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# *Determination of the Effect of Different Extraction Methods on Aloe barbadensis Miller (Aloe Vera) Extract and its Usability in Ayran*

# *Farklı Ekstraksiyon Yöntemlerinin Aloe barbadensis Miller (Aloe Vera) Ekstraktı Üzerine Etkisinin ve Ayran Üretiminde Kullanılabilirliğinin Belirlenmesi*

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## Abstract

The *Aloe barbadensis Miller (Aloe vera)* plant has been gaining increasing popularity in recent years, especially in the fields of health and cosmetics. Its use in the food industry has also been on the rise late. There is particularly growing interest in its application as an edible film and coating because of its unique structure and composition. In this study, *Aloe vera* gel and leaf parts were evaluated separately. As extraction methods, maceration and ultrasonic wave-assisted extraction methods were used, and ethanol, water, and ethanol/water (1:1) mixtures were used as solvents. The effects of different extraction methods and solvents on the extracts were investigated. The total phenolic substance content, DPPH scavenging activity, and total flavonoid substance content of the obtained extracts were calculated. The usability of the obtained *Aloe vera* gel was then investigated in ayran. Sensory analyses were performed on ayran samples obtained by adding ayran at different rates. The highest amount of phenolic substance in *Aloe vera* gel was calculated as 508.80 mg GAE /g in the extract obtained using water as the solvent and the ultrasonic wave-assisted extraction method as the method. The highest DPPH scavenging activity 27.06% was detected in *Aloe vera* gel under the same extraction conditions. When we evaluated the total amount of flavonoids, the highest amount was found to be 163.79 mgQE/g when ethanol/water mixture was used as a solvent in the maceration method. In the extract obtained from *Aloe vera* leaf, where ethanol:water (1:1) solvent was used as solvent and ultrasonic wave-assisted extraction, the highest amount of phenolic substance was 597.63 mg GAE /g, the highest DPPH activity was 66.38%, and the highest total amount of flavonoid substance was 409.20 mg QE /g. When we evaluated the

results generally, the amount of total phenolic substance, DPPH scavenging activity, and total amount of flavonoid substance increased in direct proportion to each other. Compounds with phenolic and flavonoid properties increased with DPPH scavenging activity. *Aloe vera* gel was added to ayran samples at different rates (%1.25, 2.5 and 5). Samples of ayran added at a rate of 1.25% received scores similar to those of the control group without any addition. Other samples of ayran also received high scores from the panelists. In this study, the extraction conditions of *Aloe vera* gel and leaf were optimized and their usage possibilities in ayran were evaluated.

**Keywords:** *Aloe barbadensis Miller (Aloe vera)*, Extraction, Ayran.

## Özet

*Aloe barbadensis Miller (Aloe vera)* bitkisinin son yıllarda kullanım alanı giderek artmaktadır. Özellikle sağlık ve kozmetik sektörlerinde yaygın olarak kullanılmaktadır. Gıda endüstrisindeki kullanımı da son dönemde artış göstermektedir. Bitkinin kendine özgü yapısı ve bileşimi nedeniyle, yenilebilir film ve kaplama olarak kullanımı ile ilgili çalışmalar özellikle ilgi çekmektedir. Bu çalışmada *Aloe vera* jel ve yaprak kısımları ayrı ayrı farklı olarak değerlendirilmiştir. Ekstraksiyon yöntemleri olarak maserasyon ve ultrasonik dalga destekli ekstraksiyon yöntemleri ve çözücü olarak etanol, su ve etanol:su (1:1) çözücüsü kullanılmıştır. Farklı ekstraksiyon yöntem ve farklı çözücülerin ekstraktlara etkisi araştırılmıştır. Elde edilen ekstraktların toplam fenolik madde miktarı, DPPH süpürme aktivitesi ve toplam flavonoid madde miktarı hesaplanmıştır. Sonrasın da elde edilen *Aloe vera* jelinin ayranda kullanılabilirliği araştırılmıştır. Farklı oranlarda ayrana ilave edilerek elde edilen ayran örneklerinde duyusal analizler gerçekleştirilmiştir. *Aloe vera* jelinde en yüksek fenolik madde miktarı çözücü olarak su ve yöntem olarak ultrasonik dalga destekli ekstraksiyon yönteminin kullanılmasıyla elde edilen ekstraktta 508.80 mg GAE/g olarak hesaplanmıştır. *Aloe vera* jelinde aynı ekstraksiyon şartlarında en yüksek DPPH süpürme aktivitesi %27.06 tespit edilmiştir. Toplam flavonoid miktarını değerlendirdiğimizde maserasyon yönteminde etanol/su karışımını çözücü olarak kullandığımızda en yüksek miktar olan 163.79 mg QE/g tespit edilmiştir. *Aloe vera* yaprağında ise etanol/su karışımının çözücü olarak kullanıldığı ve ultrasonik dalga destekli ekstraksiyon ile elde edilen ekstraktta en yüksek fenolik madde miktarı 597.63 mg GAE/g, en yüksek DPPH süpürme aktivitesi %66.38 ve en yüksek toplam Flavonoid madde miktarı 409.20 mg QE/g olarak tespit edilmiştir. Genel olarak değerlendirdiğimizde toplam fenolik madde miktarı, DPPH süpürme aktivitesi ve toplam flavonoid madde miktarı birbiriyle doğru orantılı şekilde artış göstermiştir. Fenolik ve flavonoid özellik gösteren bileşikler DPPH süpürme aktivitesinin artmasını sağlamıştır. Ayran örneklerine *Aloe vera* jeli farklı oranlarda % (1.25, 2.5 ve 5) eklenmiştir. %1.25 oranında eklenen ayran örnekleri hiç eklenmeyen kontrol grubuyla benzer puanlar almıştır. Diğer ayran örnekleride panelistlerden yüksek puanlar almıştır. Bu çalışma ile *Aloe vera* jel ve yaprağının ekstraksiyon şartları optimize edilmiştir ve ayranda kullanım olanakları değerlendirilmiştir.

**Anahtar Kelimeler:** *Aloe barbadensis Miller (Aloe vera)*, Ekstraksiyon, Ayran

## 1. INTRODUCTION

*Aloe barbadensis Miller (Aloe vera);* has been revered as a medicinal plant for centuries, with its use dating back to ancient civilizations (Christachi & Florou-Paneri, 2010). Discovered by Greek scientists around 2000 years ago, *Aloe vera;* has earned the moniker "universal panacea" or "cure-all" for its purported wide range of health benefits. The Egyptians, in particular, associated *Aloe vera;* with immortality (Surjushe et al., 2008).

*Aloe vera* is remarkably rich in polysaccharides (Eshun and He, 2004). Polysaccharides are a crucial class of high-molecular-weight carbohydrates derived from microorganisms, animals, and plants (Li et al., 2018). These polysaccharides exhibit a remarkable array of bioactivities, including antimicrobial (Luiz et al., 2017), antitumor (Nazeam et al., 2017), antiviral (Xie et al., 2016), and antioxidant (Chen et al., 2016) properties. Because of their exceptional and versatile properties, polysaccharides have gained widespread use in healthcare products and medicines.

*Aloe vera* is one of the most widely used medicinal plants globally for disease prevention and treatment. It is particularly effective in addressing skin disorders, metabolic diseases, cardiovascular ailments, and cancer. Research has consistently demonstrated that *Aloe vera* leaves possess immunomodulatory, antimicrobial, antiviral, anticancer, and anti-inflammatory properties (Reynolds & Dweck, 1999; Strickland, 2001; Harlev et al., 2012).

*Aloe vera* leaves are rich sources of bioactive compounds with remarkable antioxidant properties. These compounds include mannose-rich polysaccharides (mannans), anthraquinones, C-glycosides, and lectins, which have found extensive applications in the food industry (Rodriguez et al., 2010).

*Aloe vera* leaf is composed of two primary components: latex and gel. Latex, also known as "aloe juice" or "aloe extract," accounts for approximately 20-30% of the entire leaf. This bitter, yellow liquid is exuded from the pericyclic tubules located beneath the leaf's epidermis (Boudreau & Beland, 2006). *Aloe vera* latex is particularly rich in phenolic compounds and exhibits potent antibacterial activity against Gram-positive bacteria (Boudreau & Beland, 2006; Surjushe et al., 2008; Alemdar & Agaoglu, 2009).

*Aloe vera* gel is a remarkable substance extracted from the parenchymal cells of fresh *Aloe vera* leaves. This colorless, sticky gel constitutes approximately 70-80% of the *Aloe vera* product. Its notable properties include therapeutic, antibacterial, and antifungal effects, and biodegradability. Owing to its flavoring and preservative attributes, *Aloe vera* gel finds

extensive use as a functional food ingredient in beverages, ice creams, and confectionery products (Eshun & He, 2004; Boudreau & Beland, 2006).

*Aloe vera* gel's versatility extends beyond its culinary applications. It serves as an edible and bio-safe protective film and coating material for various food items (Valverde et al., 2005). The gel's rich composition includes bioactive compounds such as salicylic acid and magnesium lactate. Additionally, it harbors mucopolysaccharides, enzymes, and sterols, including the antioxidant superoxide dismutase (Vogler & Ernst, 1999).

Biologically active components from *Aloe vera* gel can be obtained through traditional methods like maceration or novel extraction techniques like ultrasound-assisted extraction. These methods enable the efficient and rapid extraction of valuable compounds from plant material (Elferjane et al., 2023).

In the maceration method, the plant material is first shredded, mixed with the appropriate solvent, and maintained at room temperature. After the process, the mixture is filtered with filter paper (Azwanida, 2015; Majekodunmi, 2015; Ingle et al., 2017). The maceration method ensures the preservation of phenolic and aroma compounds. It minimizes the loss of aroma compounds and aids in the extraction of phenolic substances by modifying plant cell walls. The extraction process takes a long time (Olejar et al., 2015). Ultrasonic sound waves are characterized by a frequency range generally exceeding 16-18 kHz. These waves propagate as mechanical vibrations in solid or liquid media (Mason & Lorimer, 2002). During the extraction process, acoustic waves propagate in the liquid medium and cause the displacement of plant particles. Cavitation occurs when mechanical vibrations are transmitted to the plant (Büyüktuncel, 2012; Turan et al., 2021). This extraction method can be applied to both solid and liquid samples. Extraction results in a shorter time and higher yield (Büyüktuncel, 2012).

This study compares the maceration method and the ultrasonic wave-assisted extraction method. Two different extraction techniques were used in this study. Additionally, three different solvents were used. The total phenolic substance content, DPPH scavenging activity, and total flavonoid content of the obtained extracts were determined. According to the results of these analyses, the most suitable solvent and extraction method for the *Aloe vera* plant was determined. In addition, *Aloe vera* gel was used in ayran at different rates, and the optimum usage rate was determined.

## 2. MATERIALS and METHOD

### 2.1. Material

*Aloe vera* plant used in the research was grown in the Laboratory of Amasya University Suluova Vocational School. The leaves and gel of the plant were separated. Then, they were used in extraction. Ethanol (Merck.Darmstadt. Germany) and water were used as solvents. Ayran was purchased from a local grocery store. All chemicals were of analytical grade and were obtained from Merck Darmstadt. Germany.

### 2.2. Method

#### 2.2.1. Preparation of Extract

The gel part and leaves of the *Aloe vera* plant were separated. Afterwards, they were ground into small pieces. Ethanol, water and ethanol-water (1:1) mixtures were used as solvents. Extraction processes were performed using the ultrasonic wave-assisted extraction method at 40 °C and 40 min. (Çalışkan Lab. Ult 4010, Turkey). In the maceration method, the extraction process was performed at room temperature for 3 days. The mixtures were then filtered. And The filtrates were evaporated using with rotary evaporator (Buchi R100, Türkiye). The total phenolic component, DPPH scavenging activity, and total flavonoid analyses were applied to the obtained extracts. *Aloe vera* plant extraction stages are shown in Figures 1 and 2.

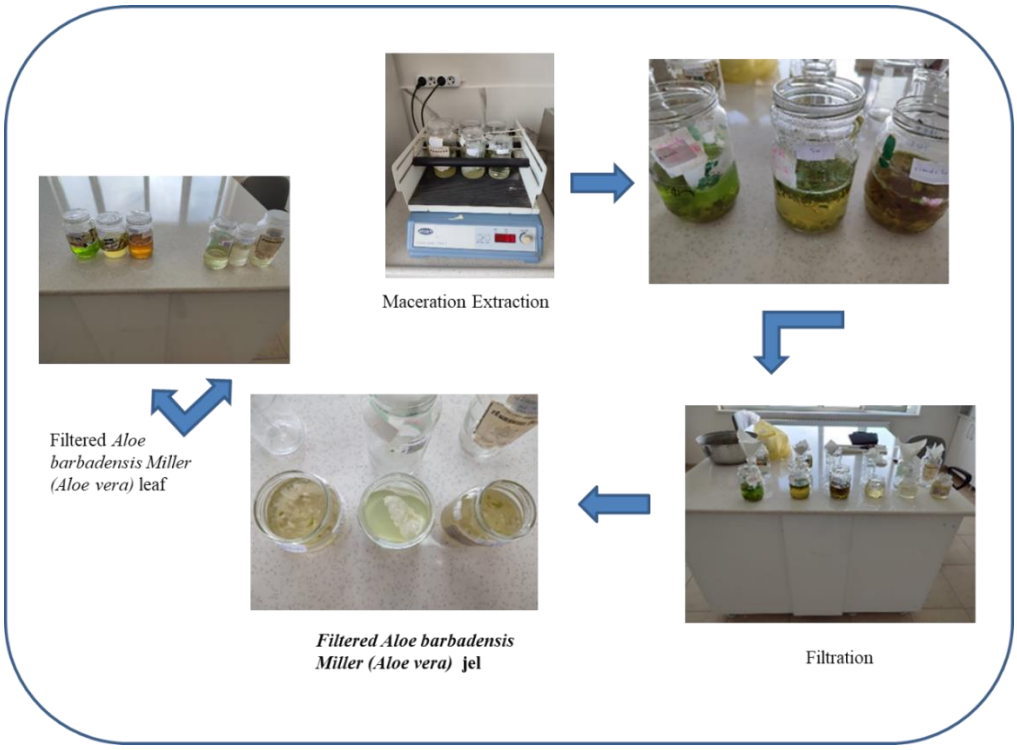


Figure 1. *Aloe vera* extraction with maceration technique

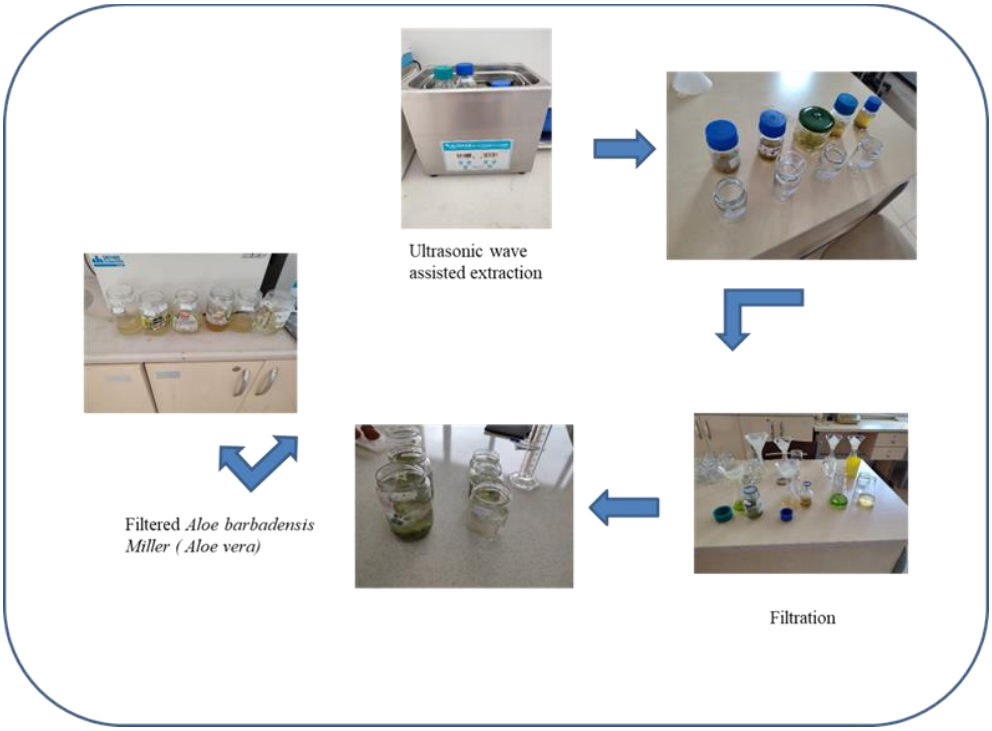


Figure 2. *Aloe vera* extraction using ultrasonic assited extraction technique stages

### 2.2.2. Total Phenolic Compounds

The total phenolic content of *Aloe vera* extracts was determined using the Folin-Ciocalteu method described by Singleton and Rossi (1965). First, 40 µL of the prepared extract (1 mg/mL) was mixed with 2.4 mL of distilled water and 200 µL of Folin-Ciocalteu reagent. After 30 seconds, 600 µL of saturated $Na_2CO_3$ and 760 µL of distilled water were added. The mixture was vortexed and incubated for 2 h at room temperature. The absorbance of the mixture was measured at 650 nm using a spectrophotometer (UV-1601 Shimadzu, Kyoto, Japan). Results: Gallic acid was given in gallic acid equivalent (GAE) using the standard calibration curve (y = 0.001x+0.0557).

### 2.2.3. DPPH Free Radical Scavenging Activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of *Aloe vera* extracts was evaluated according to Singh et al. (2002). 200 µL (1 mg/mL) of the extract was mixed with 3.8 mL of diluted DPPH solution ($1.0 \times 10^{-3}$ M). The mixture was vortexed for 15 s and kept in the dark for 60 min. At the end of the incubation period, the absorbance of the mixture was measured at 515 nm using a spectrophotometer. The DPPH scavenging activity was calculated as the percentage inhibition (Aksoylu, 2012).

### 2.2.4. Total Flavonoid Component

The total flavonoid content of the extracts was determined according to the method described by Zhishen et al. (1999). A certain volume of (1 mg/mL) was taken and mixed with 0.15 mL of 5% $NaNO_2$ solution. The mixture was incubated for 6 min, and then 0.15 mL of 10% (w/v) $AlCl_3$ solution was added and incubated for another 6 min. At the end of the incubation period, 2 mL of 1 M NaOH solution was added to the solution in the test tube, and the total volume was completed to 5 mL with pure water.The absorbance values of the extracts were read at 510 nm using a spectrophotometer at room temperature after a 15-min incubation period.Total catechin was used as the standard in the flavonoid determination studies.The total flavonoid content of the extract was calculated as mg (+) catechin/100 g sample using the calibration curve.

### 2.2.5. Production of Ayran

For the study, ayran samples obtained from the market were used. The research involved preparing control group ayran, ayran containing 1.25% *Aloe vera* extract, ayran containing 2.5% *Aloe vera* extract, and ayran containing 5% *Aloe vera* extract. Sensory analyses were performed on the prepared ayran samples.

### 2.2.6. Sensory Analysis

Sensory analysis was conducted on the prepared ayran samples by faculty members at Suluova Vocational School. The sensory analysis evaluated appearance, color, texture, odor, taste-aroma, and overall liking using a scale of 1 (very poor) to 9 (very good). Sensory analyses were performed by a panel of 10 panelists. The results were statistically evaluated (Seyrekoğlu, 2020).

### 2.2.7. Statistical Analysis

All analyses were performed in triplicate, and the mean standard deviations were calculated. The effects of the samples on the total phenolic content, DPPH scavenging activity, and total flavonoid content were determined by one-way ANNOVA analysis. All data were evaluated by variance analysis (ANOVA) using the SPSS program (SPSS 16.0). Statistical significance ($p=0.05$) was determined using Tukey's test (IBMCorp., 2011).

## 3. RESULTS and DISCUSSIONS

### 3.1. Total Phenolic Content of *Aloe vera* Extracts

In this study, two different extraction methods were used: ultrasonic-assisted extraction and maceration. Two different parts of the plant, namely the gel and leaf parts, were used in the extraction. Water, ethanol, and ethanol-water mixtures (1:1) were preferred as solvents. The total phenolic content values of *Aloe vera* extract were showed in Table 1.

Table 1. Total phenolic content of *Aloe vera* extracts

| **Samples** | **Parts of plant** | **Solvent Type** | **Extraction Method** | **Total phenolic content (mg GAE/g)** |
|---|---|---|---|---|
| **1** | *Aloe vera* gel | Ethanol | Ultrasonic wave-assisted extraction | $53.80^{g}$ ± 1.50 |
| **2** | *Aloe vera* gel | Water | Ultrasonic wave-assisted extraction | $508.80^{b}$ ± 0.50 |
| **3** | *Aloe vera* gel | Ethanol: Water (1:1) | Ultrasonic wave-assisted extraction | $208.30^{f}$ ± 2.00 |
| **4** | *Aloe vera* leaf | Ethanol | Ultrasonic wave-assisted extraction | $522.97^{b}$ ± 30.23 |
| **5** | *Aloe vera* leaf | Water | Ultrasonic wave-assisted extraction | $358.63^{e}$ ± 10.50 |
| **6** | *Aloe vera* leaf | Ethanol: Water (1:1) | Ultrasonic wave-assisted extraction | $597.63^{a}$ ± 23.96 |
| **7** | *Aloe vera* gel | Ethanol | Maceration extraction | $224.97^{f}$ ± 28.93 |
| **8** | *Aloe vera* gel | Water | Maceration extraction | $443.97^{c}$ ± 35.11 |
| **9** | *Aloe vera* gel | Ethanol: Water (1:1) | Maceration extraction | $452.80^{c}$ ± 21.36 |
| **10** | *Aloe vera* leaf | Ethanol | Maceration extraction | $329.13^{e}$ ± 44.22 |
| **11** | *Aloe vera* leaf | Water | Maceration extraction | $402.47^{d}$ ± 2.92 |
| **12** | *Aloe vera* leaf | Ethanol: Water (1:1) | Maceration extraction | $529.97^{b}$ ± 27.06 |

Different superscript letters in the same column indicate a significant difference (p>0.05).

In ultrasonic-assisted extraction of *Aloe vera* gel, the highest total phenolic content of 508.80 mg GAE/g was observed with water as the solvent, whereas the lowest total phenolic content of 53.80 mg GAE/g was observed with ethanol as the solvent. In the maceration method extraction of *Aloe vera* gel, the highest total phenolic content of 452.80 mg GAE/g was calculated when ethanol/water (1:1) was used as the solvent, whereas the lowest total phenolic content of 224.97 mg GAE/g was calculated when ethanol was used as the solvent.

In the maceration method, the total phenolic content was higher than that of the ultrasonic-assisted extraction method when water and ethanol-water mixture was used as the solvent, whereas the opposite was observed when ethanol was used as the solvent.

In ultrasonic-assisted extraction of *Aloe vera* leaves, the highest total phenolic content of 597.63 mg GAE/g was found when ethanol-water (1:1) mixture was used as the solvent, whereas the lowest total phenolic content of 358.63 mg GAE/g was found when water was used as the solvent. In the maceration method, similarly, ethanol-water (1:1) mixture was calculated as the highest phenolic compound with 529.97 mg GAE/g. When the two methods were compared, the use of ethanol and ethanol-water (1:1) mixture as the solvent provided higher total phenolic compound amounts in the ultrasonic-assisted extraction method, whereas the use of water as the solvent provided higher total phenolic compound amounts in the maceration method.

In this study, the total amount of phenolic substances in *Aloe vera* samples was calculated as 53.80-508.80 mg GAE/g in the ultrasonic wave-assisted extraction method and 224.97-452.80 mg GAE/g in the maceration method. In the *Aloe vera* leaf, it was determined as 358.63-597.63 mg GAE/g in the ultrasonic wave-assisted extraction method, whereas it was determined as 329.13-529.97 mg GAE/g in the maceration method. Elferjane et al. (2023) determined the maximum amount of phenolic compounds in, *Aloe vera* extracts as 9.95 mg GAE/g in the maceration method and 6.74 mg GAE/g in the ultrasonic wave-assisted extraction method. In another study conducted with *Aloe vera* samples, the total amount of phenolic components was determined as 56.11-93.96 mg GAE/g (Başaran, 2020).

In their study, Vega-Gálvez et al. (2011) investigated the total phenolic content of *Aloe vera* gel after subjecting it to different pressures (300, 400, and 500 MPa) for 35 days. All samples subjected to pressure (43.40±19.53–76.12±19.60 mg GAE/100), total phenol compared with the non-pressurized sample (178.45±14.76 mg GAE/100), a significant decrease

in their content was observed. Ray et al. (2013) used the freeze-drying method for *Aloe vera*. The total phenolic values in the samples were 30.11±1.89-35.77±1.07 µg GAE/mg.

Phenolic contents change during the growth periods of the plant and They also stated that there is a decrease in phenolic contents depending on the age of the plant. In their study, Miladi and Damak (2008) compared the effects of *Aloe vera* leaf on ethanol extracts. They applied distillation using hexane, ethyl acetate, chloroform-ethanol, and butanol. Chloroform-ethanol had the highest total phenolic content (40,500±0,041 µg GAE/mg) as a fraction.

In another study, *Aloe vera* peel extract exhibited the highest total phenolic content (7.99±0.26 mg GAE/g). In contrast, gel extracts displayed significantly lower phenolic content, nearly three times lower for Soxhlet extraction and four times lower for ultrasound extraction. These findings highlight the crucial role of the extraction method in determining the phenolic content. Soxhlet extraction, which involves prolonged heating, may lead to the degradation of heat-sensitive phenolic compounds (Vidic et al., 2014). Supporting this notion, previous studies (Miladi and Damak, 2008; Kammoun et al., 2011) have demonstrated that water extracts exhibit low phenolic content (2mg GAE/g), while chloroform-ethanol extracts possess a substantially higher phenolic content of approximately 40 mg GAE/g.

The total amount of phenolic compounds in both the leaf and gel parts of our *Aloe vera* samples were found to be significantly higher than that reported in the literature. Aldayel et al. (2020) showed the differences in the analysis results based on the phytochemical properties of *Aloe vera* plant composition; geography where the plant grows, climate, soil type, sun exposure, and seasonality. Changes were associated with elements such as the age of the plant. Based on the findings of this study, the most suitable extraction method for *Aloe vera* gel is ultrasound-assisted extraction using water as the solvent. For *Aloe vera* leaves, the recommended method is also ultrasound-assisted extraction, but with a solvent mixture of ethanol and water (1:1). In line with the findings of this study, numerous studies in the literature have also demonstrated that the combination of ethanol and water as solvents yields a remarkable increase in the total phenolic content of extracts. This synergy between ethanol and water can be attributed to their ability to effectively solubilize a various phenolic compounds, including both polar and non-polar compounds.

### 3.2. DPPH Scavenging Activity of *Aloe vera* Extracts

Table 2 presents the DPPH scavenging activity values of *Aloe vera* samples. When water was used as the solvent in the ultrasonic wave-assisted extraction method, the DPPH scavenging

activity of the *Aloe vera* gel reached its highest value (27.06%), which is consistent with the total phenolic content findings. The lowest value (7.25%) was obtained when ethanol was used as the solvent in the same method. In the maceration method, the highest DPPH scavenging activity (26.96%) was again achieved with water as the solvent. The lowest inhibition (4.70%) was observed when ethanol was used as the solvent in the maceration method. In both extraction methods, the use of water as the solvent increased the DPPH scavenging activity.

Table 2. DPPH scavenging activity of *Aloe vera* extracts

| **Samples** | **Parts of plant** | **Solvent Type** | **Extraction Method** | **DPPH Scavenging Activity (%)** |
|---|---|---|---|---|
| **1** | *Aloe vera* gel | Ethanol | Ultrasonic wave-assisted extraction | $7.25^{f} \pm 2.42$ |
| **2** | *Aloe vera* gel | Water | Ultrasonic wave-assisted extraction | $27.06^{b} \pm 2.86$ |
| **3** | *Aloe vera* gel | Ethanol: Water (1:1) | Ultrasonic wave-assisted extraction | $26.51^{b} \pm 3.86$ |
| **4** | *Aloe vera* leaf | Ethanol | Ultrasonic wave-assisted extraction | $21.22^{c} \pm 3.44$ |
| **5** | *Aloe vera* leaf | Water | Ultrasonic wave-assisted extraction | $11.67^{e} \pm 0.50$ |
| **6** | *Aloe vera* leaf | Ethanol: Water (1:1) | Ultrasonic wave-assisted extraction | $66.38^{a} \pm 0.23$ |
| **7** | *Aloe vera* gel | Ethanol | Maceration extraction | $4.70^{f} \pm 1.13$ |
| **8** | *Aloe vera* gel | Water | Maceration extraction | $26.96^{b} \pm 0.62$ |
| **9** | *Aloe vera* gel | Ethanol: Water (1:1) | Maceration extraction | $16.09^{d} \pm 0.43$ |
| **10** | *Aloe vera* leaf | Ethanol | Maceration extraction | $8.15^{f} \pm 1.62$ |
| **11** | *Aloe vera* leaf | Water | Maceration extraction | $0.71^{g} \pm 0.24$ |
| **12** | *Aloe vera* leaf | Ethanol: Water (1:1) | Maceration extraction | $28.51^{b} \pm 1.16$ |

Different superscript letters in the same column indicate a significant difference ($p>0.05$).

This study investigated the DPPH scavenging activity of extracts obtained from *Aloe vera* leaves using different extraction methods. The results demonstrate that the highest DPPH

scavenging activity values were achieved when an ethanol-water mixture (1:1) was used in both extraction methods. The lowest DPPH scavenging activity (11.67%) was observed when water was used as the solvent in ultrasonic-assisted extraction. In the maceration method, the lowest activity (0.71%) was also observed with water extraction.

In both ultrasonic wave-assisted extraction and maceration methods, the ethanol-water mixture provided the highest DPPH scavenging activity for *Aloe vera* leaves, whereas water decreased this value.

Antioxidants are substances that protect cells from damage caused by unstable molecules called free radicals. Free radicals can be produced by the body as a byproduct of normal metabolism, or they can be introduced from external sources such as pollution or cigaret smoke. When free radicals accumulate, they can damage cells and DNA, leading to a various of health problems, including cancer, heart disease, and neurodegenerative diseases (Wang et al., 2011).

Kumar et al. (2017) investigated the antioxidant activity of *Aloe vera* from six different agroclimatic zones in India. The researchers found that the antioxidant activity of *Aloe vera* was higher in plants collected from Northern India than in those collected from Southern India. This difference in activity was attributed to the higher content of alkaloids, glycosides, phenolic compounds, flavonoids, and saponin glycosides in Northern Indian plants (Kumar et al., 2017).

The higher antioxidant activity of *Aloe vera* from Northern India may be due to several factors, including; environmental stress**,** soil conditions, and genetic variation. The findings of this study suggest that the antioxidant activity of *Aloe vera* may vary depending on the region where it is grown. This information could be used to identify *Aloe vera* plants with the highest antioxidant activity for potential use in medicinal products or dietary supplements.

*Aloe vera* has been used for centuries for its medicinal properties. Recent research has shown that aloe vera ethanol extract has potent antioxidant properties and can protect cells from oxidative stress. In vitro studies have shown that aloe vera ethanol extract can; reduce the production of ROS (Reactive Oxygen Species), scavenge free radicals, and protect cells from damage caused by hydrogen peroxide and 4-hydroxynonenal (Cesar et al., 2018).

A clinical trial involving 53 healthy volunteers investigated the effects of 14-day supplementation with *aloe vera* gel extract on the antioxidant capacity of the subjects. The results showed that *aloe vera* supplementation significantly increased the subjects' levels of glutathione, a major antioxidant in the body (Sánchez et al., 2020).

This study compared two extraction methods: ultrasonic wave-assisted and maceration. Ultrasonic waves were significantly more effective in extracting antioxidants from both *Aloe vera* gel (7.25-27.06% DPPH scavenging activity) and leaves (11.61-66.38% DPPH scavenging activity) compared to maceration (4.70-26.96% for gel, 0.71-28.51% for leaves). Sathyaprabha et al. (2010) analyzed the antioxidant capacity of dried and powdered *Aloe vera* gel. The DPPH scavenging activity was measured at 15.8%. Çandöken (2008) investigated the water extract of *Aloe vera* pulp. The study found a substance with high hydrogen donor activity (essential for antioxidant properties) at a concentration of 60 mg/ml in the leaves. The water extract of the pulp itself exhibited a DPPH scavenging activity of 70.81 ± 0.27%. Overall, the research highlights that *Aloe vera* possesses significant antioxidant activity, and the method used for extraction can significantly impact the yield of these beneficial compounds.

The antioxidant properties of aloe vera extracts are affected by various initial developments. Changes over time vary (Hu et al., 2003). In the same study, the DPPH radical scavenging fraction of the *Aloe vera* extract was reported to be equal to or greater than that of BHT and α-tocopherol. The DPPH radical scavenging activity of *aloe vera* leaf peel ethanol extract was reported to be 39.7% (Hu et al., 2005).

The reducing power of *Aloe vera* extracts on DPPH radicals has been investigated by various researchers. López et al. (2013); This study compared the DPPH radical scavenging activity of *Aloe vera* leaf skin and flower extracts. Leaf skin extract exhibited significantly higher activity than flower extract, suggesting that the location of extraction within the plant can influence antioxidant properties. In another study the DPPH radical scavenging activity of different solvent extracts of *Aloe vera* gel was observed; both ethanolic and methanolic extracts demonstrated the highest DPPH radical scavenging activity, indicating that the choice of solvent can affect the extraction of antioxidant compounds (Khaing, 2011). In this study, while the DPPH scavenging activity of *Aloe vera* gel was similar to that reported in the literature, the inhibition of *Aloe vera* leaf was observed at lower values. Overall, these findings demonstrate that *Aloe vera* extracts possess significant DPPH radical scavenging activity, which can be influenced by various factors such as extraction conditions, growth stage, and solvent selection.

### 3.3. Total Flavonoid Content of *Aloe vera* Extracts

The total flavonoid content of *Aloe vera* gel was determined to be 80.97 mg QE/g when water was used as the solvent in the ultrasonic wave-assisted extraction method, while it was 69.73 QE mg/g when an ethanol-water (1:1) mixture was used as the solvent. In contrast, the highest

total flavonoid content was calculated as 163.79 mg QE/g when an ethanol-water (1:1) mixture was used as the solvent in the maceration method, whereas the lowest total flavonoid content was calculated as 44.91 mg QE/g when water was used as the solvent.

For *Aloe vera* gel, water is a good solvent in the ultrasonic wave-assisted extraction method, whereas an ethanol-water (1:1) mixture is a good solvent in the maceration method. In the ultrasonic wave-assisted extraction method for *Aloe vera* leaf, the highest total flavonoid content was 409.20 mg QE/g when an ethanol-water mixture was used as the solvent, whereas the lowest total flavonoid content was 157.41 mg QE/g when ethanol was used as the solvent.

Similarly, in the maceration method, the use of an ethanol-water (1:1) mixture resulted in the highest total flavonoid content of 295.18 mg QE/g, whereas the use of water as a solvent resulted in the lowest total flavonoid content of 117.89 mg QE/g. An ethanol-water (1:1) mixture was the best solvent for *Aloe vera* leaves in both the ultrasonic wave-assisted extraction method and the maceration method. The total flavonoid contents of *Aloe vera* extracts are shown in Table 3.

In this study, the total flavonoid content of *Aloe vera* samples was investigated. In the ultrasound-assisted extraction method, the values ranged from 69.73-80.97 mg QE/g for *Aloe vera* gel and 157.41-409.20 mg QE/g for *Aloe vera* leaf. In the maceration method, the values were calculated as 44.91-163.79 mg QE/g for *Aloe vera* gel and 117.89-295.18 mg QE/g for *Aloe vera* leaf.

In a study where *Aloe vera* leaf waste was extracted, the total flavonoid content was calculated as 3.48 mg QE/g using the the maceration method and 2.08 mg QE/g using the ultrasound-assisted extraction method (Elferjane et al., 2023). According to this study, the values obtained are quite high. This difference is due to the different parameters of the different parts of the *Aloe vera* plant, extraction conditions, and the solvent used. Similarly, a study conducted by Abdulbasit (2014) revealed that the methanol extract of *A. vera* exhibited the highest total flavonoid content (1958.27 mg QE/100g) compared with 10 different Arabian herbs and spices. In another study, Shashank and Vidhya (2011) reported that the total flavonoid content of *A. vera* methanol's extract was determined to be 14.10 ± 1.60 mg catechin equivalents/g. According to the results of this study, our findings are quite high. The differences in the literature can be attributed to the age of the plant, the region where it is grown, and the extraction conditions.

Table 3. Total flavonoid contents of *Aloe vera* extracts

| Samples | Parts of plant | Solvent Type | Extraction Method | Total Flavonoid content (mg QE/g) |
|---|---|---|---|---|
| **1** | *Aloe vera* gel | Ethanol | Ultrasonic wave-assisted extraction | $74.23^{h} \pm 2.00$ |
| **2** | *Aloe vera*gel | Water | Ultrasonic wave-assisted extraction | $80.97^{h} \pm 1.37$ |
| **3** | *Aloe vera* gel | Ethanol: Water (1:1) | Ultrasonic wave-assisted extraction | $69.73^{h} \pm 6.25$ |
| **4** | *Aloe vera* leaf | Ethanol | Ultrasonic wave-assisted extraction | $157.41^{d} \pm 3.44$ |
| **5** | *Aloe vera* leaf | Water | Ultrasonic wave-assisted extraction | $190.51^{c} \pm 7.16$ |
| **6** | *Aloe vera* leaf | Ethanol: Water (1:1) | Ultrasonic wave-assisted extraction | $409.20^{a} \pm 16.95$ |
| **7** | *Aloe vera*gel | Ethanol | Maceration extraction | $97.66^{g} \pm 3.56$ |
| **8** | *Aloe vera* gel | Water | Maceration extraction | $44.91^{i} \pm 2.31$ |
| **9** | *Aloe vera* gel | Ethanol: Water (1:1) | Maceration extraction | $163.79^{d} \pm 2.19$ |
| **10** | *Aloe vera* leaf | Ethanol | Maceration extraction | $143.29^{e} \pm 0.69$ |
| **11** | *Aloe vera* leaf | Water | Maceration extraction | $117.89^{f} \pm 11.98$ |
| **12** | *Aloe vera* leaf | Ethanol: Water (1:1) | Maceration extraction | $295.18^{b} \pm 12.26$ |

Different superscript letters in the same column indicate a significant difference ($p>0.05$).

According to the results published by Hu, et al. (2003), three-year-old *Aloe vera* plants exhibited significantly higher levels of polysaccharides and flavonoids compared to two- and four-year-old *Aloe vera* plants. Moreover, no significant difference in flavonoid levels was observed between 3- and 4-year -old Aloe vera plants.

### 3.4. Sensory Properties of Ayran with *Aloe vera* Extract

In the study, samples were obtained as follows: Control group (K), ayran with 5% *Aloe vera* gel added (K1), ayran with 2.5% *Aloe vera* gel added (K2), ayran with 3% *Aloe vera* gel added

(K3). The sensory properties of the ayran samples are shown in Table 4. There was no statistically significant difference in the sensory properties of any sample. The added *Aloe vera* gel did not affect the samples negatively in terms of sensory properties. In the pre-shaking appearance scores, the control group had the highest score, followed by the K3, K1, and K2 groups. In the post-shaking appearance, the K and K3 groups scored the highest, followed by the K2 and K1 groups. The same scoring was observed for color. In the consistency feature, K, K3, followed by K2, and K1 according to the score order. In odor scores, the order was K, K3, K2, and K1, while in taste scores, the order was K3, K, K1 and K2. In general liking scores, K, K3, K1, and K2 groups came from the highest to the lowest.

Table 4. Sensory Analysis Results of Ayran Samples

| **Sensory Properties** | **Samples** | | | |
|---|---|---|---|---|
| | **K** | **$K_1$** | **$K_2$** | **$K_3$** |
| **Appearance before shaking** | $8.20^a \pm 1.09$ | $7.60^a \pm 1.51$ | $7.20^a \pm 1.48$ | $8.00^a \pm 1.00$ |
| **Appearance after shaking** | $8.20^a \pm 0.83$ | $7.40^a \pm 1.81$ | $7.60^a \pm 0.89$ | $8.20^a \pm 1.09$ |
| **Color** | $8.00^a \pm 1.22$ | $7.60^a \pm 1.67$ | $7.80^a \pm 0.83$ | $8.00^a \pm 1.00$ |
| **Consistency** | $7.80^a \pm 1.64$ | $7.00^a \pm 1.87$ | $7.00^a \pm 1.87$ | $7.20^a \pm 2.04$ |
| **Smell** | $7.80^a \pm 1.78$ | $6.60^a \pm 2.88$ | $6.80^a \pm 2.58$ | $7.20^a \pm 1.64$ |
| **Taste** | $7.00^a \pm 1.58$ | $6.00^a \pm 2.23$ | $6.00^a \pm 3.24$ | $8.20^a \pm 1.30$ |
| **General appreciation** | $8.20^a \pm 1.09$ | $6.60^a \pm 2.07$ | $6.40^a \pm 2.70$ | $7.80^a \pm 1.78$ |
| **Affordability** | $1.40^a \pm 0.54$ | $2.20^a \pm 0.83$ | $2.00^a \pm 1.00$ | $1.40^a \pm 0.89$ |

K: Control Group (Sample of ayran); $K_1$: Sample of ayran with 5% *Aloe ver)* gel added; $K_2$: Sample of ayran with 2.5 % *Aloe vera* gel added; $K_3$: Sample of ayran with 1.25 % *Aloe vera* gel added.
Different superscript letters within the same row indicate a statistically significant difference (p>0.05).

In the market research, the affordability of the yogurt samples was investigated. Groups K and K3 received the same scores, indicating that the addition of up to 3% *Aloe vera* gel did not significantly impact affordability. However, K2 received a lower score, and K1, with the highest *Aloe vera* gel content (5%), received the lowest affordability score. This suggests that as the proportion of added *Aloe vera* gel increased, the affordability of the yogurt decreased.

Overall sensory scores followed a similar trend to affordability scores. As the proportion of added *Aloe vera* gel increased, the sensory scores decreased. However, the sample

with 1.25% *Aloe vera* gel received the same sensory score as the control group, indicating that this level of addition did not negatively impact sensory properties. The study suggests that adding *Aloe vera* gel to yogurt can enhance its sensory properties, but it may also affect its affordability. The addition of up to 5% *Aloe vera* gel showed positive effects on sensory scores, whereas higher concentrations negatively impacted both sensory and affordability aspects. Further research is needed to determine the optimal level of *Aloe vera* gel addition that balances sensory improvements with cost-effectiveness.

*Aloe vera* is a valuable plant for food applications because of its phenolic and flavonoid components. This study extend the shelf life of ayran and make it functional by natural means using *Aloe vera* gel. In a study by Panesar and Shinde (2012), the use of Aloe vera gel in fortified probiotic yoghurts resulted in a decrease in syneresis values from 4.7% to 8.3% during 28 days of storage at 4°C. pH values decreased from 4.03 to 3.91, *Lactobacillus acidophilus* counts decreased from 39.7x10^9 cfu/ml to 32.1x10^9 cfu/ml, and *Bifidobacterium bifidum* counts decreased from 16.9x10^9 cfu/ml to 7.3x10^9 cfu/ml. The study concluded that yoghurts with *Aloe vera* addition can be used as a sufficient probiotic product as they contain more bacteria than the recommended level.

Mudgil et al. (2016) by; *Aloe vera* juice added to ayran at the rate of 5-20% has a positive effect on the phase in ayran. It has been reported that it reduces separation and increases viscosity. Additionally, 10% *Aloe vera* juice was added to drinking yogurt, which was given the highest score by the panelists, and *Aloe vera* juice supplement was found to be nutritious, It has been emphasized that it improves physochemical and sensory properties. Bassannavar et al. (2014) reported that the addition of Aloe vera gel powder at 0.5% and 1% rates to fermented milk increased angiotensin-converting enzyme (ACE) inhibitor activity and the degree of proteolysis.

Sensory analysis was performed in a fermented yogurt-type milk drink with the addition of aloe vera crystals and granadilla (Passiflora ligularis Juss). Samples with 15% aloe vera and 5% granadilla received the highest scores in the sensory analysis (Gutiérrez-Álzate et al., 2020). The results of this study are similar to those obtained by Wijesundara and Adikari (2017), who studied different yogurt formulations with the addition of aloe vera. In their study, the best sensory results were obtained with the formulation containing 15% aloe vera. A similar result was also found by Govindammal et al. (2017), who showed that yogurt with 15% aloe vera was the most acceptable option. Similarly, in this study, no statistical difference was

observed between the control group and the other samples. The samples with 5% aloe vera added had the highest purchase intention.

## 4. CONCLUSION

This study aimed to determine the extraction conditions of *Aloe vera* that would yield the highest total phenolic content, DPPH scavenging activity, and total flavonoid content. For the *Aloe vera* gel, ultrasonic-assisted extraction with water as the solvent was the most effective. For *Aloe vera* leaves, ultrasonic-assisted extraction with a solvent mixture of ethanol and water (1:1) was the most effective. Sensory analysis showed that *Aloe vera* gel was acceptable in ayran (a yogurt-based beverage) at a concentration of up to 5%. These findings provide valuable information for the industrial extraction of bioactive compounds from *Aloe vera*. The use of *Aloe vera* gel in ayran is a promising application that could be further explored. Future research should investigate the use of different *Aloe vera* forms in various food products to expand its use in the food industry.

## DECLARATIONS

There is no conflict of interest between the authors.

## AUTHORS' CONTRIBUTIONS

The author contributes the study on his/her own.

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