendula officinalis* flower tops.

Extraction

The active components were extracted from dried *Calendula officinalis* flower tops collected from Cukurova, Turkey. These dried flowers were manually crumbled by hand, as depicted in Figure 1, and subsequently utilized for extraction employing Soxhlet, maceration, and ultrasonic methods.

Soxhlet Extraction

Soxhlet extraction is a method whereby the dried and crumbled sample is enclosed within a thimble, which is then positioned in a distillation flask containing the solvent [17-18]. In this particular study, *Calendula officinalis* flowers serve as the sample, with ethanol employed as the solvent. A total of 16 grams of dried and crumbled *Calendula officinalis* flower tops were prepared as the sample, and 200 ml of ethanol was utilized as the solvent. The extraction process was conducted at 60°C for a duration of 3 hours, as illustrated in Figure 2.

The ethanolic extract obtained with soxhlet method was evaporated using rotary evaporator for 50 minutes at 130 rpm and 45°C to obtain a pasty extract as shown in Figure 3.

Ultrasonic Extraction

The second extraction method employed in this study is ultrasonic extraction, also known as ultrasonic-assisted extraction. This technique involves the transfer of ultrasonic energy to the solvent and solid particle mixture, generating waves that exert force. In our experiment, dried and crumbled *Calendula officinalis* flowers served as the solid particles, while a mixture of distilled water and ethanol was used as the solvent. Specifically, 70 ml of ethanol and 130 ml of distilled water were added to 8 grams of *Calendula officinalis* dried and crumbled flowers. Subsequently, the mixture was placed inside an ultrasonic bath, as depicted in Figure 4. Ultrasonic extraction was carried out at 40°C for a duration of 20 minutes.

The filtered extract obtained with ultrasonic method including distilled water and ethanol was evaporated using rotary evaporator for 150 minutes at 130 rpm and 45°C to obtain pasty extract as shown in Figure 5.

Figure 3. Pasty extract of *Calendula officinalis* – Soxhlet.

Figure 4. Ultrasonic extraction of *Calendula officinalis* flower tops.

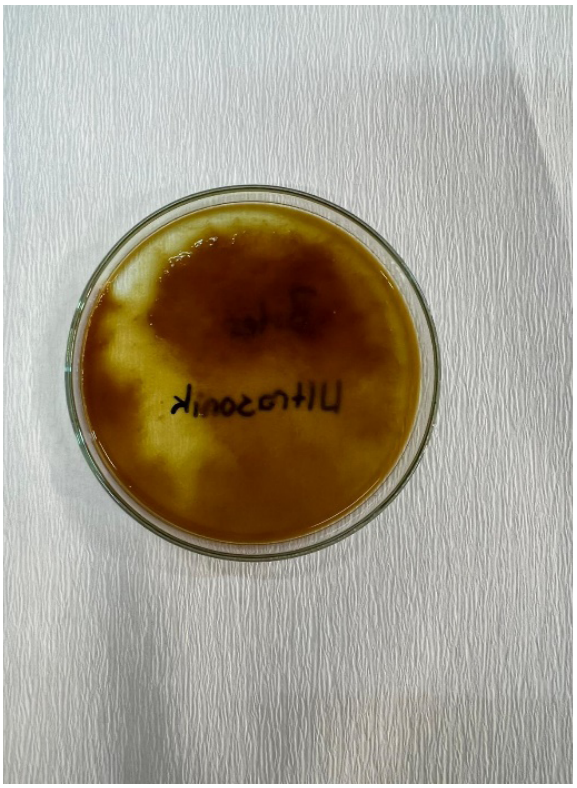

Figure 5. Pasty extract of *Calendula officinalis* - Ultrasonic.

Figure 6. Maceration extraction of *Calendula officinalis* flower tops.

Figure 7. Pasty extract of *Calendula officinalis* - Maceration.

Maceration Extraction

Maceration stands as one of the extensively employed extraction methods, involving a solvent such as methanol, ethanol, or acetone, combined with a mixture of plant material. Following this, the mixture is deposited into a maceration bath and stirred at varying time intervals [18]. In our investigation, 12 grams of powdered *Calendula officinalis* flowers were utilized as the plant material. The solvent comprised 130 ml of ethanol and 70 ml of distilled water. The mixture was then placed in the maceration bath, as illustrated in Figure 6.

The mixture underwent agitation in the maceration bath at 40°C for a duration of 50 minutes. Subsequently, the mixture was filtered, and the ethanol and distilled water content within was evaporated using a rotary evaporator. The evaporation process occurred over 110 minutes at 130 rpm and 45°C. Following evaporation, a pasty extract was obtained, as depicted in Figure 7.

RESULTS and DISCUSSION

Antioxidant Activity Measurements

The radical scavenging capacity of plant extracts can be assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, which is widely recognized as the most popular colorimetric assay for this purpose [20]. This assay offers an easy and rapid means of determining the antioxidant properties of plant extracts by observing the reduction of DPPH free radicals, which is then measured using a UV-visible spectrophotometer [21].

In accordance with the preceding sections of this paper, three distinct types of *Calendula officinalis* extracts were obtained using the Soxhlet, ultrasonic, and maceration extraction methods. The antioxidant activity of these extracts was evaluated using a DPPH solvent with a ratio of 1 mg DPPH per 25 ml of methanol. For the antioxidant activity measurements, the plant extract was prepared using a solvent consisting of 1 mg extract per 1 ml of dimethyl sulfoxide (DMSO). Furthermore, the antioxidant activities of the extracts were assessed across various extract concentrations, with the tested solution concentrations summarized in Table 1. (PE: Plant Extract)

Table 1. Solutions prepared for antioxidant measurements.

Concentration	50µL	100 µL	150 µL	200 µL	250 µL
Sample 1	50 µL PE 450 µL DMSO 500 µL DPPH	100 µL PE 400 µL DMSO 500 µL DPPH	150 µL PE 350 µL DMSO 500 µL DPPH	200 µL PE 300 µL DMSO 500 µL DPPH	250 µL PE 250 µL DMSO 500 µL DPPH
Sample 2	50 µL PE 450 µL DMSO 500 µL DPPH	100 µL PE 400 µL DMSO 500 µL DPPH	150 µL PE 350 µL DMSO 500 µL DPPH	200 µL PE 300 µL DMSO 500 µL DPPH	250 µL PE 250 µL DMSO 500 µL DPPH
Sample 3	50 µL PE 450 µL DMSO 500 µL DPPH	100 µL PE 400 µL DMSO 500 µL DPPH	150 µL PE 350 µL DMSO 500 µL DPPH	200 µL PE 300 µL DMSO 500 µL DPPH	250 µL PE 250 µL DMSO 500 µL DPPH
Blank	50 µL PE 950 µL DMSO	100 µL PE 900 µL DMSO	150 µL PE 850 µL DMSO	200 µL PE 800 µL DMSO	250 µL PE 750 µL DMSO

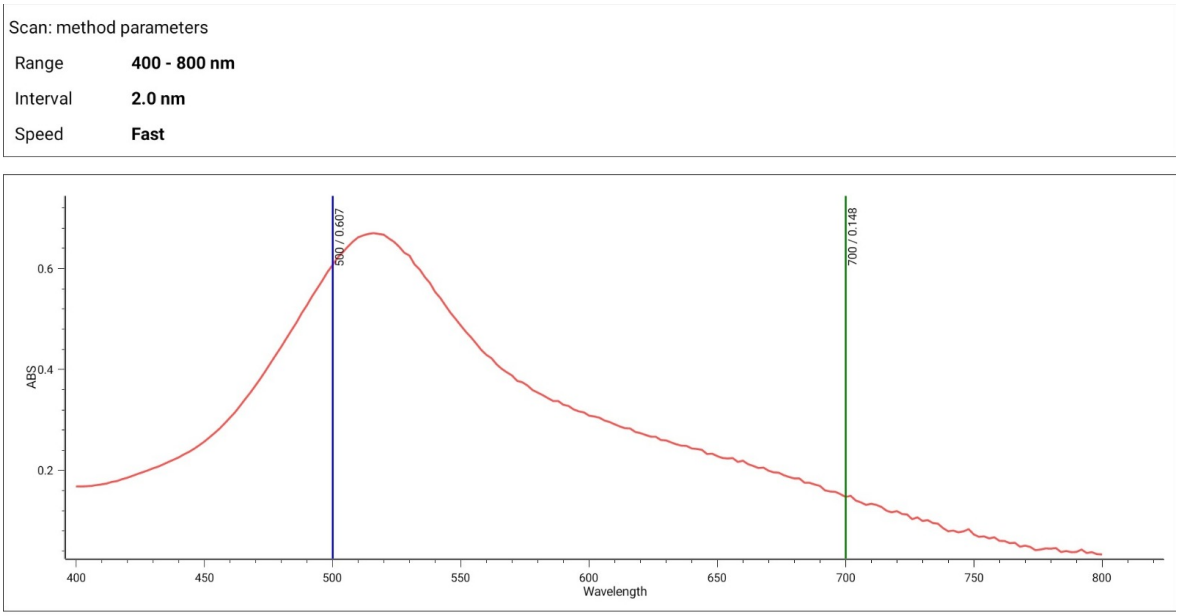

Figure 8. UV spectrum scan result for DPPH.

Figure 9. DPPH absorbance measurement set up for *Calendula officinalis* extract.

Table 2. Antioxidant Activity of *Calendula officinalis* Extract Obtained with Soxhlet, Ultrasonic and Maceration Methods (%)

Concentration	50 µL	100 µL	150 µL	200 µL	250 µL
Soxhlet Method	42.2 %	44.6%	74.4%	77.9%	84.4%
Ultrasonic Method	19.0%	32.7%	39.3%	41.1%	50.6%
Maceration Method	33.3%	41.1%	53.6%	58.3%	61.9%

The DPPH free radical scavenging capacity of each extract type obtained in this study was assessed across various concentration levels. To determine the peak of maximum UV absorbance for 2,2-diphenyl-1-picrylhydrazyl (DPPH), a UV spectrum scan was conducted, as illustrated in Figure 8. Based on the results of the UV spectrum scan for DPPH and a review of the literature, the absorbance values of the extracts were measured at 515 nm using a UV-visible spectrophotometer, as demonstrated in Figure 9.

The absorbance measurements of *Calendula officinalis* extracts obtained through Soxhlet, maceration, and ultrasonic methods at five different concentration levels were conducted. Each measurement was repeated three times for each extraction method and concentration level. The average values of the three samples for each measurement case were obtained, and the antioxidant activity of the *Calendula officinalis* extracts was calculated using an absorbance value of 0.56 for the control solution. The antioxidant activities obtained for each extract and concentration level were presented in Table 2.

Discussion

There is extensive research in the literature on the medicinal use of various plant types, with antioxidant properties being among the most notable purposes. *Calendula officinalis* stands out as one of the most renowned plants, exhibiting antioxidant, anti-inflammatory, cytotoxic, and antitumor properties. Numerous studies have delved into exploring these properties of *Calendula officinalis*. For instance, the study referenced in [22] aimed to assess the impact of extraction methods on the quality of herbal extracts and optimize the extraction of rutin from C. officinalis, with results indicating that ultrasonic-assisted extraction proved more efficient in extracting rutin. Additionally, the study referenced in [16] demonstrated that *Calendula officinalis* extract effectively scavenged superoxide, hydroxyl, and nitric oxide radicals in vitro, as well as superoxide generated in vivo.

In this study, the antioxidant properties of *Calendula officinalis* flowers were investigated, and a comparison of antioxidant activities of *Calendula officinalis* extracts obtained through different extraction methods was analyzed. Various extraction techniques were utilized to obtain extracts, and their antioxidant activity was evaluated using the DPPH test. Specifically, the DPPH radical scavenging activity of the extracts obtained through different extraction methods varied across different concentrations (ranging from 50µL to 250µL). The Soxhlet-extracted extract exhibited higher antioxidant activity, ranging from 42.2% to 84.4% across different concentrations, compared to the other methods. The ultrasonic extraction method demonstrated antioxidant activity ranging from 19.0% to 50.6%, while the maceration method showed activity ranging from 33.3% to 61.9% across different concentrations. These findings underscore the efficacy of the Soxhlet extraction method in obtaining potent antioxidant extracts from *Calendula officinalis* flowers using ethanol solvent. In conclusion, *Calendula officinalis* flowers display significant antioxidant potential, with the Soxhlet extraction method emerging as the most effective approach with ethanol solvent. Further research endeavors can explore practical applications and therapeutic benefits, potentially leading to the development of new antioxidant-based products and treatments.

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